SOUTH AFRICAN

South African Journal of Botany 85 (2013) 63-69

Contents lists available at SciVerse ScienceDirect



South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

# Effect of NaCl on growth and Cd accumulation of halophyte *Spartina alterniflora* under CdCl<sub>2</sub> stress

# M.W. Chai <sup>a,b</sup>, F.C. Shi <sup>a,\*\*</sup>, R.L. Li <sup>b,\*</sup>, F.C. Liu <sup>c</sup>, G.Y. Qiu <sup>b</sup>, L.M. Liu <sup>d</sup>

<sup>a</sup> College of Life Sciences, Nankai University, Weijin Road 94, Tianjin 300071, China

<sup>b</sup> School of Environment and Energy, Shenzhen Graduate School of Peking University, Shenzhen 518055, China

<sup>c</sup> College of Life Sciences, Cangzhou Normal University, Cangzhou 061001, China

<sup>d</sup> Environmental Health Management Center of Tianjin Port, Tianjin 300450, China

#### ARTICLE INFO

Article history: Received 1 August 2012 Received in revised form 25 October 2012 Accepted 7 December 2012 Available online 13 January 2013

Edited by TR Marks

Keywords: NaCl Cd stress Cd accumulation Spartina alterniflora

## ABSTRACT

A study quantifying the effect of NaCl on growth and Cd accumulation of Spartina alterniflora subjected to Cd stress was conducted. Seedlings were cultivated in the presence of 1 or 3 mM Cd alone, or combined with NaCl (50 or 100 mM). The results showed that NaCl magnified the phytotoxicity of moderate Cd stress (1 mM Cd) on plants due to reduced levels of plant biomass, plant height, and chlorophyll a + b, while no synergistic effects were recorded under severe Cd stress (3 mM Cd). Proline and Ca<sup>2+</sup> accumulated along with additional NaCl under moderate Cd stress, instead of reduced or unchanged levels under severe Cd stress owing to different adoption strategies caused by NaCl under different Cd stresses. NaCl reduced the oxidative stress in Cd-treated plants through increasing levels of antioxidative enzymes (catalase (CAT) and peroxidase (POD)) under moderate Cd stress. With NaCl addition, Cd<sup>2+</sup> contents in *S. alterniflora* increased and reduced under moderate and severe Cd stress, respectively. However, total Cd<sup>2+</sup> amounts increased with increasing NaCl concentration due to biological dilution. NaCl improved the increase of Cd<sup>2+</sup> translocation factor (TF) under moderate Cd stress, indicating that NaCl might improve Cd<sup>2+</sup> uptake and translocation from roots to shoots, and enhance the phytoextraction of S. alterniflora on Cd; while phytostabilization of Cd under severe Cd stress may be possible due to the reduced TF. Thus, NaCl alleviated phytotoxicity caused by Cd stress through improved management of osmotic solutes and oxidative status, and affected Cd accumulations in S. alterniflora differently under moderate and severe Cd stresses.

© 2013 SAAB. Published by Elsevier B.V. All rights reserved.

# 1. Introduction

With continuous anthropogenic activities including urbanization, industrialization, and utilization of pesticides and fertilizers, undesirable heavy metals have been discharged directly into rivers or lakes, leading to the pollution of estuary salt marshes in the last few decades (Defew et al., 2005; Han et al., 2012a). The deposited heavy metals may greatly impair plant growth and development, and finally will be especially risky to animal and human health, due to their accumulation through food chains (Zhou and Song, 2004). In China, many saline depressions in the eastern coastal region often constitute sites of accumulation of industrial effluents contaminated by heavy metals because of rapid industrial development and excessive use of fertilizers, etc. (Shi et al., 2009; Han et al., 2012a). As a widespread pollutant, heavy metal Cd not only interferes with a number of metabolic processes in plants such as photosynthesis, respiration, or nutritional status (Prasad, 1995; Sandalio et al., 2001), but also causes oxidative

\* Corresponding to: Tel.: +86 755 26033141; Fax: +86 755 26032078.

\*\* Correspondence to: Tel.:+86 22 23502477; Fax: +86 22 23502477. E-mail addresses: fcshi@nankai.edu.cn (F.C. Shi), liruili@pkusz.edu.cn (R.L. Li). stress by inducing generation of reactive oxygen species, as well as disturbances in the antioxidative systems for the detoxification of reactive oxygen species (ROS) (Rodríguez-Serrano et al., 2006; Zhang et al., 2010). Furthermore, Cd poses a potential hazard to human health by entering the food chains, causing severe illness such as Itai-itai disease in Japan (Inaba et al., 2005).

Phytoremediation is an environmentally friendly and relatively cheap technique for removal of pollutants from soils compared with other physicochemical techniques (Schwitzguèbel et al., 2009). However, many plants used for heavy metal pollution were glycophytes, such as *Thlaspi caerulescens* (Milner and Kochian, 2008) and *Brassica juncea* (John et al., 2009), not suitable in phytoremediation of heavy metals from brackish portions of coastal tidal marshes. Halophytes, different from glycophytes, not only display specific adaptations in terms of oxidative status in response to osmotic and ionic constrains caused by salinity (Flowers and Colmer, 2008; Ellouzi et al., 2011), but also possess various physiological adaptations in coping with ion toxicity, being useful for phytomanagement of heavy metal polluted salt marshes (Ruan et al., 2010; Manousaki and Kalogerakis, 2011). Salinity alleviated phytotoxicity caused by Cd stress in halophytes *Sesuvium portulacastrum* (Ghnaya et al., 2007) and *Kosteletzkya virginica* (Han et al., 2012a,b),

<sup>0254-6299/\$ -</sup> see front matter © 2013 SAAB. Published by Elsevier B.V. All rights reserved. http://dx.doi.org/10.1016/j.sajb.2012.12.004

instead of synergistic effects of salinity and Cd stress on glycophytes, including wheat (Shafi et al., 2010) and cole (Raziuddin et al., 2011), etc. There is evidence that salinity may function in the uptake and translocation of Cd from roots to shoots (Zurayk et al., 2001; Manousaki et al., 2008). Though, Smolders et al. (1998) demonstrated that additional NaCl increased twofold the contents of Cd<sup>2+</sup> in different plant-tissues of salt-tolerant plant *Beta vulgaris*, Lefèvre et al. (2009) reported that NaCl reduced rather than increased Cd accumulation in the xerohalophyte *Atriplex halimus*. Therefore, the effect of salinity on Cd accumulation is plant-specific, which is, at least partly, related to that soil salinity, especially the high content of chloride (Cl<sup>-</sup>) ligands, could change Cd solubility and bioavailability (McLaughlin et al., 1994; Gabrijel et al., 2009). Up to now, to the best of our knowledge, studies for roles of NaCl on accumulation of heavy metals in wetland halophytes were not recognized and investigated thoroughly.

Spartina alterniflora, a fast-growing perennial halophyte species, is native to the Atlantic and Gulf coast of North America, and spreading into many other countries for its powerful reproductive capacity and adaptation to complex abiotic stresses, such as salinity (Ma et al., 2011), waterlogging (Gu and Zhang, 2009), and heavy metals (Chai et al., 2012; Salla et al., 2011), etc. S. alterniflora is a promising halophyte and can be widely used for fodder, sewage treatment, bioactive material and heavy metal adsorbent (Cheng et al., 2007; Liu and Tian, 2002; Shen et al., 2008; Li and Wang, 2009). Since its first introduction into China, S. alterniflora has become established in coastal wetland from Beihai of Guangxi Province in southern China to Haihe estuary of Tianjin in northern China (Yuan and Shi, 2009). Recently, this species has been reported to be potential in soil nutrient improvement (Zhang, 2007) and phytoremediation of heavy metal-polluted wetland (Salla et al., 2011). Until now, the physiological characteristics of S. alterniflora exposed to salinity have been well described (Hester et al., 2001; Vasquez et al., 2006; Li et al., 2010; Ma et al., 2011). Plant physiological responses and Cd accumulations in S. alterniflora under Cd stress were also studied adequately (Li et al., 2009; Li et al., 2011; Chai et al., 2012). However, no information concerning the responses of S. alterniflora to NaCl under Cd stress has been fully studied. Though, Mahon and Carman designed three salinity and six heavy metals to investigate heavy metal distribution in S. alterniflroa (Mahon and Carman, 2008), little information about degree of heavy metal stress and plant's physiological responses were taken into account. Therefore, this work studies the physiological responses and Cd accumulation of S. alterniflora seedlings to Cd stress individually or in combination with NaCl. Parameters of plant growth, osmotic solutes, antioxidative enzymes, and Cd accumulation in S. alterniflora were investigated, separately.

#### 2. Material and methods

#### 2.1. Plant material and growth conditions

In June, 2011, new emerged culms of S. alterniflora were collected from the seashore (39°03'N, 117°45'E) in Tianjin, China, where S. alterniflora has occupied the naked muddy beach and formed dense vegetative colonies. Uniform seedlings (10 cm height) were transplanted into plastic pots (18 cm diameter × 15 cm depth) filled with 600 g dry and clean vermiculite for 10 days in order to minimize the influences of various stress factors in its natural habitat. To simulate the salinity and Cd pollution in natural habitat of S. alterniflora, NaCl and CdCl<sub>2</sub> were selected and added to the corresponding pots to create 7 treatments (Table 1): Control plants were cultivated on normal Hoagland's nutrient solution (Shi et al., 1998) without CdCl<sub>2</sub> and NaCl, while experimental groups include two individual Cd treatments, 1 and 3 mM CdCl<sub>2</sub> (moderate and severe Cd stresses) and four treatments combining CdCl<sub>2</sub> (1 or 3 mM) and NaCl (50 or 100 mM). Each of seven treatments had twelve sample seedlings, with one seedling per pot. All the pots were placed outdoors (day temperature 22-28 °C, night temperature 16-22 °C) and protected from the rain. Seedlings were carefully irrigated with

#### Table 1

Experimental design of different Cd and NaCl treatments.

Treatments	CdCl <sub>2</sub> concentration (mM)	NaCl concentration (mM)
Cd0Na0	0	0
Cd1Na0	1	0
Cd2Na0	3	0
Cd1Na1	1	50
Cd1Na2	1	100
Cd2Na1	3	50
Cd2Na2	3	100

100 ml Hoagland's nutrient solution every 4 days. In order to ensure the constant concentrations of Cd and NaCl, all the pots were weighed to the same weight through adding distilled water daily, with water level not lower than the vermiculite surface. Three months later, plants were collected and analyzed.

#### 2.2. Determinations

All plants were harvested carefully after 90 days of stress treatments. Plant height was measured. Shoots and roots were separated and rinsed with distilled water fully, followed by proper blotting with filter papers. During the time period from 8:00 to 11:00 in the morning, contents of chlorophyll a + b, proline, soluble sugar, and activities of antioxidant enzymes in fresh leaves were measured.

#### 2.2.1. Assays of chlorophyll a + b, proline, and soluble sugar

Leaf chlorophyll a + b content was determined according to the method described by Hao et al. (2004). The contents of proline and soluble sugar were determined using the methods of ninhydrin colorimetry (Bates et al., 1973) and phenol-sulfuric acid (Dubois et al., 1956), respectively.

#### 2.2.2. Assays of antioxidant enzymes

The fresh leaves from each treatment were homogenized in a pestle and mortar with 0.05 M sodium phosphate buffer (pH 7.5). The homogenate was centrifuged at 10,000 r/min for 20 min and the supernatant was used for analyzing superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). SOD activity was measured as described by Beyer and Fridovich (1987). CAT activity was determined by the method of Clairborne (1985). POD activity was assayed as reported by Chance and Maehly (1955).

#### 2.2.3. Cation assay

On the same day of plant harvest, shoots and roots were separately dried at 105 °C for 30 min and then at 70 °C until a constant weight was reached. Desiccated samples were ground to fine power, and then digested with  $HNO_3/HCIO_4$  (10:1 v/v). Contents of  $Cd^{2+}$ ,  $Na^+$ ,  $K^+$ , and  $Ca^{2+}$  were determined by atomic absorption spectrophotometer (Z-6100; Hitachi, Japan).

Total  $Cd^{2+}$  accumulations were then derived from the  $Cd^{2+}$  contents in shoots and roots as described above and the partitioned dry weights for shoots and roots. The translocation factor (TF) is defined as the ratio of metal concentration in shoots to that in roots, which is used to measure the effectiveness of a plant in transporting heavy metal from roots to shoots (Stoltz and Greger, 2002).

#### 2.3. Statistical analysis

Each treatment was replicated 3 times for statistical validity. All data was expressed as means  $\pm$  S.E. Analysis of variance was performed for all data sets. Tukey's HSD tests were performed to determine if the differences between individual treatments were significant (*P*<0.05).

# 3. Results

#### 3.1. Plant growth

Cd in combination with NaCl caused death of most plants in 3 mM Cd + 100 mM NaCl treatment at the end of the experiments. Therefore, the physiological data was obtained from survivor plants in the remaining 6 treatments. In Fig. 1, levels of shoot biomass, root biomass, plant height, and chlorophyll a + b were all severely reduced under 1 or 3 mM Cd stress alone compared to control (*P*<0.05). Furthermore, these parameters reduced significantly in 1 mM Cd + 100 mM NaCl treatment, compared to plants treated by 1 mM Cd stress alone (*P*<0.05), while unchanged or slightly increased trends were recorded with NaCl addition under 3 mM Cd stress.

### 3.2. Organic and inorganic osmotic solutes

In Fig. 2A and B, contents of proline and soluble sugar increased in the presence of 1 or 3 mM Cd stress alone compared to control (P<0.05). NaCl sharply enhanced the production of proline and soluble sugar under 1 mM Cd stress (P<0.05). In contrast, unchanged or slightly declining trends were observed after NaCl addition under 3 mM Cd stress.

There was no obvious change of Na<sup>+</sup> content subjected to Cd and/or NaCl (Fig. 2C). In Fig. 2D, NaCl enhanced K<sup>+</sup> contents regardless of the level of Cd stress (P<0.05). Fig. 2E showed that Na<sup>+</sup>/K<sup>+</sup> ratios were significantly reduced by NaCl under 1 and 3 mM Cd stresses (P<0.05). Furthermore, Cd stress alone markedly increased Ca<sup>2+</sup> contents (P<0.05), and NaCl dramatically increased Ca<sup>2+</sup> contents under 1 mM Cd stress (P<0.05). In addition, no significant changes of Ca<sup>2+</sup> contents were observed under 3 mM Cd stress (Fig. 2F).

#### 3.3. Activities of SOD, CAT and POD

In Fig. 3, there were significantly increased activities of SOD, CAT and POD under 3 mM Cd stress alone compared to control (P<0.05).

However, such effects on activities of these antioxidant enzymes were not recorded in the presence of 1 mM Cd stress alone, except SOD. NaCl obviously reduced SOD activities regardless of the level of Cd stress (P<0.05). Under 1 mM Cd stress, activities of CAT and POD were strongly improved with increasing NaCl concentration (P<0.05), while, reduced activities of CAT and POD with NaCl addition were detected under 3 mM Cd stress (P<0.05).

#### 3.4. Cd accumulation

In different Cd and/or with NaCl treatments,  $Cd^{2+}$  accumulated more in the roots than in the shoots (Fig. 4). For both shoots and roots, NaCl increased and reduced  $Cd^{2+}$  contents under 1 and 3 mM Cd (P<0.05), respectively (Fig. 4A, B). Under 1 mM Cd, the total Cd accumulation in shoots was unchanged, while increased significantly with additional NaCl in roots (P<0.05) (Fig. 4C). Fig. 4D showed that NaCl increased the amounts of  $Cd^{2+}$  under 3 mM Cd (P<0.05). In addition, with increasing NaCl concentration, TFs of Cd<sup>2+</sup> significantly increased and reduced under 1 and 3 mM Cd (P<0.05), respectively (Fig. 4E).

# 4. Discussion

Excess  $Cd^{2+}$  not only induces chlorosis and growth reduction of shoots and roots, but also inhibits or stimulates activities of several antioxidative enzymes before any visible symptom of toxicity appears (Pál et al., 2006; Zhang et al., 2009). In the present study, chlorosis started to appear on leaves after 30 days of Cd exposure and progressed till the end of the treatment. Under moderate Cd stress (1 mM Cd), shoot biomass, root biomass, plant height, and chlorophyll a + b content were all reduced obviously with additional NaCl due to enhancement of NaCl on CdCl<sub>2</sub> stress (Fig. 1), in agreement with previous studies (Shafi et al., 2009; Raziuddin et al., 2011). However, under severe Cd stress (3 mM Cd), unchanged or slight recovery trends indicated that NaCl did not intensify phytotoxicity caused by Cd to a certain extent. Though,



**Fig. 1.** Shoot biomass (A), root biomass (B), plant height (C), and content of chlorophyll a + b (D) in *Spartina alterniflora* seedlings under different Cd and NaCl treatments. Values represent means  $\pm$  S.E. Values at each treatment group followed by different letters are significantly different (*P*<0.05). Treatments consist of two levels of Cd (1 mM, Cd1; 3 mM, Cd2) as well as two levels of NaCl (50 mM, Na1; 100 mM, Na2). Cd0, 0 mM Cd; Na0, 0 mM NaCl.



**Fig. 2.** Contents of proline (A), soluble sugar (B),  $Na^+$  (C),  $K^+$  (D),  $Na^+/K^+$  (E), and  $Ca^{2+}$  (F) in *Spartina alterniflora* seedlings under different Cd and NaCl treatments. Values represent means  $\pm$  S.E. Values at each treatment group followed by different letters are significantly different (*P*<0.05). Treatments consist of two levels of Cd (1 mM, Cd1; 3 mM, Cd2) as well as two levels of NaCl (50 mM, Na1; 100 mM, Na2). Cd0, 0 mM Cd; Na0, 0 mM NaCl.

*S. alterniflora* is able to cope with 600 mM NaCl and grows well at 100 mM NaCl (Ma et al., 2011), the intensified or unchanged phytotoxicity occurred with NaCl addition under Cd stresses. The possible explanation may be that NaCl-induced growth stimulation of *S. alterniflora* and dilution effect may not function as combined with Cd<sup>2+</sup>, being different from those xero-halophyte plant species which are resistant to drought, salt, and heavy metals (Lutts et al., 2004; Zaier et al., 2010). Therefore, osmotic solutes, ion content, and antioxidant enzyme activities were investigated to verify whether or not NaCl could improve Cd resistance in *S. alterniflora* not for any dilution effect related to growth stimulation.

In order to deal with oxidative damages, some osmotic solutes in plant species are accumulated and often regarded as a basic strategy for protection and survival under various stresses (Parida and Das, 2005). Proline has been observed in plant species subjected to heavy metal stress, being beneficial for plants by three major actions, namely metal binding, antioxidant defense, and signaling (Sharma and Dietz, 2006). In the present study, NaCl increased and reduced proline content under moderate and severe Cd stresses (Fig. 2A), showing synergistic and antagonistic effects of NaCl on phytotoxicity caused by Cd stress, respectively. It was also noteworthy that NaCl obviously reduced soluble sugar content under moderate Cd stress (Fig. 2B), which may be due to the reason that some soluble sugar was used to synthesize proline by providing carbon skeleton and power (Manuel and Reigosa, 2001). However, the slightly reduced levels of soluble sugar under severe Cd

stress may be related to the alleviated osmotic stress with NaCl addition. Thus, the mechanism of soluble sugar in halophytes' response to NaCl under different Cd stresses may be varied and deserves further investigation.

Previous studies have shown that Cd stress reduced shoot K<sup>+</sup> concentration in halophytes, such as S. portulacastrum, Mesembryanthemum crystallinum, and K. virginica (Ghnaya et al., 2005; Han et al., 2012a). Our results showed that the significant decrease of shoot K<sup>+</sup> contents in Cd-treated plants suggested that Cd<sup>2+</sup> impaired K<sup>+</sup> nutrition to a certain extent (Fig. 2D). Under both Cd stresses, increased K<sup>+</sup> contents with NaCl addition indicated that NaCl ameliorated phytotoxicity caused by Cd through increasing K<sup>+</sup> content under Cd stresses, being inconsistent with conclusions of Han et al. (2012a) who found that NaCl reduced K<sup>+</sup> contents in stems and leaves of K. virginica under Cd stress. Thus, the mechanism of K<sup>+</sup> in responses of plant to NaCl under Cd stress may be varied and deserves to be investigated further. High Na<sup>+</sup>/K<sup>+</sup> ratio indicates metabolic disorders such as a reduction of protein synthesis and enzyme activities (Brady et al., 1984), as well as an increase in membrane permeability (Alam, 1999). Under moderate and severe Cd stresses, NaCl reduced Na<sup>+</sup>/K<sup>+</sup> ratio significantly, so as to recover protein synthesis and enzyme activities, and reduce membrane permeability (Li et al., 2010).

 $Ca^{2+}$  is involved in plant's response to various environment stresses by increasing antioxidant enzyme activities and reducing lipid peroxidation (Jiang and Huang, 2001). Under Cd stress,  $Cd^{2+}$  entered root



**Fig. 3.** Activities of SOD (A), CAT (B), and POD (C) in *Spartina alterniflora* seedlings under different Cd and NaCl treatments. Values represent means  $\pm$  S.E. Values at each treatment group followed by different letters are significantly different (*P*<0.05). Treatments consist of two levels of Cd (1 mM, Cd1; 3 mM, Cd2) as well as two levels of NaCl (50 mM, Na1; 100 mM, Na2). Cd0, 0 mM Cd; Na0, 0 mM NaCl.

cells via either higher affinity uptake system for  $Fe^{3+}$  or low affinity system for  $Ca^{2+}$  uptake (Zhao et al., 2002). In the present study,  $Ca^{2+}$ rose up to the highest level in 1 mM Cd + 100 mM NaCl treatment, indicating that the increased absorption of  $Ca^{2+}$  by NaCl may compete with  $Cd^{2+}$  in absorption and uptake of plants, and take part in detoxification of Cd stress (Fig. 2F). Analogously, elevated  $Ca^{2+}$  content was reported to alleviate the toxicity of  $Cd^{2+}$  in Arabidopsis (Suzuki et al., 2005). However, under severe Cd stress, the slightly decreased  $Ca^{2+}$  contents may not be considered as a major cause of growth impairment since NaCl reduced  $Ca^{2+}$  content to a similar extent without showing any detrimental effect on growth process (Ghanem et al., 2010; Han et al., 2012a).

Meanwhile, to reduce the oxidative damages, plant species possess scavenging systems consisting in non-enzymatic antioxidants such as glutathione (GSH), which acts as ROS scavenger by chelating metal ions (Yadav, 2010) and counteracts the harmful effects of ROS, as well as antioxidant enzymes, such as SOD, CAT, and POD (Qureshi et al., 2007; Zhang et al., 2010). As the first enzyme in detoxifying process for ROS, SOD converts  $O_2^-$  radicals to  $H_2O_2$  (Zhang et al., 2010). POD reduces  $H_2O_2$  to water using various substrates as electron donors, and CAT dismutates  $H_2O_2$  into water and oxygen (Wang et al., 2009). Han et al. (2012b) found that salinity affords efficient protection against Cd to the halophyte species *K. virginica*, in relation to an improved management of oxidative stress and hormonal status. Furthermore, the combined treatment of Cd and NaCl showed a synergistic negative effect on *Triticum aestivum* by improving activities of SOD, POD, and CAT (Shafi et al., 2009). Our data indicated that severe Cd stress alone induced an oxidative stress as observed by the increased activities of SOD, CAT, and POD (Fig. 3). Additional NaCl reduced SOD activity under moderate and severe Cd stress (Fig. 3A), suggesting that NaCl restrained the conversion of  $O_2^-$  radicals to  $H_2O_2$ . However, with increasing NaCl concentration, the activities of POD and CAT increased and reduced under moderate and severe Cd stresses (Fig. 3B, C), corresponding to the improved and inhibited scavenging processes of  $H_2O_2$ , respectively. Thus, SOD functioned differently from CAT and POD in responding to NaCl under moderate and severe Cd stresses, which should be investigated further.

Though, S. alterniflora has changed the landscape and biodiversity in some coastal regions of China, this species has received greater attention due to its buffering against storm tides, accelerating accretion and reclamation, absorbing nutrients and digesting pollutants (Wan et al., 2009). Previous studies have shown that S. alterniflora could not only desalinate salt marsh, and clean eutrophic sewage (Liu and Tian, 2002), but also restore coastal wetland polluted by heavy metals (Mahon and Carman, 2008; Salla et al., 2011). Salt has been considered to enhance the resistance of halophyte species to abiotic stress, such as water-deficit stress (Martínez et al., 2005) and heavy metals, especially Cd stress (Zurayk et al., 2001; De la Rosa et al., 2004; López-Chuken and Young, 2005). In the present study, NaCl increased  $Cd^{2+}$  contents (µg g<sup>-1</sup>) in shoots and roots under moderate Cd stress (Fig. 4A, B). Several explanations are possible: (1) increased Cd<sup>2+</sup> free ion activity through cation exchange with salt cation (Bingham et al., 1984); (2) direct uptake of chloride complexes of Cd<sup>2+</sup>, such as CdCl<sup>+</sup> and CdCl<sub>2</sub> (Smolders and McLaughlin, 1996); (3) increase in  $Cd^{2+}$  free cation concentration in the soil pore water reduced diffusion distances to root surfaces (Smolders et al., 1998). Under severe Cd stress, NaCl reduced  $Cd^{2+}$  contents (µg g<sup>-1</sup>) in shoots and roots, in agreement with previous studies (Manousaki et al., 2008, 2009). One possible hypothesis may be that a certain proportion of Cd<sup>2+</sup> was fixed by root mucilage, which deserves further investigation. In addition, the amounts of Cd<sup>2+</sup> accumulated in shoots and roots ( $\mu$ g plant<sup>-1</sup>) were enhanced by NaCl under severe Cd stress (Fig. 4C, D), so reduced  $Cd^{2+}$  content in plants in combined treatment of Cd and NaCl were essentially related to biological dilution of Cd<sup>2+</sup> by growth to some extent.

Salinity could not only affect bioavailability of heavy metals in soil due to reduced soil metal sorption, but also the translocation of metals from roots to the aerial parts of the plants (Fitzgerald et al., 2003; Wahla and Kirkham, 2008). Han et al. (2012a) reported that salinity reduced Cd<sup>2+</sup> uptake and translocation from roots to shoots in K. virginica, while Ghnaya et al. (2007) found that the translocation factor of Cd<sup>2+</sup> in S. portulacastrum increased significantly with NaCl addition. Our results showed that under moderate Cd stress, more and more Cd<sup>2+</sup> was translocated up into the shoots from roots with increasing NaCl concentration (Fig. 4E), which may be attributed to increased Cd<sup>2+</sup> uptake and translocation of complexed forms CdCl<sup>+</sup> and CdCl<sub>2</sub>; while Cd translocation factor were reduced under severe Cd stress, guaranteeing conduction of various important metabolic activities including photosynthesis in the aboveground parts. Over all, the degree of Cd stress should be considered when evaluating S. alterniflora's response to NaCl under Cd stress. The underlying reason for the inconsistent Cd<sup>2+</sup> metastatic ability in *S. alterniflora* under different Cd stresses remains elusive, which should be investigated further.

#### Acknowledgement

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



**Fig. 4.**  $Cd^{2+}$  contents in shoots (A) and roots (B), total  $Cd^{2+}$  accumulation in shoots (C) and roots (D), and translocation factor (E) in *Spartina alterniflora* seedlings under different Cd and NaCl treatments. Values represent means  $\pm$  S.E. Values at each treatment group followed by different letters are significantly different (*P*<0.05). Treatments consist of two levels of Cd (1 mM, Cd1; 3 mM, Cd2) as well as two levels of NaCl (50 mM, Na1; 100 mM, Na2). Cd0, 0 mM Cd; Na0, 0 mM NaCl.

#### References

- Alam, S.M., 1999. Nutrient uptake by plants under stress conditions. In: Pessarakli, M. (Ed.), Handbook of Plant and Crop Stress. Marcel Dekker, New York, Basel, pp. 285–313.
- Bates, L.S., Waldron, R.P., Teare, I.D., 1973. Rapid determination of free proline for water stress studies. Plant and Soil 39, 205–207.
- Beyer, W.F., Fridovich, I., 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Analytical Biochemistry 161, 559–566.
- Bingham, F.T., Sposito, G., Strong, J.E., 1984. The effect of chloride on the availability of cadmium. Journal of Environmental Quality 13, 71–74.
- Brady, C.J., Gibson, T.S., Barlow, E.W.R., Speirs, J., Wyn Jones, R.G., 1984. Salt tolerance in plants. I. Ions compatible organic solutes and the stability of plant ribosomes. Plant, Cell & Environment 7, 571–578.
- Chai, M.W., Li, R.L., Shi, F.C., Liu, F.C., Pan, X., Cao, D., Wen, X., 2012. Effects of cadmium stress on growth, metal accumulation and organic acids of *Spartina alterniflora* Loisel. African Journal of Biotechnology 11, 6091–6099.
- Chance, B., Maehly, A.C., 1955. Assay of catalase and peroxidases. Methods in Enzymology 1, 764–775.
- Cheng, X.L., Peng, R.H., Chen, J.Q., Luo, Y.Q., Zhang, Q.F., An, S.Q., Chen, J.K., Li, B., 2007. CH<sub>4</sub> and N<sub>2</sub>O emissions from *Spartina alterniflora* and *Phragmites australis* in experimental mesocosms. Chemosphere 68, 420–427.
- Clairborne, A., 1985. Catalase activity. In: Greenwald, R.A. (Ed.), handbook of methods for oxygen radical research. CRC Press, Boca Raton, pp. 283–284.
- De la Rosa, G., Peralta-Videa, J.R., Montes, M., Parsons, J.G., Cano-Aguilera, I., Gardea-Torresdey, J.L., 2004. Cadmium uptake and translocation in tumbleweed (Salsola kali), a potential Cd-hyperaccumulator desert plant species: ICP/OES and XAS studies. Chemosphere 55, 1159–1168.
- Defew, L.H., Mair, J.M., Guzman, H.M., 2005. An assessment of metal contamination in mangrove sediments and leaves from Punta Mala Bay, Pacific panama. Marine Pollution Bulletin 50, 547–552.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28, 350–356.
- Ellouzi, H., Ben-Hamed, K.B., Cela, J., Munné-Bosch, S., Abdelly, C., 2011. Early effects of salt stress on the physiological and antioxidative status of *Cakile maritima* (halophyte) and *Arabidopsis thaliana* (glycophyte). Physiologia Plantarum 142, 128–143.

- Fitzgerald, F.J., Caffrey, J.M., Nesaratnam, S.T., McLoughlin, P., 2003. Copper and lead concentrations in salt marsh plants on the Suir Estuary, Ireland. Environmental Pollution 123, 67–74.
- Flowers, T.J., Colmer, T.D., 2008. Salinity tolerance in halophytes. The New Phytologist 179, 945–963.
- Gabrijel, O., Davor, R., Zed, R., Marija, R., Monika, Z., 2009. Cadmium accumulation by muskmelon under salt stress in contaminated organic soil. The Science of the Total Environment 407, 2175–2182.
- Ghanem, M.E., Han, R.M., Classen, B., Quetin-Leclerq, J., Mahy, G., Ruan, C.J., Qin, P., Pérez-Alfocea, F., Lutts, S., 2010. Mucilage and polysaccharides in the halophyte plant species *Kosteletzkya virginica*: localization and composition in relation to salt stress. Journal of Plant Physiology 167, 382–392.
- Ghnaya, T., Nouairi, I., Slama, I., Messedi, D., Grignon, C., Abdelly, C., Ghorbel, M.H., 2005. Cadmium effects on growth and mineral nutrition of two halophytes: *Sesuvium portulacastrum* and *Mesembryanthemum crystallinum*. Journal of Plant Physiology 162, 1133–1140.
- Ghnaya, T., Slama, I., Messedi, D., Grignon, C., Ghorbel, M.H., Abdelly, C., 2007. Cd-induced growth reduction in the halophyte *Sesevium portulacastrum* is significantly improved by NaCl. Journal of Plant Research 120, 309–316.
- Gu, Z.Q., Zhang, L.Q., 2009. Physiological responses of Spartina alterniflora to long-term waterlogging stress. Acta Scientiae Circumstantiae 29, 876–881.
- Han, R.M., Lefèvre, I., Ruan, C.J., Qin, P., Lutts, S., 2012a. NaCl differently interferes with Cd and Zn toxicities in the wetland halophyte species *Kosteletzkya virginica* (L.) Presl. Plant Growth Regulation 68, 97–109.
- Han, R.M., Lefèvre, I., Albacete, A., Pèrez-Alfocea, F., Barba-Espín, G., Díaz-Vivancos, P., Quinet, M., Ruan, C.J., Hernández, J.A., Cantero-Navarro, E., Lutts, S., 2012b. Antioxidant enzyme activities and hormonal status in response to Cd stress in the wetland halophyte Kosteletzkya virginica under saline conditions. Physiologia Plantarum. http:// dx.doi.org/10.1111/j.1399-3054.2012.01667.x.
- Hao, Z.B., Cang, J., Xu, Z., 2004. Plant Physiology Experiment. Harbin Institute of Technology Press, Harbin.
- Hester, M.W., Mendelssohn, I.A., Mckee, K.L., 2001. Species and population variation to salinity stress in *Panicum hemitomon*, *Spartina patens*, and *Spartina alterniflora*: morphological and physical constraints. Environmental and Experimental Botany 46, 277–297.
- Inaba, T., Kobayashi, E., Suwazono, Y., Uetani, M., Oishi, M., Nakagawa, K., 2005. Estimation of cumulative cadmium intake causing Itai-itai disease. Toxicology Letters 159, 192–201.

- Jiang, Y.W., Huang, B.R., 2001. Effects of cadmium on antioxidant activities and water relations associated with heat tolerance in two cool-season grasses. Journal of Experimental Botany 52, 341–349.
- John, R., Ahmad, P., Gadgil, K., Sharma, S., 2009. Heavy metal toxicity: Effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea L. In*ternational Journal of Plant Production 3, 65–76.
- Lefèvre, I., Marchal, G., Meerts, P., Corréal, E., Lutts, S., 2009. Chloride salinity reduces cadmium accumulation by the Mediterranean halophyte species *Atriplex halimus* L. Environmental and Experimental Botany 65, 142–152.
- Li, K.Q., Wang, X.H., 2009. Adsorptive removal of Pb (II) by activated carbon prepared from Spartina alterniflora: Equilibrium, kinetics and thermodynamics. Bioresource Technology 100, 2810–2815.
- Li, L.X., Zhao, J.Q., Chen, S.H., Pu, Y.T., Guo, S.L., 2009. Physiological response of Spartina alterniflora L. to heavy metal Cd stress. Journal of Yantai University (Natural Science and Engineering Edition) 22, 329–332.
- Li, R., Shi, F., Fukuda, K., 2010. Interactive effects of various salt and alkali stresses on growth, organic solutes, and cation accumulation in a halophyte Spartina alterniflora (Poaceae). Environmental and Experimental Botany 68, 66–74.
- Li, J.M., Shi, G.Y., Wei, Y., 2011. Effects of cadmium on growth and physiological characteristics of *Spartina alterniflora*. Journal of Anhui Agricultural Sciences 39 (2174–2176), 2182.
- Liu, J.P., Tian, Z.K., 2002. Effect of Spartina alterniflora on sewage depuration. Hebei Environmental Science 10, 45–48.
- López-Chuken, U.J., Young, S.D., 2005. Plant screening of halophyte species for cadmium phytoremediation. Zeitschrift f
  ür Naturforschung 60, 236–243.
- Lutts, S., Lefèvre, I., Delpérée, C., Kivits, S., Dechamps, C., Robledo, A., Correal, E., 2004. Heavy metal accumulation by the halophyte species *Mediterranean saltbush*. Journal of Environmental Quality 33, 1271–1279.
- Ma, J.G., Chai, M.W., Shi, F.C., 2011. Effects of long-term salinity on the growth of the halophyte Spartina alterniflora Loisel. African Journal of Biotechnology 10, 17962–17968.
- Mahon, S., Carman, K.R., 2008. The influence of salinity on the uptake, distribution, and excretion of metals by the smooth cordgrass, *Spartina alterniflora* (Loisel.), grown in sediment contaminated by multiple metals. Estuaries and Coasts 31, 1089–1097.
- Manousaki, E., Kalogerakis, N., 2011. Halophytes present new opportunities in phytoremediation of heavy metals and saline soils. Industrial and Engineering Chemistry Research 50, 656–660.
- Manousaki, E., Kadukova, J., Papadantonakis, N., Kalogerakis, N., 2008. Phytoextraction and phyto-excretion of Cd by *Tamarix smyrnensis* growing on contaminated non saline and saline soils. Environmental Research 106, 326–332.
- Manousaki, E., Kokkali, F., Kalogerakis, N., 2009. Influence of salinity on lead and cadmium accumulation by the salt cedar (*Tamarix smyrnensis* Bunge). Journal of Chemical Technology and Biotechnology 84, 877–883.
- Manuel, J., Reigosa, R., 2001. Handbook of Plant Ecophysiology Techniques. Kluwer Academic Publishers, Dordrecht, pp. 365–383.
- Martínez, J.P., Kinet, J.M., Bajji, M., Lutts, S., 2005. NaCl alleviates polyethylene glycolinduced water stress in the halophyte species *Atriplex halimus* L. Journal of Experimental Botany 56, 2421–2431.
- McLaughlin, M.J., Palmer, L.T., Tiller, K.G., Beech, T.A., Smart, M.K., 1994. Increased soil salinity causes elevated cadmium concentrations in field grown potato tubers. Journal of Environmental Quality 23, 1013–1018.
- Milner, M.J., Kochian, L.V., 2008. Investigating heavy-metal hyperaccumulation using *Thlaspi caerulescens* as a model system. Annals of Botany 102, 3–13.
- Pál, M., Horváth, E., Janda, T., Páldi, E., Szalai, G., 2006. Physiological changes and defense mechanisms induced by cadmium stress in maize. Journal of Plant Nutrition and Soil Science 169, 239–246.
- Parida, A.K., Das, A.B., 2005. Salt tolerance and salinity effects on plants: a review. Ecotoxicology and Environmental Safety 60, 324–349.
- Prasad, M.N.V., 1995. Cadmium toxicity and tolerance in vascular plants. Environmental and Experimental Botany 35, 525–545.
- Qureshi, M.I., Abdin, M.Z., Qadir, S., Iqbal, M., 2007. Lead-induced oxidative stress and metabolic alterations in *Cassia angustifolia* Vahl. Biologia Plantarum 51, 121–128.
- Raziuddin, Farhatullah, Hassan, G., Akmal, M., Shan, S.S., Mohammad, F., Shafi, M., Bakht, J., Zhou, W., 2011. Effects of cadmium and salinity on growth and photosynthesis parameters of *Brassica* species. Pakistan Journal of Botany 43, 333–340.
- Rodríguez-Serrano, M., Romero-Puertas, M.C., Zabalza, A., Corpas, F.J., Gómez, M., del Río, L.A., Sandalio, L.M., 2006. Cadmium effect on oxidative metabolism of pea (*Pisum sativum L.*) roots. Imaging of reactive oxygen species and nitric oxide accumulation in vivo. Plant, Cell & Environment 29, 1532–1544.
- Ruan, C.J., Teixeira da Silva, J.A., Mopper, S., Qin, P., Lutts, S., 2010. Halophyte improvement for a salinized world. Critical Reviews in Plant Sciences 29, 329–359.
- Salla, V., Hardaway, C.J., Sneddon, J., 2011. Preliminary investigation of Spartina alterniflora for phytoextraction of selected heavy metals in soils from Southwest Louisiana. Microchemical Journal 97, 207–212.

- Sandalio, L., Dalurzo, H.C., Gómez, M., Romero-Puertas, M., del Río, L.A., 2001. Cadmiuminduced changes in the growth and oxidative metabolism of pea plants. Journal of Experimental Botany 52, 2115–2126.
- Schwitzguèbel, J.P., 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, M.J., Raziuddin, M., Zhang, G.P., 2009. Effect of cadmium and salinity stresses on growth and antioxidant enzyme activities of wheat (*Triticum aestivum*]. Bulletin of Environmental Contamination and Toxicology 82, 772–776.
- Shafi, M., Guoping, Z., Bakht, J., Khan, M.A., Ejaz-ul-islam, Khan, M.D.W., Raziuddin, 2010. Effect of cadmium and salinity stresses on root morphology of wheat. Pakistan Journal of Botany 42, 2747–2754.
- Sharma, S.S., Dietz, K.J., 2006. The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. Journal of Experimental Botany 57, 711–726.
- Shen, Y.M., Yang, J.S., Zeng, H., Zhou, Q., 2008. Review of studies on alien species Spartina alterniflora in China. Marine Environmental Science 27, 391–396.
- Shi, D.C., Sheng, Y.M., Zhao, K.F., 1998. Stress effects of mixed salts with various salinities on seedlings of Aneurolepidium Chinense. Acta Botanica Sinica 40, 1136–1142.
- Shi, J., Li, L.Q., Pan, G.X., 2009. Variation of grain Cd and Zn concentrations of 110 hybrid rice cultivars grown in a low-Cd paddy soil. Journal of Environmental Sciences 21, 168–172.
- Smolders, E., Mclaughlin, M.J., 1996. Effect of Cl on Cd uptake by Swiss chard in nutrient solution. Plant and Soil 179, 57–64.
- Smolders, E., Lambregts, R.M., McLaughlin, M.J., Tiller, K.G., 1998. Effects of soil solution chloride on cadmium availability to Swiss chard. Journal of Environmental Quality 27, 426–431.
- Stoltz, E., Greger, M., 2002. Accumulation properties of As, Cd, Cu, Pb and Zn by four wetland plant species growing on submerged mine tailings. Environmental and Experimental Botany 47, 271–280.
- Suzuki, T., Nakajima, S., Morikami, A., Nakamura, K., 2005. An Arabidopsis protein with a novel calcium-binding repeat sequence interacts with TONSOKU/MGOUN3/BRUSHY1 involved in meristem maintenance. Plant & Cell Physiology 46, 1452–1461.
- Vasquez, E.A., Glenn, E.P., Guntenspergen, G.R., Brown, J.J., Nelson, S.G., 2006. Salt tolerance and osmotic adjustment of Spartina alterniflora (Poaceae) and the invasive M haplotype of *Phragmites australis* (Poaceae) along a salinity gradient. American Journal of Botany 93, 1784–1790.
- Wahla, I.H., Kirkham, M.B., 2008. Heavy metal displacement in salt-water-irrigated soil during phytoremediation. Environmental Pollution 155, 271–283.
- Wan, S.W., Qin, P., Liu, J.N., Zhou, H.X., 2009. The positive and negative effects of exotic Spartina alterniflora in China. Ecological Engineering 35, 444–452.
- Wang, W.B., Kim, Y.H., Lee, H.S., Kim, K.Y., Deng, X.P., Kwak, S.S., 2009. Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. Plant Physiology and Biochemistry 47, 570–577.
- Yadav, S.K., 2010. Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. South African Journal of Botany 76, 167–179.
- Yuan, Z.N., Shi, F.C., 2009. Ecological adaptation strategies in alien species: effects of salinity, temperature and photoperiod on *Spartina alterniflora* Loisel. seed germination. Polish Journal of Ecology 54, 677–684.
- Zaier, H., Ghnaya, T., Lakhdar, A., Baioui, R., Ghabriche, R., Mnasri, M., Lutts, S., Abdelly, C., 2010. Comparative study of Pb-phytoextraction potential in *Sesuvium portulacastrum* and *Brassica juncea*: tolerance and accumulation. Journal of Hazardous Materials 183, 609–615.
- Zhang, X.L., 2007. Analysis on the advantages and disadvantages of introduced Spartina to China coastal zones. Chinese Journal of Ecology 26, 1878–1883.
- Zhang, F.Q., Zhang, H.X., Wang, G.P., Xu, L.L., Shen, Z.G., 2009. Cadmium-induced accumulation of hydrogen peroxide in the leaf apoplast of *Phaseolus aureus* and *Vicia sativa* and the roles of different antioxidant enzymes. Journal of Hazardous Materials 168, 76–84.
- Zhang, X.X., Fan, X.M., Li, C.J., Nan, Z.B., 2010. Effects of cadmium stress on seed germination, seedling growth and antioxidative in *Achnatherum inebrians* plants infected with a *Neotyphodium* endophyte. Plant Growth Regulation 60, 91–97.
- Zhao, F.J., Hamon, R.E., Lombi, E., McLaughlin, M.J., McGrath, S.P., 2002. Characteristics of cadmium uptake in two contrasting ecotypes of the hyperaccumulator *Thlaspi caerulescens*. Journal of Experimental Botany 53, 535–543.
- Zhou, Q.X., Song, Y.F., 2004. Principles and methods of contaminated soil remediation. Science Press, Beijing.
- Zurayk, R.A., Khoury, N.F., Talhouk, S.N., Baalbaki, R., 2001. Salinity-heavy metal interactions in four salt-tolerant plant species. Journal of Plant Nutrition 11, 1773–1786.