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## The effects of cadmium on germination and seedling growth of *Suaeda salsa*

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### Abstract

Cadmium ion ( $\text{Cd}^{2+}$ ) is a ubiquitous toxic heavy metal in the environment and presents a potential threat to human health via the food chain through plant root uptake systems. The halophyte, *Suaeda salsa* is the pioneer plant in the Yellow River Delta and has been widely applied as a model plant in environmental pollution assessment. In this work, the study was conducted using a liquid culture with a series of  $\text{Cd}^{2+}$  concentrations. Germination rates and activities of three enzymes (catalase (CAT), glutathione peroxidase (GPX), glutathione S-transferase (GST)) in the seedling were investigated. Results showed that the germination indices and growth inhibition indices of *S. salsa* decreased significantly ( $p < 0.05$ ) at the concentration of 0.1mg/L  $\text{CdCl}_2$ , and the inhibition effect was increased with the increasing concentration of  $\text{Cd}^{2+}$ . GPX and GST changed similarly, which reached the maximum when  $\text{Cd}^{2+}$  was 0.1mg/L, then it declined sharply with the increasing concentration of  $\text{Cd}^{2+}$ . However, for CAT, the reverse trend was observed. Overall, these results indicated that all the bio-indicators mentioned above for *S. salsa* were quite sensitive to  $\text{Cd}^{2+}$ . It could be applied to monitor  $\text{Cd}^{2+}$  pollution in Yellow River Delta.

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**Keywords :** Cadmium; *Suaeda salsa*; germination; enzyme

### 1. Introduction

Cadmium (Cd), a common and transitional metal and available in the environment, is one of highly dispersed metals by human activities [1].  $\text{Cd}^{2+}$ , naturally has been one of most serious heavy metal contaminants in the marine water body and coastal intertidal sediment along the Bohai Sea and has posed

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threat to the human health and coastal ecosystem [2]. Geochemically Cd is quite mobile in soil, water and thus freely taken up by plants. Excess  $\text{Cd}^{2+}$  could cause the decrease in plant growth [3-4], development and yield [5], even the accumulation in plants [6], which are major aspects of the studies on the eco-environmental effects. Therefore, it is necessary to assess the toxicological effects in the intertidal organisms induced by  $\text{Cd}^{2+}$  using the biomarkers.

The germination of seeds and the root of seedlings are important stages of whole plant grow circle, it is also the most sensitive stage of plants to changes of their surrounding environment [7-8]. Therefore, to study the inhibition of plants exposed to contaminants in this stage is a best way to understand the toxic mechanisms of environmental contaminants to plants.

*Suaeda salsa*, the Chenopodiaceae C3 halophyte, is the pioneer plant in the intertidal zones [9] of Yellow River Delta, where soil salt content is often higher. Considering the high tolerance to salinity and immobility, *S. salsa* becomes a bioindicator for the environmental monitoring of intertidal zones and saline soil. Therefore, *S. salsa* could be used in environmental sciences and applied for the monitoring environmental stress in the intertidal zones [10] and phytoremediation of degraded wetland with pollutions (heavy metals [11-12] and oil) or increasing salinity [13-14]. Little information is available on the effects of heavy metals on seed germination and seedling growth of *S. salsa*.

In this paper, we report a physiological study of  $\text{Cd}^{2+}$  toxicity to the germination including finally germination percentage, germination index, root and shoot length, and activities of three antioxidant enzymes (catalase (CAT), glutathione peroxidase (GPX), glutathione S-transferase (GST)) of seedling during germination.

## 2. Author Artwork

### 2.1 Germination test

Healthy seeds of *S. salsa* were selected from plants growing at Yellow River Delta in autumn, and stored at 4 °C in a refrigerator to simulate the seed dormancy. Seeds were surface sterilized using 0.5%  $\text{HgCl}_2$  for 10 min, and then washed in sterilized double distilled water for three times. Seeds with similar size were cultured in petri dishes (90 - mm diameter) on filter paper to germinate in the culture container at 25 °C. The filter paper was moistened with 10 mL of sterilized water contained  $\text{Cd}^{2+}$  concentrations of 0, 0.1, 0.5, 1.0, 2.0, 4.0 and 6.0 mg/L including 0.1 % NaCl. Each dish had 50 seeds. Each treatment had three replicates. The treatment solution was replaced every day. Germination of seeds was recorded everyday, and germination percentage, germination index were then calculated after seeds were germinated for 7 days.

Seeds of each treatment were sampled for the determination of the root length, shoot length and activities of three enzymes (CAT, GPX, GST) in the seedling.

### 2.2 Determination

Finally germination percentage was expressed as Eq.1:

$$\text{FGP} = n / N \times 100 \quad (1)$$

Where n is germinated seeds after 7 days, N is the total seeds for every petri dishes.

The germination index (GI) was calculated as described in the Association of Official Seed Analysts.

Germination index was expressed as Eq.2:

$$\text{GI} = \Sigma(\text{Gt}/\text{Dt}) \quad (2)$$

Where GI is the germination index, Gt and Dt are the amount of the germinated seeds and the germination time, respectively.

Enzyme activities of the 7-day-old seedling samples were measured. CAT activity was measured in terms of the decomposition of hydrogen peroxide, which was monitored directly by the decrease in absorbance at 240 nm [15]. The activity of GPX was measured according to Drotar et al. [16] using glutathione as substrate. The activity of GST was determined according to the method of Habig et al. [17] by evaluating the conjugation of GSH with the standard model substrate 1-chloro-2,4-dinitrobenzene.

### 2.3 Statistical Analysis

Statistical analysis was carried out using SPSS 17.0. A one-way ANOVA was carried out to determine differences among treatment groups for germination and the activity of three enzymes. Student's t test was applied to determine the significance between different treatments. Statistical significance was set at the  $p < 0.05$  confidence level.

## 3. Results and discussions

### 3.1 Germination of *S. salsa* on $Cd^{2+}$ treatment

The most critical stages in the life cycle of higher plants are seed germination and seedling establishment [18]. Germination starts with the uptake of water by the quiescent dry seed and terminates with the elongation of the embryonic axis [19]. According to the results, all studied traits were affected by the experimental factors and there was completely significant difference between control and the treated seeds (Table.1). Final germination percentage (FGP) of *S. salsa* was prevented with the increased in concentration of  $Cd^{2+}$  concentrations.  $Cd^{2+}$  inhibited seed germination significantly ( $p < 0.05$ ). The decrease in FGP ranged from 88.0% to 18% depending on the  $Cd^{2+}$ . This is further corroborated by the data of germination index (GI) which show lower values compared with control. With higher  $Cd^{2+}$  concentration, the decrease in GI values became more sensitive which indicated that *S. salsa* is relatively more sensitive to the toxic effect of  $Cd^{2+}$  than FGP. The higher cadmium concentration in the germination of *S. salsa* seeds seems to prevent water uptake and water movement in the embryo axis, and that may be the main reason for the low seedling development [20]. When the seed surrounding was contaminated with  $Cd^{2+}$ , delays in germination were often observed [21-22]. This can be associated with several disorders in the event chain of germinative metabolism.

Table 1 Germination of *S. salsa* treated with  $Cd^{2+}$

Cd(mg/L)	FGP	GI
0	88±0.0a	45.5±1.2a
0.1	78±2.0b	41.2±1.3b
0.5	70±2.0c	38.0±0.3c
1.0	70±2.0c	32.9±1.1d
2.0	68±4.0c	30.8±0.9e
4.0	46±6.0d	20.1±3.1f
6.0	18±2.0e	9.3±1.3g

Values represent the average of three samples. Significant differences at  $p < 0.05$  were showed with different letter in the same line

### 3.2 Growth of *S. salsa* on $Cd^{2+}$ treatment

Seed germination and subsequent seedling growth are important stages of the plant life and highly sensitive to  $Cd^{2+}$ , because the germinating seed is the first interface of material exchange between plant development cycle and environment [19]. For plant, the shoot and root length are the important elements

to transport water and nutrients. The results included in Fig.1 show that both shoot and root length of *S. salsa* had adversely effects to  $\text{Cd}^{2+}$  treatment. With the increasing of  $\text{Cd}^{2+}$ , the decreasing trend was obviously compared to the control. It is interesting to note that the root length was far more inhibited than shoot length and the effect was more pronounced. When the concentration of  $\text{Cd}^{2+}$  was 1 mg/L, shoot length was inhibited compared to the control ( $p < 0.05$ ). For root length, the smallest inhibited concentration was 0.1 mg/L. Root length showed a slight stronger inhibition than shoot length. It was demonstrated that shoot and root length showing some degree of tolerance to  $\text{Cd}^{2+}$  treatments. The loss of these may be due to inhibition of cell division, impairment of PSII activity, directly or indirectly inhibits physiological processes such as respiration, photosynthesis, plant–water relationships, inhibiting the activity of the cell and its enlargement, resulting in poor growth and low biomass.

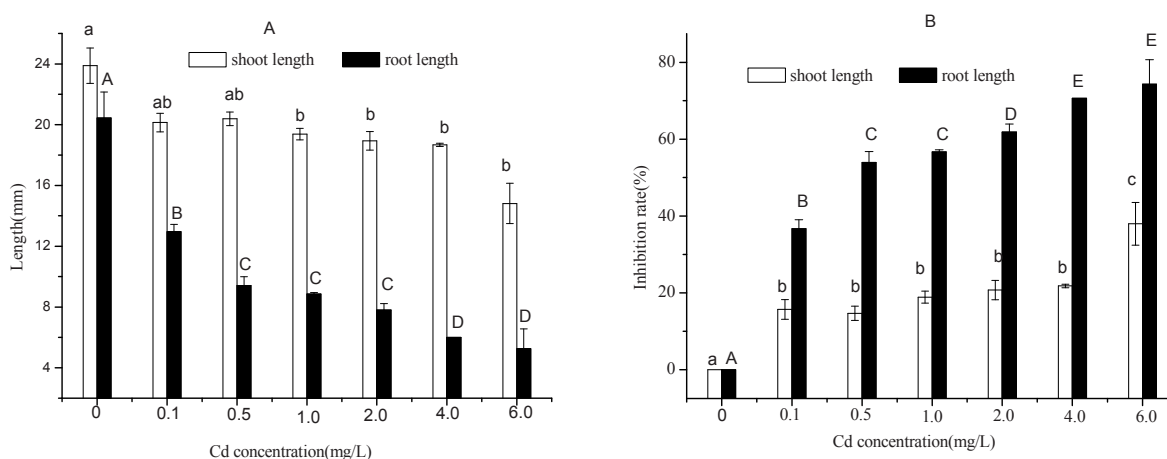


Fig.1 Effect of Cd on shoot and root length of *S. salsa* (A)root and shoot length; (B)inhibition rate

### 3.3 Responses of antioxidant enzymes to $\text{Cd}^{2+}$ treatment

$\text{Cd}^{2+}$  could induce oxidative stress and leads to cell death depending on the exposure time due to the increase of reactive oxygen species in cells [23]. Seedling enzymatic activity including CAT, GPX and

Table 2 Activity of CAT, GPX, GST treated with Cd

Cd (mg/L)	CAT(U g <sup>-1</sup> FW)	GPX(U g <sup>-1</sup> FW)	GST(U g <sup>-1</sup> FW)
0	119.9±1.5 b	27.42±0.44 c	10.88±0.07 cd
0.1	60.2±19.4 a	86.22±4.40 a	17.52±0.33 a
0.5	147.2±1.0 c	32.62±0.31 b	14.10±1.34 b
1	148.3±13.0 c	10.20±0.59 d	10.96±1.83 c
2	233.7±3.2 d	5.40±1.24 e	7.12±1.29 e
4	317.2±10.0 e	4.68±0.21 e	4.85±0.70 f
6	378.9±8.0 f	3.75±0.29 f	8.52±0.66 de

Values represent the average of three samples. Significant differences at  $p < 0.05$  were showed with different letter in the same line.

GST were determined to investigate the cellular defence response induced by  $\text{Cd}^{2+}$  (Table 2). CAT activity of *S. salsa* seedling showed a decrease significantly ( $p < 0.05$ ) compared to the control when  $\text{Cd}^{2+}$  was 0.1 mg/L, then with the  $\text{Cd}^{2+}$  concentration increasing, the activity of CAT increased correspondingly. The values of CAT were 2.64 times and 3.15 times higher than that of control when seedlings were exposed in 4 mg/L, 6 mg/L  $\text{Cd}^{2+}$ . However, the trends were different for GPX and GST, which assayed in Table 2. During the entire experimental period, 0.1mg/L  $\text{Cd}^{2+}$  induced a significant elevation in GPX and GST with 3.14 times and 1.61 times of the control respectively, and reached the max. When seedlings were activity exposed to 2.0 mg/L  $\text{Cd}^{2+}$ , the values of GPX and GST decreased to 19.7 % and 65.4 % of the control. It was showed that *S. salsa* cellular antioxidant enzymes were triggered in different degrees when exposed to  $\text{Cd}^{2+}$ . The change in antioxidant enzymes in  $\text{Cd}^{2+}$ - treated may be one of the important criteria in recognizing cadmium toxicity effect in plants that leading to seedling senescence and plant death.

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