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EFFECTS OF CADMIUM ON GROWTH AND PHYSIOLOGICAL INDEXES OF REED

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Abstract

Changes in physiological indexes of *Phragmites australis* (Cav.) Trin. *ex* Steud collected from the Panjin Liaohe estuarine wetland in China were examined during different stages of reed growth in experimental barrels, and correlations among physiological indexes and Cd (0 to 5 mg/kg) were determined. The growth of reed was restrained with increasing Cd concentrations, the plant height, root, stem and leaf biomass were minimal at 16.26 cm, 11.85, 8.69 and 3.21 g, respectively, at a Cd concentration of 5mg/kg, which decreased by 30.21, 27.76, 15.30 and 31.46% over the control respectively. The malondialdehyde (MDA) content, superoxide dismutase (SOD) and peroxidase (POD) activity rose with increasing Cd concentrations at all stages of reed growth. However, the MDA content and SOD activity all increased with-growth. POD activity, however, demonstrated an atactic change with age of the plants. The physiological indexes were significantly changed with Cd dose applied in reed leaves during different growth stages except POD activity in the germination stage.

Introduction

Cadmium (Cd) is one of the most toxic heavy-metal pollutants. Because of industrial emissions and the inappropriate control and regulation of agricultural waste, Cd contamination in soil and water has become increasingly prominent. Cd can easily be absorbed and accumulated by plants, leading to decline in production and quality. Cd can also enter the human body through the food chain and endanger human life (Zhu *et al.* 2013). Cd accumulation in the plant cell causes ion imbalance and oxidative damage, which lead to molecular damage, growth retardation and even death of plant (Pereira *et al*. 2002, Di Toppi and Gabbrielli 1999). Cd affects the activity of antioxidant enzyme and osmotic adjusting antioxidant enzymes, mainly through the excessive production of reactive oxygen species (Van Assche and Clijster 1990), which disrupt normal metabolic processes and inhibit plant growth and development. The enzymatic system for scavenging reactive oxygen species is mainly composed of superoxide dismutase (SOD) and peroxidase (POD) (Chaoui and Mazhoudi 1997), which have direct relationships with oxidative stress (Ruley *et al*. 2004). Shah *et al.* (2001) demonstrated that SOD and POD activities increased in rice when Cd concentrations increased during the seedling growth stage. Xu *et al.* (2009) argued that the inhibitory effect on SOD activity in *Salvinia natans* (L.) All. was most obvious at a high level of Cd treatment, POD activity became lower than normal, and the final product of membrane lipid peroxidation content, malondialdehyde (MDA), increased significantly. Liu *et al.* (2011) reported that Cd treatment initially increased and then decreased the SOD activity of *Zoysia japonica*, but POD activity showed an opposite trend. In reeds *(Phragmites australis* (Cav.) Trin. *ex* Steud), though, POD activity first increased and then decreased over time (Iannelli *et al*. 2002). We assume that different Cd concentrations and treatment times elicit different responses in different plants.

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As one of the pioneer species in wetland systems, reeds can effectively absorb Cd (Ren *et al*. 2010) and sustainably restore areas polluted with Cd (Dong *et al*. 2011). To provide a reference for the mechanism of how heavy metals do harm to plants and for the phytoremediation of heavy-metal contamination in soil, a simulate experiment in barrels was conducted to examine the effects of the Cd contamination in soil on growth and physiological indexes in reeds.

Materials and Methods

The selected rhizomes for transplanting were cut from non germinating reeds from the experimental area of Liaohe estuarine wetland of Panjin city in China. Soil from the same wetland was collected. The soil type was meadow soil with a pH of 8.47, organic-matter content of 1.12%, and bulk density of 1.03 g/cm^3.

A canopy was constructed over barrel pots as protection from rain and to control for ventilation and light. The test soil was dried, processed and placed into white steel barrels at 40 kg per barrel. Analytically pure $CdCl_2 \cdot 2.5H_2O$, in triplicate, Cd at six concentrations were added: 0mg/kg (C_0), 1 mg/kg (C_1), 2 mg/kg (C_2), 3 mg/kg (C_3), 4 mg/kg (C_4) and 5 mg/kg (C_5). Ten healthy reeds of similar size were transplanted to the barrels and were marked for identification. Residential drinking water containing no Cd was added to the barrels and maintained a depth of 5 cm above the surface of the soil to simulate wetland conditions.

The fresh reed leaves were collected in the germination, blade-expansion, rapid-growth, heading and maturity stages, respectively, and each treatment with three repeats. 0.5 of fresh reed leaves in 5 ml of thiobarbituric acid were ground in a mortar. MDA was extracted in 98ºC water bath and followed by centrifugation. The supernatant fluid was taken to determine absorbance values at 450, 532 and 600 nm. MDA content was calculated with the formula of [6.45 (OD_{532} - OD_{600}) - 0.56 × OD_{450}].

One of fresh reed leaves in phosphate buffer was ground in a mortar. Enzymes were extracted in ice bath and followed by centrifugation. The nitroblue tetrazolium (NBT) method (Dhindsa 1981) for determining enzymatic activity was adopted, where 50 per cent inhibition of NBT photochemical reduction per unit time is regarded as one unit of activity.

Enzymes were extracted as above. The supernatant was then cooled, the residue was again extracted with phosphate buffer, and the supernatants were combined. POD activity was measured with the guaiacol colorimetric assay (Zhang 1990).

Healthy leaves from three reeds per barrel were selected for drying and grinding and were preprocessed by Wang's method (Wang 2005) Cd concentration was measured by inductive coupling plasma emission spectrography.

Data were analysed by two-way ANOVAs with SPSS version 17.0 (statistical significance was set at $p < 0.05$; a significance level of 0.01 was deemed to indicate very high significance). Data was expressed with the form that average ± standard error ($n = 3$).

Results and Discussion

MDA, the final product of membrane lipid peroxidation, is used to measure the degree of membrane lipid peroxidation. MDA content in reed leaves was maximal at C5 in each growth stage (Table 1). At the same growth stage, MDA content at the various Cd concentrations were significantly different from the control except C_1. Compared to C_0, MDA content at germination, blade-expansion, rapid-growth, heading and maturity stages was increased to 77.92, 64.04, 76.44, 69.09 and 80.93% at C_5, respectively, and the differences were significant ($p < 0.05$). It was showed that a certain concentration of Cd stress could lead to the increasing of MDA content.

MDA content in reed leaves varied depending on the stage of growth and development, with the lowest content at germination, following the blade-expansion, rapid-growth, heading, and maturity stages. Compared to germination stage, MDA content at maturity, heading, rapid-growth and blade-expansion stages was increased to 55.84, 35.77, 22.99 and 6.57 % at C_5, respectively.

Table 1. MDA content (nmol/g Fw) in reed leaves at different growth stages.

Growth stages	C_0	C_1	C_2	C_3	C_4	C_5
Germination	*154d	169cd	183c	224b	257a	274a
Blade-expansion	178d	185d	219c	249b	279a	292a
Rapid growth	191f	217e	237d	263c	296b	337a
Heading	220e	233e	256d	285c	328b	372a
Maturity	236e	248e	272d	314c	355b	427a

*Treatments without common letters in the same column indicate significance at the 0.05 level.

SOD, a protective enzyme that can scavenge and reduce destructive reactive oxygen species in plants, can neutralise superoxide and convert O_2^- into H_2O_2. Cd stress is generally considered to induce the expression of SOD, which depends on concentration, duration, plant species, size of plant, etc. (Sun *et al.* 2009). SOD activity in reed leaves consistently increased with increasing concentrations of Cd in all stages of growth (Fig. 1). SOD activity in the germination stage in the absence of Cd was significantly different than the activities in the presence of Cd ($p < 0.05$), but the activities at the various Cd concentrations were not significantly different from each other ($p > 0.05$). SOD activity was maximal at C_5 at 10 U· (g Fw· h)$^{-1}$.

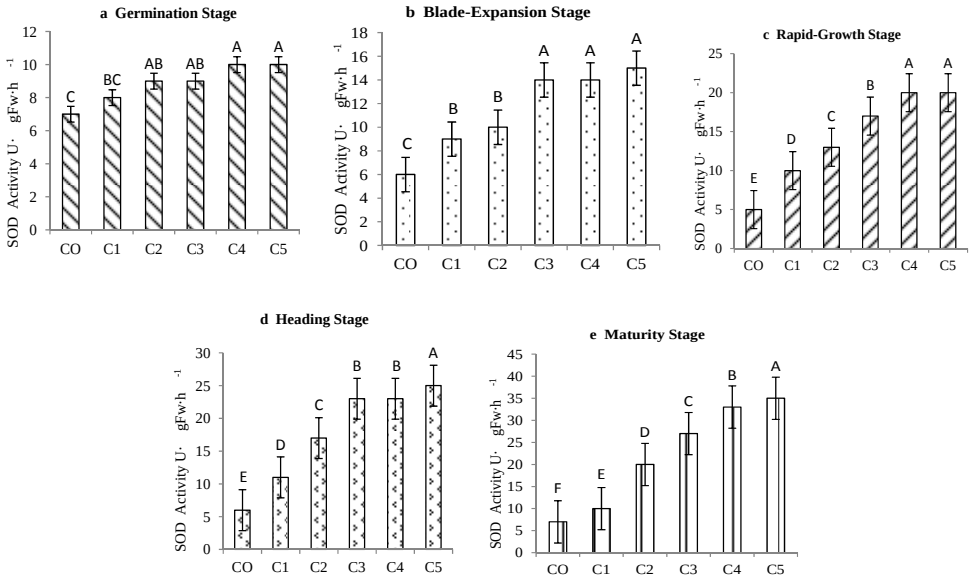

Fig.1. The influence of Cd on SOD activity of reed leaves at different stages.

In the blade-expansion stage, SOD activity differed little at C_3, C_4 and C_5 and at C_1 and C_2, but activities at C_3, C_4 and C_5 differed significantly from those at C_1 and C_2. A maximal activity of 15 U (g/Fw/h) was measured at C_5. In the rapid-growth stage, SOD activity differed significantly

at all concentrations except C_4 and C_5, where activity was maximal at 25 U· (g Fw· h)$^{-1}$. In the heading stage, SOD activity was significantly different at all concentrations except C_3 and C_4, a pattern that was similar to that seen in the rapid-growth stage. In the maturity stage, SOD activities were significantly different ($p < 0.05$) at all concentrations and was maximal at C_5 at 35 U·(g Fw· h)$^{-1}$. SOD activities at C_1, C_2, C_3, C_4, and C_5, compared to the base reference at C_0, increased by 0.43, 1.86, 2.86, 3.71 and, 4 times, respectively, which were all significantly different from the base reference ($p < 0.05$). These results indicate that Cd contamination led to increased SOD activity.

SOD activity in reed leaves varied depending on the stage of growth and development, with the highest activity at maturity, following by the heading, rapid-growth, blade-expansion, and germination stages. At C_5, SOD activity in the blade-expansion, rapid-growth, heading, and maturity stages, compared to the germination stage, increased by about 0.5, 1, 1.5, and 2.5 times, respectively. SOD activities differed significantly during the different growth stages under different levels of Cd stress. SOD activities at C_3, C_4, and C_5 were similar, which in the maturity stage was significantly higher than in the heading stage and was lowest in the germination stage. At C_2, SOD activity varied dramatically during the rapid-growth, heading, and maturity stages, but the variation was minor in the germination and blade-expansion stages. The differences in SOD activity at C_0 and C_1 during the different growth stages were not significant. SOD activity varied from higher to lower values at C_1 and C_2, which was typical of plants under stress. Plants under stress have defensive mechanisms for adapting to changes in the environment, but if the amount of stress exceeds the endurance of the plant, defensive measures will weaken accordingly before death.

POD is an important enzyme in the response of reeds to stress. It can effectively help plants to scavenge peroxides and free radicals and has important functions in the elimination of superoxide radicals, the control of lipid peroxidation, and the protection of cell-membrane metabolism (Fig. 2).

Except for the germination stage, POD activity increased as Cd contamination increased. POD activity was highest at C_5. In the germination stage, pod activity did not significantly vary with Cd concentration ($p > 0.05$). POD activity differed significantly among all concentrations ($p < 0.05$) in the blade-expansion stage, reaching a maximum of 19 U· (g Fw· h)$^{-1}$ at C_5. In the rapid-growth stage, the differences in activity between C_2 and C_3 and between C_0 and C_1 were not significant, but the differences among the remaining concentrations were significant. POD activity reached a maximum of 25 U·(g Fw· h)$^{-1}$ at C_5. Activity was significantly different among all concentrations ($p < 0.05$) in the heading stage and reached its maximum of 22 U· (g Fw· h)$^{-1}$ at C_5. Similar to the trend in the blade-expansion stage. In the maturity stage, POD activity was significantly different among all concentrations except C_0 and C_1. POD activity at C_1, C_2, C_3, C_4, and C_5, compared to the base reference, increased by 0.12, 1.5, 2.38, 3.12, and 3.38 times, respectively. All the results except that at C_1 were significantly different from that of the C_0 ($p < 0.05$). These results suggest that Cd contributes to increased POD activity at high concentration.

The changing trend of POD activity in reeds during growth was complicated. For example, POD activity at C_5 rose from 11 U·(g Fw· h)$^{-1}$ to 25 U·(g Fw· h)$^{-1}$ from the germination to the rapid-growth stage, decreased to 22 U·(g Fw· h)$^{-1}$ in the heading stage, and then increased to 35 U·(g Fw· h)$^{-1}$ in the maturity stage. This behaviour may be related to the metabolic activity of reeds in the different stages. Further studies on the mechanism of POD activity are needed.

Cd contamination had a number of effects on physiological indexes in reed leaves. As Cd concentration in the soil increased, MDA content, SOD activity and POD activities rose at all stages of reed growth.

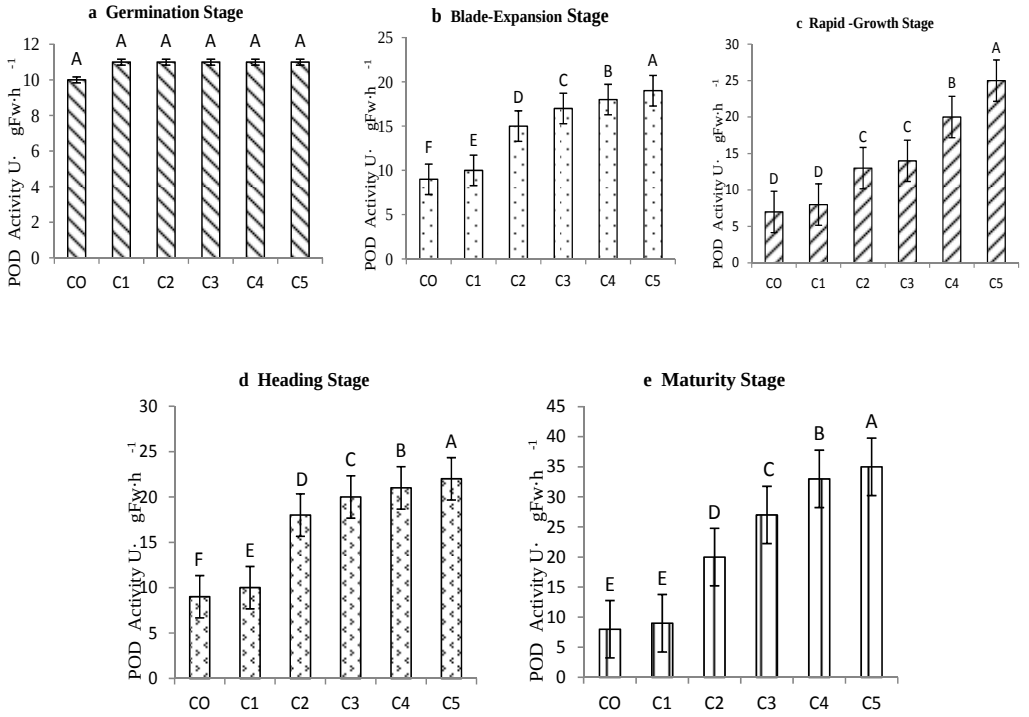

Fig. 2. The effects of Cd on POD activity in reed leaves of different growth of age.

When the Cd concentration in the soil was under 5 mg/kg, MDA content, SOD activity and POD activity was positively correlated with Cd content in reed leaves ($p < 0.05$), respectively.

Our team will further investigate the thresholds of tolerance to stress from heavy metals and the changes in physiological and biochemical indexes throughout the growth stages of these plants. This information will provide a sound theoretical basis for the mechanisms of heavy-metal toxicity and tolerance in plants.

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