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Effects of Cadmium on Superoxide Dismutase Activity in Reed Leaves

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ABSTRACT

Industrial polluted water has become an important water source for irrigation in the majority of constructed wetlands or even natural wetlands in China. The shortage of clean water resources has raised concerns about the potential accumulation of heavy metals, such as cadmium. Plants stressed by high levels of cadmium can increase the activity of superoxide dismutase. We have identified a positive correlation between the superoxide dismutase activity and the cadmium content of reed leaves in the wetland. Regression analysis confirmed that the superoxide dismutase activities fit a logistic curve. The logistic model was then applied to describe the superoxide dismutase activity, estimating parameters under different levels of cadmium stress. According to the findings, higher cadmium concentrations would cause the superoxide dismutase activity to increase at a higher intrinsic rate, have a lower environmental capacity *k*, and have lower inflection points. The dynamic model predicted an acceptable cadmium concentration of less than $3mg.kg^{-1}$. At this concentration, reeds could develop and grow satisfactorily in the presence of cadmium. Therefore, the concentration of cadmium in the irrigated water of polluted water in mine sites, papermaking wastewater, or other industrial wastewater should be less than or equal to $3mg.kg^{-1}$ to ensure the normal growth of reeds in related wetlands.

INTRODUCTION

The rapid development of industry and agriculture has led to a shortage of water resources for irrigation, especially because agricultural practices account for 70% of the total water withdrawn from freshwater resources (Koehler 2008, Xu et al. 2020). Using wastewater for irrigation can thus help in minimizing freshwater use for agriculture. Irrigation with treated wastewater improved plant growth, reduced mold infection rate, and increased the productivity of poor soils (AlMomany et al. 2019). Consequently, treated industrial wastewater has been increasingly used to irrigate wetlands. However, the remaining contaminants present in wastewater can threaten the growth of wetland plants as well as the environment (Qadir et al. 2007). Such heavy metals in polluted water are not prone to microbial or chemical degradation, which may not only lead to the accumulation of metals in soil but also may result in excessive uptake of the contaminants by plants (Dhiman et al. 2020, Khan et al. 2015). Plants uptake metals via roots and transport them into various plant tissues, where their presence triggers physiological, as well as genetic changes (Ahmad et al. 2016).

Superoxide dismutase (SOD) and peroxidases are antioxidant enzymes that protect cells against the damaging effects of reactive oxygen species. Antioxidant enzymes mainly include superoxide dismutase (SOD), superoxide dismutase (POD), catalase (CAT), ascorbate peroxidase (APX), etc. The SOD is responsible for the disproportionation of excessive production of H_2O_2, and O_2. The POD, CAT, and APX are then responsible for decomposing H_2O_2 into H_2O and O_2 (Bowler et al. 1992, Elster 1982). It shows that SOD activity is the first response indicator of plant resistance to stress compared with other antioxidant enzymes, which can indicate the feedback of plants to environmental stress. The environmental factors that influence SOD activities in plants include season changes, illumination, and moisture content. One of the promptest effects, when plant cells are exposed to toxic concentrations of heavy metals, is the production of reactive oxygen species (ROS), the generation of O^{-2} leads to the formation of H_2O_2 via the action of SOD and in the cell wall class III peroxidases catalyze the oxidation of various substrates (via the peroxidative cycle), which results in cell wall cross-linking and growth arrest (Passardi et al. 2004).

Heavy metal cadmium (Cd) influences antioxidant activity in plants mainly by the production of high levels of reactive oxygen (Assche and Clijsters, 1990). This effect varies with factors such as concentration, duration of exposure, and plant size (Sun et al. 2009). High levels of Cd increase the activities of SODs and peroxidases, which are thought to serve an antiperoxidative function in reeds (Wang et al. 2002). SOD activity under Cd stress is also influenced by the stage of growth in reeds. An increase in Cd^{2+} concentration from 0.2 to 0.6 $mmol.L^{-1}$ considerably reduces antioxidant activity in reed seedlings (Di et al. 2007). A concentration of 17.6$\mu mol.L^{-1}$ significantly increased SOD activity in reed leaves during the heading period (Alfadul and Al-Fredan, 2013). Concentrations up to 100 $\mu mol.L^{-1}$ significantly increased SOD activity in the leaves of broad beans during germination (Issam et al. 2012). A Cd concentration of 10 $\mu mol.L^{-1}$ inhibited growth and increased SOD activity in Arabidopsis seedlings, but a concentration of 1 $\mu mol.L^{-1}$ had no significant effect on SOD activity (Issam et al. 2012). Other studies have shown that excessive nickel stress leads to the peroxidation of the cell wall of basmati rice seedlings, which causes a reduction in the germination rate of rice (Khan et al. 2015). Therefore, determining the tolerable concentration of heavy metals in plants is the key to the application of wastewater irrigation wetlands.

Reeds are the main species in wetlands and can efficiently absorb such pollutants as heavy metals and nutrients from the water imported by the rivers or slope runoff (Ren et al. 2010). In the previous studies of our research group, it was found that the concentration of cadmium treatment, the duration, and the growth period of reeds all have significant effects on SOD and POD activities in reed leaves. Among them, POD activity exhibits a linear change characteristic, which is difficult to indicate the feedback of plants to stress. However, SOD activity presents more complex changes. Therefore, an in-depth study of the kinetic characteristics of SOD activity can analyze the response of plants to external stress and determine the mechanism of cadmium resistance in reeds.

In this study, we experimentally investigated the effects of different Cd concentrations on the activities of SOD in reed leaves. We developed a dynamic model to determine the maximum reaction rates and inflection points of SOD activity at different Cd concentrations. The study aimed to explore the responses of reeds to Cd stress and to provide a theoretical basis and technical support for the wise use of the polluted water in mine sites, papermaking wastewater, or other industrial wastewater to irrigation wetlands and the restoration of degraded wetlands.

MATERIAL AND METHODS

Materials

The reeds as the heavy metal hyperaccumulator plant were used in this study to explore the impact of Cd pollution on plant growth. The experimental material reeds were collected in April 2019 from the core area of the Liaohe estuarine wetland in the city of Panjin, Liaoning Province, China. Ungerminated reed rhizomes with healthy buds were also collected, cut into pieces 30 cm in length, wrapped in soil-laden sacks, and sprinkled with water to keep the roots wet. The plants were taken to the test field at Shenyang Agricultural University and transplanted into experimental buckets. The soil was collected from the Water Conservancy Comprehensive Experimental Site of Shenyang Agricultural University. The soil type was meadow soil, with a pH of 8.47, organic-matter content of 1.12%, and a density of 1.03 $g·cm^{-3}$. The Cd content in the soil was lower than the detection limit by ICP and has not been detected, which showed there was no Cd in the soil.

Experimental Design

To clarify the effect of different concentrations of Cd stress on the growth of reeds, this study set 6 concentration gradients as C1(1$mg.kg^{-1}$), C2(2 $mg.kg^{-1}$), C3(3 $mg.kg^{-1}$), C4(4 $mg.kg^{-1}$), C5(5 $mg.kg^{-1}$) and control concentration C0(0$mg.kg^{-1}$) according to the preliminary soil and wastewater investigation in Fushun West Open-pit Mine. A certain amount of $CdCl_2.2.5H_2O$ was mixed into air-dried soil in each barrel based on the set concentration gradient to explore the effect of Cd pollution on the growth of reeds more effectively.

Three replicates were set for each concentration, and the collected reed rhizomes were planted in 18 white steel buckets marked with the corresponding concentration under a ventilated and photo permeable rain shelter. The planting density of reeds was controlled at an average of 10 plants per experimental bucket. The reeds were transplanted into each barrel from the wetland test facility on April 12th, 2019. Tap water was added to the barrels and maintained at a level 5 cm above the soil. The tap water contained no Cd.

Six fully expanded healthy reed leaves were collected randomly from each experimental bucket according to the corresponding concentration markers at the end of each growth stage of reeds. The collected samples were stored in an ice bag and brought back to the laboratory for pretreatment quickly.

The reed's growth stages were divided as follows: germination stage (Mid-April to early May), leaf expansion stage (Mid-May to early June), rapid growth stage (Mid-August-early September), heading stage (Mid-August-early September) and maturation stage (Mid-September-October) according to the growth and development process of reeds under local climate conditions of Shenyang.

Sample Collection and Processing

SOD activity: One gram of fresh leave of reeds in phosphate buffer was ground in a mortar in an ice bath. The homogenate was centrifuged, and enzymes were extracted from the supernatant. Enzymatic activity was determined by the nitroblue tetrazolium (NBT) method, where 50% inhibition of NBT photochemical reduction per unit time is regarded as one unit of activity (Wang et al. 2002).

Cd content: Three healthy leaves from each barrel were steamed, dried, crushed (refer to Wang 2005) for information on phytotreatment and decomposition), and placed into three 100 mL plastic bottles individually. The Cd concentrations of the samples were determined by inductively coupled plasma atomic emission spectrometry.

An inductively coupled plasma emission spectrometer (Thermo iCAP 7000 Series) was used to determine the concentrations of Cd in the wastewater and plant samples. A national standardized solution containing 32 metal elements was purchased from a Chinese standard material website.

Data Processing and Analysis

In a limited environment, the physiological changes in plants follow a logistic pattern (Ding et al. 2009), and its differential form is:

$$\frac{df}{dt} = rf((k-f)/k) \qquad \ldots(1)$$

In the above formula, $f(t)$ is the enzyme activity at the time t, which represents the SOD activity in this study specifically. r is the intrinsic growth rate, which represents the potential growth capacity, k is the environmental capacity, which represents the upper limit of enzyme activity.

When $f(0) = f_0$, let $a = \frac{k - f_0}{f_0}$, which represents the influence of initial conditions on enzyme activity and integrate (1) into an explicit functional form:

$$f(t) = 1/(1/k + e^{a}/\mathrm{k}) \cdot e^{-rt} \qquad \ldots(2)$$

Introduce parameter t_m instead of a, let

$$a = rt_m \qquad \ldots(3)$$

Obtained Bartlett form:

$$f = k/(1 + \mathrm{e}^{-r(t-t_m)}) \qquad \ldots(4)$$

When $t = t_m$, $f = k/2$, $k/2$ is the maximum sustained activity of the enzyme, and the moment t_m is the time when the inflection point of the Logistic curve is located. The inflection point is always reached $f = k/2$.

RESULTS AND DISCUSSION

The Effects of Cd on SOD Activity in Reed Leaves

SOD activity is affected by such factors as illumination, temperature, water content, and the presence of heavy metals. The influence of the different Cd concentrations on SOD activity in reed leaves at the five stages of growth is shown in Fig. 1.

SOD activity in the leaves generally increased with Cd concentration. The variation in SOD activity increased as the plants matured.

In the whole stage of physiological, with levels C0 and C1, the changes in SOD activity were not significant, with levels C2, C3, C4, and C5, SOD activity increased and have a significant difference from the contraposition ($P<0.05$),

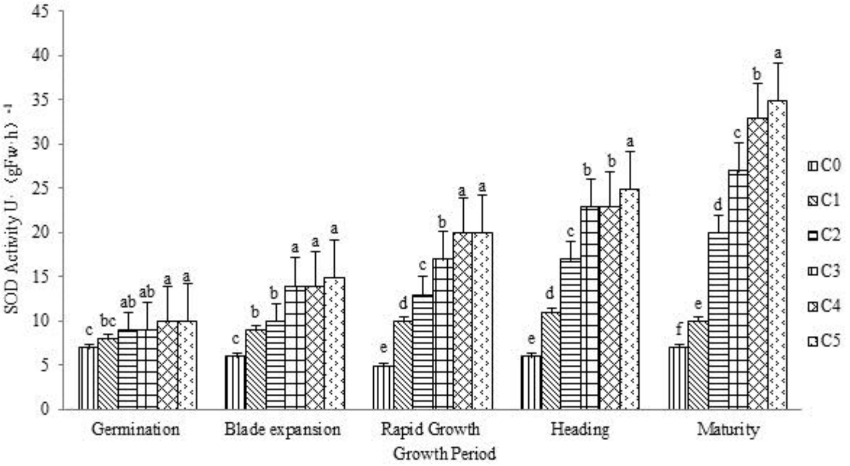

Fig. 1: The influence of Cd contamination on SOD activity in reed leaves. Different letters indicate significant differences. “FW” means fresh weight.

indicating that factors such as illumination did not cause significant changes in SOD activity. The significant changes in SOD activity were thus likely caused by Cd concentration.

Different concentrations of Cd led to significant changes in SOD activity at different stages of growth. The variation in SOD activity was similar at C3, C4, and C5, and SOD activity was higher at maturation than at the heading stage. SOD activity was lowest at germination. The increases in SOD activity were significant at C2 in the rapid-growth, heading, and maturation stages. SOD activity changed little at C0 and C1 in the germination and leaf expansion stages. At C1 and C2, SOD activity tended to first increase and then decrease. Plants under Cd stress typically resort to resistance measures to adapt to adverse environments. If the stress is too large and exceeds the limit of tolerance of the plants, the health of the plants will weaken until death occurs.

The Kinetic Analysis of SOD Activity in Reed Leaves under Cd Stress

Cd concentration was used as an impact factor and then for trend analysis of the intrinsic rate of increase in SOD activity and carrying capacity. These analyses allowed us to determine the quantitative relationships between Cd concentration and carrying capacity or the intrinsic rate of increase. Regression equations were derived from the logistic curves (Table 1).

The relationship between Cd concentration and carrying capacity is:

$k = -0.0528x^3 + 7.3488x + 12.4$ (R^2=0.972)

The relationship between Cd concentration and intrinsic rate of increase is:

$v_x = -0.0005x^3 + 0.0438x + 0.1229$ (R^2=0.991)

Applying these values to equation (4), we can derive the dynamic equation of SOD activity under Cd stress:

$$f = \frac{-0.0528x^3 + 7.3488x + 12.4}{1 + e^{(a-(0.0005x^3+0.0438x+0.1229))\cdot t}} \qquad \ldots(5)$$

where x is Cd concentration (in units), t is time, a is a constant representing initial conditions, and v_x is the intrinsic rate of increase.

Comparing equation (4) with the regression equations (Table 1), we could derive the parameters a, v_x, and k of SOD activity in reed leaves at different Cd concentrations and then use equation (5) to derive the parameters tm and k/2 (Table 2).

Table 2 indicates a positive correlation between Cd concentration and intrinsic rate of increase in SOD activity in reed leaves. In contrast, Cd concentration is negatively correlated with carrying capacity, Michaelis constant, and inflection point. The ability of the reeds to react to Cd stress thus increased as Cd concentrations increased. If concentrations are too high, though, the reaction of the plants to Cd will lower the maximum continuous activity. At a Cd concentration of 5 mg.kg^{-1}, the inflection point (7.85) occurred at the end of July when the rate of increase in SOD activity was highest. At a Cd concentration of 4 mg.kg^{-1}, the inflection point (8.60) occurred in the middle of August. Reeds are at the heading stage at this time, and the plants' resistance to Cd stress is at its peak. Beyond this point, resistance declines, and biomass and absorption of Cd increase. At concentrations of 3 or 2 mg.kg^{-1}, the inflection points were 10.04 or 12.07, respectively, which occurred in October or December. Reeds are mature at this time and are ready

Table 1: Parameters of SOD activity at different concentrations of Cd.

Concentration	Regression equation	Correlation
C5	$Y=1/(1/27.188+e^{2.307/27.188})*e^{-0.294*t}$	0.994
C4	$Y=1/(1/31.608+e^{2.433/31.608})*e^{-0.283*t}$	0.984
C3	$Y=1/(1/39.696+e^{2.479/39.696})*e^{-0.247*t}$	0.977
C2	$Y=1/(1/42.153+e^{2.593/42.153})*e^{-0.215*t}$	0.987

Table 2: Parameters of SOD activity from regression equations at different Cd concentrations.

Concentration	a	The intrinsic rate of increase v_x (maximum rate of increase)	Carrying capacity k (maximum activity)	Michaelis constant $k/2$ (maximum continuous activity)	Inflection point (t_m)
C5	2.307	0.294	27.188	13.5940	7.8469
C4	2.433	0.283	31.608	15.8040	8.5972
C3	2.479	0.247	39.696	19.8480	10.0364
C2	2.593	0.215	42.153	21.0765	12.065

to be harvested, so Cd will have little impact on biomass. The limiting concentration of Cd on SOD activity is thus 3 $mg.kg^{-1}$. At this concentration, the reeds still respond to Cd, which will have little effect on increases in biomass. Cd is fully absorbed at a time when biomass increases the most and economic benefits are highest.

CONCLUSIONS

(1) SOD activity in reed leaves increased as Cd concentrations increased under laboratory conditions. The variation in SOD activity was significant at different concentrations of Cd and different growth stages. Over the entire growth period, SOD activity reacted similarly to Cd concentration. SOD activity at the various growth stages showed the general trend of C5>C4>C3>C2>C1>C0, illustrating that Cd stress favored increases in SOD activity in reed leaves.

(2) The changes in SOD activity at different Cd concentrations followed a logistic curve. The experimental results indicated that the intrinsic rate of increase rose with increasing Cd concentration, but the variation in carrying capacity decreased, and the inflection point was extended.

(3) The quantitative relationships between Cd concentration and the intrinsic rate of increase and the carrying capacity of SOD activity in reed leaves were determined. These were expressed as logistic curves to establish the dynamic equation of state for the variation of SOD activity under Cd stress. This equation identified a limiting Cd concentration of $3mg.kg^{-1}$ on SOD activity. At this concentration, reeds can effectively respond to Cd stress and grow satisfactorily. Therefore, the concentration of cadmium in polluted water used to irrigate reed wetland should be less than or equal to $3mg.kg^{-1}$ to ensure the normal growth of wetland plants. The result has an important practical guiding role in the environmental remediation of chromium-polluted mining areas, the purification of papermaking wastewater, and other biological treatment of industrial polluted water bodies.

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