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Original Research

Effects of Cadmium on Mineral Metabolism and Antioxidant Enzyme Activities in *Salix matsudana* Koidz

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Abstract

Salix matsudana Koidz was exposed to different concentrations of Cd (0, 10, 50, and 100 μmol/L) to study the effects of Cd on mineral metabolism and antioxidant enzyme activities. The results showed that plant height and root length were inhibited by 50 and 100 μmol/L Cd, except the one under 10 μmol/L Cd treatment. The Cd content accumulated in different organs of *S. matsudana*, gradually increasing with increased Cd concentrations and prolonged treatment times. The root was the main organ for absorbing and accumulating Cd. Cd inhibited the accumulation of Fe, Zn, Mn, and Cu. In addition, the activities of antioxidant enzymes and the contents of reactive oxygen species were also changed by different concentrations of Cd. The results obtained here can provide scientific and objective data for the use of *S. matsudana* in the remediation of Cd-contaminated soil.

Keywords: *Salix matsudana* Koidz, cadmium, minerals metabolism, antioxidant enzymes, reactive oxygen species (ROS)

Introduction

Nowadays, heavy metal pollution of soils has become a serious environmental concern and a potential threat to human health. Cadmium (Cd) is one of the most toxic nonessential elements for plants. High Cd concentrations in soils inhibit photosynthesis and growth as well as diminish water and nutrient uptake [1]. Normally, heavy metals only change their morphology and valence when entering the environment. They cannot be degraded biologically and are difficult to clean up. A clean and effective method of removing heavy metals from soil, called phytoremediation, has been widely studied [2]. Plants are essential components in ecosystems because they transfer elements from the abiotic environment to the biotic one [3]. Some woody plants are widely used in phytoremediation, which could absorb, accumulate, and transport heavy metals to roots, stems, branches, and leaves of woody plants [4]. All willow species can accumulate high concentrations of Cd. They can serve as a prospecting guide for metal ions and be utilized in phytoremediation of heavy-metal polluted soils [5].

Salix matsudana Koidz is a fast-growing, productive, and deeply rooted tree of the willow species that adapts to temperate region climatic conditions and has the ability to tolerate high Cd [4, 6]. Therefore, it has been discovered that Cd is concentrated by *S. matsudana*,

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which is believed to be a comparatively simple and easy method relative to simulating multi-metal pollution in hydroponic conditions [7]. It is of vital importance to explore the reaction mechanism of *S. matsudana* exposed to different Cd concentrations. The inhibition of photosynthesis leads to the accumulation of reduced plastoquinone, ferredoxin, and triplet state chlorophyll, which in turn facilitates the production of reactive oxygen species (ROS) [8]. ROS is identified as an indispensable process when plants have normal aerobic metabolism. It exists in the form of four major types, including singlet oxygen ($^1O_2$), superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) [9]. ROS accumulation aggravates membrane lipid peroxidation and oxidative damage [10-11]. Antioxidant enzymes eliminate ROS and act as a set of antioxidant enzymatic defense systems [12]. Cd can inhibit the metabolism processes such as photosynthesis, carbohydrate and nitrate metabolism, water balance, and DNA and lipid matrix, resulting in growth inhibition, morphological alterations, and plant senescence or even death [6, 13]. Cd interferes with the uptake, transport, and use of different macro- and micronutrients such as zinc (Zn), iron (Fe), manganese (Mn), and selenium (Se) [6, 14].

In order to understand the physiological and biochemical changes mechanism and the resistance mechanism of *S. matsudana* to Cd, the effects of Cd on mineral metabolism, activities of antioxidant enzymes, and ROS contents in *S. matsudana* under different concentrations of Cd stress were studied in this investigation. The results obtained here can provide scientific and objective data for the use of *S. matsudana* in the remediation of Cd-contaminated soil.

Material and Methods

Plant Material and Growth Conditions

Healthy and fresh woody cuttings (25 cm long) were chosen from *S. matsudana* grown in the campus of Tianjin Normal University, Tianjin, China. They were immersed into four plastic containers with 2.5 L half Hoagland's nutrient solution mixed 0, 10, 50, and 100 μmol/L $CdCl_2$ for 28 d at a constant temperature, respectively. Hoagland's solution was composed of 0.5 mol/L $Ca(NO_3)_2$, 0.5 mol/L KNO_3, 0.1 mol/L KH_2PO_4, 0.1 mol/L $MgSO_4{\cdot}7H_2O$, 10 mmol/L $FeCl_3{\cdot}6H_2O$, 10 mmol/L Na_2-EDTA, 50 mmol/L H_3BO_3, 4.5 mmol/L $MnCl_4{\cdot}4H_2O$, 0.3 μmol/L $CuSO_4{\cdot}5H_2O$, 0.1 mmol/L $(NH_4)_6Mo_7O_{24}{\cdot}7H_2O$, and 3.8 mmol/L $ZnSO_4{\cdot}7H_2O$, adjusted to pH 5.5. The solutions were aerated by pumps and changed regularly every 7 d. And in the four treatment groups, the plant heights and root lengths were measured by the same ruler at the end of each time interval (7 d). All treatments were done in five replicates.

Estimating Total Cd and Several Minerals

Ten plants from control and each treatment were harvested at the end of each time interval (7 d). These plants, which were washed thoroughly with deionized water, were divided into roots, new stems, leaves, xylem of old stems, and phloem of old stems. All plant samples were dried to a constant weight (45ºC for 3 d, 80ºC for 1 d, and 105ºC for 12 h) and prepared using the wet-digestion method using concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) [13] (Wu et al., 2017). Concentrations of Cd, Cu, Fe, Mn, and Zn were measured using inductively coupled plasma atomic emission spectrometry (ICP-AES, Leeman Labs Inc., New Hampshire, USA).

Analysis of the Activities of Antioxidant Enzymes

Fresh leaves (0.1 g) and roots (0.05 g) were harvested and washed with deionized water. These samples were ground into homogenates with 5 mL chilled sodium phosphate buffer (50 mmol/L, pH 7.8) at the end of each time interval (7 d). The homogenates were centrifuged at 12,000 rpm for 15 min. The supernatants were stored at 4ºC and applied to analyzing the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT).

SOD Assay

The SOD activity was estimated according to the method of Wu et al. (2017). The reaction mixture consisted of 81 mL methionine, 60 μL EDTA-Na_2, 0.3 mL riboflavin, 3 mL nitroblue tetrazolium chloride (NBT), and 5.64 mL sodium phosphate buffer (50 mmol/L, pH 7.8). The reaction started by placing tubes below two 15 W fluorescent lamps for 12 min. Absorbance was recorded at 560 nm using a UV–Vis spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan). One unit of enzyme activity was defined as the quantity of the SOD required to produce a 50% inhibition of reduction of NBT under the experimental conditions, and the specific enzyme activity was expressed as units per g fresh weight of roots and leaves (FW). All steps were kept in the dark.

POD Assay

The reaction mixtures were composed of 100 mL sodium phosphate buffer (0.1 M, pH 6.0), 50 μL guaiacol, and 38 μL H_2O_2 (30 %). The absorbance was measured immediately at 470 nm at 0.5 min intervals up to 2 min using a UV-Vis spectrophotometer (UV-2550, Shimadzu Japan) after enzyme extract was added to the reaction mixture. Enzyme-specific activity is defined as units (one peroxidase activity unit defined as absorbance at 470 nm changes per minute) per g of FW [15].

CAT Assay

The activity of CAT was measured instantly when 200 µL enzyme extract and 300 µL H_2O_2 (0.1 mol/L) were added to the reaction mixture containing 1.5 mL sodium phosphate buffer (50 mmol/L, pH 7.8) and 1 mL deionized water. The method of reading the results was the same as POD. However, the absorbance was monitored at 240 nm until H_2O_2 was consumed completely. Activity was expressed as units (one catalase activity unit defined as absorbance at 240 nm changes per minute) per g of fresh weight [15].

Analysis of Reactive Oxygen Species

O_2^- Assay

Fresh roots (0.2 g) and leaves (0.2 g) were reaped and homogenized in 2 mL sodium phosphate buffer (50 mmol/L, pH 7.8) with a pestle and mortar at the end of each time interval (7 d) of the Cd treatment. Homogenates were centrifuged at 12,000 rpm for 15 min. The supernatant (0.5 mL) was added to two kinds of reagents, including 0.5 mL sodium phosphate buffer (50 mmol/L, pH 7.8) and 1 mL hydroxylamine hydrochloride (1 mmol/L). After the mixture reacted completely for 60 min at 25ºC, 1 mL sulphanilic acid (17 mmol/L) and 1mL α-naphthylamine (7 mmol/L) were fully integrated into the reaction system. The whole reaction system continued to react for 20 min at 25ºC and were mixed with 4 mL chloroform. Pink supernatant was obtained and centrifuged at 12,000 rpm for 3 min. Absorbance was recorded at 530 nm using a UV–Vis spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan). The reaction of oxidation hydroxylamine was expressed as the content of O_2^- in nmol/L per g of fresh weight [13].

H_2O_2 Assay

Fresh roots (0.5 g) and leaves (0.5 g) were harvested and homogenized in 5 mL cooling acetone at the end of each time interval (7 d) of the Cd treatment. Homogenates were centrifuged at 12,000 rpm for 20 min. Two kinds of reagents contained 0.1 mL titanium sulphate (5%) and 0.2 mL concentrated ammonia water were added to the supernatant (1 mL). The reaction mixture was centrifuged at 12,000 rpm for 15 min. The sediments were washed a few times by adding cooling acetone until the pigmentum of sediments disappeared. Finally, the sediments and 5 mL sulfuric acid (2 mol/L) were completely dissolved. The absorbance of mixture was surveyed at 415 nm using a UV–Vis spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan). The peroxide was expressed as the content of H_2O_2 in nmol/L per g of fresh weight [13].

Statistical Analysis

Each treatment was triplicated for statistical validity. Data from this investigation were analyzed with standard statistical software (Sigma Plot 10.0) using means ±standard error (SE). For equality of averages the t-test was applied. Results were considered statistically significant at $P<0.05$.

Results

Macroscopic Effects of Cd Treatment on Plant Growth

The different concentrations of Cd and treatment time had a great effect on plant height and root length. The leaves of *S. matsudana* exhibited chlorosis and

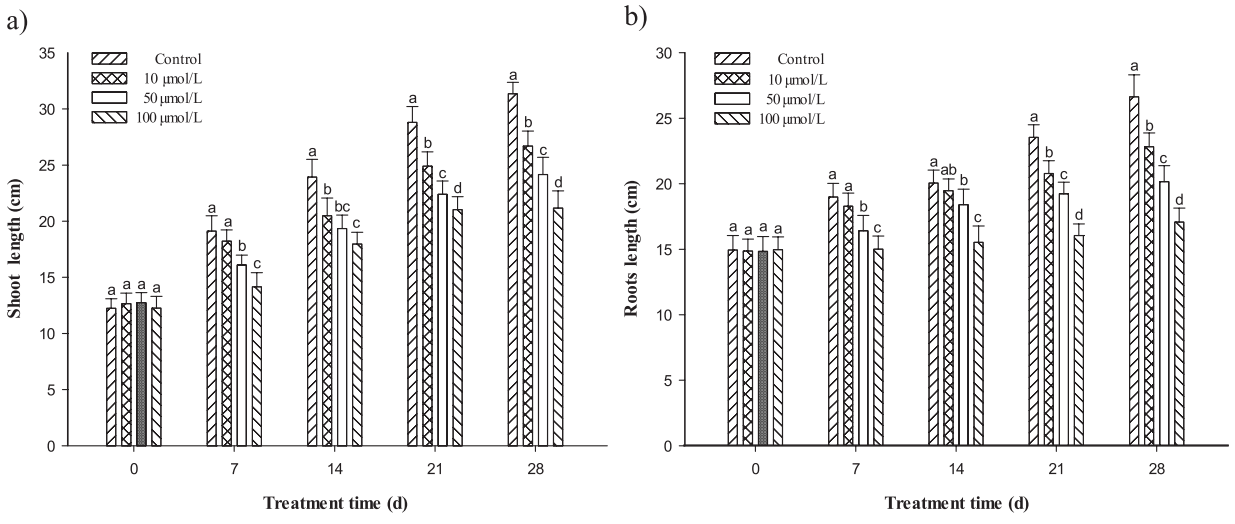

Fig. 1. Effects of different Cd concentrations on plant height and root length of *S. matsudana* under different Cd concentrations for 0, 7, 14, 21 and 28 d. Error bars represent SE of the mean, n = 5. Values with different letters differ significantly from each other ($P<0.05$, *t*-test).

Table 1 Cd accumulation in different organs of *S. matsudana* after treatments with different concentrations and duration of Cd.

Time (d)	Treatment (μmol/L)	Cd (μg/g, DW±SE)					TF (%)
		Root	New stem	Leaf	Xylem of old stem	Phloem of old stem	
7	0	0.00a	0.00a	0.00a	0.00a	0.00a	0
	10	324.24±60.26b	46.24±5.87b	10.84±1.37b	11.03±0.98b	49.52±4.42b	26.6
	50	636.65±83.21c	124.94±7.63c	24.81±2.35c	16.44±0.68b	58.01±4.89b	26.0
	100	1643.73±90.47d	148.83±7.97c	59.65±2.09d	26.34±2.72c	91.89±5.24c	16.6
14	0	0.00a	0.00a	0.00a	0.00a	0.00a	0
	10	568.93±82.19b	95.21±6.31b	29.85±1.59b	15.13±0.96b	83.33±5.59b	28.2
	50	1232.26±93.59c	126.80±7.89c	45.64±2.89c	54.76±3.63c	111.83±6.15c	21.6
	100	2602.41±195.57d	163.38±8.34d	65.18±2.27d	89.66±3.65d	137.17±6.34d	14.9
21	0	0.00a	0.00a	0.00a	0.00a	0.00a	0
	10	631.06±86.29b	120.86±7.59b	56.80±3.37b	16.21±0.79b	106.68±5.83b	32.3
	50	1508.30±94.58c	179.94±8.58c	62.89±3.02b	54.94±3.75c	133.92±6.58c	22.3
	100	2769.62±196.27d	201.61±8.86c	77.63±2.49c	98.35±4.76d	161.25±6.55d	16.9
28	0	0.00a	0.00a	0.00a	0.00a	0.00a	0
	10	759.53±93.58b	129.94±7.23b	60.83±1.08b	17.32±1.08b	120.56±6.25b	30.2
	50	1817.04±105.28c	214.37±8.69c	84.67±3.89c	58.39±3.89c	154.04±7.01c	22.0
	100	2984.54±198.89d	253.24±9.25d	103.75±5.25d	129.36±5.25d	181.59±7.56d	18.3

the roots become black after 28 d of exposure to 100 μmol/L Cd. At 10 μmol/L Cd there were slight effects on leaf growth during the whole growth course. In comparison with control, the plant height of the *S. matsudana* seedlings treated with 10 μmol/L Cd was inhibited at the 28th d. At the same treated time, the plant height in the seedlings exposed to 50 and 100 μmol/L Cd had obvious alterations in contrast with control. The growth trend was suppressed during the whole course of growth (Fig. 1a). The root length of plants treated with 10, 50, and 100 μmol/L Cd were inhibited when compared to control during the whole course growth. The inhibition degree was more and more serious with increasing the concentration of Cd and treatment time (Fig. 1b). The roots of *S. matsudana* were more sensitive than the leaves under Cd stress.

Cd Uptake and Accumulation in Different Organs

The uptake and accumulation of Cd amount in *S. matsudana* roots, new stems, leaves, xylem of old stems, and phloem of old stems varied with Cd concentration and treatment time. As shown in Table 1, the organs of *S. matsudana* in the control group had no detectable Cd, and levels of Cd accumulation were enhanced significantly (P<0.05) with increasing Cd concentration and expanding duration of treatment. The root was the initial organ of Cd entering plant, and has an obvious enrichment effect of Cd. The Cd content in roots reached the maximum value of 2984.54 μg/g after 28 days of treatment. The levels of Cd in *S. matsudana* were in the order as follows: roots > phloem of old stems > new stems > xylem of old stems > leaves when treated with 10 μmol/L for 7 d, roots > new stems > phloem of old stems > leaves > xylem of old stems at 50 μmol/L Cd during nearly all course growth, and roots > new stems > phloem of old stems > xylem of old stems > leaves at 100 μmol/L Cd after the 14th d. The translocation factor(TF) (the percentage of Cd from underground to the ground part) of *S. matsudana* in 10 μmol/L Cd treatment group was 26.6% after 7 d, 28.2% after 14 d, 32.3% after 21 d, and 30.2% after 28 d. TF were 26%, 21.6%, 22.3%, and 22% under 50 μmol/L Cd stress for 7-28 d, and 16.6% and 18.3%, respectively, in 100 μmol/L Cd treatment group for 7 d and 28 d.

Effects of Cd on Cu, Fe, Mn, and Zn Contents in Different Organs

The uptake and distribution of Cu, Fe, Mn, and Zn in *S. matsudana* were affected by different concentrations of Cd solution (Table 2-5). The results in Table 2 indicated that a low concentration of Cd (10 μmol/L) was able to promote the uptake and accumulation of Cu in contrast with control. At 100 μmol/L Cd, there were inhibitory effects on absorbing Cu in new stems and xylem of old stems. Table 3 showed that the uptake and accumulation of Fe in the organs were inhibited significantly (P<0.05) under Cd stress, and the inhibition was stronger and

Table 2 Effect of different concentrations Cd on Cu contents in the different organs of *S. matsudana.*

Time (d)	Treatment (μmol/L)	Organs (μg/g, DW±SE)				
		Roots	New stems	Leaves	Xylem of old stems	Phloem of old stems
7	0	18.48±1.37a	21.50±0.61a	24.74±1.45a	12.98±1.34a	16.30±1.21a
	10	23.09±1.75ab	22.82±1.64ab	21.57±1.38a	23.20±1.46b	19.67±1.59a
	50	26.76±1.38b	26.09±1.64b	24.55±0.85a	18.76±1.63c	15.20±1.37a
	100	21.67±1.72c	24.67±1.72ab	20.28±1.58a	12.87±1.32a	10.63±1.35b
14	0	14.18±0.82a	16.31±1.27a	16.93±1.02a	20.10±1.74a	16.35±1.38a
	10	30.63±1.38b	32.62±1.59b	17.52±1.64a	22.65±0.95a	17.77±1.54a
	50	28.17±1.27 b	22.66±0.92c	21.75±0.65b	17.95±2.71a	16.60±1.38a
	100	27.56±1.16b	14.08±1.32a	18.87±1.39a	11.28±0.91b	11.47±0.63b
21	0	17.63±0.68a	12.49±0.55a	13.62±1.32a	11.92 ±0.38a	15.14±1.20a
	10	34.56±1.04b	28.52±1.19b	13.65±1.3a7	16.52±0.82b	17.02±0.16a
	50	32.70±1.76b	20.64±1.83c	21.67±1.57b	17.39±0.69b	16.07±0.76a
	100	26.69±1.35c	13.85±1.28d	13.31±1.27a	10.80±0.80a	16.13±0.69a
28	0	16.34±1.82a	13.85±1.06a	13.29±1.43a	10.73±0.48a	12.89±1.07a
	10	27.86±1.41b	19.65±0.78b	12.05±0.79a	12.00±061a	14.84±0.67a
	50	33.02±1.81c	16.40±0.67b	18.37±0.85b	17.40±0.67b	15.93±2.41a
	100	27.65±0.34b	10.26±0.46a	12.21±0.73a	9.41± 0.43a	14.26±0.86a

Table 3 Effect of different concentrations Cd on Fe contents in the different organs of *S. matsudana.*

Time (d)	Treatment (μmol/L)	Organs (μg/g, DW±SE)				
		Roots	New stems	Leaves	Xylem of old stems	Phloem of old stems
7	0	1260.47±56.21a	1991.31±84.20a	1420.18±36.87a	911.59±12.63a	1096.29±50.41a
	10	1449.04±25.53b	1872.13±16.52b	1123.31±12.64b	832.52±16.58a	926.65±15.21b
	50	619.17±18.96c	471.14±14.23c	385.32±12.27c	405.29±12.68b	405.11±12.54c
	100	651.56±18.92c	408.83±12.34c	278.07±10.32d	192.01±8.97c	360.87±10.56c
14	0	1146.82±26.97a	1253.05±21.34a	905.35±18.97a	786.54±15.67a	853.06±16.84a
	10	1209.90±12.64a	931.89±11.68b	684.41±18.24b	621.65±12.10b	610.36±13.28b
	50	603.09±12.64b	399.44±15.75c	342.74±12.38c	330.79±11.69c	318.66±10.52c
	100	630.35±18.92b	214.83±10.20d	272.82±8.64c	283.57±6.82c	275.78±6.82c
21	0	1016.28±16.52a	883.72±12.94a	741.81±12.37a	467.36±8.92a	694.86±14.61a
	10	1103.21±63.24a	665.52±36.57b	565.54±52.74b	309.86±7.86b	407.66±12.81b
	50	525.01±12.85b	291.34±13.57c	332.48±12.46c	233.21±26.41c	313.66±62.41c
	100	608.38± 62.41b	199.77±16.24d	255.61±12.58d	243.15±9.61c	248.28±21.17d
28	0	832.00±16.74a	689.98±21.84a	421.67±2.95a	244.06±12.58a	470.09±43.79a
	10	621.36±12.58b	641.44±13.47a	355.58±10.46b	183.26±10.32b	347.66±10.55a
	50	554.14±12.57bc	268.24±13.86b	275.55±10.73c	158.46±10.82bc	226.94±10.81b
	100	495.19±10.85c	135.27±16.79c	188.49±13.82d	109.27±6.70c	238.43±10.34b

Table 4 Effect of different concentrations Cd on Mn contents in the different organs of *S. matsudana.*

Time (d)	Treatment(μmol/L)	Organs (μg/g, DW±SE)				
		Roots	New stems	Leaves	Xylem of old stems	Phloem of old stems
7	0	43.42±3.25a	40.16±2.48a	68.46±3.63a	22.25±1.84a	35.78±2.10a
	10	111.76±8.94b	52.96±3.86a	56.67±3.75b	19.55±1.98a	46.81±3.42b
	50	29.45±2.41c	28.14±2.10ab	44.12±3.12c	19.40±2.15a	24.57±2.34c
	100	16.63±1.22d	19.52±1.34b	29.49±2.47d	11.16±1.04b	9.18±1.41d
14	0	45.08±3.42a	36.86±2.03a	55.28±3.67a	16.50±1.79a	34.64±2.63a
	10	74.67±5.43b	45.62±3.15a	47.41±3.04ab	33.10±2.36b	29.57±1.27a
	50	28.82±1.30c	25.74±1.05b	43.67±2.74b	13.81±0.94ac	23.14±1.22b
	100	14.76±0.55d	18.94± 0.84c	23.07±1.24c	10.40±0.67c	8.04±0.51c
21	0	40.45±2.54a	30.06±2.46a	53.70±3.21a	17.06±1.05a	32.09±2.13a
	10	65.24±3.42b	37.55±2.16b	38.28±2.29b	25.42±1.64b	29.23±1.37a
	50	23.95±1.32c	23.58±1.28c	42.77±2.71b	14.20±0.64a	20.90±0.83b
	100	10.79±0.61d	10.97±0.45d	16.43±0.73c	8.00±0.24c	5.76±0.18c
28	0	34.88±2.43a	29.81±2.16a	47.95±3.10a	18.62±1.07a	35.14±1.84a
	10	38.26±1.84a	31.77±1.29a	44.04±2.57a	18.02±1.21a	20.79±1.36b
	50	22.08±1.23b	17.21±1.08b	32.49±1.87b	10.00±0.76b	7.03±0.24c
	100	2.73±0.12c	9.92±0.37c	13.82±0.78c	7.05±0.16b	1.71±0.12d

Table 5 Effect of different concentrations Cd on Zn contents in the different organs of *S. matsudana.*

Time (d)	Treatment (μmol/L)	Organs (μg/g, DW±SE)				
		Roots	New stems	Leaves	Xylem of old stems	Phloem of old stems
7	0	90.64±6.46a	152.58±7.17a	120.57±9.79a	76.96±6.47a	250.62±9.51a
	10	133.71±10.59b	207.25±10.64b	129.21±8.71a	84.04±6.44a	247.26±7.56a
	50	126.37±13.54b	161.31±12.64a	162.51±14.47b	74.53±5.68a	237.80±15.27a
	100	158.77±3.75b	173.53±9.24a	141.20±3.45c	67.87±3.75a	229.91±12.64a
14	0	92.77±2.34a	141.53±5.37a	96.51±8.42a	74.87±3.45a	248.01±3.78a
	10	157.39±2.37b	158.98±6.72a	111.67±3.42a	60.00±1.37b	239.55±15.34a
	50	124.72±1.34c	127.58±1.57b	130.32±2.30b	59.03±1.02b	227.11±12.09a
	100	111.06±1.85c	126.77±1.24b	115.46±1.38a	58.67±1.38b	226.22±13.01a
21	0	74.76±2.17a	105.95±1.38a	91.82±2.37a	60.09±1.34a	246.41±13.25a
	10	122.35±10.34b	135.54±10.58a	102.35±1.35a	58.13±1.36a	219.55±10.38a
	50	119.76±9.65b	112.79±6.54a	111.65±4.65a	51.56±1.59b	214.21±6.24b
	100	107.07±6.14b	118.66±5.37a	99.76±4.65a	40.39±3.54c	210.73±3.54b
28	0	66.64±1.37a	87.23±1.52a	82.08±1.45a	47.38±1.38a	204.81±10.34a
	10	103.39±6.37b	117.51±4.68b	84.4±1.38a	52.09±2.20a	190.23±4.38ab
	50	110.39±3.81b	81.16±2.37a	80.01±2.41a	50.24±1.35a	188.70±6.27b
	100	86.30±1.38c	64.40±1.39c	92.14±6.34b	44.09±1.25a	137.95±4.85c

stronger with the elongation of treatment time, which was consistent with the phenomena of leaves turning yellow due to the lack of Fe (Table 3). After 10 μmol/L Cd treatment, the contents of Mn in roots and new stems gradually increased to a different extent, but were reduced under 50 μmol/L and 100 μmol/L Cd in overall organs of *S. matsudana,* and the reduction increased with increasing Cd concentration when compared to control values (Table 4). Versus control, Zn contents in roots increased significantly and Zn contents in phloem of old stems decreased significantly under three Cd treatments (10, 50, and 100 μmol/L) during the whole treatment cycle. Zn levels in new stems were also affected by different concentrations of Cd in the nutrient solution. The general trend in new stems was that low concentrations of Cd promoted the Zn levels while high concentrations inhibited them. However, Cd had no impact on accumulating Zn in leaves and xylem of old stems (Table 5).

Effects of Cd on the Activities of SOD, POD, and CAT

The effects of Cd on SOD, POD, and CAT activities of roots and leaves in *S. matsudana* varied with the different concentrations of Cd (Fig. 2). The results showed that the SOD activity in leaves exposed to 10, 50, and 100 μmol/L Cd and that in roots exposed to 50 and 100 μmol/L Cd were observed to significantly improve during the whole treatment when compared with control (Figs 2a-b). However, the SOD activities in leaves had a sharp decline when treated with 100 μmol/L Cd on the 28th d (Fig. 2a). The SOD activities in the seedlings exposed to 10 μmol/L Cd for 14 d began to reduce significantly (P<0.05) in comparison with that in control (Fig. 2b).

As shown in Fig. 2c, the POD activities in leaves exposed to 10 and 50 μmol/L at 7th d were slightly higher (P<0.05) than that in control, but decreased significantly (P<0.05) between 14 and 21 d of Cd exposure and were lower than that in control. The POD activities in leaves treated with 100 μmol/L Cd and in roots treated with 10, 50, and 100 μmol/L Cd were noted to be significantly higher (P<0.05) than that in control (Fig. 2d). The increased percentage of POD activities was higher in roots than that in leaves during the 21 d of Cd exposure.

The CAT activities were quite different between leaves and roots. The CAT activities in leaves exposed to 10, 50, and 100 μmol/L Cd were lower than those in control during the whole treatment (Fig. 2e). On the contrary, the CAT activities in roots had no obvious distinction between the treatments and control during the 21 d period of the experiment. The CAT activities in Cd treatments increased significantly and were far higher than that in control during the 21 to 28 d (Fig. 2f).

In summary, the SOD, POD, and CAT activities in roots and the SOD activities in leaves of *S. matsudana* could be improved under Cd stress. The ability to accelerate the antioxidant enzymes (SOD, POD, and CAT) was stronger in high Cd concentration than in low concentration in *S. matsudana* under Cd stress.

Effects of Cd on the Contents of O_2^- and H_2O_2

The contents of O_2^- and H_2O_2 had a close connection with concentration of Cd and treatment time (Fig. 3). The H_2O_2 contents in leaves and roots saw a similar trend when treated with different Cd concentrations (Figs 3a-b). The H_2O_2 contents in leaves treated with 50 and 100 μmol/L Cd were greatly higher than that in control, but the H_2O_2 contents at 10 μmol/L Cd were not significantly different from that in control. Cd was capable of promoting the production of H_2O_2 compared with control. In the leaves, the contents of O_2^- in the seedlings exposed to 10 and 50 μmol/L Cd were less than in control during the 14 d of treatment. At the 21st d, the O_2^- contents were higher than that in control. At 100 μmol/L Cd concentration, the O_2^- content was increased significantly (P<0.05) when compared with that in control during the entire 28-d treatment (Fig. 3c). The O_2^- contents in roots were all enhanced under 10, 50, and 100 μmol/L Cd for 28 d, and the O_2^- contents increased with the increasing Cd concentration (Fig. 3d). Therefore, it seemed that *S. matsudana* had the ability to resist the low concentration of Cd, whereas there was a limit to struggling against the high concentration of Cd.

Discussion

As one of the most dangerous and extensively distributed pollutants, Cd had a severe threat to the environment and human health. Although Cd is not the essential element for plants, it is also readily taken in and accumulated by plants and influenced the normal metabolism of plants [16]. The most tangible effects of Cd toxicity in plants are stunted growth and leaf chlorosis [6, 17]. In this investigation, the reductions of plant height and root length were observed with increasing Cd concentrations and extending the treatment time. After being stressed for a long time, the leaves of *S. matsudana* turned slightly yellow and the roots turned black and excreted mucus at high Cd concentrations.

A recent study has shown that heavy metal Cd can be accumulated in the order roots, stems, branches, and leaves. The Cd accumulation capability of different woody plants is different and that in different parts of the same kinds of woody plants are also different [4]. Concentrations of Cd and other elements are almost significantly higher in the roots than in above-ground tissues [18]. Data from the present investigation indicated that *S. matsudana* had the ability to accumulate Cd primarily in their roots (about 70%), with lower concentrations in the shoots. However, TF increased with increasing the Cd concentration and prolonging the treatment time, and reached the maximum after 21 d of Cd stress. There are several definitions on hyperaccumulators [19-20]. Most recognized standard criteria were based on metal concentrations in

aboveground tissues of plant material sampled from its natural habitat [21]. The currently accepted 0.01% (w/w) Cd concentration in the shoot defines hyperaccumulation [22]. Data from the present investigation implied that *S. matsudana* could be efficient phytoextraction plants with a considerable ability to accumulate Cd. This was in accordance with the findings of Zárubová et al. [23].

Accumulating Cd to different organs had an obvious impact on the uptake of Cu, Fe, Mn, and Zn. Plants have a complex metal homeostasis network system that regulates the uptake and distribution of some basic elements in order to ensure the normal metabolic processes. The interactions among all the elements are well documented for herbaceous, woody, and hyperaccumulator plants

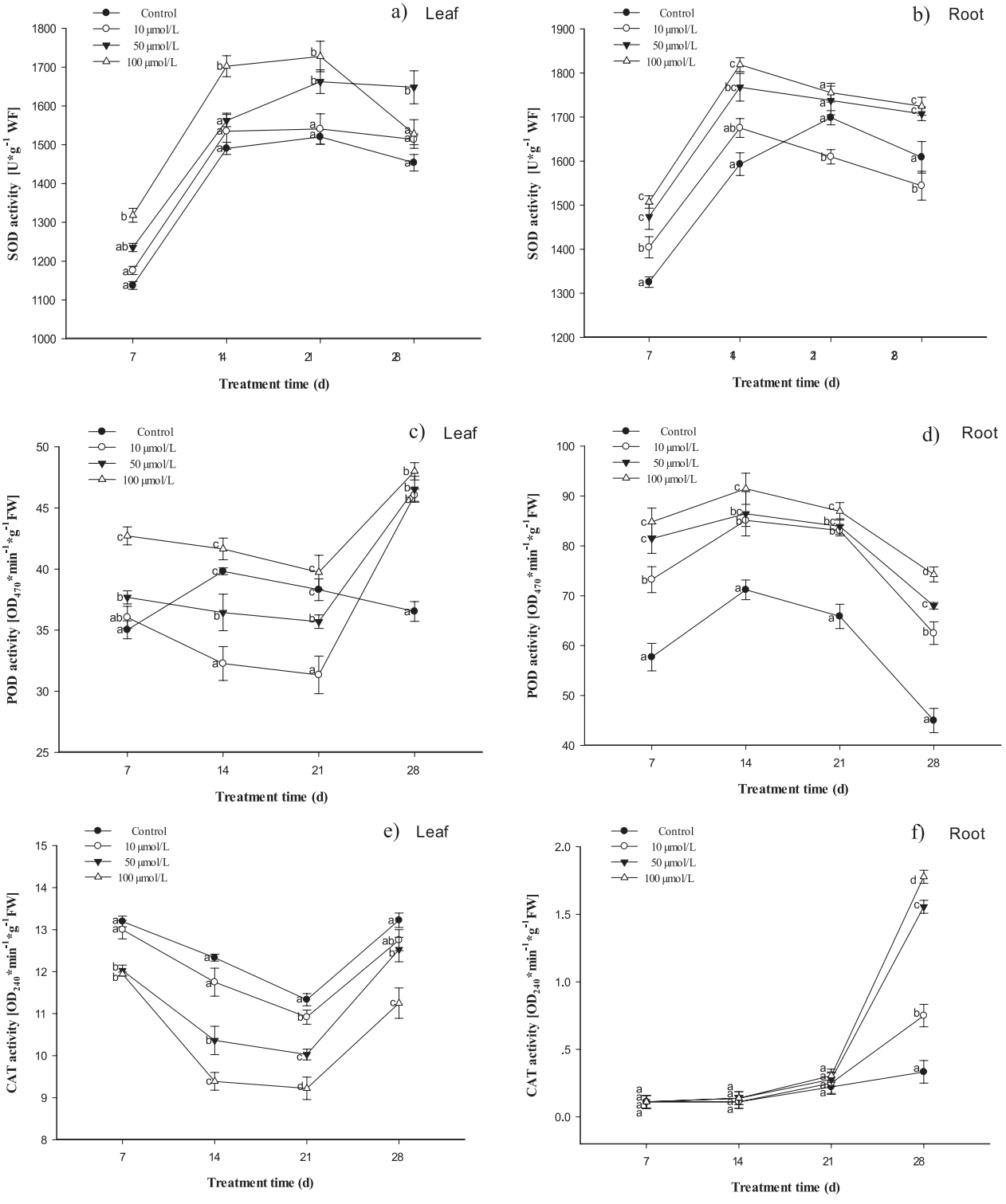

Fig. 2. Effect of different concentrations Cd on the activities of three antioxidant enzymes in leaves and roots of *S. matsudana* stressed for 7, 14, 21 and 28 d. Vertical bars denote SE, n = 5. Values with different letters differ significantly from each other (P<0.05, t-test).

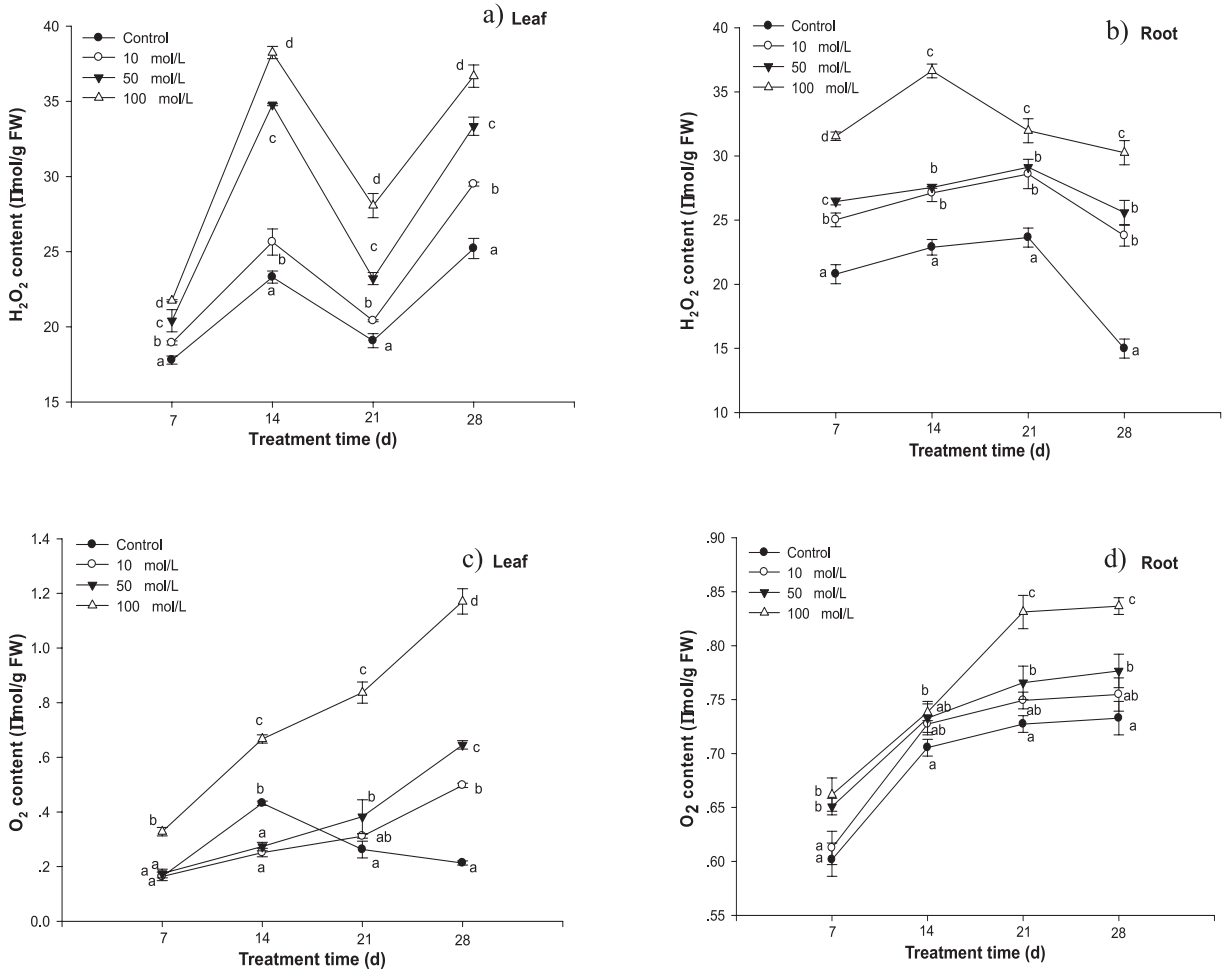

Fig. 3. Effect of different concentrations Cd on the activities of two reactive oxygen species in leaves and roots of *S. matsudana* stressed for 7, 14, 21 and 28 d. Vertical bars denote SE, n = 5. Values with different letters differ significantly from each other ($P<0.05$, t-test).

[24-26]. The Cu, Fe, Mn, and Zn are employed by organisms to perform a remarkable array of functions that are critical to life. They are indispensable elements of photosynthesis, which act as cofactors mediating diverse biochemical processes including CO_2 fixation, the synthesis of chlorophyll, the uncoupling of electron transport, ROS detoxification, and signaling events that stimulate molecular, cellular, and systemic responses [27]. In plants, these metal ions are absorbed from the soil through the roots and subsequently export from xylem parenchyma cells into xylem vessels responsible for long-distance transport to the stem [28]. The results from this investigation explained that high Cd concentration restrains the accumulation of Cu, Mn, and Zn. On the contrary, low Cd concentration was able to accelerate these elements' absorption. During the whole treatment, Fe was still inhibited by Cd. Moreover, some works have reported that the accumulation of Fe decreased under Cd stress [16].

Cd always causes disorders in mineral nutrition, and reduction in photosynthesis and oxidative stress by inducing ROS production in plants [29-30]. Normally, over-accumulation of ROS such as O_2^-, H_2O_2, hydroxyl radicals (HO-), and singlet oxygen ($^1O_2$) have a serious effect on normal metabolism by disrupting cellular macromolecules like degradation of proteins, crosslinks in DNA, and membrane fatty acid peroxidation [31]. The antioxidant enzymatic defense systems in plants are good at eliminating ROS and protecting plants against oxidative damage and indicating metal toxicity [5, 32]. The antioxidant enzymatic defense systems consist of SOD, POD, and CAT, representing a defensive strategy that plants use against Cd stress. The results also illustrated that prolonged exposure to Cd increased activities of the antioxidative enzymes, such as SOD, POD, and CAT. However, CAT activity in leaves at Cd treatment decreased its activity as exposure time increased. These antioxidative defense enzymes of *S. matsudana* could resist the Cd stress. Moreover, the results also showed that the content of O_2^- and H_2O_2 were restrained at low Cd concentrations. The activity of antioxidant enzymes and the ROS contents in *S. matsudana* can serve as useful biomarkers in ecotoxicological tests with Cd.

The results obtained in this investigation can provide valuable information for understanding the tolerance and detoxification mechanisms to Cd in *S. matsudana* and other woody plants and for choosing *S. matsudana* as one of the phytoremediation species for Cd-polluted soils.

Conclusions

Based on the information provided in this article, it is concluded that: *S. matsudana* can be considered an efficient phytoextraction plant as it has considerable ability to accumulate Cd (Cd concentration in shoot exceeding 0.01% (w/w)). Cd can decrease the uptake and accumulation of Cu, Fe, Mn, and Zn in *S. matsudana*. Cd induces high activities of SOD in roots and shoots and POD, CAT in roots, indicating that antioxidant enzymes provided a better defensive mechanism against Cd-induced oxidative damage in *S. matsudana*. In the presence of Cd, the contents of $O_2^{\cdot-}$ and H_2O_2 of *S. matsudana* increase. $O_2^{\cdot-}$ and H_2O_2 were important ROS, which causes damage to cellular plasma membrane lipids and other biomolecules.

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Conflict of Interest

The authors declare no conflict of interest.

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