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Original Research

Cadmium Effects on Mineral Accumulation and Selected Physiological and Biochemical Characters of *Salix babylonica* L.

Jie Ouyang, Binbin Li, Chonghao Li, Xiaoshuo Shang, Jinhua Zou*

Tianjin Key Laboratory of Animal and Plant Resistance, College of Life Sciences, Tianjin Normal University, Tianjin 300387, China

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Abstract

To understand the phytoremediation capability of Cd by *Salix babylonica* L. we studied Cd accumulation and translocation, antioxidant enzyme activities, lipid peroxidation, and soluble protein contents in *S. babylonica* exposed to 10, 50, and 100 μM Cd for 7, 14, 21, and 28 d. The results indicated that seedling growth was accelerated by 10 μM Cd, and significantly inhibited by 50 and 100 μM Cd. The contents of Fe and Mn decreased significantly. The superoxide dismutase (SOD) activity in roots exposed to Cd was significantly higher than that in leaves. The level of peroxidase (POD) was significantly higher than that of control except for the roots treated with 10 and 50 μM Cd on day 28. POD activity in leaves was lower than that in roots. The level of catalase (CAT) was significantly lower than that of control. At 100 μM Cd, malondialdehyde (MDA) content increased significantly during the whole experiment. 50 μM Cd could induce high content of MDA in leaves. In general, the contents of hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot-}$), and soluble protein showed an increasing trend. *S. babylonica* could be an efficient phytoextraction plant as it had considerable ability to accumulate Cd.

Keywords: accumulation, antioxidant enzymes, reactive oxygen species (ROS), malondialdehyde (MDA), soluble protein

Introduction

Cadmium (Cd) is thought to be one of the most toxic elements to all living organisms, even at low concentrations. In plants, Cd can severely influence several physiological and biochemical processes such as mineral uptake, photosynthesis, and respiration [1-2]. It has been found that Cd can interfere with microelements such as iron (Fe) and manganese (Mn), which leads to nutrient imbalance in plants [3].

Considerable attention has been attached to problems associated with Cd pollution, with rapid expansion of industrialization and the heavy use of chemical fertilizer, pesticides, and herbicides in agriculture [4]. Thus, developing efficient techniques for Cd removal from the environment is urgent and imperative. However, most conventional remediation approaches, such as excavation and chemical leaching of metals, are expensive and do not provide acceptable solutions to toxic metal pollution. The idea of using specific plants that hyperaccumulate

*e-mail: zjhmon@126.com

metals to selectively remove and recycle excessive soil metals was introduced by Chaney [5]. Hyperaccumulators can accumulate several hundred-fold certain metals comparing normal plant species, with no adverse effects on their growth [6]. Plant potential for Cd extraction generally depends on shoot Cd concentration and shoot biomass yield [7-9]. However, hyperaccumulators are species that often have a low biomass and a slow growth rate, leading to a slow time frame for metal uptake and soil decontamination [10]. The ideal plant for metal phytoextraction should be highly productive in biomass, and assimilate and translocate to shoots a significant part of the metals. Besides, favorable characteristics such as fast growth, easy propagation, and a deep rooting system should also be needed.

The Salicaceae family with its genera *Salix* (willows) and *Populus* (poplars) comprises a large number of woody species and hybrids, of which many adapted to ecological niches such as nutrient-poor, dry, wet, or metal-contaminated environments [11-14]. Willows are known to have several characteristics that make them ideal plant species for phytoremediation application, including easy propagation and cultivation, large biomass, fast growth, deep root systems, a high transpiration rate, tolerance to hypoxic conditions, and high metal accumulation capability [15-16]. *S. babylonica* is a species of willow that has adapted to waterfront and wet environment with a great potential for restoration of soil environments or contaminated water, and is one of the most widely distributed and commonly cultivated willow species in China [17]. It has been reported that *S. babylonica* could tolerate and accumulate Cd at low concentration (10 μmol/L), and was suitable for potential phytoremediation [18-19].

Oxidative damage and ROS production can be promoted by Cd in the shoots and roots of plants [20-21]. Oxidative stress is accelerated by the increasing production of ROS, and comprises both free radicals ($O_2^{\cdot-}$ and $OH^{\cdot}$) and non-radical/molecular forms (H_2O_2 and singlet oxygen) [22]. At a certain level, ROS plays an important role as signaling molecules in regulation of biological process and influences the activity of enzymes [23]. The ROS at higher concentrations are harmful to plants. For maintaining the compatible level of ROS, plants run defensive system (SOD, POD, and CAT) to scavenge ROS [24].

$O_2^{\cdot-}$ and H_2O_2 are important ROSs that cause damages to cellular plasma membrane lipids and other biomolecules [25]. It is essential to prevent oxidative damage by detoxificating $O_2^{\cdot-}$ and H_2O_2. SOD, as the major $O_2^{\cdot-}$ scavenging enzyme, catalyzes the disproportionation of $O_2^{\cdot-}$ radicals into H_2O_2 and O_2 in the cytosol, chloroplasts, and mitochondria, and it is usually considered as the first line of antioxidant defense systems, while both CAT and POD catalyze the degradation of H_2O_2 to H_2O and O_2 [26-27]. Generally, these enzymes play a cooperative role in protecting plants. MDA content is generally used as an indicator of oxidative damage to lipid peroxidation [28]. The content of soluble protein in plants exposed to Cd is changed, which plays an important role in avoiding harmful stress. There are several reports about the changes of soluble protein under Cd stress [29-31].

In order to better understand tolerant mechanisms under Cd stress in woody plants we investigated the effects of Cd on growth, Cd accumulation, and its effects on other mineral accumulation, antioxidant enzymes, MDA content, oxidative stress, and antioxidative response of *S. babylonica* plants under Cd stress.

Experimental

Culture Conditions and Cadmium Treatment

Healthy and equal-sized woody cuttings (20 cm long) from shoots of *S. babylonica* grown in the campus of Tianjin Normal University, Tianjin, China were collected and fully rinsed with distilled water before starting the experiment. They were germinated in distilled water in plastic containers for two weeks. Then the plants were grown in containers with 6 L half-strength Hoagland's solution for a week. The solution (pH 5.5) consisted of 5 mM KNO_3, 5 mM $Ca(NO_3)_2$, 1 mM KH_2PO_4, 50 μM H_3BO_3, 10 μM FeEDTA, 4.5 μM $MnCl_2$, 3.8 μM $ZnSO_4$, 0.3 μM $CuSO_4$, and 0.1 μM $(NH_4)_6Mo_7O_{24}$. The plants were randomly divided into four groups. Control groups were grown in the solution and the other three groups were treated with three different Cd concentrations of 10, 50, and 100 μM $CdCl_2$ solutions for 28 d. Cd was provided as cadmium chloride ($CdCl_2$). The solutions were continuously aerated with an aquarium air pump every day.

Plant Harvest

The plants were harvested respectively on days 7, 14, 21, and 28. Root length and shoot length were measured. At the time of harvest, the plants exposed to 0, 10, 50, and 100 μM Cd were washed thrice with distilled water and finally with deionized water. The intact plants were divided into roots and leaves for further analysis. New Hoagland's solution and different concentrations of Cd solutions were added to the remaining plants after harvest.

Determining Cd and Other Minerals

Plants exposed to 10, 50, and 100 μM Cd solutions for 28 d and control were harvested respectively based on uniformity of size and color (removing the greatest and the smallest plants and then selected randomly). The plants were washed thoroughly with running tap water, and then with deionized water. After removal of necrotic and putrid tissue, the roots were immersed in 20 mM EDTA-Na_2 for 15 min and rinsed in tap water and deionized water to remove traces of nutrients and Cd ions from the root surfaces. The plants were divided into roots and shoots (leaves, new stems, and old stems). They were dried at

45ºC for 72 h, 80ºC for 48 h, and 105ºC for 12 h, and then ground with a cutting mill (IKA-Werke GMBH & CO. KG, Germany). All dried plant samples were prepared using the wet-digestion method [32]. Concentrations of Cd, Mn, and Fe were analyzed using an atomic absorption spectrometer (PerkinElmer AAnalyst 400, USA).

Examining Antioxidant Enzyme Activities

The fresh roots (0.05 g) and leaves (0.1 g) of *S. babylonica* from each treatment were harvested at the end of each time interval (7 d), and were homogenized in a mortar and pestle with 0.05 M sodium phosphate buffer (PBS, pH 7.8). The homogenate was centrifuged at 12,000 rpm for 10 min and the supernatant liquid was used for analyzing SOD, POD, and CAT. All above steps were carried out at 4ºC.

The activities of SOD, POD, and CAT were determined by the method of Qin et al. [33], and absorbance was recorded (UV/VIS Spectrometer Lambda 35, PerkinElmer, USA).

Determining H_2O_2

Hydrogen peroxide (H_2O_2) content in *S. babylonica* leaves and roots were estimated according to the method of Hu et al. [34], with some modifications. At the end of each time interval (7 d) of the Cd treatment, the fresh leaves (0.5 g) or roots (0.5 g) were harvested and homogenized in a pestle and mortar with 5 mL cold acetone (-20ºC) and centrifuged at 12,000 rpm at 4ºC for 10 min. One milliliter of supernatant extracts was mixed thoroughly with 0.15 mL of 5% titanium sulfate and 0.2 mL of ammonia, and the reaction mixture was then centrifuged at 12,000 rpm at 4ºC for 10 min. Five milliliters of 2 M H_2SO_4 was added to dissolve the yellow precipitate. The absorbance of the yellow color solution was measured at 415 nm. The level of H_2O_2 was calculated from a standard curve generated with known concentrations of H_2O_2. The H_2O_2 content was expressed as μmol per g of fresh weight.

Determining $O_2^{\cdot-}$

Superoxide anion ($O_2^{\cdot-}$) assay was according to the method of Zhou et al. [35], with some modifications. The fresh leaves (0.2 g) and roots (0.2 g) were homogenized in a pestle and mortar with 2 mL 0.05 M PBS (pH 7.8). The homogenate was centrifuged at 12,000 rpm at 4ºC for 10 min. 0.5 mL supernatant extract was mixed thoroughly with 0.5 mL of 0.05 M PBS and 1 mL hydroxylamine hydrochloride at 25ºC for 20 min. 1 mL of 17 mM sulfanilic acid and 1mL of 7 mM alpha-Naphthylamine were added to the mixed solution for another 20 min at 25ºC. After the same volume (4 mL) of trichloromethane was added to the solution, the pink supernatant was measured at 530 nm. The $O_2^{\cdot-}$ content was expressed as μmol per g of fresh weight.

Measurement of MDA Content

The level of lipid peroxidation was expressed as the content of MDA according to Qin et al. [33]. The roots (0.15 g) and leaves (0.15 g) from each treatment were homogenized with 5 mL 10% trichloroacetic acid (TCA) with a pestle and mortar at the end of each time interval (7 d). Homogenates were centrifuged at 12,000 rpm at 4ºC for 10 min. 2 mL of 0.6% 2-thiobarbituric acid (TBA) in 10% TCA was added to each 2 mL aliquot of the supernatant. The mixtures were heated in boiled water for 15 min and then quickly cooled in an ice bath. After centrifuging at 5,000 rpm for 10 min the absorbance of the supernatant was recorded at 532 nm and 450 nm. Lipid peroxidation was expressed as the MDA content in nmol per g of fresh weight.

Determining Soluble Protein Content

Measuring soluble protein content in this investigation was carried out according to Bradford's [36] method

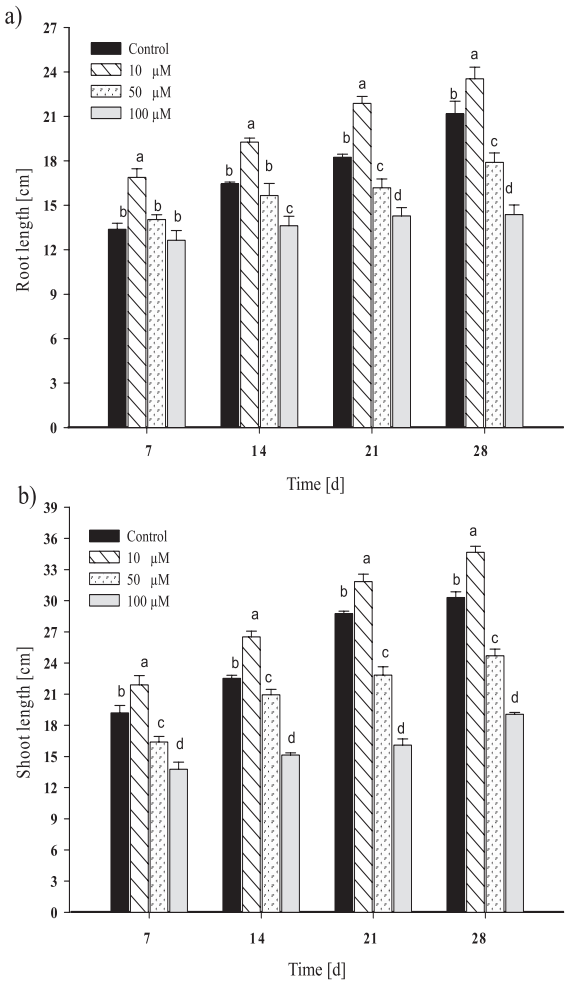

Fig. 1. Effects of different Cd concentrations on root and shoot length of *Salix babylonica* exposed to Cd stress for 28 d. a) root length, b) shoot length. Vertical bars denote SE and values with different letters differ significantly from each other (P<0.05, t-test).

Table 1. Cd, Fe, and Mn concentrations of different organs in *S. babylonica* under Cd stress for 28 d.

μg/g dry weight ±SE				
Treatment (μM)	Organs	Element		
		Cd	Fe	Mn
Control	Roots	0.00±0.00a	584.88±0.33a	401.25±0.07a
10		898.79±1.30b	501.41±0.32b	123.47±0.16b
50		1027.16±1.12c	411.13±0.71c	50.41±0.52c
100		1069.78±0.46d	399.19±2.92d	34.69±0.94d
Control	Shoots	0.00±0.00a	530.88±1.19a	172.75±0.19a
10		241.00±1.01b	379.21±0.81b	90.33±0.41b
50		688.33±1.26c	328.45±1.02c	87.21±1.27c
100		1070.33±2.90d	277.58±1.00d	77.25±0.13d

Values followed by different letters differ significantly from each other (P<0.05). Means±SE, n = 6

using bovine serum albumin (BSA) as a standard. The fresh roots (0.05 g) and leaves (0.1 g) from each treatment were washed with distilled water and homogenized in a mortar and pestle with 5 mL 0.05 M PBS (pH 7.8) at the end of each time interval (7 d) of the Cd treatment. The homogenate was centri-fuged at 12,000 rpm for 10 min and the absorbance of 0.1 mL supernatant mixed with 3 mL Coomassie Brilliant Blue G-250 reagent recorded at 595 nm after 6 min, and was used for analyzing soluble protein content. The soluble protein content was expressed as mg per g of fresh weight.

Statistical Analysis

Each treatment was replicated five times for statistical validity. Statistical analysis was performed with statistical package SPSS (version 17.0). Data were tested at a significance level of P<0.05 by one-way analysis of variance (ANOVA) completed with t-test.

Results

Effects of Cd on Seedling Growth

The effects of Cd on root and shoot growth of *S. babylonica* varied with concentration and treatment time (Fig. 1). Root length increased significantly (P<0.05) at 10 μM Cd during the whole experiment in comparison with control and the other treatment groups. 50-100 μM Cd showed the significantly inhibitory effect during the 14-28 d period treatment, except for the group exposed to 50 μM Cd on day 14 when compared with control (Fig. 1a). At 100 μM Cd, root growth was inhibited severely and stopped completely. Shoot length was exposed to 50 and 100 μM Cd decreased significantly (P<0.05) during the whole experiment time when compared with control (Fig. 1b). 10 μM Cd played a significant role in promoting shoot growth in comparison with control (P<0.05).

Cadmium Accumulation and its Effects on Other Minerals

Statistical analysis showed the presence of significant correlations between the concentration of Cd and microelements (Fe and Mn). The accumulation of Cd in *S. babylonica* roots and shoots varied with Cd concentration. The Cd content of the roots and shoots increased with increasing Cd concentration (Table 1). At low Cd concentration (10 μM), Cd ions primarily accumulated in roots, and small amounts of Cd were transported to shoots. At high Cd concentration (100 μM) there was no obvious difference between roots and shoots. In the presence of Cd, the contents of Fe and Mn in roots and shoots of *S. babylonica* de-creased significantly (P<0.05) with increasing Cd concentration.

Effects of Cd on Activities of Antioxidant Enzymes

The changes of antioxidant enzyme activities (SOD, POD, and CAT) in *S. babylonica* roots and leaves exposed to Cd at different concentrations were presented in Fig. 2. In roots the SOD activity exposed to 10 and 50 μM Cd was significantly high (P<0.05) during the whole experiment time when compared with control and 100 μM Cd treatment (Fig. 2a). There was no obvious change in activity between control and the 100 μM Cd treatment during 21-d period of treatment. The activity of SOD in roots exposed to 100 μM Cd on day 28 was lower than control. In leaves, SOD activity of all Cd treatments was significantly higher than that of control during the 28-d period (P<0.05) (Fig. 2b). SOD activity increased with increasing Cd concentration and prolonged treatment time during the 21-d experiment and declined sharply at day 28. Both controls and Cd treatments showed higher SOD activity in roots than in leaves. The effects of Cd on POD activities of *S. babylonica* roots and leaves varied

with the different Cd concentrations and the duration of treatment. The POD activity in roots exposed to all Cd concentrations was significant (P<0.05) during the whole experiment except for the groups treated with 10 and 50 μM Cd on day 28 (Fig. 2c). Cd induced significantly high POD activity in leaves during the whole Cd period treatment in comparison with control (Fig. 2d). The POD activity in leaves was lower than that in roots. The CAT activities in roots and leaves of *S. babylonica* were present in Figs 2(e-f). The activities of CAT in roots and leaves exposed to Cd decreased significantly (P<0.05) during the 28-d treatment when compared with control. Both controls and Cd treatments showed higher CAT activity in leaves than in roots.

Effects of Cd on H_2O_2 and $O_2^{\cdot-}$ Content

Information on H_2O_2 and $O_2^{\cdot-}$ contents of *S. babylonica* stressed by Cd is shown in Figs 3-4. In roots the H_2O_2 contents in all Cd treatments were significantly higher

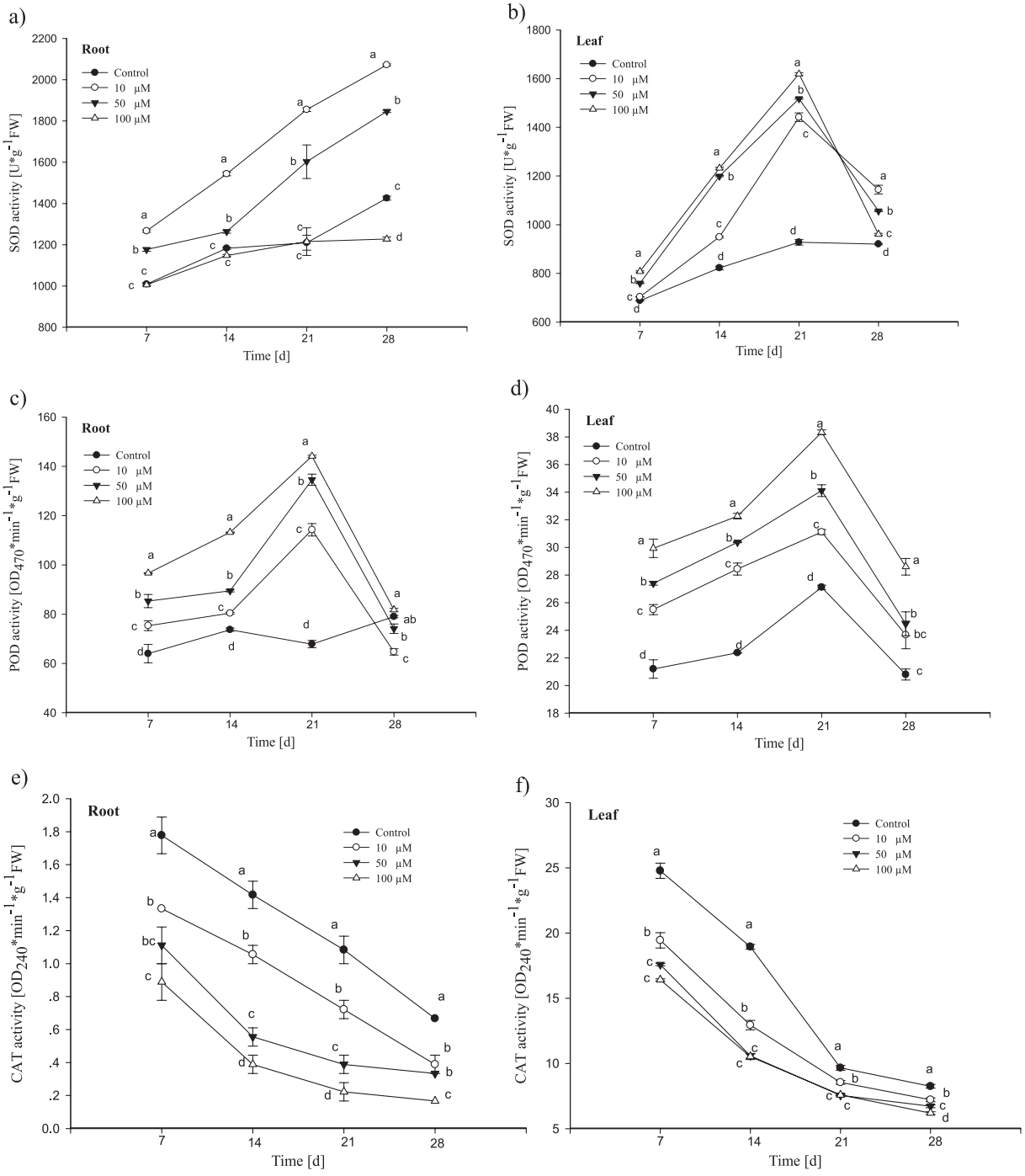

Fig. 2. Effects of different Cd concentrations on the activities of three antioxidant enzymes in *Salix babylonica* exposed to Cd stress for 28 d. a) SOD in roots, b) SOD in leaves, c) POD in roots, d) POD in leaves, e) CAT in roots, f) CAT in leaves. Vertical bars denote SE and values with different letters differ significantly from each other (P<0.05, t-test).

(P<0.05) during the whole experiment than that in control, except for the group exposed to 10 μM Cd on days 7 and 28 (Fig. 3a). The contents of H_2O_2 in leaves increased significantly (P<0.05) with increasing Cd concentration and prolonged treatment time, except for the group treated with 10 μM Cd on days 21 and 28 (Fig. 3b). The $O_2^{\cdot-}$ contents in roots exposed to Cd increased significantly (P<0.05) in comparison with control during the 28-d treatment (Fig. 4a). In leaves, the $O_2^{\cdot-}$ contents rose significantly (P<0.05) during days 14 and 21 when compared with control (Fig. 4b). There were no obvious changes in the $O_2^{\cdot-}$ contents of leaves exposed to Cd except for the groups treated with 100 μM Cd on days 7 and 28.

Effects of Cd on Lipid Peroxidation

The effects of Cd on MDA contents of *S. babylonica* are presented in Fig. 5. The MDA contents in roots exposed to 100 μM Cd are significantly high (P<0.05) during the whole experiment when compared with control and the other treatment groups (Fig. 5a). There were no obvious differences in root MDA contents between control and the 10 and 50 μM Cd treatment groups. Data from Fig. 5b) revealed that 50 and 100 μM Cd could raise MDA content significantly (P<0.05) in leaves when compared with control and other treatment groups during the whole experiment. 10 μM Cd had no obvious effects on MDA content during the whole experiment in comparison with control.

Effects of Cd on Soluble Protein Contents

The effects of Cd on soluble protein contents are presented in Fig. 6. Soluble protein contents of roots and leaves varied with different Cd concentrations. The content of soluble protein in roots exposed to 50 μM Cd was the highest among all the groups (Fig. 6a). The soluble protein contents in roots of all Cd treatments were significantly higher (P<0.05) than that in control during the whole 28-d period (Fig. 6a), and the contents

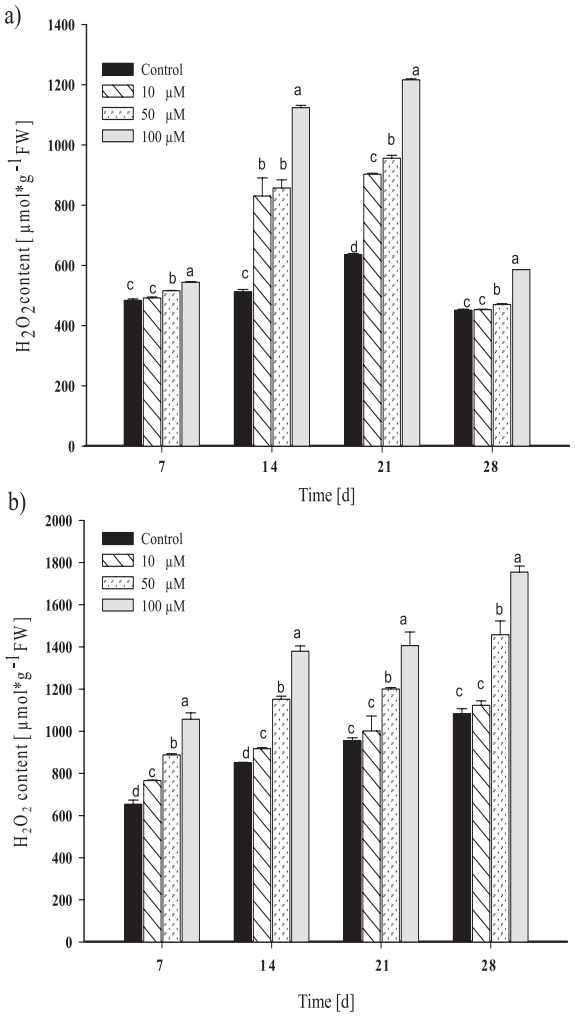

Fig. 3. Effects of different Cd concentrations on the H_2O_2 contents in *Salix babylonica* exposed to Cd stress for 28 d. a) H_2O_2 content in roots, b) H_2O_2 content in leaves. Vertical bars denote SE and values with different letters differ significantly from each other (P<0.05, t-test).

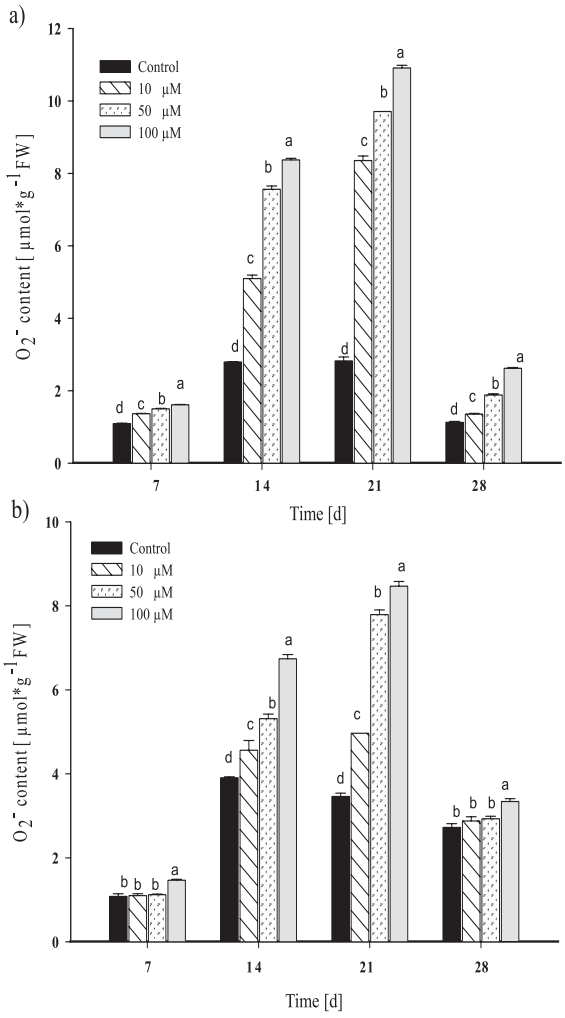

Fig. 4. Effects of different Cd concentrations on the $O_2^{\cdot-}$ content in *Salix babylonica* exposed to Cd stress for 28 d. a) $O_2^{\cdot-}$ content in roots, b) $O_2^{\cdot-}$ content in leaves. Vertical bars denote SE and values with different letters differ significantly from each other (P<0.05, t-test).

of soluble protein in leaves exposed to all Cd treatment concentrations increased significantly (P<0.05) during the 21-d treatment period, except for the 10 μM Cd group on days 7 and 21 (Fig. 6b).

Discussion

The results in the present investigation show that during the whole experiment Cd accelerated plant growth of *S. babylonica* significantly (P<0.05) at 10 μM concentration, and 50 and 100 μM Cd had significantly inhibitory effects (P<0.05). This is in agreement with the findings of Yang and Chen [18].

There have been many reports since then on the use of some crop plants and forest species (including poplar and willows) to remove Cd from contaminated soils [4, 10, 25, 37-40]. It has been reported that a few clones of willows have high heavy metal tolerance [13-14, 41]. Plants growing on heavy metal-contaminated soils had to develop adaptation strategies requiring complex alterations in the plant metal homeostasis network, including metal uptake, chelation, transport, storage, biochemical detoxification, and tolerance [42]. Data from the present investigation showed that *S. babylonica* had the ability to accumulate Cd primarily in their roots (79%) at low Cd concentration (10 μM) with lower concentrations in the shoots, while at high Cd concentration (100 μM) Cd accumulation was more or less the same between roots and shoots. There were several definitions on hyperaccumulators [9, 42]. Most recognized standard criteria were based on metal concentrations in aboveground tissue of plant material sampled from its natural habitat [4]. According to the currently accepted shoot concentration defining hyperaccumulation being 0.01% (w/w) for Cd [8], *S. babylonica* could be considered an efficient phytoextraction plant with considerable ability to accumulate Cd. This was in accordance with the findings of Dos Santos Utmazian and Wenzel [41], Yang and Chen [18], Wang et al. [19], and Ling et al. [39]. But its

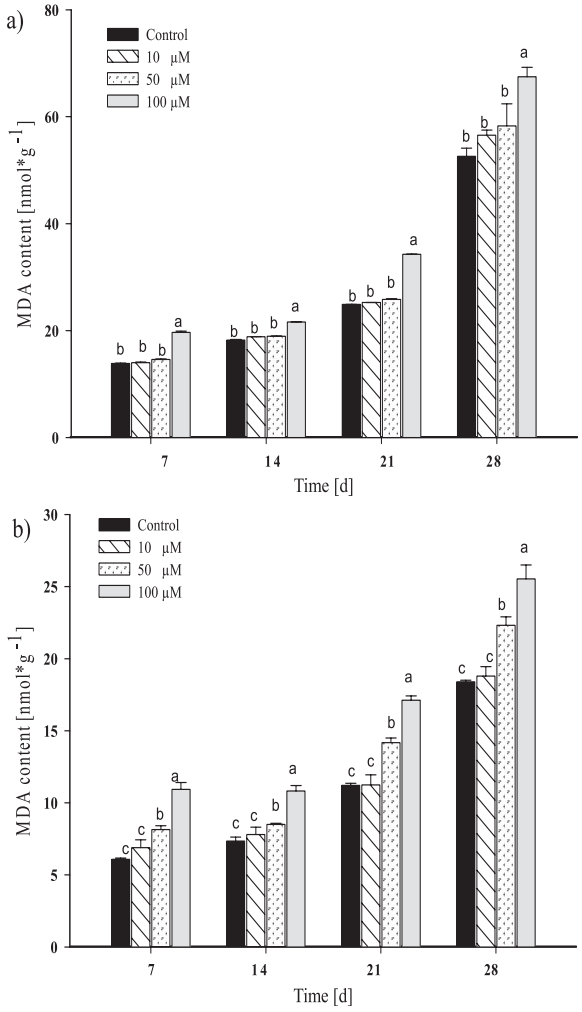

Fig. 5. Effects of different Cd concentrations on the MDA content in *Salix babylonica* exposed to Cd stress for 28 d. a)MDA content in roots, b) MDA content in leaves. Vertical bars denote SE and values with different letters differ significantly from each other (P<0.05, t-test).

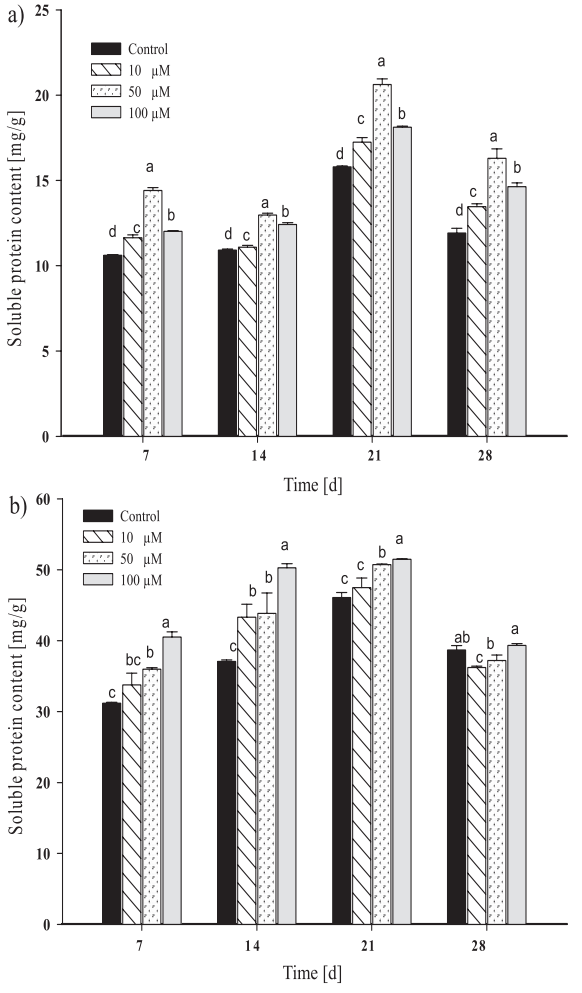

Fig. 6. Effects of different Cd concentrations on the soluble protein content in *Salix babylonica* exposed to Cd stress for 28 d. a) soluble protein content in roots, b) soluble protein content in leaves. Vertical bars denote SE and values with different letters differ significantly from each other (P<0.05, t-test).

accumulating Cd ability was low when compared with *Salix smithiana, Salix dasyclados*, and *Salix matsudana* [14, 19, 39, 41, 43].

Fe and Mn play a vital role in mitigating Cd stress on plants by activating certain Cd avoidance and/or tolerance mechanisms in plants [43-46]. The results in the present investigation indicated that in the presence of Cd and the contents of Mn and Fe in roots and shoots of *S. babylonica* decreased significantly ($P<0.05$) with increasing Cd concentrations during the whole treatment period. Some research has indicated that Fe and Mn were conducive to internal defensive mechanisms in plants [3]. The two elements compete with Cd in the active transporters and can minimize Cd transportation into plants. So the concentrations of Fe and Mn, and Cd levels in the seedlings showed a negative correlation during the whole experiment. This may explain why concentrations of Fe and Mn gradually declined with increased Cd concentrations in the seedlings. Adding Mn to the solution containing Cd can significantly improve plant growth and reduced the concentrations of Cd in all organs of the plant [47]. Evidence from Kovács et al.'s work [48] demonstrated that Fe ions might compete with Cd ions for the same membrane binding (transport) sites in plants. Adding an adequate Fe ion to plants exposed to Cd can result in increased activity of antioxidative enzymes against oxidative stress [20].

Plants possess a number of antioxidant systems that protect them from oxidative damage [49]. These defensive systems are composed of enzymatic scavengers of activated oxygen such as SOD, POD, and CAT [50]. Data from the investigation showed that the SOD activities in the leaves of *S. babylonica* exposed to all the Cd treatments and in the roots exposed to 10 and 50 μM Cd were significantly higher ($P<0.05$) than those in controls. High SOD activity has been associated with stress tolerance in plants, which may be attributed to the increased production of superoxide, resulting in the activation of existing enzyme pools or increased expression of genes encoding SOD [50]. The activity of SOD in the roots exposed to 100 μM Cd was significantly lower ($P<0.05$) compared to control in the present investigation, suggesting that reduced SOD might be due to an inactivation of the enzyme by H_2O_2 produced in different compartments, where SOD catalyzes the disproportionation of superoxide radicals [51]. POD is also an important enzyme that is able to scavenge H_2O_2, which is a major substance degraded by SOD. The activity of POD in *S. babylonica* increased significantly ($P<0.05$) except for the roots treated with 10 and 50 μM Cd and the leaves exposed to 10 μM Cd at day 28 in comparison with controls in the present investigation. Increased POD activity might be due to Cd directly causing excessive production of H_2O_2 in plants. Thus, increased POD activity, in turn, scavenged excessive H_2O_2 and damage was limited [4]. CAT is an antioxidant enzyme that degrades hydrogen peroxide into water and oxygen [52-53]. The activity of CAT in roots and leaves of *S. babylonica* declined during the whole experiment when compared with control. The decline in CAT activity was supposedly due to inhibition of enzyme synthesis or a change in assembly of enzyme subunits [53]. CAT is possibly a less efficient H_2O_2 scavenger than POD because of its low substrate affinity and is more sensitive to high Cd levels than SOD and POD as seen by the decline in CAT activity. In the present work, CAT activity decreased, suggesting that eliminating ROS by CAT was limited. Cd induced higher SOD and POD activities than CAT activity, indicating that SOD and POD provided a better defensive mechanism against Cd-induced oxidative damage in *S. babylonica*.

MDA formation is used as the general indicator of the extent of lipid peroxidation resulting from oxidative stress. The major consequence of Cd stress in plants is an enhanced production of high ROS, which are toxic to living systems and usually cause oxidative damage, inducing lipid peroxidation [54]. Data from the present work showed that the content of MDA increased significantly ($P<0.05$) only at 100 μM Cd in roots and 50 and 100 μM Cd in leaves compared with controls, indicating that Cd indirectly leads to the production of superoxide radicals, resulting in increased lipid peroxidative products and oxidative stress in *S. babylonica*. Consequently, ROS-induced cellular damage induces local programmed cell death, which generally affects plant growth and development [55]. Some studies indicated that Cd toxicity was proven to induce the formation of various ROS and increase MDA content as a result of lipid peroxidation [21]. The present investigation showed that Cd exposure caused an increase in $O_2^{\cdot-}$ and H_2O_2 concentrations associated with impaired integrity of the plasma membrane in *S. babylonica* [56].

The soluble protein content of *S. babylonica* increased under Cd stress in the present investigation. This can be explained by the fact that soluble protein is related to a variety of metabolic processes in cells, and Cd stress can induce related stress protein gene expression, which is a defensive mechanism of plants to environmental stress [40, 57].

Conclusions

Based on the information provided in this article, we conclude that:

1. *S. babylonica* can be considered as 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 Fe and Mn in *S. babylonica*.
2. In the presence of Cd, the contents of $O_2^{\cdot-}$ and H_2O_2 of *S. babylonica* increase. The MDA content increases significantly. Meanwhile, Cd induces higher activities of SOD and POD than that of CAT, indicating that SOD and POD provide a better defensive mechanism against Cd-induced oxidative damage in *S. babylonica.*
3. The soluble protein content of *S. babylonica* increases under Cd stress.

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