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Full Length Research Paper

Uptake of cadmium from hydroponic solutions by willows (*Salix* spp.) seedlings

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Willow (*Salix* sp.) has large biomass production and high resistance to environmental stress. As an important multipurpose tree species in afforestation, it has been proved to be effective in the uptake and accumulation of metals from contaminated waters and soils. Suliu172 (*Salix × jiangsuensis* 'J172'), Hanliu (*Salix matsudana*), Weishanhu (*Salix integra* 'Weishanhu') and Yizhibi (*S. integra* 'Yizhibi') were chosen as model plants to evaluate their potential for uptake of cadmium from hydroponic culture and relative uptake mechanism. Cadmium uptake showed a linear increase in the short time course, and a nonlinear and slow increase in the long time course. After one week cultivation, cadmium accumulation in different parts of willows generally followed the order of root > stem > leaf. Cadmium influx in willow roots increased with the increase of cadmium concentration in hydroponic solutions. A modified Michaelis-Menten equation was employed to describe the concentration-dependent kinetics of cadmium uptake through the roots. Cadmium influx could be resolved into linear and saturable components under concentration–dependent kinetics. The saturable component followed Michaelis–Menten kinetics, which indicated that cadmium uptake across the plasma membrane was transporter–mediated. The uptake capacity (V_{max}/K_m) jointly decided by the V_{max} and K_m followed descending order of Hanliu > Weishanhu ≈ Yizhibi > Suliu172, indicating that their inherent potential of cadmium uptake reduced in turn. Low temperature and metabolic inhibitor inhibited the apparent uptake of cadmium in willow. Both active absorption and passive absorption occurred in the cadmium uptake by willow roots.

Key words: Cadmium, willow, uptake kinetics.

INTRODUCTION

With the rapid development of mining, smelting, electroplating industries and agricultural activities such as the application of fertilizers and pesticides, cadmium pollution has become a severe and growing problem of concern (Taylor, 1997; Waalkes, 2000). Due to its harmful effects, the World Health Organization (WHO) has set a maximum limit concentration of 0.003 mg/L for cadmium in drinking water (WHO, 2008). Cadmium is a toxic metal without any known physiological function in plants and can be transferred efficiently from soil to plants. Cadmium in plant disrupts antioxidant enzyme system and causes metabolite modifications (Zhao, 2011; Zoghlami et al., 2011). Moreover, special consideration should be paid to cadmium pollution in water-soil-plant systems because of its high mobility and low toxic concentrations in organisms (Moreno et al., 2000). Cadmium may pose a risk to human and animal health due to the transfer of a high level of cadmium from agricultural soils or aquatic system to the human food chain (Jackson and Alloway, 1992). Therefore, cadmium is one of the most important metals to be considered in terms of food-chain contamination.

Phytoremediation is a promising approach for *in situ* cleanup of contaminated sites. However, choosing a plant species that can remove metal ions from contaminated soil or water depends on three variables in the practice: plant growth, biomass and metal levels (Williams, 2002). Willow is an important multipurpose tree species in afforestation, which shows high resistance to salt and alkali, drought and water logging stress, and can grow well in all kinds of waters and soils. Willow has large

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biomass production and is not directly associated with the food chain, which qualifies it for removing the soil pollutants gradually through short-rotation cultivation and harvesting periodically (Jaconette et al., 2005). Willow has remarkable capacity to concentrate elements including toxic heavy metals (Greger and Landberg, 1999; Dickinson and Pulford, 2005), especially cadmium (Berndes et al., 2004; Meers et al., 2007). Willow also has deep root system compared to grasses which can act as biological filter. These traits make it a potential ideal candidate for phytoremediation of cadmium contaminated waters and soils. Dickinson and Pulford, (2005) also reported that willow is a hyperaccumulator, and extracting cadmium with willow is an effective and low-cost phytoremediation method. Hence, it is necessary to understand the cadmium accumulation mechanism in order to assess the potential.

Many researchers have focus on growth performance and cadmium uptake by willow adopting soil culture or field experiments, while kinetics of cadmium uptake by willow are rarely investigated. Willow's cadmium uptake characteristics can be assessed rapidly using hydroponics or nutrient solutions culture (Watson et al., 2003). Furthermore, willow has high resistance to water logging stress, a primary evaluation of its phytoremediation ability towards water pollution by cadmium. The objective of this study was to examine whether willow is able to accumulate cadmium from hydroponic solutions. Hence, accumulation and uptake kinetics of cadmium by Suliu172 (*Salix* × *jiangsuensis* 'J172'), Hanliu (*Salix matsudana*), Weishanhu (*Salix integra* 'Weishanhu') and Yizhibi (*S. integra* 'Yizhibi') were investigated. The roles of bivalent cations, cation channel inhibitor (La and Cs), low temperature and metabolic inhibitor in the uptake of cadmium by Yizhibi and Suliu172 were also studied.

MATERIALS AND METHODS

Plant culture and pre-treatments

Four species of willows, Suliu172 (*Salix* × *jiangsuensis* 'J172'), Hanliu (*S. matsudana*), Weishanhu (*S.alix integra* 'Weishanhu') and Yizhibi (*S. integra* 'Yizhibi'), were collected from a nursery in Hangzhou, Zhejiang Province, China. Culture vessel was a 40 × 20 × 15 cm plastic box. One year old willow branches collected from the nursery were cut into about 10 cm length, then uniform and healthy cuttings were selected and cultivated in the basal nutrient solution containing (mg/L): KNO_3, 510; $Ca(NO_3)_2$, 820; $MgSO_4·7H_2O$, 490; KH_2PO_4, 136; $FeSO_4$, 0.6; H_3BO_3, 2.86; $MnCl_2·4H_2O$, 1.81; $ZnSO_4·7H_2O$, 0.22; $(NH_4)Mo_7O_{24}$, 0.45; EDTA, 0.744; $CuSO_4·5H_2O$, 0.08. The nutrient solution was aerated continuously and renewed every seven days. Nutrient solution pH was adjusted daily to 5.8 with 0.1 mol/L NaOH or HCl. Plants were grown under glasshouse conditions with natural light, day/night temperature of 26/20°C and day/night humidity of 70/85%. After four weeks of cultivation, the heights of willows seedlings were 0.8 to 1.5 m high depending on different species, which were the applicable size for this study.

Willow plants with uniform size for each species were selected and rinsed in deionized water, and then treated with a pre-treatment solution containing 2 mmol/L MES-TRIS (pH 5.8) and 0.5 mmol/L $CaCl_2$ (Lasat et al., 1996). After 12 h pre-treatment, the plants were used for different experiments as subsequently described. All the experiments were carried out in vessels filled with an uptake solution identical to the pre-treatment solution. Cadmium was added as $CdCl_2·2.5H_2O$ into the uptake solution 24 h before each experiment and stirred to ensure complete mixing. Before uptake experiment, 1 ml of uptake solution was collected and cadmium content was measured.

Time-course dynamics of cadmium uptake and accumulation

Willow roots were immersed in 400 ml uptake solution containing 2 mmol/L MES-TRIS (pH 5.8), 0.5 mmol/L $CaCl_2$, and 10 μM/L $CdCl_2$, at each time interval (0 to 90 min for short term, and 2 to 72 h for long term time course experiments, respectively), willow was harvested and desorbed in ice-cold desorption solutions (2 mmol/LMES-TRIS, and 5 mmol/L $CaCl_2$, pH 5.8) for 15 min in order to remove most of the cadmium adsorbed on cell walls of roots (Lasat et al., 1996). After desorption, the plants were separated roots from shoots, the roots and shoots were oven-dried at 65°C for 72 h, and weighed. Dried plant materials were ground using a mill. Plant materials were digested with $HNO_3/HClO_4$ (87/13, v/v) and the total concentrations of cadmium were determined using flame or graphite atomic absorption spectrometry (SOLAAR–M6, Thermo Fisher Scientific). For the long-term experiments, uptake solution was replaced every 8 h to keep the concentrations of cadmium in the uptake solutions unchanged. All treatments were performed in three replicates with two willow plants in each pot.

Cadmium accumulation in roots, stems and leaves

Four plants each were planted in 10 L plastic box containing 10 L solutions with low concentration (2 μmol/L $CdCl_2$) and high concentration (100 μmol/L $CdCl_2$) of cadmium for one week. The uptake solutions were replaced every two days. All treatments were performed in three replicates with four willow plants in each 10 L plastic box. After one week growth, willows were harvested and desorbed for 15 min, separated into roots, stems and leaves, and then oven-dried at 65°C for 72 h. Dried plant materials were ground and digested, and cadmium concentrations were determined using flame or graphite atomic absorption spectrometry.

Concentration-dependent kinetics of cadmium uptake

Willow was transferred to hydroponic pots containing 400 ml of uptake solution. Ten different concentrations of cadmium (0.25, 0.5, 1, 2, 5, 10, 15, 30, 50 and 100 μmol/L) were used to study the influx kinetics of cadmium, and each treatment was replicated three times and each replicate had one pot with two willow plants. After 60 min of uptake, the plants were quickly rinsed with the pretreatment solution, and then desorbed in ice-cold desorption solutions (2 mmol/LMES-TRIS, and 5 mmol/L $CaCl_2$, pH 5.8) for 15 min. The plants were separated into roots and shoots, blotted dry with paper tissue, and dried at 65°C for 72 h. Cadmium concentrations were determined as previously described.

Effects of Zn, Mg, Mn, Fe, Cu, La and Cs on cadmium influx

To investigate the effects of metal cations and cation channel inhibitors on cadmium uptake, the experiment was conducted using Suliu172 and Yizhibi as model plants. The uptake solutions containing (μmol/L) 10 $CdCl_2$, 10 $CdCl_2$ +10 $ZnCl_2$, 10 $CdCl_2$ + 10 $MgCl_2$, 10 $CdCl_2$ + 10 $MnCl_2$, 10 $CdCl_2$ + 10 $FeCl_2$, 10 $CdCl_2$ + 10

$CuCl_2$ and 10 $CdCl_2$ + 50 $LaCl_3$, and 10 $CdCl_2$ + 50 CsCl, respectively, were used. After 4 h of uptake, the willow plant roots were desorbed as previously described and then the roots and shoots were separated, oven-dried at 65°C for 72 h, and weighed for the determination of cadmium. All experiments were in three replicates for each treatment and each replicate had one pot with two willow plants.

Effects of low temperature or metabolic inhibitors on cadmium uptake

Plants were cultured in the uptake solution containing 2 mmol/L MES-TRIS (pH 5.8), 0.5 mmol/L $CaCl_2$, and 10 mol/L $CdCl_2$ for different treatments: control, 2,4-dinitrophenol (DNP) (100 mol/L), and 2°C. For the 2°C treatment, plants were transferred to ice-cold pre-treatment solution for 30 min prior to the uptake, and then the uptake containers were placed in an ice bath and shaded from light. At each time interval (0.5, 1, 2, 4, 8, 16 and 24 h), water loss caused by transpiration was measured by weighing and compensated by addition of deionized water. A 2.0 ml aliquot of the uptake solution was taken out from each pot for the determination of cadmium concentrations, and 2.0 ml of deionized water was then added to each pot. Total amounts of cadmium removed by sampling of the uptake solution were < 2% of the initial amounts of cadmium in each pot. After 24 h of treatment, plants were rinsed, separated into roots and shoots, blotted dry with tissue paper, then oven-dried and weighed. Cumulative uptake of cadmium by willows for each treatment was calculated from total cumulative depletion of cadmium in the uptake solution. All treatments were performed in three replicates with two willow plants in each pot.

Statistical analysis

All statistical analyses (ANOVA and LSD test for mean comparisons) were conducted with SPSS 16.0. Differences at $p < 0.05$ were considered significant. Independent-samples *T*-tests were adopted to compare the metal cations treatments with control.

RESULTS AND DISCUSSION

Time–course dynamics of cadmium uptake and accumulation

The uptake time is one of the most important factors affecting the uptake of heavy metals by plants. Uptake solutions of 10 μmol/L cadmium were chosen to study the short-term and long-term cadmium influx for four willow species, as willow could retain normal growth under the concentration of cadmium at 10 μmol/L in solution. The short- and long-term uptake periods were defined as 5 to 90 min and 2 to 72 h, respectively (Figures 1 and 2). Figure 1 shows that the influx of cadmium in roots of willow was more or less linear within 90 min. The observation that linear, time-dependent cadmium accumulation intersected the y-axis above the origin in four willow species indicated that quite an amount of cadmium was not completely removed from roots with the desorption regime used in these experiments (Lu et al., 2008). The slope of Suliu172 with the value of 0.006 μmol/g root dry weight/min was the lowest among the four cultivars. The slope k values of Hanliu, Yizhibi and Weishanhu showed no significant differences, which implied that the influx rates of the three willow species were almost alike during the short-term period within 90 min. Moreover, after 90 min uptake, the cadmium concentration in the roots of Hanliu, Yizhibi and Weishanhu exhibited similar value, about 1.6 μmol/g root dry weight (DW), while Suliu172 was the lowest with the value of 1.1 μmol/g root dry weight.

Furthermore, Figure 2 shows that the cadmium uptake gradually increased with increasing uptake time of 2 to 72 h. After 72 h of uptake, the four willow species showed a significant difference in the cadmium uptake and accumulation (Figure 2). Also, after 72 h uptake of cadmium, the total cadmium accumulated in Hanliu was the highest (9.3 μmol/g root DW). The total cadmium accumulated in Suliu172 and Weishanhu were found to be 6.4 and 6.1 μmol/g root DW, respectively; and the total cadmium accumulated in Yizhibi was the lowest (5.2 μmol/g root DW). The uptake rate became slower for all species at about 4 h for Hanliu and Weishanhu, while at about 8 h for Yizhibi and Suliu172. After the slower uptake rate period, they however returned to a high uptake rate.

Cadmium accumulation in roots, stems and leaves

The results of cadmium accumulation in willow roots, stems and leaves are given in Table 1. Cadmium content in different parts generally followed the order of root > stem > leaf. The result suggests that cadmium was mainly accumulated in willow roots. Cadmium content in roots showed a descending order of Hanliu > Suliu172 > Yizhibi > Weishanhu, which varied from 1.011 to 1.310 μmol/g (2 μmol/L) and from 4.535 to 5.432 μmol/g (100 μmol/L). Cadmium content in stems were not significantly different among the four willow species at solution of cadmium concentration of 2 μmol/L, while Suliu172 was significantly higher than Hanliu, Weishanhu and Yizhibi at 100 μmol/L. Cadmium content in willow leaves of Suliu172 and Yizhibi were significantly higher than Weishanhu and Hanliu at 2 and 100 μmol/L.

Willows have been shown to be promising for cadmium phytoextraction. Utmazian et al. (2007) reported that the cadmium concentrations in leaves varied between 11.9 and 315 mg/kg (0.156 and 2.802 μmol/g), and the corresponding cadmium concentrations in roots between 237 and 2610 mg/kg (2.108 and 23.219 μmol/g) among 20 willow clones when they were exposed in 4.45 μmol/L cadmium solutions for four weeks. Cosio et al. (2006) reported that cadmium content were 39 mg/kg (0.347 μmol/g) in leaves and 313 mg/kg (2.784 μmol/g) in roots at 3 μmol/L nutrient, which increased to 260 mg/kg (2.313 μmol/g) in leaves and 798 mg/kg (7.099 μmol/g) in roots at 100 μmol/L for *S. viminalis* cultivated within six weeks in uptake solutions. Compared to the aforementioned

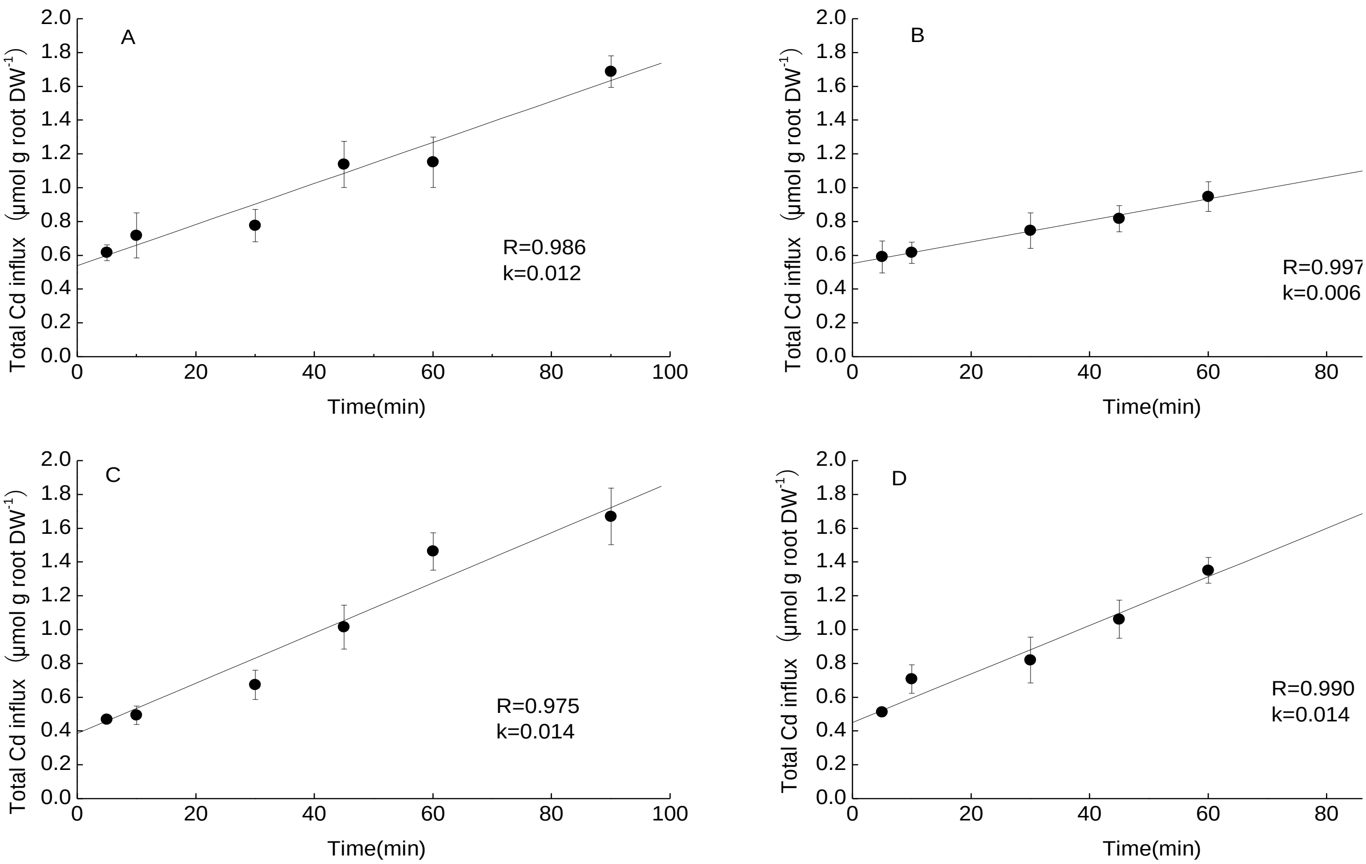

Figure 1. Short-term (5-90 min) cadmium influx of Yizhibi (A), Suliu172 (B), Hanliu (C) and Weishanhu (D). Data points and error bars represent means and SE, respectively. Error bars do not extend outside some symbols. DW, dry weight.

results, cadmium accumulations of the four species in this experiment were relatively lower in general, which can be attributed to the shorter uptake time and different willow species. The variability in both cadmium accumulation and tolerance specificity exists between willow species (Hakmaoui et al., 2006; Utmazian et al*.,* 2007).

Concentration–dependent kinetics of cadmium uptake

Figure 3 shows that the concentration-dependent cadmium influx kinetics of four willow species was characterized by smooth, non-saturating curves. The experimental curves could be graphically resolved into saturable and linear components by a modified Michaelis-Menten kinetics model:

$$V = V_{max}[C] / (K_m + [C]) + k[C]$$

Where, V_{max} is the maximum influx rate of plant root cells, reflecting the inherent potential of uptake by plant roots (higher V_{max} value indicates a higher inherent potential); K_m is the characteristic constant which shows the relationship between plant cells and the elements absorption; a small K_m indicates high affinity. The linear component represents cell-wall-bound cadmium that remained after desorption procedure. The saturable component of uptake probably indicates carrier-mediated transport across the root cell plasma membranes. Similar concentration-dependent kinetics has been reported for Zn (Lasat et al., 1996; Cohen et al., 1998; Hart et al., 1998; Lombi et al., 2001).

Analysis of the kinetic constants for cadmium uptake indicated that influx characteristics were different (Table 2). The V_{max} values of the four willow species varied between 2.31 to 11.67 μmol/g DW/h and followed the order of Hanliu > Weishanhu > Yizhibi > Suliu172, which indicated that their inherent potential of cadmium uptake reduced in turn. Uptake capacity of Hanliu was about four times that of Suliu172, and about two times that of Weishanhu and Yizhibi, while the uptake capacity of Weishanhu and Yizhibi's was similar. The K_m value of the four willow species varied between 27.03 to 79.04 μmol/L; the K_m values of the four willow species at the same order of magnitude mean similar affinity.

It is generally recognized that the uptake capacity of plants for ions from soils is also affected by the surface area and morphology of roots, rhizosphere pH, root exudates and other factors (Jones et al., 1996;

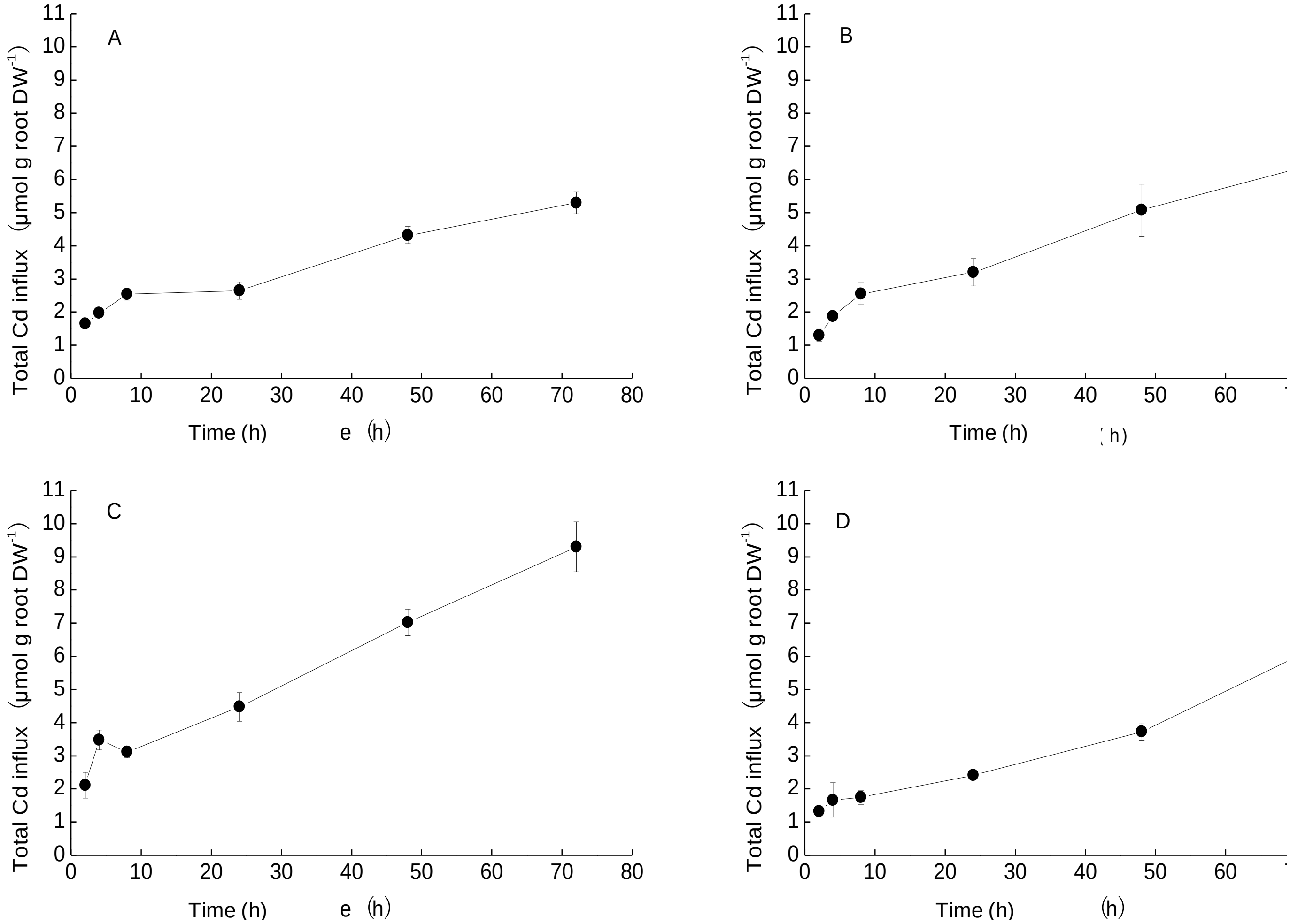

Figure 2. Long-term (2-72 h) cadmium influx of Yizhibi (A), Suliu172 (B), Hanliu (C) and Weishanhu (D). Data points and error bars represent means and SE, respectively. Error bars do not extend outside some symbols. DW, dry weight.

Table 1. Cadmium accumulation (μmol/g) in roots, stems and leaves of the four willow species.

Treatment	Plant part	Hanliu	Weishanhu	Yizhibi	Suliu172
Cadmium content (2 μmol/L)	Root	1.310 ± 0.034^{a}	1.011 ± 0.043^{c}	1.123 ± 0.004^{b}	1.127 ± 0.009^{b}
	Stem	0.016 ± 0.001^{a}	0.015 ± 0.000^{ab}	0.014 ± 0.000^{b}	0.016 ± 0.000^{a}
	Leaf	0.010 ± 0.000^{b}	0.012 ± 0.000^{b}	0.017 ± 0.001^{a}	0.016 ± 0.000^{a}
Cadmium content (100 μmol/L)	Root	5.432 ± 0.105^{a}	4.535 ± 0.132^{c}	4.768 ± 0.159^{bc}	5.142 ± 0.203^{ab}
	Stem	0.799 ± 0.028^{b}	0.860 ± 0.020^{b}	0.846 ± 0.021^{b}	0.972 ± 0.015^{a}
	Leaf	0.019 ± 0.001^{b}	0.020 ± 0.000^{b}	0.026 ± 0.000^{a}	0.027 ± 0.001^{a}

Different letters indicate significant differences between the means with $P < 0.05$ (mean ± SE).

Krishnamurti et al., 1997). The accumulation of mineral elements in plants is not only related to the uptake ability of plant roots, but also affected by the transfer efficiency of mineral elements in plants (Clemens. 2006). The uptake capacity is jointly decided by the V_{max} and K_m, hence the V_{max}/K_m represents the absorption capacity of willow better. The V_{max}/K_m values (Table 2) indicated that the cadmium uptake capacity of willow followed Hanliu > Yizhibi > Weishanhu > Suliu172. The slopes of the linear components of Weishanhu and Yizhibi were also similar, but higher than that of Suliu172, while the slopes of the linear components of Hanliu were the lowest (Table 2).

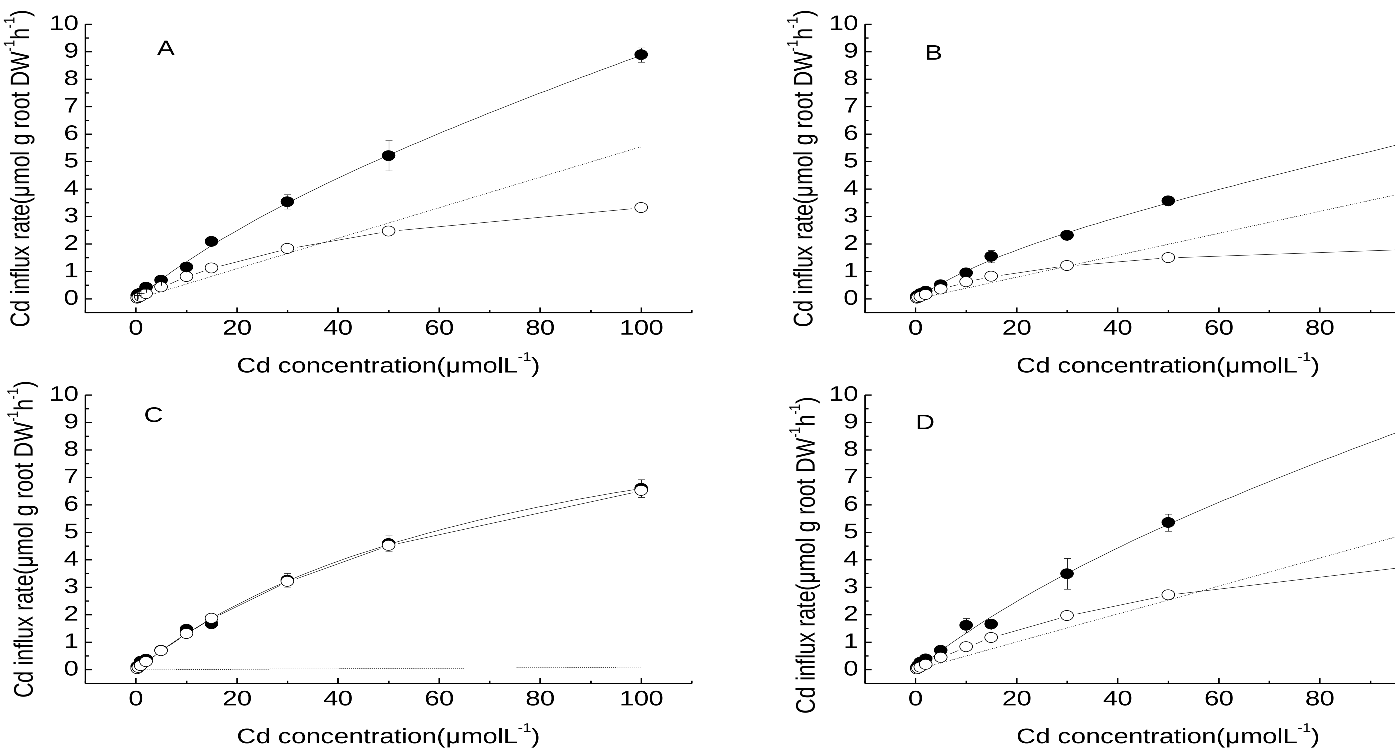

Figure 3. Concentration-dependent cadmium influx kinetics in roots of Yizhibi (A), Suliu172 (B), Hanliu (C) and Weishanhu (D). Linear (dotted line) and saturable (open circles) components were derived from experimental data (filled circles) by mathematically resolving these curves using Origin 7.5. Data points and error bars represent means and SE, respectively. Error bars do not extend outside some symbols. DW, Dry weight.

Table 2. Kinetic parameters for root cadmium influx of the four willow cultivars.

Willow cultivar	V_{max}	K_m	V_{max}/K_m	k	R^2
Yizhibi	5.09	53.09	0.096	0.055	0.998
Suliu172	2.31	27.03	0.085	0.039	0.998
Hanliu	11.67	79.04	0.147	0.001	0.998
Weishanhu	6.36	66.83	0.095	0.051	0.998

R^2, coefficient determination.

Therefore, we could conclude that Hanliu's cell wall had a relatively strong cadmium binding capacity, while Weishanhu and Yizhibi had similar but relatively lower capacity. The cadmium uptake ability of willows from hydroponic solutions depends on different willow species, size of willow seedlings and environmental conditions. Suliu172 is an interspecific hybrid and expresses well; fast-growing. Hanliu shows high resistance to salt and alkali drought but its water logging tolerance is not good. Yizhibi and Weishanhu are cultivars of *S. integra* and prefer adequate illumination. Particular physiological characteristics of willow species resulted in different cadmium uptake ability. The four weeks cultivation of willow seedlings partly reflected cadmium uptake ability of the four willow species.

As the first barrier of metal ions across membrane into the cytoplasm, cell wall plays an important role in the process of resistance to metal ion toxicity (Nishzono, 1987). Cell level reduces metal ion toxicity of plant roots in two ways: restriction of the metal from crossing the plasma membrane and detoxification of metal ions within the cell (Macfie et al., 2000; Hall, 2002). In other words, root uptake of divalent cations typically exhibits two phases: apoplastic binding and symplastic uptake (Hart et al., 1998; Zhao et al., 2002). To analyze cadmium influx into the symplast, apoplastic binding to reactive apoplastic sites of root cells must be taken into consideration and minimized by the desorption steps. According to Zhao et al. (2002), however, complete removal of apoplastically bound cadmium by desorption,

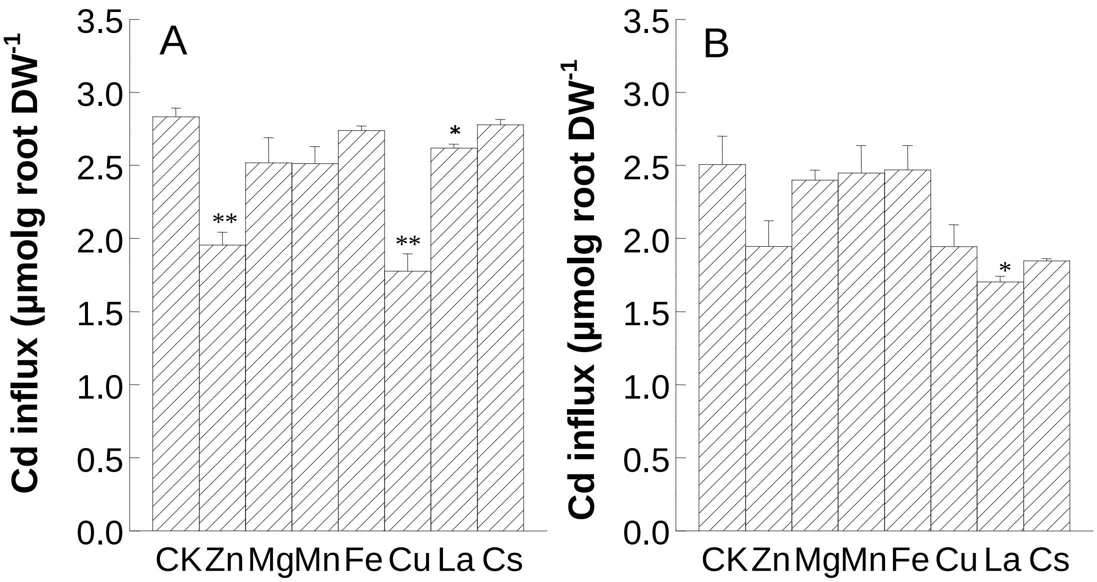

Figure 4. Effects of $ZnCl_2$, $MgCl_2$, $MnCl_2$, $FeCl_2$, $CuCl_2$, $LaCl_3$ and CsCl on cadmium uptake by the two cultivars of willow, Yizhibi (A), and Suliu172 (B). Error bars represent SE. Means marked with * indicate significant difference between control and treatments at P<0.05 and ** at P<0.01. DW, Dry weight.

without risking efflux of symplastic cadmium, is probably unachievable. In this study, desorption step did not fully remove apoplastic cadmium. The slopes of the linear components (Table 2) showed that desorption did not remove apoplastically bound cadmium completely, although the slope was not high, indicating that most of the apoplastically bound cadmium had been removed. Saturable kinetics of concentration-dependent process suggested that cadmium was taken up via a carrier-mediated system.

Effects of Zn, Mg, Mn, Fe(II), Cu, La, and Cs on cadmium influx

The effects of common divalent ions and essential elements; Zn, Mg, Mn, Fe(II), Cu and ion channel inhibitor (La and Cs) on cadmium influx into roots of willow were investigated by adding ions to the cadmium uptake solutions. The results (Figure 4) showed that the addition of equal molar (10 µmol/L) Mg, Mn and Fe(II) had no significant effect on cadmium influx. However, Zn and Cu decreased the cadmium influx of Yizhibi by 31%(P<0.01) and by 37% (P<0.01), respectively in comparison with the control, while Zn and Cu decreased the cadmium influx of Suliu172 by 22% (non-significant; ns) and by 22% (non-significant). Treatments with 50 µmol/L $LaCl_3$ decreased the cadmium uptake influx by 8% for Yizhibi (P<0.05) and by 32% for Suliu (P<0.05), and 50 µmol/L CsCl decreased cadmium uptake influx by about 2% (ns) for Yizhibi and by 26% (ns) for Suliu172.

Numerous studies have shown that Zn, Cu or Mn suppresses the uptake of cadmium by plants or algae (Lu et al., 2008; Tripathi et al., 1995). As a non-essential element, it is generally believed that cadmium uptake by non-accumulator plants represents opportunistic transport by a carrier for essential elements such as Zn, Cu, Mg, or Fe(II), or via cation channels for Ca, which is a consequence of a lack of specificity of these transport proteins (Welch and Norvell, 1999). Members of the ZIP (ZRT/IRT-like protein) gene family are competent of transporting transition metals including Fe(II), Zn, Mn and Cd (Gurinot, 2000). In the cadmium-hyperaccumulator *Arabidopsis halleri*, cadmium uptake partly occurs through the Zn pathway (Zhao et al., 2006). However, cadmium uptake in *Thlaspi caerulescens* is probably mediated by specific cadmium transporters (Lombi et al., 2001; Zhao et al.,2002), as well as by ZNT1, a high-affinity Zn transporter that mediates low-affinity uptake of cadmium (Lasat et al., 2000; Pence et al., 2000). The results observed here for willow suggest that this species may use the Zn and Cu pathway, although there are not enough literature report on Cu pathway for cadmium uptake. Further researches about the effect of Zn and Cu deficiency on cadmium uptake are therefore necessary to verify the observed results.

La and Cs are ion channel inhibitors for Ca and K, respectively. Many studies show that addition of La or an increase in the Ca concentration decreases cadmium uptake (Zhao et al., 2002, Lu et al., 2008). Cadmium influx into roots of Yizhibi and Suliu172 was significantly suppressed by addition of Ca channel inhibitor La,

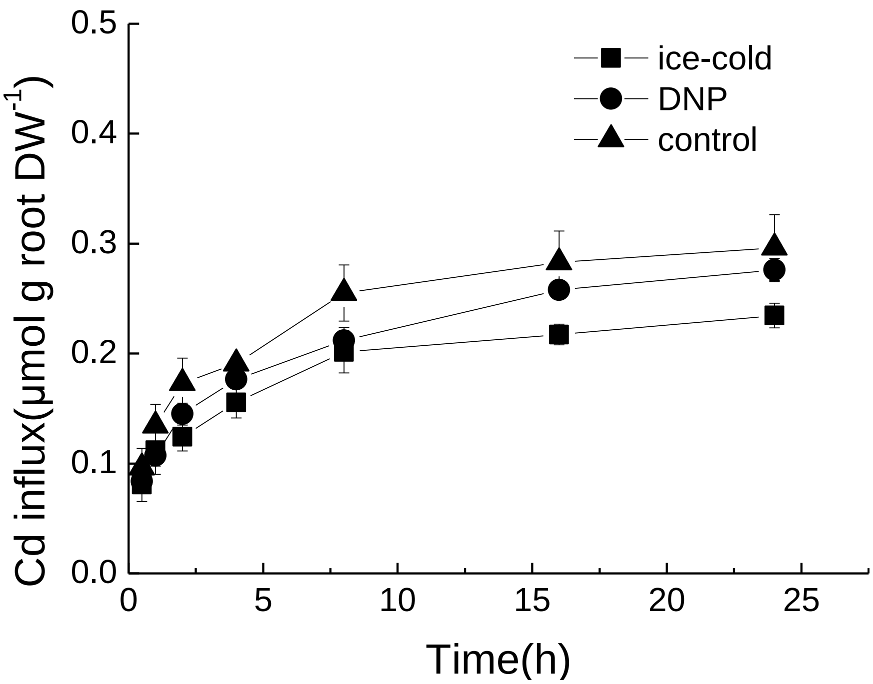

Figure 5. Cumulative uptake of cadmium by Yizhibi with treatments of control (triangles), ice-cold (squares), and+100 mol/L DNP (circles), as determined from the depletion of cadmium in the uptake solution. Data points and error bars represent means and SE, respectively. Error bars do not extend outside some symbols. DW, dry weight.

suggesting that cadmium uptake by willow was probably regulated by Ca transporters or channels in root cell plasma membranes. For K ion channel inhibitor Cs, however, no significant inhibition of willow cadmium uptake was observed.

Effects of low temperature or metabolic inhibitors on cadmium uptake

Figure 5 exhibits the results of cadmium uptake of Yizhibi under low temperature and metabolic inhibitors (2, 4-DNP). Both treatments inhibited cadmium uptake of Yizhibi, and the inhibition effect of low temperature was more significant than that of DNP's. After 24 h of treatment at low temperature, cadmium uptake decreesed by 23% compared to that of the control, while metabolism inhibitor only decreased the cadmium influx by 6% compared with the control. At cold treatment, the process of cadmium uptake by willow had two phases: within 0 to 8 h, the cadmium uptake increased linearly with increasing uptake time; when the treatment time was longer than 8 h, the uptake process reached a saturation state. When the metabolic inhibitor, 2, 4-DNP, was supplied in the solution, the uptake rate gradually reduced until it leveled off after 16 h. The results observed here suggest that 2, 4-DNP not only inhibited the cadmium absorption, but also postponed the time of uptake rate to be saturated.

Active symplastic translocation would be inhibited when roots are treated under cold condition or under the presence of metabolic inhibitors. Temperature had a close relationship with uptake process regulated by metabolism. At an appropriate growth temperature, all kinds of physiological metabolisms ran normally, while under low temperature, plant's metabolism slowed down and enzyme activity decreased, then plant growth was almost at a standstill, which had a negative influence on the cadmium active uptake. Through studying the impact of low temperature on cadmium uptake of *Sedum alfredii*, Lu et al. (2009) discovered that uptake of cadmium was significantly inhibited by low temperature treatment (4°C) in hyperaccumulating ecotype plants. In living cells, DNP acts as a proton ionophore, an agent that can shuttle protons (hydrogen ions) across biological membranes. It defeats the proton gradient across mitochondria and chloroplast membranes, collapsing the proton motive force that the cell uses to produce most of its ATP; hence, instead of producing ATP, the energy of the proton gradient is lost as heat. Sequentially, it restrains active absorption, and because the passive absorption of plants does not need energy, metabolic inhibitors have no impact on passive absorption. Figure 5 shows that the cadmium absorption of willow had the participation of active transport. So, we could infer that active absorption took part in cadmium uptake by willow roots.

Passive transport is the diffusion of substances across a membrane. This is a spontaneous process and cellular energy is neither expended nor related to metabolism. However, active absorption process depends on the energy that is produced by respiration, and has connection with metabolic activities (Wolterbeek et al., 1988; Lu et al., 2009). Without removal of apoplastically bound cadmium by the desorption steps, cumulative uptake of cadmium by willows were calculated from total cumulative depletion of cadmium in the uptake solution in this experiment. Results reveal that cadmium uptake was only inhibited by 23% at low temperature treatment, while metabolism inhibitor only decreased the cadmium uptake influx by 6%. This illustrated two problems: first, active transport was an important way for cadmium absorption of willow, which was in accordance with conclusion of concentration-dependent kinetics experiment; secondly, root cell wall had bound a large number of cadmium, thus implying that the negative groups of cell wall had restricted cadmium ions by precipitation, adsorption and complexation in the process of passive absorption. Hence, the passive absorption was another important way to absorb cadmium for willow.

Conclusion

Cadmium accumulation by the four willow species increased when uptake time and initial cadmium concentration was increased. After one week cultivation, cadmium accumulation in different parts of willows generally followed the order of root > stem > leaf. Under hydroponics conditions, concentration-dependent cadmium influx could be resolved into linear and saturable components. The saturable component followed Michaelis-Menten kinetics, which indicated that cadmium uptake across the plasma membrane was transporter-mediated. In contrast, Hanliu had the highest cadmium uptake capacity, Weishanhu and Yizhibi had medium, while Suliu172 had the lowest. More also, time course dynamic results revealed that cadmium accumulation linearly increased during a short time course, but increased with prolong uptake time and became nonlinear, while a slow absorption rate phase were observed during a long time course.

In addition, Zn and Cu decreased the cadmium uptake of Yizhibi significantly, while other cations had no significant effect. However, the cadmium influx into roots of Yizhibi was significantly suppressed by Ca channel inhibitor La, implying that Ca transporters or channels were responsible for the cadmium uptake of willow. Results also indicate that K ion channel inhibitor Cs had no obvious inhibition effect on the cadmium uptake of willow. Low temperature and metabolic inhibitor inhibited the apparent uptake of cadmium in willow via root cadmium active transport. Both active absorption and passive absorption took part in the cadmium uptake by willow roots.

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