

## EFFECT OF SILICON-MEDIATED ALLEVIATION OF CADMIUM TOXICITY WITH A CYPRESS VARIETY: *JUNIPERUS FORMOSANA* HAYATA

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*Key words:* Cadmium, Rhizosphere root exudation, Silicon, *Juniperus formosana*

### Abstract

Interaction of silicon and root exudates on cadmium bioavailability in the rhizosphere was assessed in a pot experiment with a species of cypress: *Juniperus formosana* Hayata. Potted soil was treated with 100 mg/kg Cd and/or 400 mg/kg Si. The results showed that added Si increased Cd tolerance in *J. formosana*. Si significantly increased soil pH and Cd absorption capacity in the rhizosphere, and enhanced Cd retention of the roots of *J. formosana*, but effectively inhibited growth. Moreover, Si mobilized Cd from the rhizospheric soil by stimulating phenolic exudation from the roots suggesting that Cd-chelation combined with Si-induced phenolics were involved in the Cd detoxification.

### Introduction

Heavy metal contamination is a global problem that is adversely affecting both human and environmental health. Cadmium (Cd) has been shown to disrupt nutrient uptake and photosynthesis in plants which inhibits growth and even causes death (De Filippis and Ziegler 1993, Sandalio *et al.* 2001). Moreover, food crops grown in Cd contaminated soils may pose a major risk to human health.

Silicon is beneficial for healthy plant growth and development (Ma and Yamaji 2006), and it has been shown to enhance the resistance of some plants to heavy metals including Mn, Zn, Al, and Cd (Savant *et al.* 1997). Liang *et al.* (2007) reported that Si has a ability to increase tolerance of Cd toxicity by restricting its accumulation via solution chemistry and internal plant mechanisms.

In past years, hydroponic research showed that high concentrations of Si, deposited in cell walls of the endodermis and epidermis, contribute to the restriction of Cd transport in bypass flow from roots to shoots (Rizwan *et al.* 2012, Vaculík *et al.* 2012, Ye *et al.* 2012). Moreover, a series of pot experiments conducted by Liang *et al.* (2005), using maize, showed that the Si transformation of Cd phytotoxicity was due to both Si action inside the plant and a decrease in Cd bioavailability caused by a silicate-induced rise in soil pH. In contrast, da Cunha and do Nascimento (2009) had contradictory results and reported that Si exposure increased growth and led to higher Cd accumulation. Those conflicting results about Cd assimilation indicate that the Si-mediated alleviation of Cd toxicity remains hardly understood and the mechanism of Si-enhanced Cd tolerance is still controversy.

Recently, Song *et al.* (2009) found that Si-enhanced resistance to Cd toxicity in *Brassica chinensis* L. could be attributed to a Si-enhanced antioxidant defense capacity. Supplying plants with Si can produce phenolics which respond to fungal infection (Ma and Yamaji 2006). This phenomenon has also been observed in plants under Al stress (Kidd *et al.* 2001). Some studies

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have reported that the process of root exudation contributes to metal detoxification through the formation of soluble complexes (Lux *et al.* 2011, Li *et al.* 2011). However, little information is available on the effect of Si on Cd availability in the rhizosphere, and, it is still not clear what the root exudation stimulated by Cd stress inhibits the Si-induced immobilization of Cd in the rhizosphere.

Cypresses have many virtues, such as evergreen, long-lived, large biomass, rapid perennial growth and asexual propagation. Such plants are ideal subjects for the study of phytostabilization. Thus a pot experiment was conducted to evaluate the characteristics of the rhizosphere and bulk soil and Cd adsorption and translocation *Juniperus formosana* Hayata, as the test plant. The findings will provide information: to gain better insight into the possible mechanisms involved in Si-mediated detoxification of Cd in plants, and to provide a basis for field-scale application of Si during phytostabilization.

### Materials and Methods

A greenhouse experiment was conducted from March 11 to October 6, 2014 at Hangzhou Normal University, China, where the daily photoperiod was 12 hrs and the maximum temperature was 33°C. The minimum temperature at night was adjusted to 20°C. One-year-old plants of the Cypress species *J. formosana* was purchased from Zhejiang Weijin Seeds Co., Ltd., Hangzhou, China. Their roots were washed thoroughly with distilled water before the specimens were planted.

Soil sample was collected at a depth of 0 - 20 cm from paddy field near Hangzhou city, Zhejiang Province, China. The soil had a pH of 6.46 and contained 9.32 g/kg of organic matter, 1.16 g/kg of total-N, 10.2 mg/kg of Olsen-P and 122.4 mg/kg of NH<sub>4</sub>AC extractable-K. The Cd level in the soil was below the detection limit (< 0.02 mg/kg). The soil was mixed well with slow-release fertilizer at a rate of 20 g/kg (APEX, Simplot Co., Ltd., USA.).

Uniformly sized plants were planted directly into nylon bags (80 µm nylon mesh, 6 cm diameter and 15 cm height), which were then transferred to plastic pots (25 cm diameter and 30 cm height). The nylon bag and plastic pot held a total of 8.0 kg of pretreated soil. The root growth was limited to the volume within the nylon bag, and it was considered to be rhizosphere soil. The experiment involved three treatments, each repeated five times, namely (1) control with non-Cd contaminated soil, (2) Cd (100 mg/kg Cd), and (3) as Cd plus Si (100 mg/kg Cd and 400 mg/kg Si). Silicon and cadmium were added as Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O and CdCl<sub>2</sub>·H<sub>2</sub>O, respectively. To ensure homogeneous distribution of Si and Cd in the soil, some soils were pretreated at 10,000 mg/kg Cd and 40,000 mg/kg Si, before being air dried, passed through a 2 mm sieve, and then mixed thoroughly with the non-treated soil. Less than 2 mmol/kg of sodium silicate was added. This amount would not significantly increase the sodium concentration within the soil, and so should not have influenced the soil pH (Arjouni *et al.* 2015). Distilled water was used to keep soil moisture at approximately 60%.

The soil solution was extracted using the method of Jones and Willett (2006). After harvesting, the rhizosphere soil (within the nylon bag) and bulk soil (soil in non-rhizosphere zones) were separated and sampled. Moist potting soil was extracted with deionized-distilled water using a soil: water ratio of 1 : 2.5 (w/v) on a dry weight basis, and shaken at 200 rpm for 2 hours at 20°C in a reciprocal shaker. The suspension was centrifuged at 4,000 g for 25 mins, and the supernatant was filtered through a 0.45 µm membrane filter. The resulting samples of soil solution were stored in the dark at 4°C for subsequent determination of DOC, Cd, simple sugars, phenolics, and Cd adsorption.

A 1.0 g sample of air-dried soil was placed into a 50 ml polyethylene centrifuge tube and equilibrated with 25 ml of the DOC solution derived from the rhizosphere for each treatment. Three levels of Cd (25, 50 and 100 mg/l) were used. The pH of all suspensions was adjusted to 6.50, and added two drops of 1.0 mol/l  $\text{NaN}_3$  to inhibit DOC decomposition during the adsorption experiment. The soil suspensions were shaken on a reciprocal shaker at 200 rpm and 25°C for 4 hrs, and then equilibrated in the dark for 2 hrs. The tubes were centrifuged at 5000 g and filtered. The quantity of Cd adsorbed was calculated by subtracting the Cd concentration in the equilibrium solution from the total initial concentration.

Plants were separated into three parts: leaves, stems, and roots. The taproot epidermis and endodermis were carefully scraped; the remaining part of the taproot was considered as stele. The plant tissues were washed thoroughly with distilled water, oven dried for 72 hrs at 70°C, and then ground and passed through a 2.0 mm sieve. Dried samples (up to 0.10 g) were digested with 10 ml of nitric acid at 150°C for 24 hrs, to determine Cd.

The level of  $\text{NH}_4\text{NO}_3$  extractable Cd in the rhizosphere and bulk soils were determined, using a soil to 1 mol/l  $\text{NH}_4\text{NO}_3$  solution ratio of 1 : 2.5 (w/v). The extracted solution was shaken at 200 rpm for 1 hr, centrifuged for 15 mins at 4,000 g, and filtered through a 0.45  $\mu\text{m}$  membrane filter. The DOC and Cd concentrations were determined using a total organic carbon analyzer (HACH TOC-600) and an atomic absorption spectrophotometer (PerkinElmer AAS-800), respectively.

The phenolic content of the soil solution derived from the rhizosphere was measured using a Folin-Ciocalteu reagent (Ainsworth and Gillespie 2007). 2.0 ml of the extract was mixed with 1.0 ml Folin-Ciocalteu reagent and 5.0 ml saturated  $\text{Na}_2\text{CO}_3$  solution for 1 hr at 25°C. Absorbance of phenolic content was measured at 760 nm using ferulic acid as a standard. The simple sugar content in the soil solution was analyzed using dinitrosalicylic acid (DNS) assay. 2.0 ml extraction was mixed with 1 ml of DNS reagent, boiled for 5 min, cooled and absorbance was recorded at 540 nm using glucose as a standard.

Differences among treatments were evaluated by Duncan's New Multiple Range Test at a 0.05 probability level. Analyses were conducted using SPSS software 17.0.

## Results and Discussion

The addition of 100 mg/kg of Cd had significantly inhibited the growth of *J. formosana*. Further more the results showed that the leaves and roots of *J. formosana* were sensitive to Cd toxicity. The growth of leaves and roots decreased by 26.5 and 21.9%, respectively. Compared to the Cd treatment, the Cd plus Si treatment significantly increased the biomass of *J. formosana* (Table 1).

Before treatment, the Cd concentration and content in cypress roots was negligible. However, after treatment, *J. formosana* assimilated large amount of Cd, retaining it mainly in the roots (Table 1). The total Cd content in the roots was 62 times higher than that found in the leaves of *J. formosana*. The addition of 400 mg/kg of Si influenced the Cd uptake of *J. formosana*, Si addition did not change the Cd concentration in the roots, but decreased it in leaves and stems. The total Cd content of *J. formosana* for the Si with Cd treatment was 13.92% higher than single application of the Cd.

The pH of the rhizosphere soil was lower than that of the bulk soil in the control treatment, This change was pronounced in *J. formosana*, which decreased by 0.39 (Table 2). The addition of Cd further lowered the pH of the rhizosphere. Si addition significantly increased the pH values of the bulk soil (7.32) and rhizosphere (6.42) of *J. formosana* compared to the control. In the Cd treatment,  $\text{NH}_4\text{NO}_3$ -extractable Cd (mobile Cd) made up approximately 65% of the total amount of Cd in the bulk soils (Table 2) which indicated that it was the predominant form of Cd. In the

rhizosphere of *J. formosana*, however, the mobile Cd concentrations decreased significantly as compared to the bulk soil, where the decrease was 12.1 mg/kg.

**Table 1. Plant biomass, Cd concentration and total Cd content of *J. formosana* after 210 days of growth in Cd-contaminated soil.**

	Treatment	Total biomass	Leaf	Stem	Root
Dry weight (g/plant)	Control	14.92 a	4.31 a	3.94 a	6.67 a
	Cd	11.20 c	3.07 b	2.97 c	5.16 b
	Si+Cd	13.85 b	4.12 a	3.45 b	6.28 a
Concentration (mg/kg)	Cd	230 a	12 a	69 a	455 a
	Si+Cd	214 a	7 b	42 b	446 a
Total content (g/plant)	Cd	2607 b	38 a	208 a	2361 b
	Si+Cd	2970 a	29 b	147 b	2794 a

Means followed by different letters within the same column are significantly different at the level of  $p < 0.05$  according to a DMRT ( $n = 5$ ).

**Table 2. Soil pH,  $\text{NH}_4\text{NO}_3$  extractable-Cd and DOC in the bulk and rhizosphere soil of *J. formosana* after 210 days of growth in Cd-contaminated soil.**

Soil	Treatment	pH	$\text{NH}_4\text{NO}_3$ -Cd (mg/kg)	DOC (mg/kg)
Bulk soil	Control	6.52 b	nd	67.1 c
	Cd	6.45 ab	65.4 a	61.3 d
	Si+Cd	7.32 a	41.0 c	58.8 d
Rhizosphere	Control	6.13 c	nd	80 b
	Cd	5.89 d	53.3 b	91 a
	Si+Cd	6.42 b	52.0 b	88 ab

Means followed by different letters within the same column are significantly different at the level of  $p < 0.05$  according to a DMRT ( $n = 5$ ).

The DOC concentrations in the bulk soil decreased slightly with the Cd treatment, and decreased further with the Si plus Cd treatment (Table 2); in the rhizosphere though, the DOC concentrations were greater than those observed in the bulk soil. After exposure to Cd for 210 days, *J. formosana* released 13.8% more DOC into the rhizosphere, compared to their corresponding control treatments. Moreover, the addition of Si decreased the DOC level 3.01 mg/kg in the rhizosphere of *J. formosana* compared to the Cd treatment alone.

Table 3 showed that the Cd exposure over 210 days significantly enhanced the dry weight of the epidermis and endodermis tissues of *J. formosana*. Furthermore, Cd treatment significantly lowered the biomass of the stele, by 19.4% for *J. formosana*. Compared to the Cd treatment, the addition of Si significantly increased the dry weight of the epidermis plus endodermis.

The addition of Si to the Cd treatment affected Cd accumulation in *J. formosana*, the Cd concentration of the stele was 21.9% lower than that of Cd treatment alone. Moreover, the total content of the stele decreased nearly 5.32%, but increased exceeding 25.2% of the epidermis and endodermis tissues, due to addition of Si than the Cd treatment alone.

However, it was different in the simple sugar content of the *J. formosana* rhizosphere with the Cd treatment and the addition of Si to the Cd treatment. Treatment of Cd decreased this content, while the Si addition slightly reversed the Cd-induced effect.

**Table 3. Dry weights and Cd concentrations in the root tissues of epidermis plus endodermis, and stele, of *J. formosana* after 210 days of growth in Cd-contaminated soils.**

Parts	Treatment	Dry weight	Cd concentration	Total content
Epidermis plus endodermis	Control	0.655 d	nd	nd
	Cd	0.873 d	1284 a	1208 b
	Si+Cd	1.224 c	1136 a	1513 a
Stele	Control	5.87 a	nd	nd
	Cd	4.73 b	273.1 b	1146 bc
	Si+Cd	4.98 b	213.0 c	1085 c

Means followed by different letters within the same column are significantly different at the level of  $p < 0.05$  according to a DMRT ( $n = 5$ ).

The simple sugar concentration in the *J. formosana* rhizosphere was 23.6, Cd 17.3 and 20.8 mg/kg in the control, Cd and Si treatments, respectively (Fig. 1).

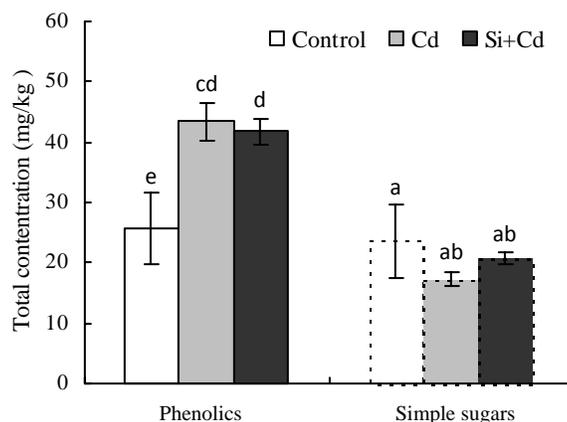


Fig. 1. The concentrations of simple sugars and phenolics in the rhizosphere of *J. formosana*.

The Cd treatment increased the phenolic concentration significantly in the *J. formosana* rhizosphere over the control. The phenolic concentration in the *J. formosana* rhizosphere was only 25.8 mg/kg in the control treatment comprising approximately 68.2% lower than that of Cd treatment. Fig. 1 showed that the Cd treatment significantly enhanced the phenolic exudation, while no such difference was observed due to Si addition. However, Si addition slightly reversed the Cd-induced effect.

The Cd adsorption on the paddy soil was directly related to the concentration of Cd added initially; that is, there was a linear correlation between the equilibrium Cd concentrations and the amount of Cd adsorption (Fig. 2, Table 4). The Cd adsorption was calculated by linear regression, where the gradient ( $K$ ) was positively related to the soil metal adsorption capacity.

The  $K$  value obtained was higher in the control treatment ( $K = 13.58$ ) than that Cd treatment ( $K = 12.22$ ) and in association with Si ( $K = 12.84$ ) for *J. formosana*. The Cd treatment slightly decreased the  $K$  values of Cd adsorption in comparison to other two treatments. Furthermore, the addition of Si promoted the  $K$  values, in the rhizosphere of in *J. formosana*. Regarding the ratios of Cd adsorbed, the highest Cd adsorption ratio was measured from the control treatment (36.5%), followed by its Si plus Cd treatment (33.7%).

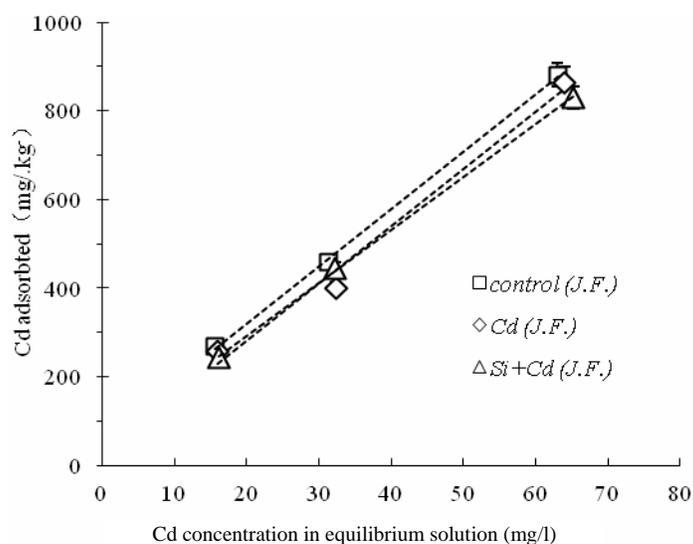


Fig. 2. The kinetics of Cd adsorption influenced by DOC derived from the rhizosphere of *J. formosana*.

**Table 4. Linear regression for the effect of DOC on Cd adsorption derived from the rhizosphere of *J. formosana*.**

Treatment	$K$	$R^2$	*Adsorbed ratios (%)
Control	13.58	0.9865	36.5 ± 2.1%
Cd	12.22	0.9776	33.7 ± 2.7%
Si+Cd	12.84	0.9957	34.9 ± 1.8%

Adsorbed ratio: the ratio (%) between the total Cd content in the equilibrium solution and the total initial content.

The forms of Cd present in the rhizosphere soil have an important influence on the absorption of Cd by plants and potted plants rhizosphere migration, as a high exchange state Cd of rhizosphere could inhibit and damage plant growth. When the concentrations of different Cd forms in the rhizosphere soils were compared, most of the Cd was found in the exchange form (Table 5). The addition of Si had the greatest impact on the exchange state of Cd in which the concentration of exchange state Cd decreased significantly. However, the variations in other forms of Cd viz. Carbonato bound, Fe/Mn oxide bound and organic bound were found to be not significant.

The results demonstrated that *J. formosana* acidified the soil rhizosphere, resulting lower pH values as compared to those observed in bulk soil (Table 2). In general, rhizosphere pH changes

are dominated by the inorganic cation-anion balance in the plant and the associated root excretion of  $H^+$  or  $OH^-$ . The reduction in solution pH might have resulted from the increased release of root exudates (Hinsinger *et al.* 2003), consistent with the enhanced DOC derived from rhizosphere root exudation (Table 2). Moreover, the exudation process was accelerated, in response to the Cd toxicity. DOC in the root exudates includes different organic compounds, which can trigger a range of chemical reactions and biological transformation in the rhizosphere. The cypress roots possibly exhibited - directly, or indirectly via exudation - a greater ability to mobilize Cd. It is evident that the root exudates from cypress mobilized greater amounts of Cd from the Cd-contaminated rhizosphere soil (Fig. 2, Table 4). The Cd-mobilization due to high root exudation plays an important role in Cd uptake by the cypress variety, demonstrated by the assimilation of large amount of Cd (Table 1). This eventually reduced the mobile Cd in the rhizosphere to a level below that of the bulk soil (Table 2), this findings agrees favourably well with results of previous studies (Hu *et al.* 2011, Li *et al.* 2011).

**Table 5 Distribution of Cd forms in rhizosphere soil of *J. formosana*.**

Cd forms in rhizosphere (mg/kg)	<i>J. formosana</i>	
	Cd	Cd + Si
Exchange	40.5 ± 1.7 a	38.9 ± 0.8 b
Carbonate-bound	9.2 ± 1.1 a	9.4 ± 0.7 a
Fe/Mn oxide-bound	2.5 ± 0.6 a	2.7 ± 0.9 a
Organic bound	1.1 ± 0.1 a	1.0 ± 0.2 a

The addition of Si significantly decreased the amount of  $NH_4NO_3$ -extractable Cd in the bulk soils. It might have been caused by the Si-induced rise in soil pH (Table 2) possibly due to the deprotonation of surface-bound  $H^+$  on the soil-exchange sites. This is consistent with Liang *et al.* (2005), who also reported that adding 400 mg/kg of Si as sodium metasilicate effectively immobilized Cd in acidic soil. Accordingly, adding Si-rich compounds (such as fly ash and steel slag) to Cd contaminated soil has been used effectively to reduce the environmental risk of Cd (Gu *et al.* 2011).

Although the addition of Si slightly increased pH in the *J. formosana* rhizosphere, it did not lower Cd solubility in the rhizosphere (Fig. 2, Table 4), and hence did not decrease the total Cd uptake of the plant. In contrast, the total Cd content in the roots was significantly higher than that of the Cd treatment alone. Furthermore, the Si addition lowered the total Cd content in the stem and leaf of *J. formosana*, compared to the Cd treatment alone (Table 1). This regulation is consistent with the findings of a previous study that demonstrated a 30~50% decrease in the shoot Cd concentration of rice, following the addition of Si into paddy soil (Zhang *et al.* 2008). These results suggest that the beneficial effect of Si can be attributed to both the immobilization of Cd in soils and also the retention of Cd in roots, leading to reduced Cd translocation to the shoots. Si plays role in protecting the root stele as a mechanical barrier, by hardening the cell wall of the epidermis and endodermis tissues (Hattori *et al.* 2003, Lux *et al.* 2003), resulting in higher Cd retention in these tissues and lower Cd permeation in the stele, as evident from the results (Table 3). The strengthening of cortical tissues through Si deposition may reduce apoplastic bypass flow and provide binding sites for Cd, resulting in decreased uptake and translocation of this toxic metal from the roots to the shoots (Gong *et al.* 2006), as has also been reported in wheat and maize (Vaculík *et al.* 2012).

The outcome of. This work reinforces the usefulness of Si for increasing the resistance of plants to toxic metals. The amendment of Si into Cd-contaminated soil effectively alleviated the Cd toxicity in *J. formosana*, and that the mechanisms were species-specific. The Si-mediated Cd tolerance in *J. formosana* was due mainly to the Cd immobilization both in soil and plant roots. Furthermore, the addition of Si and Cd treatment significantly increased the concentration of DOC in rhizosphere, but decreased the in bulk soil of *J. formosana*. Thus, an avenue for future work would be to apply these methods to known Cd-tolerant and Cd-sensitive lines.

Because the chelating effect of Si was confined to the rhizosphere, the exudation of phenolics that it stimulated in plants may be more effective and pose lower potential risks compared to the *in situ* application of chelating agents, because such substances can cause groundwater pollution through uncontrolled metal dissolution and leaching. Further molecular studies are needed to precisely control this Cd-chelating effect in order to better understand the roles of Si in the biochemical synthesis and exudation of phenolics from cypress roots. Finally, this understanding could enrich phytoextraction methods and make Si-mediated detoxification more feasible.

### Acknowledgements

This study was supported jointly by the National Natural Science Foundation of China (No. 21207030), the Science Foundation of the Zhejiang Province Department of Education (No.Y201224448), the Science and Technology Research Projects of Hangzhou, China (No. 20120433B15) and the Science Foundation of Qianjiang College of Hangzhou Normal University (No. 2016QJXS15).

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(Manuscript received on 16 March, 2016; revised on 18 September, 2016)