

## Silicon-mediated alleviation of Cadmium toxicity on *Thujaopsis dolabrata*

La presencia de Sílice reduce la toxicidad del Cadmio en *Thujaopsis dolabrata*

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**Abstract.** We conducted pot experiments on the cypress *Thujaopsis dolabrata* (Linn. f.) Sieb. et Zucc. in order to study the interaction of silicon (Si) and root exudates on cadmium (Cd) bioavailability in the rhizosphere. Each variety was planted with 100 mg/kg Cd and/or 400 mg/kg Si for 210 days. The results showed that adding Si increased Cd tolerance in *T. dolabrata*, but that the mechanism was specific. In *T. dolabrata*, Si did not prevent Cd translocation from roots to shoots, and it significantly enhanced Cd accumulation without inhibiting 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 Cd detoxification.

**Keywords:** Cadmium; Rhizosphere; Root exudation; Silicon; *Thujaopsis dolabrata*.

**Resumen.** Se condujeron experimentos en potes con el ciprés *Thujaopsis dolabrata* (Linn. f.) Sieb. et Zucc. para estudiar la interacción del silicio (Si) y exudados radicales sobre la biodisponibilidad de cadmio (Cd) en la rizósfera. Cada variedad se plantó con 100 mg/kg de Cd y/o 400 mg/kg de Si durante 210 días. Los resultados mostraron que el agregado de Si incrementó la tolerancia al Cd en *T. dolabrata*, pero que el mecanismo fue específico. En *T. dolabrata*, el Si no previno la movilización de Cd desde las raíces a los tallos, y mejoró significativamente la acumulación de Cd pero sin inhibir el crecimiento. Más aún, el Si movilizó el Cd desde el suelo rizosférico estimulando la exudación de compuestos fenólicos desde las raíces, sugiriendo que el ligamento de Cd combinado con los compuestos fenólicos exudados desde las raíces estuvieron implicados en eliminar la toxicidad del Cd.

**Palabras clave:** Cadmio; Rizósfera; Exudación desde raíces; Silicio; *Thujaopsis dolabrata*.

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## INTRODUCTION

Toxic metal contamination is a global problem, because of it is harmful to both human and environmental health. For instance, Cadmium (Cd) has been proved to disrupt nutrient uptake and photosynthesis in plants, which restrain growth and even cause death (Gussarson et al., 1996; Sandalio et al., 2001). What's worse, food crops grown in Cd contaminated soils may pose a major risk to human health. The presence of silicon (Si) is good for a healthy plant growth and development (Ma & Yamaji, 2006), and increasing the resistance of some plant species to toxic metals [e.g., zinc (Zn), manganese (Mn) and cadmium]. Liang et al. (2007) reported that Si has the ability to increase the tolerance to Cd toxicity by restricting its accumulation via chemical solutions and internal plant mechanisms.

Some researchers reported that high concentrations of Si in cell walls of the endodermis and epidermis can reduce Cd transport from roots to shoots (Shi et al., 2005; Zhang et al., 2008; Song et al., 2009; Rizwan et al., 2012; Ye et al., 2012). Furthermore, Liang et al. (2005) proved that the Si could ameliorate Cd phytotoxicity and decrease Cd bioavailability. This was because of Si and silicate inside maize plants could induce a rise in soil pH. However, Vaculík et al. (2009) obtained contradictory results. They concluded that exogenous Si not only increased total Cd uptake, but also induced Cd translocation from roots to shoots. Similarly, da Cunha and do Nascimento (2009) discovered that exposure to Si increased growth and led to a higher Cd accumulation. These conflicting results indicate that the Si-mediated alleviation of Cd toxicity remains poorly understood, and that the mechanism of Si-enhanced Cd tolerance is unclear. Lately, Song et al. (2009) found that Si-enhanced resistance to Cd toxicity in *Brassica chinensis* L. could be due to a Si-enhanced antioxidant defense capacity. Addition of Si to plants can produce phenolics, which respond to fungal infection (Ma & Yamaji, 2006). This may explain the Si-mediated Cd tolerance in plants, but the hypothesis needs to be further tested at the rhizosphere level.

Approximately 20-40% of the plant's photosynthetic products are excreted into the soil, causing significant changes in soil chemistry (e.g., nutrient availability). This process of root exudation contributes to metal detoxification through the formation of soluble complexes (Pellet et al., 1996; Yang et al., 1997; Li et al., 2011; Lux et al., 2011). However, little research has investigated the effect of Si on Cd availability in the rhizosphere. It is not clear if root exudation, stimulated by Cd stress, inhibits the Si-induced immobilization of Cd in the rhizosphere.

Cypresses are valued for (1) being evergreen, (2) long-lived, (3) having large biomass and a rapid perennial growth, and (4) possessing asexual propagation. This makes these plants ideal subjects for the study of phytostabilization. Only a few

researchers have reported the relation of Si and Cd toxicity in cypress. Guo et al. (2015) found that Si alleviates Cd toxicity. It was shown that Si alleviated growth inhibition induced by Cd toxicity in *Juniperus chinensis* and *Platyclusus orientalis*. This paper focused on *Thujaopsis dolabrata* (Linn. f.) Sieb. et Zucc. Pot experiments were conducted to evaluate characteristics of the rhizosphere and the bulk soil, and Cd adsorption and translocation in plants. This was made (1) to gain better insight into the possible mechanisms involved in Si-mediated detoxification of Cd in plants, and (2) to provide a basis for field-scale applications of Si during phytostabilization.

## MATERIALS AND METHODS

**Plants and soil.** This study was conducted from 11 March to 6 October 2013 (210 days) in a greenhouse at Hangzhou Normal University, China. The daily photoperiod was 12 h, and temperature was controlled between 20 °C-30 °C. One-year-old plants of *T. dolabrata* were purchased from Zhejiang Weijin Seeds Co., Ltd, Hangzhou, China. Their roots were washed thoroughly with distilled water before the specimens were planted.

Soil was collected at a depth of 0-20cm in the vicinity of Hangzhou, Zhejiang Province, China. It had a pH of 6.47, and contained 9.41 g/kg of organic matter, 1.13 g/kg of total-N, 10.2 mg/kg of Olsen-P and 131.4 mg/kg of extractable-K. The Cd level in the soil was below the detection limit (<0.02 mg/kg). The soil was mixed well with a slow-release fertilizer at a rate of 20 g/kg (APEX, Simplot Co., Ltd, USA.).

**Pot experiments and extraction of soil solution.** Similar-size plants were each planted directly into nylon bags (80 μm nylon mesh, 7 cm diameter × 15 cm height), which were then each transferred into plastic pots (25 cm diameter × 30 cm height). Together, the nylon bag and plastic pot held a total of 7.0 kg of pretreated soil. Root growth was limited to the volume of the nylon bags, which was considered to be rhizospheric soil. The experiment consisted of three treatments, each repeated five times: (1) a control (non-Cd contaminated soil); (2) a Cd treatment (100 mg/kg Cd), and (3) a Cd plus Si treatment (100 mg/kg Cd and 400 mg/kg Si). Silicon was added in the form of Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O, and cadmium was added as CdCl<sub>2</sub>·xH<sub>2</sub>O. The required amounts of Cd and Si were determined using the experimental soil weight (7 kg), and the desired concentrations of Cd (100 ppm) and Si (400 ppm). Distilled water was used daily to keep soil moisture at approximately 70% field water holding capacity.

We extracted the soil solution using the method of Jones and Willett (2006). After harvesting, the rhizosphere and bulk (soil in non-rhizosphere zones), soil were separated and sampled. Moist potting soil was extracted with deionized-distilled water using a water:solid ratio of 1:2.5 (w/v) on a dry weight basis. After extraction, it was shaken at 200 rpm for

2 h at 20 °C in a reciprocal shaker. The suspension was centrifuged at 10000g for 25 min, and the supernatant was filtered through a 0.45 mm membrane filter. The resulting samples of soil solution were stored in the dark at 4 °C for (1) subsequent determinations of DOC, Cd, simple sugars, and phenolics, and (2) the Cd adsorption experiment.

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

We determined the level of  $\text{NH}_4\text{NO}_3$  extractable Cd in the rhizosphere and bulk soil using a soil to 1M  $\text{NH}_4\text{NO}_3$  solution ratio of 1:2.5 (w/v). The extracted solution was shaken at 200 rpm for 1 h, centrifuged for 15 min at 4000 g, and filtered through a 0.45 mm membrane filter. The DOC and Cd concentrations were determined using a total organic carbon analyzer (HACH TOC-600) and an atomic absorption spectrophotometer (Perkin Elmer AAS-800 r), respectively.

The phenolic content of the soil solution derived from the rhizosphere was measured using a Folin–Ciocalteu reagent (Ainsworth and Gillespie 2007), which contained 10%  $\text{Na}_2\text{WO}_4$ , 2.5%  $\text{Na}_2\text{MoO}_4$ , 4.25%  $\text{H}_3\text{PO}_4$ , 3.7% HCl, and 15%  $\text{Li}_2\text{SO}_4$ . A 2 mL extraction was mixed with 1 mL Folin–Ciocalteu reagent and 5 mL saturated  $\text{Na}_2\text{CO}_3$  solution for 1 h at 25 °C. The total phenolic content was detected by absorption at 760 nm using ferulic acid as a standard. A 2 mL extraction was mixed with 1 mL of DNS reagent, boiled for 5 min, and then cooled and detected by 540 nm absorption using glucose as a standard. The enzyme activity and MDA level were calculated according to the methods of Liu and Huang (2000).

**Statistical analysis.** Potential differences among treatments were evaluated using analysis of variance. Means were compared with Duncan's New Multiple Range Test at a 0.05 probability level. Analyses were conducted using SPSS software 17.0.

## RESULTS

**Plant growth and Cd uptake.** After 210 experimental days, the addition of 100 mg/kg of Cd significantly inhibited growth of *T. dolabrata*. Further analysis showed that the leaves and roots of *T. dolabrata* were sensitive to Cd toxicity (growth of leaves and roots decreased by 29.1% and 26.1%, respectively) (Table 1). Before treatment, the Cd concentration and content in cypress roots were negligible. However, after treatment, the species assimilated large amounts of Cd, retaining it mainly in the roots (Table 1). For example, the total Cd contents in the roots were 33 and 8 times higher than those in the leaves and stems of *T. dolabrata*, respectively (Table 1). For *T. dolabrata*, the addition of Si significantly increased the Cd concentration in all parts, compared to the Cd treatment alone.

### Soil levels of pH, $\text{NH}_4\text{NO}_3$ extractable-Cd and DOC.

For the control treatment, the pH of the rhizosphere was significantly lower than that of the bulk soil in *T. dolabrata*, which decreased by 0.76 (Table 2). The addition of Cd further lowered the pH of the rhizosphere of *T. dolabrata*. By contrast, the rhizosphere pH of *T. dolabrata* (5.42) was still lower than that of the control, even after Si addition.

For 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 soil (Table 2), which indicated that it was the predominant form of Cd in the rhizosphere of *T. dolabrata*. However, the mobile Cd concentrations in *T. dolabrata* decreased significantly compared to that in the bulk soil. The addition of Si further lowered the concentration of mobile Cd in the rhizosphere.

**Table 1.** Plant biomass, Cd concentration and total Cd content of *T. dolabrata* after 210 days of growth in Cd-contaminated soils.

**Tabla 1.** Biomasa vegetal, concentración de Cd y contenido total de Cd de *T. dolabrata* después de 210 días de crecimiento en suelos contaminados con Cd.

	Treatment	Total Biomass	Leaf	Stem	Root
Dry weight (g/plant)	Control	14.64 a	4.12 a	4.46 a	6.06 a
	Cd	10.61 b	2.90 b	3.27 b	4.44 b
	Si+Cd	10.71 ab	3.04 b	3.11 b	4.56 b
Concentration (mg/kg)	Cd	291 b	28 b	99 b	606 b
	Si+Cd	333 a	41 a	132 a	732 a
Total content (g/plant)	Cd	3130 b	82 b	334 b	2705 b
	Si+Cd	3872 a	125 a	420 a	3327 a

Means followed by different letters within the same column are significantly different at the level of  $P < 0.05$  according to a Duncan's New Multiple Range Test ( $n=5$ ).

Los promedios seguidos por letras diferentes dentro de una misma columna son significativamente diferentes a  $P < 0,05$  de acuerdo a la prueba de rango múltiple nueva de Duncan ( $n=5$ ).

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, the DOC concentrations of *T. dolabrata* were greater than those observed in the bulk soil. After exposure to Cd for 210 days, *T. dolabrata* released 31.9% and 13.8% more DOC into the rhizosphere, respectively, compared to control treatments. Moreover, the addition of Si significantly increased the DOC level in the rhizosphere of *T. dolabrata* (by 17.7%) compared to the Cd treatment alone.

**Dry weights and Cd concentrations in the root tissues of the epidermis plus endodermis and stele.** Table 3 showed that Cd exposure during 210 days significantly enhanced the dry weight of the epidermis and endodermis tissues of *T. dolabrata*. Furthermore, Cd treatment significantly lowered the biomass of the stele by 34.5% on *T. dolabrata*. Compared to the Cd treatment, the addition of Si significantly increased the dry weight of the epidermis plus

endodermis for *T. dolabrata*. After Cd treatment, the Cd concentration of the epidermis plus endodermis was 12% higher in *T. dolabrata*. For the latter, the Cd concentration of the stele was 20.9% lower than that measured after Cd treatment alone, whilst no difference was observed for *T. dolabrata*.

**Simple sugars and phenolics in the rhizosphere and effect of DOC derived from rhizosphere.** Figure 1 showed that the Cd treatment decreased this content in *T. dolabrata*, while Si addition slightly reversed the Cd-induced effect. The Cd treatment significantly enhanced the phenolic exudation of *T. dolabrata*. However, the increases were greater in *T. dolabrata* when Si was added to the Cd treatment: this increased the phenolic concentration by a further 42.2%.

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 sorption (Fig. 2, Table 4). As such, Cd adsorption was calculated by linear regression,

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

**Tabla 2.** pH del suelo, Cd extraído con  $\text{NH}_4\text{NO}_3$  y DOC en el suelo y la rizosfera de *T. dolabrata* después de 210 días de crecimiento en suelos contaminados con Cd.

Soil	Treatment	pH	$\text{NH}_4\text{NO}_3$ -Cd (mg/kg)	DOC (mg/kg)
Bulk soil	Control	6.38 b	nd	67 d
	Cd	6.38 b	65.6 a	54 e
	Si+Cd	7.39 a	43.8 b	52 e
Rhizosphere	Control	5.62 c	nd	94 c
	Cd	5.23 d	52.1 b	124 b
	Si+Cd	5.42 cd	42.4 b	146 a

Means followed by different letters within the same column are significantly different at the level of  $P < 0.05$  according to a Duncan's New Multiple Range Test (n=5).

Los promedios seguidos por letras diferentes dentro de una misma columna son significativamente diferentes a  $P < 0,05$  de acuerdo a la prueba de rango múltiple nueva de Duncan (n=5).

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

**Tabla 3.** Pesos secos y concentraciones de Cd en la epidermis más la endodermis, y la estela, de los tejidos radicales de *T. dolabrata* después de 210 días de crecimiento en suelos contaminados con Cd.

Parts	Treatment	Dry weight	Cd concentration	Total content
Epidermis plus endodermis	Control	0.583 e	nd	nd
	Cd	0.742 d	1962 a	1456 b
	Si+Cd	1.045 c	1798 a	1879 a
Stele	Control	5.39 a	nd	nd
	Cd	3.53 b	306 b	1083 c
	Si+Cd	3.93 b	327 b	1285 c

Means followed by different letters within the same column are significantly different at the level of  $P < 0.05$  according to a Duncan's New Multiple Range Test (n=5).

Los promedios seguidos por letras diferentes dentro de una misma columna son significativamente diferentes a  $P < 0,05$  de acuerdo a la prueba de rango múltiple nueva de Duncan (n=5).

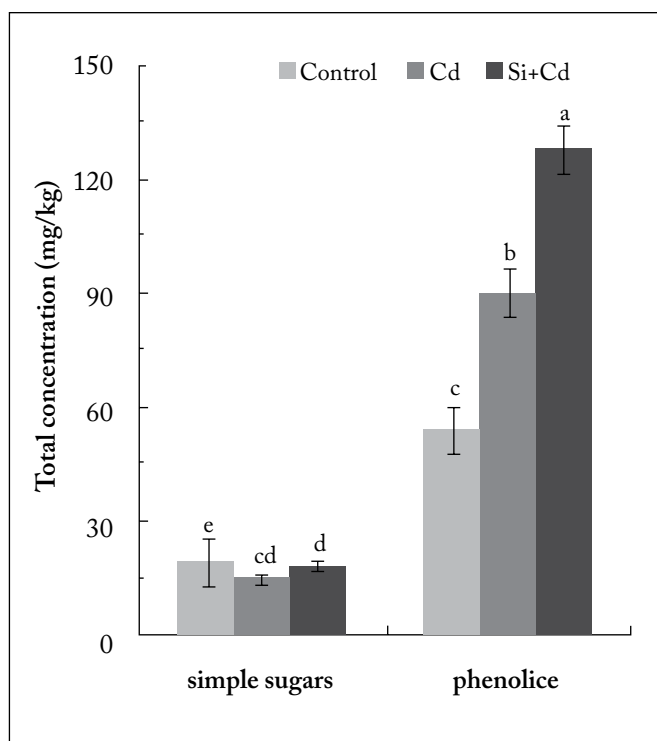


Fig. 1. Concentrations of simple sugars and phenolics in the rhizosphere of *T. dolabrata* after 210 days of growth.

Fig. 1. Concentración de azúcares simples y compuestos fenólicos en la rizosfera de *T. dolabrata* después de 210 días de crecimiento.

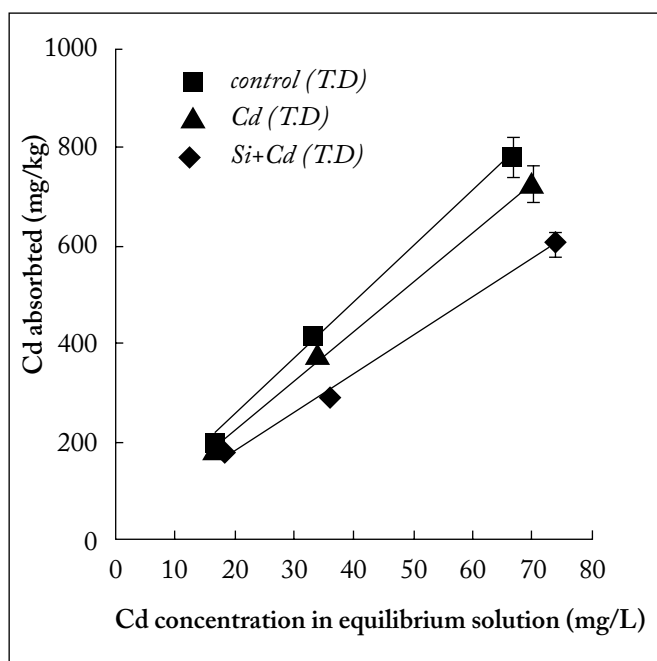


Fig. 2. The kinetics of Cd adsorption influenced by DOC derived from the rhizosphere of *T. dolabrata*.

Fig. 2. Cinética de adsorción de Cd influenciada por DOC, derivado de la rizosfera de *T. dolabrata*.

where increasing concentrations of Cd in the equilibrium solutions ( $K$ ) were positively related to the soil metal adsorption capacity. The Cd adsorption was slightly below values in the control under increasing  $K$  values in *T. dolabrata*. However, the addition of Si to the Cd treatment further decreased Cd adsorption, below values in the Cd treatment (Fig. 2).

**MDA and antioxidant concentration in the leaves.** Under the influence of Cd, the analysis of malondialdehyde (MDA) and antioxidase content in the leaves of *T. dolabrata* can help to explain the action mechanism of Si in the rhizosphere (Li et al., 2015) (Table 5). The addition of Cd alone significantly increased MDA concentrations in the leaves of *T. dolabrata*. However, the further addition of Si had the opposite effect, with the MDA concentrations decreasing by 30.71% in *T. dolabrata*.

Compared to the control group, the addition of Cd alone significantly increased the superoxide dismutase (SOD) activity in the leaves of *T. dolabrata*. After the addition of Si, the SOD activity decreased significantly. In the control group, the addition of Cd significantly inhibited the POD activity in *T. dolabrata*. However, after treatment with Si, the POD activity in the leaves of *T. dolabrata* increased; the POD activity on *T. dolabrata* increased to 36.6% of values in the control group. Treatment with Cd alone significantly inhibited catalase (CAT) activity in *T. dolabrata*. After the addition of Si, the CAT activity significantly increased in *T. dolabrata*.

**Dynamic pH and Silicic acid concentration in the rhizosphere and bulk soil.** It is well established that pH is an important indicator of soil physical and chemical properties. These properties have a direct influence on plant growth, and the transportation of nutrients to the rhizosphere. The dynamic rhizosphere and bulk soil pH were investigated for *T. dolabrata* (Fig. 3). We found that the pH of soils in the control experiment decreased over time for *T. dolabrata*. For *T. dolabrata*, the rhizosphere pH also decreased (by 1.24 units) upon the addition of Cd. However, this effect was not mitigated by the addition of Si (Fig.3), where the rhizosphere pH on *T. dolabrata* decreased by 1.05 units.

The silicic acid concentration directly affects the pH value and root exudates in the rhizosphere and non-rhizosphere soils, which further influences the action mechanism of the plant to Cd. The addition of 400 mg/kg of silicic acid was equivalent to 1.407 mM of  $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ . Figure 4 showed the variation of silicic acid concentration in the rhizosphere and bulk soil for *T. dolabrata*. These data should be considered alongside the soil pH data presented in Fig. 3, and will be discussed in the subsequent section.

**Table 4.** Linear regression for the effect of DOC on Cd adsorption derived from the rhizosphere of *T. dolabrata*.**Tabla 4.** Regresión lineal entre el efecto de DOC y la adsorción de Cd derivado de la rizósfera de *T. dolabrata*.

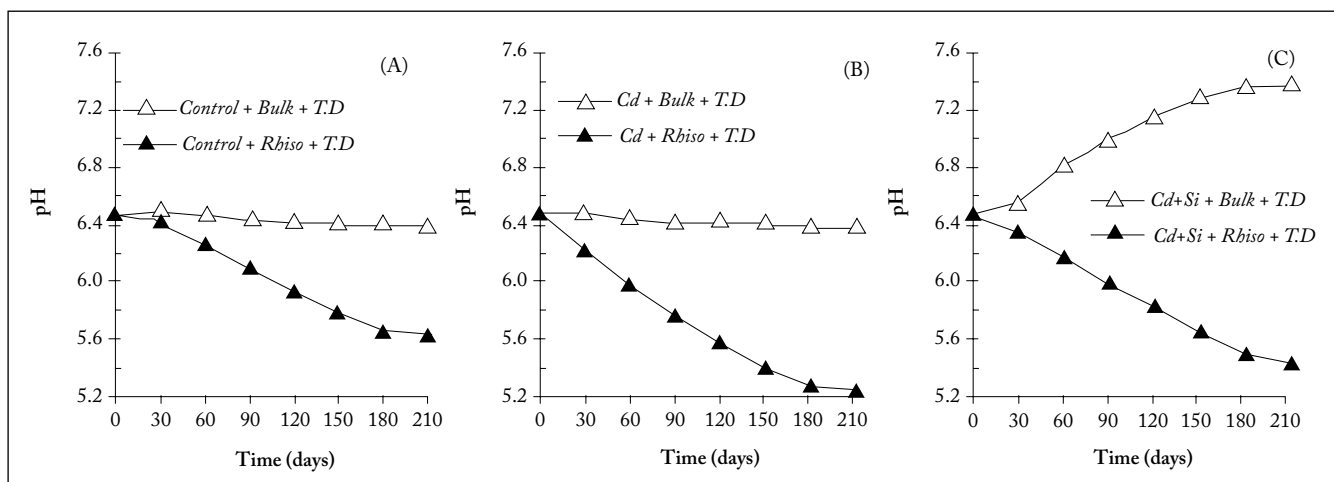
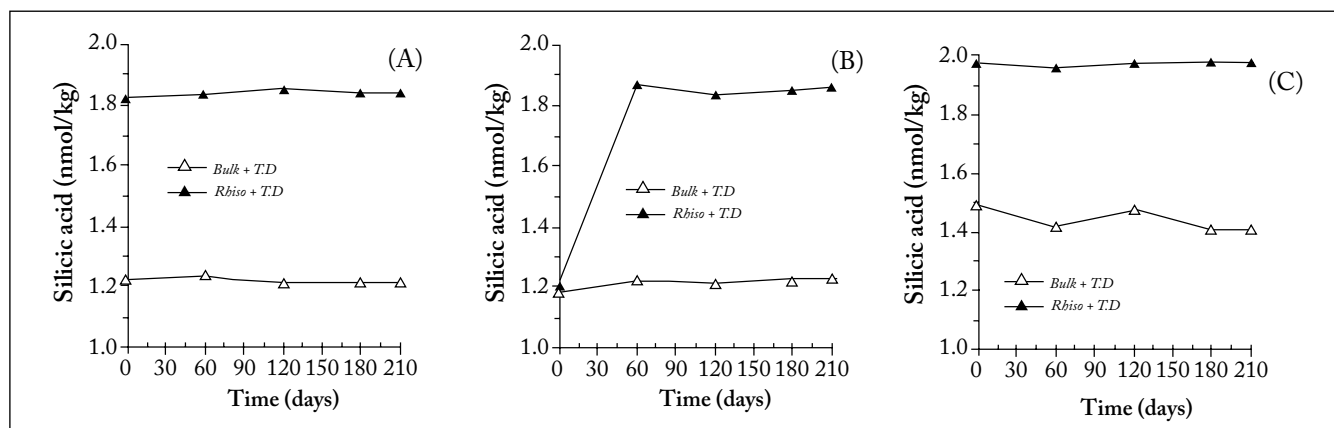
Treatment	<i>K</i>	<i>R</i> <sup>2</sup>	Adsorbed ratios
Control	11.33	0.9901	31.7% ± 1.3%
Cd	10.11	0.9940	29.3% ± 0.9%
Si+Cd	8	0.9987	24.2% ± 0.6%

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

Relación de adsorción: la relación (%) entre el contenido total de Cd en la solución de equilibrio y el contenido inicial total.

**Table 5.** MDA and antioxidase concentrations in leaves of *T. dolabrata*.**Tabla 5.** MDA y concentraciones de antioxidasa en las hojas de *T. dolabrata*.

Items	control	Cd	Cd+Si
MDA (malondialdehyde concentration nmol/g)	13.01 ± 1.26 b	23.41 ± 3.16 a	16.22 ± 2.02 b
POD (peroxidase U/g protein)	0.41 ± 0.06 ab	0.32 ± 0.05 b	0.56 ± 0.12 a
SOD (superoxide dismutase U/g protein)	1.22 ± 0.16 c	1.91 ± 0.15 a	1.25 ± 0.14 c
CAT (catalase U/g protein)	0.18 ± 0.06 a	0.09 ± 0.03 a	0.16 ± 0.03 a

**Fig. 3.** Soil pH of *T. dolabrata* in the pot experiment. (A) Control soil; (B) soil with addition of Cd; (C) soil with addition of Cd and Si.**Fig. 3.** pH del suelo de *T. dolabrata* en el estudio en macetas. (A) Suelo control; (B) suelo con agregado de Cd; (C) suelo con agregado de Cd y Si.**Fig. 4.** Silicic acid concentration distribution in rhizospheric soil. (A) Control soil; (B) soil with addition of Cd; (C) soil with addition of Cd and Si.**Fig. 4.** Distribución de la concentración de ácido silícico en la rizósfera. (A) Suelo control; (B) suelo con agregado de Cd; (C) suelo con agregado de Cd y Si.

## DISCUSSION

**Differential rhizospheric Cd mobilization.** This study demonstrated that *T. dolabrata* acidified the rhizosphere, with lower pH values compared to those observed in the 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<sup>+</sup> or OH<sup>-</sup>. In this research, the reduction in solution pH may have resulted from the increased release of root exudates (Hinsinger et al., 2003), consistent with the enhanced DOC derived from the rhizosphere root exudation (Table 2). Moreover, the exudation process was accelerated in *T. dolabrata* in response to Cd toxicity. DOC in the root exudates includes different organic compounds, which can trigger a range of chemical reactions and biological transformations in the rhizosphere. In this work, roots of *T. dolabrata* exhibited directly, or indirectly via exudation, a greater ability to mobilize Cd. In fact, we found that the root exudates from *T. dolabrata* mobilized greater amounts of Cd from the Cd-contaminated rhizospheric soil (Fig. 2 and Table 4). The Cd-mobilization due to high root exudation plays an important role in Cd uptake by *T. dolabrata*, demonstrated by the assimilation of a 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), similar to the results of previous studies (Lin et al., 2003; Li et al., 2011).

*Thujopsis dolabrata* exudated a higher amount of DOC into the rhizosphere, Cd addition further increased this discrepancy (Table 2). That likely caused the higher Cd solubility in the rhizosphere of *T. dolabrata* (Fig. 2, Table 4), and possibly explains why *T. dolabrata* assimilated greater quantities of Cd from the rhizosphere, as more Cd was translocated from the roots to the shoots (Table 1).

**Si reduced oxidative damage, stimulated root exudation and enhanced Cd uptake in *T. dolabrata*.** MDA is the product of membrane lipid peroxidation, and its concentration is positively correlated with the level of oxidative damage observed in plants (Guo et al., 2007). With the addition of Cd, the MDA content in the leaves of *T. dolabrata* increased significantly, indicating that Cd resulted in an increased oxidative damage to *T. dolabrata*. The rhizosphere pH of *T. dolabrata* in the Si plus Cd treatment was 0.24 units lower than that in the corresponding control. This suggests that the root exudates from this plant caused strong pH buffering, minimizing the effect of the Si-induced increase of pH in the rhizosphere.

It has been reported that Si-treated plants can produce phenolics in response to fungal infection (Ma & Yamaji, 2006). The Si-mediated synthesis of phenolics may also occur in plants under Cd stress. Characteristics of the root exudates in our study indicated that higher amounts of phenolics were exudated from *T. dolabrata* in the Cd plus Si treatment. This showed that the Si-induced efflux of phenolics from roots is

species-specific. Similarly, Kidd et al. (2001) demonstrated that an Al-exposed maize species pretreated with Si exuded up to 15 times more phenolics than the untreated controls. However, the phenolic exudation from *T. dolabrata* was unaffected by Si pretreatment. It also was confirmed in *Juniperus chinensis* and *Platyclusus orientalis* by Guo et al. (2015).

Due to the complexity of the phenolic structure, the stability constant for the complex formation of Cd is higher than that for a low molecular organic acid. The log *K* values of Cd complexes with phenolic groups (log *K* = 8.7–9.4) are much higher than those with carboxylic and phosphoric groups (log *K* = 5.7–6.6) when assessing the metal biosorption of extra-cellular polymeric substances extracted from the activated sludges (Comte et al., 2008). Similarly, Yang and Pan (2013) found that Cd adsorption of root exudates from sunflower was log *K* = 9.2 for phenolic groups and log *K* = 4.7 for carboxylic groups, suggesting that the aromatic groups were mainly responsible for Cd adsorption. In this study, the high amount of phenolics released into the rhizosphere significantly increased Cd mobility (Fig. 2 and Table 4), and consequently, significantly increased the total Cd uptake and content of *T. dolabrata* shoots (Table 1). Although the mechanism of Si-mediated synthesis of phenolics remains unknown, the higher assimilation and translocation of Cd in *T. dolabrata* did not aggravate the growth inhibition effect compared to the Cd treatment alone. This indicates that phenolics derived from the roots of Si-treated *T. dolabrata* may play an important role in Cd detoxification in plants.

This work showed the usefulness of Si for increasing the resistance of plants to toxic heavy metals. The amendment of Si into Cd contaminated soil effectively alleviated the Cd toxicity in *T. dolabrata*, and we found that the mechanism was specific. In *T. dolabrata*, Si mediated the Cd detoxification by stimulating phenolic exudation from roots with a high Cd-chelating ability of roots with Si. Further, the addition of Si significantly increased POD and CAT activities and inhibited SOD activity in leaves of *T. dolabrata*, further mitigating the oxidative damage induced by Cd stress. 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 other chelating agents, such as EDTA. Further, this understanding could enrich phyto extraction methods and make Si-mediated detoxification more feasible.

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## REFERENCES

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- Ainsworth, E.A. & K.M. Gillespie (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nature Protocols* 2: 875–877.
- Comte, S., G. Guibaud & M. Baudu (2008). Biosorption properties of extracellular polymeric substances (EPS) towards Cd, Cu and Pb for different pH values. *Journal of Hazardous Materials* 151: 185–193.
- da Cunha, K. P.V. & C.W.A. do Nascimento (2009). Silicon effects on metal tolerance and structural changes in maize (*Zea mays* L.) grown on a cadmium and zinc enriched soil. *Water, Air, and Soil Pollution* 197: 323–330.
- Guo, B., Y.C. Liang, Y.G. Zhu & F.J. Zhao (2007). Role of a salicylic acid in alleviating oxidative damage in rice roots (*Oryza sativa* L.) subjected to cadmium stress. *Journal of Environmental Pollution* 147: 743–749.
- Guo, B., C. Liu, N.F. Ding, Q.L. Fu, Y.C. Lin, H. Li & N.Y. Li (2015). Silicon alleviates Cadmium toxicity in two cypress varieties by strengthening the exodermis tissues and stimulating phenolic exudation of roots. *Journal of Plant Growth Regulation* 34: 809–828.
- Gussarson, M., H. Asp, S. Adalsteinsson & P. Jensen (1996). Enhancement of cadmium effects on growth and nutrient composition of birch (*Betula pendula*) by buthioninesulphoximine (BSO). *Journal of Experimental Botany* 47: 211–215.
- Hinsinger, P., C. Plassard, C. Tang & B. Jaillard (2003). Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. *Plant and Soil* 248: 43–59.
- Jones, D.L. & V.B. Willett (2006). Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. *Soil Biology and Biochemistry* 38: 991–999.
- Kidd, P.S., M. Llugany, C.H. Poschenrieder, B. Gunse & J. Barcelo (2001). The role of root exudates in aluminium resistance and silicon-induced amelioration of aluminium toxicity in three varieties of maize (*Zea mays* L.). *Journal of Experimental Botany* 52: 1339–1352.
- Liang, Y., W. Sun, Y.G. Zhu & P. Christie (2007). Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review. *Environmental Pollution* 147: 422–428.
- Liang, Y., J.W.C. Wong & L. Wei (2005). Silicon-mediated enhancement of cadmium tolerance in maize (*Zea mays* L.) grown in cadmium contaminated soil. *Chemosphere* 58: 475–483.
- Lin, Q., Y.X. Chen, H.M. Chen, Y.L. Yu, Y.M. Luo & M.H. Wong (2003). Chemical behavior of Cd in rice rhizosphere. *Chemosphere* 50: 755–761.
- Li, T., Z. Di, X. Yang & D.L. Sparks (2011). Effects of dissolved organic matter from the rhizosphere of the hyperaccumulator *Sedum alfredii* on sorption of zinc and cadmium by different soils. *Journal of Hazardous Materials* 192: 1616–1622.
- Li, W.D.; B. Guo, Q.L. Fu, N.F. Ding, N.Y. Li & C.H. Liu (2015). Mechanisms of Silicon Mediated Cadmium Tolerance on two Cypress Varieties. *Journal of Nuclear Agricultural Science* 29: 1225–1231 (in Chinese).
- Liu, X.Z. & B. Huang (2000). Heat stress injury in relation to membrane lipid peroxidation in creeping bent grass. *Crop Science* 40: 503–510.
- Lux, A., M. Martinka, M. Vaculik & P.J. White (2011). Root responses to cadmium in the rhizosphere: a review. *Journal of Experimental Botany* 62: 21–37.
- Ma, J.F. & N. Yamaji (2006). Silicon uptake and accumulation in higher plants. *Trends in Plant Science* 11: 392–397.
- Pellet, D.M., L.A. Papernik & L.V. Kochian (1996). Multiple aluminium resistance mechanisms in wheat. Role of root apical phosphate and malate exudation. *Plant Physiology* 112: 591–597.
- Rizwan, M., J.D. Meunier, H. Miche & C. Keller (2012). Effect of silicon on reducing cadmium toxicity in durum wheat (*Triticum turgidum* L. cv. Claudio W.) grown in a soil with aged contamination. *Journal of Hazardous Materials* 209: 326–334.
- Sandalio, L.M., H.C. Dalurzo, M. Gomez, M.C. Romero-Puertas & L.A. Del Rio (2001). Cadmium-induced changes in the growth of oxidative metabolism of pea plants. *Journal of Experimental Botany* 52: 2115–2126.
- Shi, X.H., C.C. Zhang, H. Wang & F.S. Zhang (2005). Effect of Si on the distribution of Cd in rice seedlings. *Plant and Soil* 272: 53–60.
- Song, A., Z. Li, J. Zhang, G. Xue, F. Fan & Y. Liang (2009). Silicon-enhanced resistance to cadmium toxicity in *Brassica chinensis* L. is attributed to Si-suppressed cadmium uptake and transport and Si-enhanced antioxidant defense capacity. *Journal of Hazardous Materials* 172: 74–83.
- Vaculik, M., A. Lux, M. Luxová, E. Tanimoto & I. Lichtscheidl (2009). Silicon mitigates cadmium inhibitory effects in young maize plants. *Environmental and Experimental Botany* 67: 52–58.
- Yang, J. & X. Pan (2013). Root exudates from sunflower (*Helianthus annuus* L.) show a strong adsorption ability toward Cd (II). *Journal of Plant Interactions* 8: 263–270.
- Yang, X.E., V.C. Baligar, J.C. Foster & D.C. Martens (1997). Accumulation and transport of nickel in relation to organic acids in ryegrass and maize grown with different nickel levels. *Plant and Soil* 196: 271–276.
- Ye, J., C. Yan, J. Liu, H. Lu, T. Liu & Z. Song (2012). Effects of silicon on the distribution of cadmium compartmentation in root tips of *Kandelia obovata* (S., L.) Yong. *Environmental Pollution* 162: 369–373.
- Zhang, C., L. Wang, Q. Nie, W. Zhang & F. Zhang (2008). Long-term effects of exogenous silicon on cadmium translocation and toxicity in rice (*Oryza sativa* L.). *Environmental and Experimental Botany* 62: 300–307.