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Technical Note

Hyperaccumulation of zinc by *Corydalis davidii* in Zn-polluted soils

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ABSTRACT

A field survey was conducted to identify potential Zn accumulators from an artisanal Zn smelting area in southwest China's Guizhou Province. Hydroponic and soil culture experiments were performed to investigate the accumulation ability of Zn in *Corydalis davidii*. Zn concentrations in roots, stems and leaves of *C. davidii* in the smelting site were 1.1–3.5, 1.2–11.2, and 3.3–14 mg g^{-1}, respectively, whereas Zn concentrations in roots, stems and leaves of *C. davidii* in the contaminated site impacted by the Zn smelting were 1.0–2.4, 1.9–6.5, and 3.0–1.1 mg g^{-1}, respectively. Zn concentrations in leaves and stems of *C. davidii* were observed at above 10 mg g^{-1} that refers to the threshold of Zn hyperaccumulator. The concentration distribution of Zn in *C. davidii* was leaf > stem > root, and the Zn bioaccumulation factors of *C. davidii* were above 1. It is concluded that *C. davidii* has high tolerance to concentrate Zn stress, and that *C. davidii* is a newly discovered Zn-hyperaccumulator with high biomass in the aboveground parts. Based on the cultivation experiments, *C. davidii* could reduce Zn concentration by 26.6, 21.2, and 10.2 mg $kg^{-1}yr^{-1}$ by phytoextraction from the smelting slag, Zn-contaminated soil, and background soil, respectively.

1. Introduction

Large areas of soils have been contaminated by heavy metals, which are detrimental to the growth, reproduction and development of living organisms including plants, animals and microorganisms (Liu et al., 2008; Fritsch et al., 2010). This phenomenon has even threatened the health of ecosystems and human beings (Xiao et al., 2004; Tang et al., 2009), and heavy metal pollution has become an urgent problem throughout the world. Phytoextraction is a new technology that uses metal-accumulating plants to extract the metals from contaminated soils (CS), groundwater or surface water, and has been proposed as an effective and affordable solution to clean up heavy metal contamination (Pulford and Watson, 2003; Xu et al., 2009). The application of hyperaccumulators may be one of the best choices for phytoextraction of soil contaminated by heavy metals because such hyperaccumulators can accumulate large amounts of metals in their harvestable parts which are relatively easy to dispose (McGrath and Zhao, 2003).

However, phytoextraction technology is not yet widely used in remediation practice, although more than 400 plant species have been identified as natural metal hyperaccumulators (Freeman et al., 2004). The problem is that most of the hyperaccumulators were native species restricted to specific sites, and had only very low biomass as well (Li et al., 2003). The amount of phytoextraction for heavy metals in CS is usually small by hyperaccumulators because of their low biomass, even though the contents of heavy metals in the harvested parts are high enough. Therefore, systematic screening of plants from the heavy metal polluted areas is of high importance, and may allow the identification of appropriate plant species for phytoremediation of metal-contaminated soils (Wang et al., 2009).

It is no doubt that metal smelters are the important anthropogenic sources of heavy metal pollution to local soils through metal releases of smelting slags (SL) and smelting fume depositions. Among them, artisanal zinc smelting activities using an indigenous method that have produced considerable metal pollution in China have aroused high concerns (Feng et al., 2004; Bi et al., 2006; Yang et al., 2006; Li et al., 2008). An excellent site that experienced long-term of artisanal zinc smelting activities using an indigenous method is from Hezhang County (104°10′–105°03′ E, 26°46′–27°28′ N), western Guizhou Province, China, where the Zn smelting activities were back to the 17th century but completely ceased in 2004 due to serious metal pollution. The local smelting activities applied with coal to burn zinc ores, sphalerite (ZnS) and/or calamine ($ZnCO_3$), in column furnaces of ceramic jars under around 800 °C without any pollution control devices utilized during the whole zinc smelting processes (Feng et al., 2004). Such indigenous methods for Zn smelting has produced 20 Mt of open dumped SL and 1200 ha farmland polluted with Zn as well as Pb, Cd and Hg (Yang et al., 2006). Zn is

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an essential nutrient for normal growth and development of plants, however, excess Zn in soils caused by anthropogenic activities may retard the growth and development of plants, and induce damage to the ecosystem (An et al., 2006). Zn contamination is occurred widely in soil and water environments, and remediation for Zn contamination is an urgent issue to solve. For purpose of phytoremediation for Zn pollution in soils, an ecological survey was performed in the artisanal Zn smelting area in Hezhang County, and screened 14 dominant species including *Buddleja lindleyana*, *Ixeris gracilis*, *Artemisia annua*, *Rhododendron simsi*, *Litsea cubeba*, *Sambucus Chinensis*, *Querus fabric*, *Senecio scandens*, *Smilax china*, *Polygonum posumbu*, *Corydalis davidii*, *Artemisia argyi*, *Sorbus megalocarpa*, and *Anemone hupehensis* (Lin, 2009). Among them, the species of *C. davidii* was identified to have high potential to hyperaccumulate Zn. For the first time, we investigated the characteristics of growth, tolerance and Zn accumulation of *C. davidii* in both soil and hydroponic cultures. This paper aims to identify the hyperaccumulation of *C. davidii* and to estimate the remediation efficiency of *C. davidii* for Zn-polluted soils.

2. Materials and methods

2.1. Field survey

The study area is located in the Magu smelting site (104°30′–104°58′ E, 26°45′–27°30′ N) is located in Hezhang County, Guizhou Province, China. The area exhibits a sub-tropical continental monsoon climate, with an annual average precipitation of 1200 mm and an annual average temperature of 12 °C. The study area is part of a karst terrain attaining an elevation of 2000–2200 m above sea level.

Three sites from the study area were selected for field survey and sample collection. The artisanal Zn smelting site was targeted the substrates mainly composed of SL that contained high contents of Zn. A contaminated site with elevated Zn in soil from smelting fume deposition was selected 1 km away from the smelting site, and a control site (background area) devoid of major source of Zn pollution was 2 km away from the smelting site. Properties of the three types of substrates, i.e. SL, CS and background soils (BS), collected from the above three sites were analyzed at the laboratory.

During the field survey, 12 samples of *C. davidii* were collected from the smelting site, eight samples from the contaminated site, and five samples from the background area. Each plant sample was collected the whole plant that contains the root system. The rhizosphere soils were also sampled at the depth of 10–30 cm when collecting the plant samples of *C. davidii*. The samples of SL, CS and BS were measured for both total and diethylenetriamine pentaacetic acid (DTPA)-extractable Zn concentrations at the laboratory. Zn concentrations in roots, stems and leaves of *C. davidii* were also determined.

2.2. Hydroponic culture experiment

The seedlings of *C. davidii* were collected from the Zn smelting site of the study area for purpose of clone and culture in greenhouse. The composition of the nutrients in solution (in μM) were composed of 2000 $Ca(NO_3)_2$, 100 KH_2PO_4, 500 $MgSO_4$, 100 KCl, 700 K_2SO_4, 10 H_3BO_3, 0.5 $MnSO_4$, 1 $ZnSO_4$, 0.2 $CuSO_4$, 0.01 $(NH_4)_6Mo_7O_{24}$, and 100 Fe-EDTA (Ye et al., 2003). The seedlings of *C. davidii* were precultured for 30 d for initiation of new roots. Average Zn concentrations in roots, stems and leaves of precultured *C. davidii* were 1.08, 1.34 and 2.68 mg g^{-1}, respectively, before exposure to Zn treatments. Then they were exposed to treatments at various Zn supply levels for 20 d. The treatments included control (without addition of Zn), 50, 100, 200, 400, and 800 mg Zn L^{-1} supplied as $Zn(NO_3)_2$. The nutrient solution was aerated and renewed once every 3 d. At each harvest time, the biomass in roots, stems and leaves were recorded. The parameters of superoxide dismutase (SOD) activity, catalase (CAT) activity, peroxidase (POD) activity, malondialdehyde (MDA) contents, and soluble protein (SP) contents in the shoots of *C. davidii* were measured. Zn concentrations in roots, stems and leaves of *C. davidii* were also determined.

2.3. Soil culture experiment

The precultured *C. davidii* from the hydroponic culture experiment was also applied to soil culture experiment. *C. davidii* were transplanted in January into the three types of substrates of SL, CS and BS that were collected from the smelting site, contaminated site and background area, respectively. Each substrate contained three plots (1.5 m × 2 m). A total of 50 precultured *C. davidii* were transplanted in each plot (20 cm × 30 cm). Aboveground parts of *C. davidii* were harvested when the coverage reached to around 80%. During the year, the aboveground parts of *C. davidii* were harvested in May, August and November, respectively.

2.4. Samples analyses

Measurement of properties of soil and slag substrates was based in the methods of Lu (2000). Electrical conductivity (EC) was determined by an EC meter (DDS-307, Shanghai, China) (solid:de-ionized water = 1:2), and pH by pH meter (320-S, Chengdu, China) (solid:de-ionized water = 1:5). The samples were digested by $K_2Cr_2O_7$–H_2SO_4 for measurement of total Kjelahl nitrogen (TKN) by the Kjeldahl method. The samples were fused by NaOH and measured for ortho-phosphorus (OP) by acid molybdenum blue method. Total carbon (TC) was measured by dichromate oxidation method, total potassium (TK) by flame spectrophotometry method, and cation exchange capacity (CEC) by ammonium acetate displacement method.

Plant samples were firstly rinsed with tap water and then with de-ionized water. The rinsed samples were dried at 105 °C for 5 min and then at 70 °C in an oven until completely dry and weighed. The dried plant samples were ground to a powder and passed through a 1 mm sieve. The powder samples were digested with a solution of concentrated HNO_3 and $HClO_4$ (4/1). Soil samples were ground to a powder and passed through a 0.15 mm sieve. Soil samples were digested with 2:1:2 (V/V/V) HCl:HNO_3:$HClO_4$ mixture for analysis of total Zn. DTPA-extractable Zn in soils was extracted by 0.005 M DTPA + 0.1 M TEA + 0.01 M $CaCl_2$ (soil/liquid: 1/5).

Zn concentration was determined using inductively coupled plasma mass spectrometry (ICP MS, ELAN DRC-e, PerkinElmer, USA). The analytical precision, determined based on the standard quality control procedures of the laboratory using the China EPA Standard Reference Materials (SRMs) of soil and plant samples, internal standards (Rh at 500 μg L^{-1}), duplicates, and reagent blanks, was better than ±5%. The recovery rates of SRMs in this study were 95–103% for Zn. The detection limit for Zn was 0.5 mg kg^{-1} in solid substrates, and 2 μg L^{-1} in liquid substrates. All the determined data of Zn are reported as dry weight (DW).

Fresh leaves of *C. davidii* were homogenized in 50 mM cold NaH_2PO_4 + Na_2HPO_4 buffer (pH 7.8) using a prechilled mortar and pestle in an ice bath. The homogenate was centrifuged at 4000 rpm for 20 min by a high speed refrigerated centrifuge (GL-20C, Guangzhou, China) at 4 °C for further analyses. SOD activity was measured as described by Somashekaraiah et al. (1992). POD activity and CAT activity were determined using guaiacol and H_2O_2 substrates, respectively (Wu and von Tiedemann, 2002). MDA was measured as described by Liu et al. (2004). Soluble

protein content of the enzyme extracted was determined through the method of Bradford (1976), with bovine serum albumin used as standard.

2.5. Statistical analysis

Statistical analyses were performed using SPSS (version 13.0). Data with replicates are expressed as the means ± standard deviation (SD). Fisher's least significant difference (LSD) was used at $p < 0.05$ for mean separations.

3. Results

3.1. Substrate properties

The summary of properties of the three types of substrates, i.e. SL, CS and BS, is listed in Table 1. The pH values in SL, CS and BS are 6.72–9.05, 4.34–4.96 and 4.86–5.94, respectively. The lower pH in BS is consistent with the acidic red soils in southwest China. Compared to others, pH values in CS declined due to SO_2 deposition from the smelting fumes. SL was alkaline and attributable for the fact that large amounts of carbonate (host rock) in Zn ores were decomposed into soda lime during smelting (Lin et al., 2007). Low pH value may increase mobility of Zn (Lombnfes et al., 2008), and it may also affect the DTPA-extractable fraction of Zn. The DTPA-extractable concentrations are reliable for predicting bioavailability of metals and bioavailability of metals in soils (Hseu, 2006; Khan and Jones, 2009). It is interesting to observe that the percentage of DTPA-extractable Zn in CS was higher than that in SL, although DTPA-extractable Zn in CS were lower than that in SL owing to high Zn pollution in SL. EC value in SL was 1.42 dS m^{-1}, significantly higher than the level (0.5 dS m^{-1}) that may negatively affect the growth of vegetation (Casselman et al., 2006). The mean CEC value in SL was 8.4 cmol kg^{-1}, significantly lower than those in BS (12.5 cmol kg^{-1}) and CS (14.1 cmol kg^{-1}). The parameter of CEC implies for the ability of maintaining fertility, and the low CEC value in SL indicated that SL was adverse to revegetation. TC and TK contents in SL were substantially higher than those in CS and BS, whereas concentrations of TKN and OP in SL were lower.

3.2. Accumulation of Zn in C. davidii from field observations

Growth properties and Zn concentration distribution of *C. davidii* from the field observation are listed in Table 2. Mean height and root length of *C. davidii* from the smelting site were 41.5 and 20.1 cm, respectively, and the average biomass in roots, stems and leaves of per plant of *C. davidii* were 2.88, 4.97, and 3.21 g DW. Growth properties of *C. davidii* in the contaminated site and the background area were similar to the smelting site. It is clearly shown that the species of *C. davidii* has high biomass with the majority focusing on the aboveground parts (stems and leaves). The average percentages of Zn contents in root, stems and leaves of *C. davidii* in the smelting site accounted for 13%, 42%, and 45%, respectively, and similar distributions were observed from the contaminated site and the background area.

The contents of Zn in the various parts (roots, stems and leaves) of *C. davidii* and in the rhizosphere soils are listed in Table 3. Zn concentrations in roots, stems and leaves of *C. davidii* in the smelting site were 1.1–3.5, 1.2–11.2, and 3.3–14 mg g^{-1}, respectively, and those in the contaminated site were 1.0–2.4, 1.9–6.5, and 3.0–11 mg g^{-1}, respectively. However, Zn concentrations in roots, stems and leaves of *C. davidii* in the background area were lower to 0.6–0.8, 0.9–2.0, and 1.5–2.9 mg g^{-1}, respectively. It is shown that the order of Zn concentrations in *C. davidii* is as follows: SL > CS > BS, in accord with the order of DTPA-extractable Zn (Table 1). It is clear to show the accumulation of Zn decreases in the following order regarding to mean values: leaf > stem > root in all samples, indicating that Zn was liable to translocate from root to leaf.

The bioconcentration factors (BFs) in roots, stems and leaves of *C. davidii* in the smelting site were all lower than 1 with respect to the total Zn concentration, but were much higher than 1 with respect to DTPA-extractable Zn (Table 3). The BFs in roots, stems and leaves of *C. davidii* in the contaminated site and the background area were all above 1 with respect to the total Zn and DTPA-extractable Zn (Table 3). The higher BFs further demonstrated that the plant species of *C. davidii* has high potential of accumulating Zn from the Zn-polluted soils. It is interesting to observe that Zn concentrating in the aboveground parts of *C. davidii* accounted for more than 80% of the total Zn concentrations in the whole plant. Therefore, the species of *C. davidii* was identified

Table 1
General properties of substrates.

Samples	pH range	Bulk density (g cm^{-3})	Total Zn (mg kg^{-1})	DTPA-extractable Zn (mg kg^{-1})	CEC (cmol kg^{-1})	EC (dS m^{-1})	TC (%)	TKN (mg g^{-1})	OP (mg g^{-1})	TK (mg g^{-1})
SL ($n = 8$) [A]	6.72–9.05	1.51 ± 0.18b [B]	9847 ± 983c	312 ± 39c	8.4 ± 2.3a	0.42 ± 0.14a	11.8 ± 1.98a	0.7 ± 0.03a	0.96 ± 0.13a	2.6 ± 0.3a
CS ($n = 8$)	4.34–4.96	1.14 ± 0.23a	1568 ± 134b	186 ± 12b	12.5 ± 3.2b	0.15 ± 0.02b	1.48 ± 0.08b	1.1 ± 0.1b	0.85 ± 0.12a	6.3 ± 0.3b
BS ($n = 8$)	4.86–5.94	1.18 ± 0.25a	118 ± 45a	25 ± 11a	14.1 ± 2.42b	0.07 ± 0.01c	1.18 ± 0.20b	1.0 ± 0.2b	1.0 ± 0.10a	6.2 ± 0.2b

[A] *n*: Number of samples.
[B] a, b and c refer to significant differences at $p < 0.05$ according to the LSD test in the same column.

Table 2
Growth properties of *C. davidii* from field observations.

Samples		Biomass of *C. davidii* (g DW)			Zn percentage in various parts of *C. davidii* (%)			Height of *C. davidii* (cm)	Root length (cm)
		Root	Stem	Leaf	Root	Stem	Leaf		
Smelting area ($n = 12$) [A]	Range	1.37–4.32	1.24–9.94	2.30–6.92	4–22	18–66	29–63	21.2–82.4	14.3–31.6
	Mean ± SD[B]	2.88 ± 0.81	4.97 ± 2.09	3.21 ± 1.29	13 ± 7	42 ± 14	45 ± 13	41.5 ± 17.8	20.1 ± 4.85
Contaminated area ($n = 8$)	Range	2.45–4.43	3.42–5.93	2.13–5.27	7–22	22–62	31–68	21.3–66.7	11.4–34.2
	Mean ± SD	3.17 ± 0.70	4.67 ± 0.91	3.34 ± 1.23	12 ± 5	43 ± 11	45 ± 11	41.5 ± 14	20.4 ± 8.30
Background area ($n = 5$)	Range	2.13–5.45	3.45–7.56	2.45–4.34	6–20	41–52	37–43	22.7–76.5	13.2–31.3
	Mean ± SD	3.43 ± 1.22	5.33 ± 1.50	3.11 ± 1.15	15 ± 6	45 ± 5	40 ± 3	44.3 ± 20.2	20.9 ± 7.04

[A] *n*: Number of samples.
[B] SD: standard deviation.

Table 3
Concentrations of Zn (DW) in *C. davidii* from field observations.

Samples		Zn in *C. Davidii* (mg g^{-1})			Zn in rhizosphere soils (mg g^{-1})		BF (with respect to the total Zn)			BF (with respect to DTPA-extractable Zn)		
		Root	Stem	Leaf	Total	DTPA	Root	Stem	Leaf	Root	Stem	Leaf
Smelting area (n = 12) [A]	Range	1.14–3.46	1.22–11.2	3.30–14.0	12.0–26.7	0.31–0.70	0.06–0.20	0.07–0.79	0.19–0.94	2.58–6.50	4.00–17.7	10.8–23.2
	Mean ± SD [B]	2.25 ± 0.70	6.64 ± 0.38	9.45 ± 3.81	19.1 ± 5.89	0.52 ± 0.14	0.13 ± 0.04	0.36 ± 0.21	0.53 ± 0.24	4.43 ± 1.15	12.1 ± 4.91	17.7 ± 3.70
Contaminated area (n = 8)	Range	0.99–2.43	1.91–6.53	3.01–11.0	0.54–3.55	0.04–0.26	0.60–2.52	0.69–4.32	0.92–5.77	5.85–34.6	13.9–50.2	18.7–82.3
	Mean ± SD	1.70 ± 0.47	3.55 ± 1.68	5.81 ± 3.36	2.09 ± 1.16	0.16 ± 0.08	1.20 ± 0.84	2.19 ± 1.20	3.44 ± 1.73	13.9 ± 9.11	25.7 ± 11.4	41.0 ± 20.2
Background areas (n = 5)	Range	0.56–0.79	0.97–2.02	1.45–2.87	0.10–0.15	0.02–0.03	4.27–7.63	8.62–13.3	13.0–21.7	18.2–43.7	60.3–93.2	87.2–141
	Mean ± SD	0.68 ± 0.08	1.48 ± 0.40	2.20 ± 0.57	0.12 ± 0.02	0.02 ± 0.01	5.64 ± 1.38	11.9 ± 2.68	17.7 ± 3.40	33.9 ± 10.3	69.7 ± 13.7	104 ± 22.3

[A] n: Number of samples.
[B] SD: standard deviation.

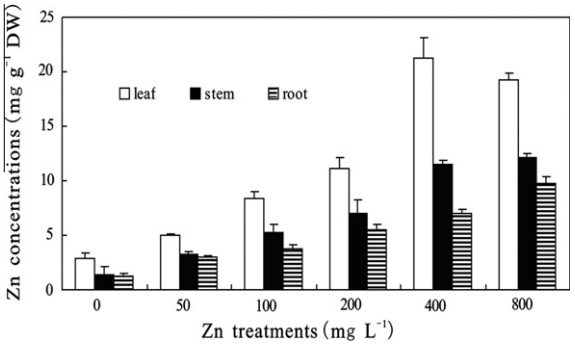

Fig. 1. Accumulation of Zn in various parts of *C. davidii* in hydroponic culture experiment.

to be an appropriate Zn-hyperaccumulator that is suitable for harvesting to phytoextract Zn from the SL and CS.

3.3. Zn accumulation and physiological response of *C. davidii* from hydroponic culture observations

Based on the hydroponic culture experiments, Zn accumulations in roots, stems and leaves of *C. davidii* in all treatments showed a distribution of leaf > stem > root (Fig. 1), in accordance with those from the field observations. The accumulated contents of Zn in roots and stems of *C. davidii* increased with the elevated Zn concentrations in all the treatments (Fig. 1). Among the treatments, Zn contents in leaves reached to the maximum at the treatment of 400 mg Zn L^{-1}, i.e. 21.3 and 12.1 mg g^{-1} in leaves and stems, respectively.

There was no significant difference for biomass in leaf, stem and root between the different treatments (Table 4). *C. davidii* has high tolerance to Zn as it can grow normally at 800 mg Zn L^{-1}. The concentrations of SOD, CAT, POD and MDA in *C. davidii* increased significantly ($p < 0.05$) starting at the spiked Zn concentration of 200, 400, 800 and 200 mg L^{-1}, respectively, compared with the control. There was no significant difference in SP concentrations between different treatments.

3.4. Estimation on Zn elimination from soil culture experiment

Based on the soil culture experiments, the aboveground parts of *C. davidii* (a total of 150 plants transplanted in the three plots of each substrate, see Section 2.3.) were harvested in May, August and November. In each harvest, the Zn concentration and the plant biomass were measured. Combined with the parameters of the soil bulk density of each plot and the depth of rhizospheric soil, the ability of phytoextraction for Zn was estimated.

In this study, the soil bulk density in the substrates of SL, CS and BS was measured at 1.51, 1.14 and 1.18 g cm^{-3}, respectively (Table 1). The depth of rhizospheric soil was observed at 0.15 m, and referred to the depth of plough layer of Zn-polluted substrates for phytoextraction. Assuming that Zn-phytoextraction follows a linear pattern, the eliminated amount of Zn per square meter per year in top soil layer (D_{Zn}: g Zn kg^{-1} yr^{-1}) can be quantitatively estimated by using the following equation:

$$D_{Zn} = C_{Zn} \times W \times 10^{-3}/(BD \times 0.15)$$

where C_{Zn} are Zn concentrations in the harvested aboveground parts based on DW (mg Zn kg^{-1}); W, DW of the harvested parts (kg m^{-2}); BD, bulk density (g cm^{-3}).

In this study, the amounts of phytoextracted Zn in the plough layers of substrates of SL, CS and BS were estimated at 6.03, 3.62, and 1.80 g m^{-2} yr^{-1}, respectively (Table 5). With respect to the

Table 4
Physiological response of *C. davidii* in various Zn treatments.

Treatments ($mg\ L^{-1}$)	SP ($mg\ g^{-1}$, FW)[A]	SOD ($U\ g^{-1}$, FW)	CAT ($mg\ kg^{-1}\ min^{-1}$, FW)	POD ($U\ g^{-1}$, FW)	MDA ($nmol\ g^{-1}$, FW)	Root (g DW)[B]	Stem (g DW)	Leaf (g DW)
0 ($n = 4$)[C]	6.9 ± 0.2a[D]	15.5 ± 0.8a	62 ± 6a	0.62 ± 0.12a	32.8 ± 7.1a	0.23 ± 0.02a	0.35 ± 0.04a	0.22 ± 0.03a
50 ($n = 4$)	6.9 ± 1.0a	16.3 ± 0.9a	57 ± 4a	0.68 ± 0.24ab	34.5 ± 1.1a	0.24 ± 0.04a	0.37 ± 0.06a	0.24 ± 0.04a
100 ($n = 4$)	6.7 ± 1.5a	16.4 ± 0.8a	64 ± 7a	0.83 ± 0.06abc	38.0 ± 3.0ab	0.23 ± 0.03a	0.38 ± 0.04a	0.23 ± 0.03a
200 ($n = 4$)	6.7 ± 1.2a	18.0 ± 0.6b	65 ± 21ab	0.78 ± 0.03abc	43.9 ± 3.5bc	0.24 ± 0.05a	0.36 ± 0.07a	0.23 ± 0.04a
400 ($n = 4$)	6.3 ± 0.1a	17.9 ± 0.4b	72 ± 11ab	0.85 ± 0.05bc	44.3 ± 4.9bc	0.23 ± 0.04a	0.35 ± 0.04a	0.24 ± 0.03a
800 ($n = 4$)	6.6 ± 0.4a	17.9 ± 0.4b	85 ± 11b	0.90 ± 0.08c	46.8 ± 3.4c	0.26 ± 0.04a	0.38 ± 0.02a	0.24 ± 0.04a

[A] FW: fresh weight.
[B] DW: dry weight.
[C] n: Number of samples.
[D] a, b and c refer to significant differences at $p < 0.05$ according to the LSD test in the same column.

Table 5
Phytoextraction of Zn in the aboveground parts of *C. davidii* from the soil culture experiments.

Substrate cultures	May		August		November		Phytoextracted Zn ($g\ m^{-2}\ yr^{-1}$)	Zn concentration decreased in top soils ($mg\ kg^{-1}\ yr^{-1}$)
	Zn ($mg\ kg^{-1}$)	Biomass ($kg\ m^{-2}$ DW)	Zn ($mg\ kg^{-1}$)	Biomass ($kg\ m^{-2}$ DW)	Zn ($mg\ kg^{-1}$)	Biomass ($kg\ m^{-2}$ DW)		
Plot of smelting slag ($n = 3$)[A]	5895 ± 463c[B]	0.41 ± 0.09a	5542 ± 352c	0.33 ± 0.08a	6523 ± 412c	0.27 ± 0.08a	6.03 ± 0.67c	26.6 ± 3.9b
Plot of contaminated soil ($n = 3$)	3635 ± 563b	0.40 ± 0.08a	3245 ± 213b	0.33 ± 0.07a	3875 ± 265b	0.28 ± 0.07a	3.62 ± 0.52b	21.2 ± 2.4c
Plot of background soil ($n = 3$)	1863 ± 234a	0.42 ± 0.11a	1632 ± 185a	0.34 ± 0.08a	1613 ± 201a	0.29 ± 0.07a	1.80 ± 0.52a	10.2 ± 1.8a

[A] n: Number of substrate culture plots.
[B] a, b and c refer to significant differences at $p < 0.05$ according to the LSD test in the same column.

Zn contents in substrates before phytoextraction, Zn concentrations in substrates of SL, CS, and BS were estimated to decrease by 26.6, 21.2, and 10.2 $mg\ kg^{-1}\ yr^{-1}$ by phytoextraction of *C. davidii*, respectively (Table 5).

4. Discussions

In literature, a so-called metal hyperaccumulator should meet with the following four criterions: (1) accumulating capability, i.e. the threshold values of metal concentrations in plants have been used to define metal hyperaccumulators including 10 $mg\ g^{-1}$ (DW) in shoots for Zn (Baker and Brooks, 1989; Salt et al., 1995), (2) BF index, i.e. the ratio of metal concentration in plants to that in soil is greater than 1.0 (Brooks et al., 1998), (3) translocation factor (TF) index, i.e. the ratio of metal concentration in shoots to that in roots is greater than 1.0 (Chaney et al., 1997; Vogel-Mikus et al., 2005), and (4) tolerance capability, i.e. hyperaccumulator has high tolerance capability to heavy metals, even does not show visible toxic symptoms under a certain concentration (Sun et al., 2009).

In this study, Zn concentrations in leaf and stem of *C. davidii* were observed over the threshold value (10 $mg\ g^{-1}$ DW) of Zn hyperaccumulator from both field survey (Table 3) and hydroponic culture (Fig. 1). BF indexes with respect to DTPA-extractable Zn in soils were all much higher than 1. BF indexes with respect to total zinc were higher than 1 in the smelting site with low Zn concentration, while lower than 1 in the contaminated site with high Zn concentration (Table 3). However, the criterion of BF > 1 might not necessarily be required to account for hyperaccumulation (Wang et al., 2009), as the total concentrations of metals in soils may not reflect the ions available for plant uptake or for immediate contamination (Branquinho et al., 2007). Instead, the portion of DTPA-extractable metal was commonly used to estimate heavy metal phytoavailability (Khan and Jones, 2009), and the BF indexes with respect to the available metals were more appropriate to identify hyperaccumulator (Branquinho et al., 2007). In this study, the BF indexes in leaves and stems of *C. davidii* with respect to DTPA-extractable Zn were much higher than 1 (Table 3), and implied that the species of *C. davidii* highly accumulates Zn that exists in the available fractions in soils.

In this study, Zn accumulation in *C. davidii* was leaf > stem > root from both field survey (Table 3) and hydroponic culture (Fig. 1), with clearly higher TF indexes than 1. The higher TF indexes indicated that *C. davidii* could not only absorb Zn from soil and water but can also effectively translocate Zn from roots to stems and leaves. Thus, *C. davidii* is an appropriate plant to phytoextract Zn from soils through harvesting the aboveground parts of stems and leaves.

As a hyperaccumulator, the plant should have high tolerant capability and exhibit a variety of responses to mechanical stresses that enable it to tolerate and evolve resistance to adverse conditions that are toxic to most other plants (Sun et al., 2009). In this study, no significant difference in biomass of *C. davidii* under the soil and hydroponic cultures was observed, indicating that *C. davidii* has high tolerance to Zn stress. The oxidative stress in plants was induced by high concentrations of heavy metals by generating O_2^-, O_2, H_2O_2 and OH. (Sun et al., 2007). The oxidative stress was prevented by antioxidant enzymes such as SOD, POD and CAT. SOD is one of the stress-resistant enzymes and can catalyze the disproportionation of two O_2^- radicals to H_2O_2 and O_2 (Srivastava et al., 2005). H_2O_2 is also toxic to plant cells, which can be removed by CAT and POD (Yu and Gu, 2007; Asthir et al., 2009). Therefore, the combination of SOD, CAT and POD plays an important role in the resistance of a plant to environmental stress. Increase of antioxidant enzymes activity usually meant oxidative stress. In this study, the antioxidant enzymes activity in *C. davidii* increased significantly at higher Zn concentration ($\geqslant 200\ mg\ L^{-1}$) compared with the control, suggesting that *C. davidii* was exposed to oxidative stress. The result showed that Zn-induced oxidative stress occurred in the hyperaccumulator tissues even though the growth

was unaffected by Zn stress, similar to the previous findings of Boominathan and Doran (2003) and Sun et al. (2007). It was suggested that *C. davidii* was exposed to oxidative stress at high Zn concentration ($\geqslant$200 mg L^{-1}), while oxidative enzymes activity enhanced and effectively scavenged active oxygen species. Therefore the growth of *C. davidii* was unaffected even though it was exposed to high concentrations of Zn.

In summary, the plant species of *C. davidii* was a newly identified Zn-hyperaccumulator for the first time, based on the field survey and the hydroponic and soil culture experiments. *C. davidii* has high biomass with the majority focusing on the aboveground parts (stems and leaves), and also has high ability to phytoextract Zn from soils with high values of both BF and TF. *C. davidii* accumulates the majority of Zn in the aboveground parts of stems and leaves without any obvious toxication symptoms, Thus, *C. davidii* is an appropriate plant to phyoextract Zn from soils through harvesting the aboveground parts of stems and leaves. Based on the cultivation experiments, *C. davidii* may reduce Zn concentration by 26.6, 21.2, and 10.2 mg kg^{-1} yr^{-1} by phytoextraction from SL, CS and BS, respectively.

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