

## Research Article

# Growth Responses and Metal Accumulation in an Ornamental Plant (*Osmanthus fragrans* var. *thunbergii*) Submitted to Different Cd Levels

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Received 24 February 2011; Accepted 7 April 2011

Academic Editors: A. Chappelka and S. Loppi

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To characterize the effects of Cd supplies on the accumulation efficiency of Cd, Pb, Zn, and Cu in an ornamental plant (*osmanthus*, *Osmanthus fragrans* var. *thunbergii*), a pot experiment using current-year *osmanthus* in field was carried out in western China. Biomass and its components showed a decreasing tendency as Cd supply increased, though insignificant differences were observed between treatments with a low and no Cd supply. Cd supplies increased the concentrations of Cd and Pb in plants, but the concentrations of Zn and Cu in plant showed a decreasing tendency with the increase of Cd supplies. Cd supplies also increased Cd accumulation for the plant, although the highest Cd accumulation was observed at a low Cd supply (T1). A higher Pb accumulation in the *osmanthus* was also detected in T1 than the other treatments. However, Zn and Cu accumulations decreased with the increase of Cd supplies. The examined *osmanthus* showed relative higher Cd and Pb transfer efficiencies in the presence of Cd supplies, but Cd supplies can limit the transfer of Zn and Cu. The results suggested that the examined *osmanthus* has potential for use in metal-contaminated environments due to phytoremediation application in the soil.

## 1. Introduction

One of the main environmental problems in many developing countries is the increasing pollution by toxic metals related to increased industrial activity and the heavy use of chemical fertilizer, pesticides, and herbicides in agriculture [1–3]. Bioaccumulation of toxic metals in humans can result in several harmful symptoms in gastrointestinal, neurological, and immunological systems [4, 5]. The most dangerous metals include the so-called “toxic trio”: Cd, Pb, and Hg, for which no biological function has been found [6–8]. Besides them, there is a long list of other metals which although essential in low doses, become toxic in high doses, such as Zn, Cu, and Mn [5].

Many soils are receiving lots of toxic metals with high pollution, but many others only display pollution with one or two metal. Compared with other toxic metals, Cd is not an essential nutrient in higher plants, and the exposure to relatively low concentrations results in high toxicity to plants and animals [9]. Unfortunately, Cd is the most common

toxic metal in many areas worldwide. Since Cd stress often decreases plant growth [10], exposure to Cd can decrease accumulation of other toxic metals (such as Pb, Zn, and Cu) in plants. Moreover, the study on tobacco found that there were negative correlations as Cd accumulation increased, Zn accumulation decreased, implying that some heavy metals were antagonistic [11]. However, a few studies have found that a low concentration of Cd can stimulate plant growth [12], consequently increasing the accumulation of other metals. As yet, it is not only the presence of various toxic metals that might enlarge the toxicity of metals, but also the influence that one heavy metal can have in facilitating or limiting the accumulation of the others [13]. The lack of a mean for removing these complicated toxic metals from soil is the crux of the current problem.

A variety of the engineering and biological technologies have been developed to remedy the contaminated ecosystems [10, 14, 15]. Phytoextraction, the use of plants to extract, sequester, and/or detoxify hazardous heavy metals from different media (soil, water, and air), is regarded as

a practical and affordable alternative for remediation of polluted sites [16–18]. According to previous studies, there are two different ways in phytoextraction. One is the use of a hyperaccumulator that accumulates toxic metals in plant organs with high biomass production such as poplar and willow [19–21]; the other is the use of a phytoextractor that concentrates toxic metals in the organisms with high metal concentration such as *Lonicera japonica* [22]. However, the problem lies in harvesting and disposal of the metal-contaminated biomass. If animals consume the contaminated biomass, biomagnification occurs on each level of the trophic chain and can pose harm to humans as the final consumers in using both remediation methods [4]. Many ornamental wood-plants can tolerate the environment with high toxic metal concentration and long growth time [13], showing relatively high growth characteristics and metal accumulation. Additionally, the ornamental wood-plants are not part of the human food chain, but they can extract several toxic metals from soil at the same time, resulting in particular economic and ecological values for polluted environment. Accordingly, the use of ornamental wood-plants could provide an improved method for remedying polluted environment.

*Osmanthus* (*Osmanthus fragrans* var. *thunbergii*) is a valuable ornamental wood-plant popular in urban gardens and landscaping in many cadmium-contaminated regions of the Yangtze River Basin. With the expansion of industry and agriculture, increasing contamination of Cd, Pb, Zn, and Cu has limited the economic and social development in this region [23, 24]. Little information has been available on the adaptation of osmanthus to Cd contamination, and far less has focused on the effects of Cd contamination on the accumulation of other metals, such as Pb, Zn, and Cu. Therefore, it is hypothesized that Cd contamination could limit the growth of osmanthus, and subsequently decrease accumulation of other metals. The objectives of this study were (1) to characterize the effects of different cadmium levels on the growth of osmanthus and (2) to understand the accumulation characteristics of Cd, Pb, Zn, and Cu in osmanthus under different levels of cadmium stress.

## 2. Material and Methods

**2.1. Field Site and Soil Characterization.** The field pot-culture experiment was located at the State Key Laboratory of Forestry Eco-engineering in Sichuan Agricultural University (102°59' E, 29°58' N, a.s.l. 620 m). The site is subtropical with a warm and moist climate, 16°C average annual temperature, 1732 mm average annual precipitation, 838 mm average transpiration, and 294 frostless days per year [25]. Samples of purple soil (dystric purple-udic cambisols) were collected from the surface (0–20 cm) in a field near the university and near the Qingyi River, respectively. The sampled soil characteristics were measured with 4.85 for pH, 20.03 g kg<sup>-1</sup> for organic carbon, 1.28 g kg<sup>-1</sup> for total N, 0.45 g kg<sup>-1</sup> for total P, 3.05 g kg<sup>-1</sup> for total K, 2.95 mg kg<sup>-1</sup> for Cd, 54.91 mg kg<sup>-1</sup> for Pb, 123.52 mg kg<sup>-1</sup> for Zn, and 29.51 mg kg<sup>-1</sup> for Cu.

**2.2. Experimental Design.** 25 kg soil samples were air dried and sieved by a 4-mm plastic sieve, and then placed into porcelain pots (25 cm height and 36 cm in diameter) after mixing with a Cd solution. In order to analyze the tolerance ability and growth adaptation of osmanthus to Cd contamination, five levels of Cd (CK: 0, T1: 25, T2: 50, T3: 100, and T4: 200 mg Cd kg<sup>-1</sup> dry soil) as CdCl<sub>2</sub>·2·5H<sub>2</sub>O solution was applied to the pots. Meanwhile, 6 g urea and 3 g KH<sub>2</sub>PO<sub>4</sub> were applied to each pot to avoid nutrient limitation. Current year osmanthus with around 12 cm height and 1.80 mm basal diameter from a noncontaminated nursery in the Key Laboratory of Forestry Eco-engineering in Sichuan Agricultural University were transferred directly to the porcelain pots on March 3, 2008. Five similar osmanthus in the same living condition were used to determine the average dry mass of the potted plants. The plants were grown outdoors to simulate field conditions. Each treatment was arranged in five replicates. The experiment was terminated at the end of the growing period on November 28, 2008. The total growth time was 270 days.

**2.3. Measurements and Calculations.** Fallen leaves were collected every month during the experiment. Biomass was determined after being oven dried at 70°C for at least 36 h. The plants were harvested in each treatment when the experiment was terminated. The harvested plants were rinsed with tap water, and the roots were immersed in 20 mM Na<sub>2</sub>-EDTA for 15 min to remove cadmium that adhered to the root surface [22, 26], and then the whole plant was rinsed with deionized water. The leaves, roots, and shoots were divided and dried in an oven for at least 48 h at 70°C to constant weight for biomass determination. Initial average dry mass of the plants was subtracted from final dry mass for biomass determination. Total plant biomass was the sum of the litter, leaf, root and shoot. The oven-dried samples were ground finely by a porcelain mortar for metal analysis.

The powder of samples were digested with a concentrated acid mixture of HNO<sub>3</sub>–HClO<sub>4</sub> (3:1, v/v) and heated at 160°C for 5 h. After cooling, the extract was diluted, filtered, and made up to 25 mL with 5% HNO<sub>3</sub> [27]. The concentrations of Cd, Pb, Zn, and Cu in the extract were determined by Inductive Coupled Plasma Atomic Emission Spectroscopy (ICP-AES IRIS IntrepidXSP; Thermo Electron Company, USA). The analysis was carried out in triplicate.

The bioaccumulation coefficient (BC) or enrichment factor was described by Liu et al. [22] and Tanhan et al. [28]: BC = the metal concentration in the whole plant/the metal concentration in the soil.

The translocation factor (TF) indicated the ability of plants to translocate cadmium from the roots to the shoots [29]. TF from metal concentration was calculated according to Liu et al. [22]: TF = the metal concentration in shoots/the metal concentration in roots; TF from metal accumulation (TF') = the metal accumulation in shoots/the metal accumulation in roots.

The tolerance index (Ti) was calculated to measure the ability of the plant to grow in the presence of a given concentration of metal [3, 30], calculated as Ti = dry weight

TABLE 1: Biomass and its components (means  $\pm$  SD,  $n = 5$ ) of *O. fragrans* var. *thunbergii* under different Cd supplies treatments.

	Leaf biomass (g)	Stem biomass (g)	Root biomass (g)	Litter biomass (g)	Total (g)	R/S
CK	17.96 $\pm$ 1.46a	25.38 $\pm$ 3.99a	20.72 $\pm$ 4.06a	1.61 $\pm$ 0.60a	65.67 $\pm$ 4.58a	0.82 $\pm$ 0.08a
	27.35%	38.65%	31.55%	2.45%	100%	
T1	14.82 $\pm$ 2.08a	22.28 $\pm$ 4.44ab	20.65 $\pm$ 3.75a	1.28 $\pm$ 0.44a	59.03 $\pm$ 5.12a	0.93 $\pm$ 0.05b
	25.11%	37.74%	34.98%	2.17%	100%	
T2	5.62 $\pm$ 2.97b	5.83 $\pm$ 1.53bc	6.05 $\pm$ 1.79b	1.27 $\pm$ 0.54a	18.77 $\pm$ 3.43b	1.04 $\pm$ 0.09b
	29.94%	31.06%	32.23%	6.77%	100%	
T3	4.70 $\pm$ 2.10b	5.53 $\pm$ 1.76bc	5.60 $\pm$ 0.96b	1.26 $\pm$ 0.31a	17.09 $\pm$ 3.54b	1.01 $\pm$ 0.12b
	27.50%	32.36%	32.77%	7.37%	100%	
T4	3.61 $\pm$ 1.73b	2.52 $\pm$ 0.52c	2.55 $\pm$ 1.11c	1.25 $\pm$ 0.47a	9.93 $\pm$ 2.18c	1.01 $\pm$ 0.11b
	36.35%	25.38%	25.68%	12.59%	100%	

Different letters within a column indicate the significant differences among the treatments ( $P < .05$ ).

of the plants growing in cadmium supplies/dry weight of the plants growing in control.

**2.4. Statistical Analysis.** The results reported are average values. Standard deviation and standard error of the mean were also calculated. Differences between treatments were tested by ANOVA followed by a LSD test after a check of normal data distribution. All statistical analysis was conducted using the SPSS (Standard released version 11.5 for Windows, SPSS Inc., IL, USA) software package.

### 3. Results

**3.1. Biomass Production.** Plant biomass and its components showed a decreasing tendency with the increase of Cd supplies, but which were not significantly ( $P > .05$ ) influenced at low levels of Cd supplies (T1; Table 1). The total biomass decreased to about 72.5%, 74.2%, and 80.8% in T2, T3, and T4 compared with CK, respectively. Few differences in litter biomass were observed among the treatments with different amounts of Cd supplies, but the proportion of litter biomass to total increased with the increase of Cd supplies. The proportion of leaf biomass was higher in T4, but stem and root occupied larger proportions in other treatments. In addition, Cd supplies significantly increased the root-to-stem ratio (R/S).

**3.2. Metal Concentration.** Cd concentration in plant organs increased with the increase of Cd supplies, especially in leaf and stem (Figure 1). Cd concentration in root was not significantly different among T1, T2, T3, and T4. Compared with other components, stem and leaf showed higher Cd concentration in treatment with higher Cd supplies. Cd supplies increased Pb concentration in stem, but there were few differences among the treatments with differing Cd supplies. Stem and litter had higher Pb concentration regardless of Cd treatments compared with other components. In contrast, Zn and Cu concentration in plant organs showed a decreasing tendency with the increase of Cd supplies, except for an insignificant difference between T1 and CK.

Zn and Cu concentrations in stem were not significantly different among the treatments. Root had higher Zn and Cu concentration compared with other components.

**3.3. Cd, Pb, Zn, and Cu Accumulation.** In comparison with other Cd supplies, T1 showed higher Cd accumulation in the total plant and its components except for litter, but T2 showed lower Cd accumulations (Table 2). Litter Cd accumulation increased with the increase of Cd supplies. T1 also displayed higher Pb accumulation in leaf, stem, and total plant than the other treatments. The other three Cd supply levels (T2, T3, and T4) decreased Pb accumulation in leaf, stem, and the total plant, though T3 and T4 increased Pb accumulation in root and litter. Zn and Cu accumulations in leaf, stem, and the total plant decreased with the increase of Cd supply levels, but Zn and Cu accumulations in root were insignificantly different between CK and T1.

Cd supplies increased the proportion of Cd accumulation in leaf to the total plant but decreased the proportion in root (Figure 2). Pb accumulation in stem represented a majority of the accumulation in plant, though T4 increased the proportion of Pb accumulation in litter and root to the total plant and decreased the proportion in stem. The proportion of Zn accumulation in leaf increased with the increase of Cd supplies but decreased the proportion in roots. Cu accumulation in root represented a majority of the accumulation in the plant. Compared with the other treatments, T4 increased the proportion of Cu accumulation in litter, but decreased Cu accumulation in root.

Additionally, BC of Cd in plant decreased with the increase of Cd supplies, but BC of Pb increased (Table 3). Compared with CK, T1 had few effects on BC of Zn and Cu, both of which displayed a decreasing tendency with the increase of Cd supplies in other treatments. There were insignificant differences in Tf of Cd between T1 and CK, but Tf of Cd increased with the increase of other Cd supplies. T1 and T2 increased the Tf of Pb, but T3 and T4 decreased it. Tf of Zn and Cu increased with the increase of Cd supplies. There were few differences in Tf of Cd among CK, T2, T3,

TABLE 2: The accumulation of Cd, Pb, Zn, and Cu (Means  $\pm$  SD,  $n = 5$ ) of *O. fragrans* var. *thunbergii* under different Cd supplies treatments.

		Leaf ( $\mu\text{g}$ )	Stem ( $\mu\text{g}$ )	Root ( $\mu\text{g}$ )	Litter ( $\mu\text{g}$ )	Total ( $\mu\text{g}$ )
Cd	CK	15.10 $\pm$ 2.52	73.52 $\pm$ 16.86	50.18 $\pm$ 9.54	1.13 $\pm$ 0.32	139.93 $\pm$ 28.55
	T1	267.17 $\pm$ 18.40	415.32 $\pm$ 171.01	384.60 $\pm$ 68.71	1.54 $\pm$ 0.49	1068.63 $\pm$ 218.21
	T2	130.31 $\pm$ 45.71	234.49 $\pm$ 103.86	162.07 $\pm$ 46.97	6.30 $\pm$ 1.25	533.17 $\pm$ 169.76
	T3	262.82 $\pm$ 113.29	271.26 $\pm$ 106.70	189.23 $\pm$ 82.67	8.72 $\pm$ 3.23	732.03 $\pm$ 303.44
	T4	231.89 $\pm$ 92.64	236.19 $\pm$ 139.83	168.18 $\pm$ 89.85	11.93 $\pm$ 4.11	648.19 $\pm$ 314.43
Pb	CK	7.84 $\pm$ 1.52	42.90 $\pm$ 14.84	5.94 $\pm$ 1.39	1.83 $\pm$ 0.45	58.51 $\pm$ 16.75
	T1	14.66 $\pm$ 1.13	77.16 $\pm$ 43.64	11.26 $\pm$ 3.61	1.22 $\pm$ 0.44	104.31 $\pm$ 46.38
	T2	4.40 $\pm$ 2.09	25.23 $\pm$ 11.88	3.73 $\pm$ 0.66	1.76 $\pm$ 0.59	35.11 $\pm$ 13.62
	T3	5.96 $\pm$ 2.78	29.46 $\pm$ 21.20	6.80 $\pm$ 4.68	3.11 $\pm$ 1.22	45.34 $\pm$ 29.51
	T4	4.46 $\pm$ 1.84	13.19 $\pm$ 7.89	11.93 $\pm$ 6.78	3.36 $\pm$ 0.99	32.95 $\pm$ 15.48
Zn	CK	653.30 $\pm$ 121.58	480.55 $\pm$ 194.63	817.69 $\pm$ 199.47	48.46 $\pm$ 18.64	2000.00 $\pm$ 483.37
	T1	612.63 $\pm$ 56.66	387.52 $\pm$ 177.96	862.69 $\pm$ 244.22	63.61 $\pm$ 16.08	1926.44 $\pm$ 439.41
	T2	123.47 $\pm$ 54.24	104.25 $\pm$ 53.36	192.83 $\pm$ 34.63	32.00 $\pm$ 11.29	452.56 $\pm$ 119.87
	T3	111.97 $\pm$ 60.01	101.48 $\pm$ 69.52	203.90 $\pm$ 103.99	26.87 $\pm$ 15.09	444.22 $\pm$ 244.16
	T4	51.05 $\pm$ 27.55	49.81 $\pm$ 30.92	139.99 $\pm$ 97.52	18.25 $\pm$ 9.86	259.10 $\pm$ 148.78
Cu	CK	120.45 $\pm$ 22.64	250.74 $\pm$ 102.30	749.71 $\pm$ 182.63	12.35 $\pm$ 4.61	1133.24 $\pm$ 294.19
	T1	104.10 $\pm$ 13.91	202.95 $\pm$ 80.54	769.73 $\pm$ 218.17	8.15 $\pm$ 3.62	1084.93 $\pm$ 283.74
	T2	23.92 $\pm$ 9.52	54.26 $\pm$ 28.27	174.90 $\pm$ 29.50	8.44 $\pm$ 2.42	261.52 $\pm$ 60.21
	T3	18.54 $\pm$ 6.30	52.52 $\pm$ 28.57	175.47 $\pm$ 114.84	8.21 $\pm$ 4.65	254.74 $\pm$ 153.63
	T4	5.29 $\pm$ 2.99	21.21 $\pm$ 13.63	37.95 $\pm$ 26.03	5.49 $\pm$ $\pm$ 1.75	69.95 $\pm$ 41.78

TABLE 3: Bioaccumulation coefficient (BC), transport factor from concentration (Tf) and from accumulation (Tf'), and tolerance index (Ti) of (Means  $\pm$  SD,  $n = 5$ ) of *O. fragrans* var. *thunbergii* under different Cd supplies treatments.

		BC	Tf	Tf'	Ti
Cd	CK	0.77 $\pm$ 0.05	1.20 $\pm$ 0.06	1.47 $\pm$ 0.04	1.00
	T1	0.66 $\pm$ 0.04	1.19 $\pm$ 0.43	1.08 $\pm$ 0.09	0.86 $\pm$ 0.05
	T2	0.60 $\pm$ 0.13	1.40 $\pm$ 0.23	1.45 $\pm$ 0.23	0.24 $\pm$ 0.06
	T3	0.37 $\pm$ 0.12	1.59 $\pm$ 0.31	1.43 $\pm$ 0.32	0.18 $\pm$ 0.03
	T4	0.27 $\pm$ 0.02	2.39 $\pm$ 0.13	1.40 $\pm$ 0.10	0.11 $\pm$ 0.03
Pb	CK	0.03 $\pm$ 0.01	5.68 $\pm$ 0.10	7.22 $\pm$ 0.63	—
	T1	0.05 $\pm$ 0.01	7.60 $\pm$ 0.66	6.85 $\pm$ 0.59	—
	T2	0.06 $\pm$ 0.00	6.66 $\pm$ 0.32	6.75 $\pm$ 0.15	—
	T3	0.07 $\pm$ 0.02	4.68 $\pm$ 0.42	4.34 $\pm$ 0.18	—
	T4	0.08 $\pm$ 0.01	1.87 $\pm$ 0.16	1.11 $\pm$ 0.04	—
Zn	CK	0.26 $\pm$ 0.00	0.45 $\pm$ 0.00	0.59 $\pm$ 0.04	—
	T1	0.27 $\pm$ 0.00	0.51 $\pm$ 0.00	0.45 $\pm$ 0.05	—
	T2	0.22 $\pm$ 0.01	0.52 $\pm$ 0.00	0.54 $\pm$ 0.03	—
	T3	0.19 $\pm$ 0.01	0.51 $\pm$ 0.01	0.50 $\pm$ 0.04	—
	T4	0.18 $\pm$ 0.02	0.64 $\pm$ 0.01	0.36 $\pm$ 0.01	—
Cu	CK	0.62 $\pm$ 0.01	0.26 $\pm$ 0.02	0.33 $\pm$ 0.08	—
	T1	0.63 $\pm$ 0.24	0.30 $\pm$ 0.08	0.26 $\pm$ 0.07	—
	T2	0.52 $\pm$ 0.09	0.31 $\pm$ 0.09	0.31 $\pm$ 0.08	—
	T3	0.45 $\pm$ 0.04	0.34 $\pm$ 0.02	0.30 $\pm$ 0.02	—
	T4	0.20 $\pm$ 0.08	1.00 $\pm$ 0.23	0.56 $\pm$ 0.05	—

and T4, but Tf' of Cd decreased in T1. Tf' of Pb and Zn decreased with the increase of Cd supplies. Tf' of Cu was higher in T4, although it showed few differences in the other four treatments. Ti of Cd decreased with the increase of Cd supplies, but it reached 0.86 in T1.

#### 4. Discussion

The hypothesis that Cd supplies would decrease the growth of osmanthus and decrease the accumulation of other metals was only partly demonstrated in this study. Biomass

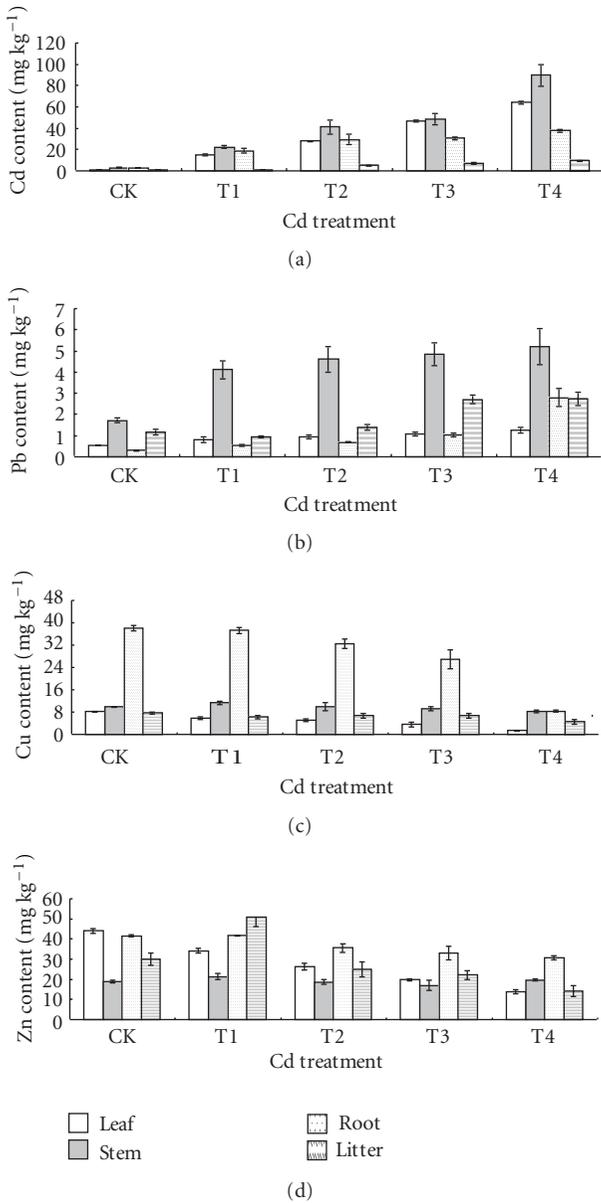


FIGURE 1: Cd, Pb, Zn, and Cu concentrations of *O. fragrans* var. *thunbergii* under different treatments with different Cd supplies. Bars indicate SD,  $n = 5$ .

and its components showed a decreasing tendency as Cd supply increased, but no significant differences were observed between the treatments with the low Cd supply (25 mg Cd kg<sup>-1</sup>) and no Cd supply. Furthermore, the low Cd supply (T1) clearly increased the accumulation of Cd and Pb, but the other Cd supplies decreased the accumulation of Pb, Zn, and Cu. This is in agreement with the observation from Liu et al. [13] that one heavy metal could facilitate the accumulation of another. However, this only occurred in the treatment with low Cd supply, the higher Cd contamination significantly decreased the accumulation of other metals because of the obvious decrease in the production of plant biomass.

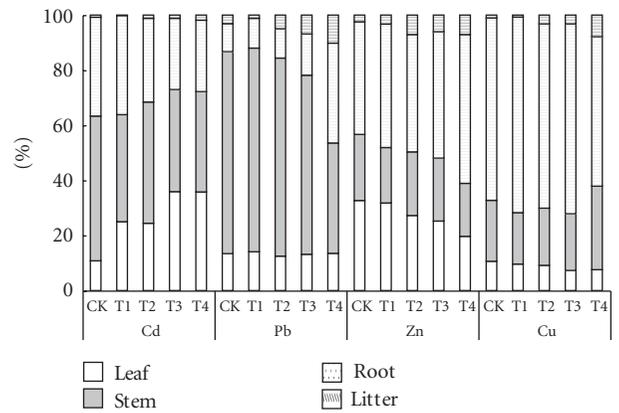


FIGURE 2: Cd, Pb, Zn, and Cu partitioning of *O. fragrans* var. *thunbergii* under different treatments with different Cd supplies.

Adaptive responses in biomass production and its distribution may be a primary mechanism by which the species can cope with the environmental characteristics of their respective habitats [31]. Since heavy metals often induce low availability of soil resources and limit the absorption ability of root, decreased biomass production has often been detected in habitats with heavy metal contamination [6, 16, 20]. The results here also support this theory, but the low Cd supply (T1) did not significantly decrease biomass production and its components, suggesting the examined osmanthus could adapt to the Cd-contaminated environment with a little harm. It is essential to note that Cd supplies significantly increased the ratio of *R/S*, which is the direct evidence of adaptation responses on the part of osmanthus to the Cd contaminated habitat. Additionally, leaf biomass represented a higher proportion of total plant biomass in the highest Cd supplies (T4), a finding which indicated a maximum carbon-fixation adaptation mechanism in respond to a heavy environmental factor stress. Wu et al. [31] have also observed similar phenomenon in the heavy drought stress environment.

Metal accumulation of plant was determined by biomass production and metal concentration in plant components. Although biomass production was decreased in response to Cd supplies, the concentration of examined metals in plant components did show inconsistent responses to different levels of Cd supplies. Cd and Pb concentration in the plant components showed an increasing tendency with the increase of Cd supplies. Many previous studies have observed that Cd concentration in plant components increased with the increase of Cd supplies except for excluder plants [16, 32], which suggested that the plant employs the accumulation mechanism [33]. Higher Pb concentration in stem was also observed in the treatment with Cd supplies compared with no Cd supply in this study, which is further support for the theory that one heavy metal could facilitate the accumulation of another [13]. Moreover, due to higher Pb concentration in shoot compared with that in root (Figure 1), osmanthus might have an efficient translocation ability that transferred Pb from root to shoot. In contrast to Cd and Pb, Zn and Cu

concentration in plant components displayed a decreasing tendency as Cd supplies increased. The observations here agree with the results on tobacco from Vasiliadou and Dordas [11], indicating that Cd contamination could limit the absorption of Zn and Cu, and could also affect the transfer efficiency from root to shoot due to relative higher root Zn and Cu concentration. Tf of Zn and Cu also showed a similar pattern (Table 3). Zn and Cu are essential micronutrients required for proper physiological and metabolic functioning of plants, and are only toxic at high concentrations [34]. Heavy Cd contamination might lead to a deficiency of Zn and Cu, which could be one of the main reasons for the limited growth of osmanthus in the Cd contaminated environment.

Low Cd supply treatment (T1) had insignificant effects on biomass production, but significantly increased Cd and Pb concentration in plant organs, osmanthus in T1 accumulated more Cd and Pb compared with that in other treatments. This suggested that metal phytoextraction using the examined osmanthus could potentially be applied to clean up soils moderately contaminated by Cd and Pb in this environment. Meanwhile, the results also showed that Cd supplies increased the proportion of leaf Cd accumulation, and the proportion of stem Pb accumulation that occupied the majority of total Pb accumulation, implying that Cd and Pb have relatively higher transfer ability in this examined osmanthus, and Cd toxicity is primarily harmful to leaves, which agreed with the previous studies [3, 20]. However, high Cd contamination level could decrease Pb accumulation and limited Pb transfer from root to shoot in osmanthus by limiting plant growth and stem Pb accumulation. As mentioned above, Cd supplies limited the transfer of Zn and Cu in osmanthus, so that the above-ground accumulation of Zn and Cu decreased with the increase of Cd supplies. Insignificant differences of root Zn and Cu accumulation between CK and the low Cd supply treatment could be related to the insignificant differences in root biomass.

Many previous studies have documented that four indicators (the threshold value of metal, BC, Tf, and Ti) could be used to determine the metal accumulation efficiency of plant [16, 17, 35]. The threshold value of metal was not examined here, only Ti of Cd was examined, the results from the BC, Tf, Tf' were inconsistent among Cd, Pb, Zn, and Cu in response to different Cd supplies. Even so, the synthesized analysis implied that osmanthus had an efficient transfer efficiency of Cd and Pb since both Tf and Tf' > 1.0, even Tf and Tf' of Pb > 4.0 in relatively low Cd supplies treatments (CK, T1, T2, and T3). Both Tf and Tf' of Pb were higher than those of Cd, Zn, and Cu, which could be explained by two different explanations. First, the appearance of Cd promoted the transfer of Pb from root to shoot since BC of Pb increased with the increase of Cd supplies. Second, the examined osmanthus could be a potential Pb hyperaccumulator with high Pb accumulation efficiency. These two issues warrant further study. In contrast, decreased Zn and Cu accumulation in osmanthus mainly resulted from Cd limitation, since both Tf and Tf' were far less than 1.0, and BC of Zn and Cu decreased with the increase of Cd supplies. Although osmanthus would not be a potential Cd hyperaccumulator in this environment

since BC < 0.8 and decreased with the increase of Cd concentration in the soil, osmanthus could grow well under the region with low Cd contamination (25 mg Cd kg<sup>-1</sup> dry soil), since Ti reached 0.86.

## 5. Conclusions

Cd supplies decreased biomass and its components in osmanthus, but there were no significant differences between the low Cd supply treatment (25 mg Cd kg<sup>-1</sup>) and no Cd supply. Low Cd supply also significantly increased the accumulation of Cd and Pb, although the other Cd supplies decreased the accumulation of Pb, Zn, and Cu. The examined osmanthus showed efficient Cd- and Pb-transfer efficiency in the presence of Cd supplies. Cd supplies could limit the absorption and transfer of Zn and Cu. Therefore, the osmanthus has potential for phytoremediation application in Cd and Pb contaminated soil, and the plant could grow well in the low Cd-contaminated soil. More work should be done to reveal the responses of metal accumulation and plant growth under low metal-contamination level.

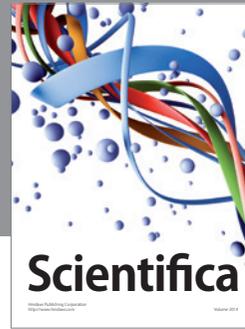
## Acknowledgments

The paper was financially supported by the programs of the Key Project of Public Welfare Research of Sichuan (no. 2007NGY006), Sichuan Provisional Key Technologies R & D, China (no. 2010SZ0051), and the Key Cultivation Programs of the Education Department of Sichuan Province (07ZZ024 and 09ZZ023).

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