Tolerant mechanisms of *Rorippa globosa* (Turcz.) Thell. hyperaccumulating Cd explored from root morphology

Shuhe Wei a,⇑, Yunmeng Li a,b,⇑, Jie Zhan c, Shanshan Wang a,b, Jiangong Zhu a,b

a Key Laboratory of Pollution Ecology and Environment Engineering, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, PR China

b Graduate School of Chinese Academy of Sciences, Beijing 100039, PR China

c College of Liaoning Professional Sanitation Technology, Shenyang 110101, PR China

**HIGHLIGHTS**

- Hyperaccumulative properties of *R. globosa* to Cd was further affirmed.
- *Rorippa palustris* (Leyss.) Bess. was a Cd non-hyperaccumulator.
- Cd tolerant mechanism of plant was relative with root morphology.

**ABSTRACT**

Hoagland solution was used to determine the root morphology properties of *Rorippa globosa* (Turcz.) Thell. and *Rorippa palustris* (Leyss.) Bess. Under the conditions of Cd spiked at 2.5 and 5 mg kg⁻¹, *R. globosa* showed all hyperaccumulative characteristics and was a Cd-hyperaccumulator. In contrast, *R. palustris* was a non-hyperaccumulator. The total root lengths, total root surface areas and total root volumes of *R. globosa* were not significantly decreased (*p* < 0.05) compared to the control when 2.5 and 5 mg kg⁻¹ of Cd added. However, these 3 indexes of *R. palustris* were all significantly decreased (*p* < 0.05) when 2.5, 5, 10, 20 and 40 mg kg⁻¹ Cd added compared its control. The average root diameters of *R. palustris* and *R. globosa* were not affected by Cd. These results showed that root morphology might be a factor of plant with strong tolerance to Cd.

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**1. Introduction**

Cd is one of heavy metals which are toxic not only to soil microflora, fauna and vegetation but also to human being through food chain. In environmental science, the remediation of soil contaminated with Cd is a challenging and difficult problem. Phytoextraction mainly using hyperaccumulator or accumulator is a newly developing and promising phytoremediation technology besides some traditional physical and chemical remediation methods. Till now, some hyperaccumulators have been comprehensively researched such as the Cd and Zn hyperaccumulator *Thlaspi caerulescens* (Xiao et al., 2010), the arsenic hyperaccumulator *Pteris vittata* (Ma et al., 2001; Mathews et al., 2010; Wan et al., 2010), Zn and Cd hyperaccumulator *Sedum alfredii* (Li et al., 2011), and Cd hyperaccumulator *Solanum nigrum* (Wei et al., 2010). However, phytoremediation technology using these hyperaccumulators has not been applied in a large scale to remove excessive heavy metals from contaminated soil partly due to limited remediation efficiencies. Thus, hyperaccumulator identification and relative hyperaccumulative mechanism exploration are still the key step of phytoextraction.

Usually, hyperaccumulator refers to some plants that can accumulate exceptionally high quantities of heavy metals in their stems or leaves. The main characteristics of hyperaccumulative plants can be summarized as follows: (1) accumulation property, i.e. the minimum concentration in the shoots of a hyperaccumulator for As, Pb, Cu, Ni, and Co should be greater than 1000 mg kg⁻¹ dry mass, and Zn and Mn 10,000 mg kg⁻¹, Au is 1 mg kg⁻¹, and Cd is 100 mg kg⁻¹, respectively (Baker and Brooks, 1989); (2) translocation property, elemental concentrations in the shoots of a plant should be higher than those in roots, i.e., TF > 1 (translocation factor, concentration ratio of shoots to roots) (Chaney et al., 1997; Ma et al., 2001); (3) enrichment property (enrichment factor-EF, concentration ratio of plant to media), EF value in shoots of plants should be higher than 1 (Wei et al., 2005); and (4) tolerance...
property, a hyperaccumulator should have high tolerance to heavy metal. As for the plants tested under experimental conditions, their shoot biomass should not decrease significantly when growing in contaminated media (Wei et al., 2005).

In our previous published papers, Rorippa globosa (Turcz.) Thell. showed some Cd hyperaccumulative characteristics compared to Taraxacum mongolicum and Conyza canadensis when tested 13 and 24 weed species tolerant and accumulative properties to Cd (Wei et al., 2008; Wei et al., 2009). But T. mongolicum and C. canadensis are belonging to different plant family (Asteraceae) with R. globosa, which may be not felicitous comparing them together. The aim of this experiment was to further affirm Cd hyperaccumulative characteristics of R. globosa compared to its same family (Cruciferae) plant Rorippa palustris (Leyss.) Bess. and to explore their tolerant mechanism from root morphologies under hydroponic conditions.

2. Methods

2.1. Plant seed collection and seedling culture

The seeds of R. globosa and R. palustris were collected from the field of Shenyang Ecological Experimental Station, Chinese Academy of Sciences (41°31′N and 123°41′E). All seeds were sterilized by 0.1% of NaClO. After that, same size seeds were sown to soil in seedling pot. The soil was also collected from the field in Shenyang Ecological Experimental Station with background Cd concentration at 0.16 mg kg⁻¹. After the seeds of R. globosa and R. palustris germinated (10 days) in a warm room, 10 cm height seedlings were randomly relocated in a temperature controlled glasshouse was aired continuously and renewed every week. All the pots were sampled for each replication) (Li et al., 2011).

2.2. Hydroponic experiment and plant culture

The experimental design for the pot experiment was made up of 6 Cd treatments for R. globosa and R. palustris. These were control (Cd 0) without Cd addition, and treatments T₁–T₅, with Cd (CdCl₂·2.5H₂O) added at 2.5, 5, 10, 20 and 40 mg kg⁻¹, respectively. Polyethylene pots were filled with 1.0 L Hoagland solution. pH of Hoagland solution was adjusted by 0.1 M HCl and NaOH to 6.8 and 10 μM of FeEDDHA and FeNaEDTA were used to keep pH approaching to 6.8 in the Hoagland solution. The pot solution was aired continuously and renewed every week. All the pots were random relocated in a temperature controlled glasshouse (20 ± 5 °C). A 14-h photoperiod with a daily photosynthetic photon flux of 350 mmol m⁻² s⁻¹ was supplied by cool-white fluorescent lamps. All treatments were replicated four times. The plants were harvested after 2 weeks of growth.

2.3. Sample analysis

A root automatism scan apparatus (MIN Mac, STD1600+) was used to determine root morphological parameters. WinRHIZOTM 2000 was used to recognize digital root images and analyzes root parameters (length, surface area, volume and diameter). WinRHIZOTM2000 software offered by Regent Instruments Inc. Roots was stained for 5 min in crystal violet (1 g per 100 ml water) at 50 °C. Root segments were then placed on STD1600+ with a transparent plastic tray filled with 0.01 M NaOH. A white plastic plate served as image background. Image record was performed at a resolution of 800 dpi and images were saved as TIFF (tagged image file format). Total root length, surface area, volume and average root diameter per plant were analyzed (roots of the 10 plants were analyzed for each replication) (Li et al., 2011).

Plant samples were separated into roots, stems and leaves, then rinsed with tap water and carefully washed with deionized water later. These samples were oven dried at 105 °C for 5 min, after that at 70 °C until completely dry (near 2 days). Near half of stems and leaves were mixed together to examine Cd concentration in shoots. The dried plant samples were ground to a powder and passed through a sieve. Plant samples were digested using a solution containing 87% of HNO₃ and 13% of HClO₄ to determine total metal concentration (Wei et al., 2009). Cd concentration was determined by using an atomic absorption spectrophotometer (AAS, Hitachi 1547, peach leaves) obtained from the National Institute of Standards and Technology (Gaithersburg, USA). The value of pH was determined using a pH meter (PHS-3B). The enhancement factor (EF) was calculated as the ratio of Cd concentration in plant to concentration of Cd in solution, and the translocation factor (TF) was calculated by the ratio of Cd concentration in shoots to the concentration of Cd in roots (Wei et al., 2009).

2.4. Data processing

Data mean and standard deviation were calculated using Microsoft EXCEL. Treatment comparison of mean was made with SPSS using one-way ANOVA with the Duncan’s multiple range tests to separate means. Differences were considered significant at the p < 0.05 level (Wei et al., 2009).

3. Results

3.1. Effects of Cd on R. globosa and R. palustri biomasses

Usually, plant shoot biomass is an important character to exhibit plant growth (Yang, 2002). In phytoextraction, higher biomass is often associated with better growth and greater tolerance to heavy metals (Li et al., 2011). As shown in Fig. 1, the root and shoot biomasses of R. globosa were not significantly decreased (p<0.05) when 2.5 and 5 mg kg⁻¹ Cd added, indicating its strong tolerance to Cd (Li et al., 2011). However, their biomasses were significantly decreased (p<0.05) by 12%, 20%, and 39.5% respectively compared to the control when 10, 20 and 40 mg kg⁻¹ Cd added, which expressed their tolerance limitation (Li et al., 2011).

In contrast, all plant root and shoot biomasses of R. palustri under different Cd levels of 2.5, 5, 10, 20 and 40 mg kg⁻¹ gradually decreased (p<0.05) by 19.8%, 36.1%, 54.2%, 61.8% and 73.3% respectively compared to its control, indicating a weak tolerance of it to Cd (Fig. 1) (Li et al., 2011).

3.2. Cd hyperaccumulative characteristics of R. globosa

Table 1 showed the characteristics of R. globosa and R. palustri accumulating Cd. Cd concentration in roots, stems, leaf and shoots of R. globosa were all higher than 100 mg kg⁻¹ the typical concentration what Cd hyperaccumulator should accumulate. Particularly, the Cd concentrations in shoots were 326.2 and 461.3 mg kg⁻¹ when Cd additions were 2.5 and 5 mg kg⁻¹, indicating Cd typical concentration property (Li et al., 2011). In addition, the TFs and EFs of R. globosa were all greater than 1, expressing its TF and EF properties of Cd hyperaccumulator either.

Thus, when Cd additions were 2.5 and 5 mg kg⁻¹, R. globosa showed tolerance property, typical concentration property, TF and EF property, which was a Cd hyperaccumulator (Fig. 1, Table 1) (Li et al., 2011).
In contrast, though Cd concentrations in roots, stems, leaf and shoots in *R. palustri* were higher than 100 mg kg\(^{-1}\) and the EFs higher than 1 either, the TFs were all lower than 1. Combined the weak shoot biomass and low TF characteristics, *R. palustri* should be a non Cd hyperaccumulator (Fig. 1, Table 1) (Li et al., 2011).

### 3.3. Effects of Cd on total root lengths of *R. globosa* and *R. palustri*

As shown in Fig. 2, total root lengths of *R. globosa* in Cd 2.5 mg kg\(^{-1}\) and 5 mg kg\(^{-1}\) treatments were not significantly decreased (\(p < 0.05\)) compared to the control, indicating its strong tolerance (Li et al., 2011). When Cd addition were 10, 20 and 40 mg kg\(^{-1}\), the total root lengths were decreased by 39.0%, 41.8% and 46.3% respectively, expressing its tolerance limitation.

Compared to the control, total root lengths of *R. palustri* were decreased by 32.6%, 49.2%, 55.3%, 64.1% and 64.4% gradually when Cd treatments as 2.5, 5, 10, 20 and 40 mg kg\(^{-1}\), indicating its weak tolerance (Li et al., 2011).

In contrast, total root lengths of *R. globosa* were higher than that of *R. palustri*, and the former was with stronger tolerance than the latter to Cd.

### 3.4. Effects of Cd on total root surface areas of *R. globosa* and *R. palustri*

The total root surface area changes of *R. globosa* and *R. palustri* showed same trends with their total root lengths. When 2.5 mg kg\(^{-1}\) and 5 mg kg\(^{-1}\) of Cd added, total root surface areas of *R. globosa* were not significantly decreased (\(p < 0.05\)) compared to its control (Fig. 3). But its total root surface areas sharply decreased by 38.9%, 42.9% and 45.4% when 10, 20 and 40 mg kg\(^{-1}\) of Cd were added. Fig. 3 also showed the total root surface areas of *R. palustri* were respectively decreased by 21.7%, 39.3%, 57.4%, 61.2% and 62.3% compared to its control when Cd added at 2.5, 5, 10, 20 and 40 mg kg\(^{-1}\).

Likewise, total root surface areas of *R. globosa* were higher than that of *R. palustri*, and the former showed stronger tolerance than the latter to Cd (Li et al., 2011).

### Table 1

Accumulative characteristics of *R. globosa* and *R. palustri* to Cd.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Treatment</th>
<th>Root</th>
<th>Stem</th>
<th>Leaf</th>
<th>Shoot</th>
<th>EF</th>
<th>TF</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. globosa</em></td>
<td>Cd0</td>
<td>1.9 ± 0.06</td>
<td>2.3 ± 0.02</td>
<td>3.0 ± 0.02</td>
<td>2.6 ± 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cd2.5</td>
<td>141.8 ± 11.26</td>
<td>319.2 ± 24.86</td>
<td>343.8 ± 18.05</td>
<td>326.2 ± 29.04</td>
<td>130.5</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>Cd5</td>
<td>313.0 ± 11.26</td>
<td>458.9 ± 18.98</td>
<td>468.6 ± 6.86</td>
<td>461.3 ± 33.21</td>
<td>92.3</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Cd10</td>
<td>721.5 ± 47.98</td>
<td>847.0 ± 68.61</td>
<td>911.5 ± 39.02</td>
<td>902.0 ± 87.32</td>
<td>90.2</td>
<td>1.3</td>
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<tr>
<td></td>
<td>Cd20</td>
<td>1032.1 ± 77.06</td>
<td>1122.5 ± 73.71</td>
<td>1224.0 ± 35.70</td>
<td>1198.0 ± 96.74</td>
<td>59.9</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Cd40</td>
<td>1659.3 ± 92.59</td>
<td>1667.4 ± 87.97</td>
<td>1993.6 ± 47.28</td>
<td>1745.7 ± 58.16</td>
<td>43.6</td>
<td>1.1</td>
</tr>
<tr>
<td><em>R. palustri</em></td>
<td>Cd0</td>
<td>2.9 ± 0.02</td>
<td>2.0 ± 0.01</td>
<td>2.8 ± 0.03</td>
<td>2.4 ± 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cd2.5</td>
<td>311.2 ± 17.25</td>
<td>289.6 ± 11.94</td>
<td>317.8 ± 23.17</td>
<td>295.5 ± 26.46</td>
<td>118.2</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Cd5</td>
<td>469.1 ± 27.97</td>
<td>374.0 ± 42.29</td>
<td>415.9 ± 37.51</td>
<td>389.4 ± 30.92</td>
<td>77.9</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Cd10</td>
<td>617.9 ± 46.40</td>
<td>447.5 ± 36.98</td>
<td>485.3 ± 65.37</td>
<td>465.2 ± 39.83</td>
<td>46.5</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Cd20</td>
<td>969.9 ± 60.46</td>
<td>756.7 ± 41.42</td>
<td>788.0 ± 43.49</td>
<td>768.4 ± 40.17</td>
<td>38.4</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Cd40</td>
<td>1325.6 ± 96.06</td>
<td>931.8 ± 69.20</td>
<td>980.8 ± 23.08</td>
<td>960.7 ± 35.61</td>
<td>24.0</td>
<td>0.7</td>
</tr>
</tbody>
</table>

(Note: Cd0, Cd2.5, Cd5, Cd10, Cd20 and Cd40 for Cd treatments mean 0, 2.5, 5, 10, 20 and 40 mg L\(^{-1}\) were added, respectively.)
Cd pollution (Fig. 4). When Cd addition were 2.5 and 5 mg kg⁻¹, total root lengths and total root surface areas of \( R. \) globosa were decreased by 30.0%, 52.4%, 57.1% and 61.9% respectively compared to its control (Fig. 4). The results further showed that total root volumes of \( R. \) globosa showed obvious different from the trends of total root lengths, total root surface areas and total root volumes, and average root diameters of \( R. \) globosa was with stronger tolerance than that of \( R. \) palustri to Cd.

3.5. Effects of Cd on total root volumes of \( R. \) globosa and \( R. \) palustri

Plant root volume was relative with root length and surface area. There were similar trends in total root volumes with total root lengths and total root surface areas of \( R. \) globosa and \( R. \) palustri to Cd pollution (Fig. 4). When Cd addition were 2.5 and 5 mg kg⁻¹, total root volumes of \( R. \) globosa were not significantly decreased \((p < 0.05)\) compared to its control, but decreased by 47.4%, 54.4% and 57.9% in the conditions of 10, 20 and 40 mg kg⁻¹ of Cd added. In contrast, total root volumes of \( R. \) palustri in all Cd treatments were decreased by 30.0%, 52.4%, 57.1%, 61.9% and 66.7% respectively compared to its control (Fig. 4). The results further showed that \( R. \) globosa was with stronger tolerance than that of \( R. \) palustri to Cd.

3.6. Effects of Cd on average root diameter of \( R. \) globosa and \( R. \) palustri

The changes of average root diameters of \( R. \) globosa and \( R. \) palustri showed obvious different from the trends of total root lengths, surface areas and volumes. The average root diameters of \( R. \) globosa in the treatment of 2.5, 5, 10, 20 and 40 mg kg⁻¹ Cd addition were not impacted \((p < 0.05)\) compared to its control (Fig. 5). However, the average root diameters of \( R. \) palustri were significantly decreased by 24.5% and 27.1% only when 20 and 40 mg kg⁻¹ Cd added compared to its control (Fig. 5). The results suggested that the effects of Cd to plant average diameters were weak (Li et al., 2011).

4. Discussions

In our previous experiment of soil pot culture about \( R. \) globosa accumulating Cd, shoot biomasses were not significantly decreased \((p < 0.05)\) under 25 and 50 mg kg⁻¹ Cd spiked (Wei et al., 2009). However, shoot biomasses of \( R. \) globosa were significantly decreased \((p < 0.05)\) when Cd addition higher than 10 mg kg⁻¹ in this experiment. Furthermore, the plant accumulated very high Cd in latter. The reason may lie in Cd bio-activities was much higher in solution than of in soil due to almost all Cd can be used in hydroponic condition (Su et al., 2009). But the accumulative characteristics of \( R. \) globosa to Cd were basically same in the soil and the solution experiments, indicating its hyperaccumulative properties.

Usually, the studies on the mechanisms of plant accumulating heavy metals were mainly focused on the tolerance (Mathews et al., 2010), transfer (Page et al., 2006) and subcellular distribution (Page et al., 2006), while seldom research was made in root morphology. Actually, root was the main interface of plant obtaining nutrients from environment, thus root played a key role even in each process (Verbruggen et al., 2009). The changes of root morphology may directly affect the usages of nutrients and thus influence plant growth and its biomass. Obviously, root length, surface area, volume and diameter respond the capacity to acquire nutrients, and therefore heavy metal accumulation ability. However, root biomass is the whole response to be as the index for Cd toxicity due to it includes root length, surface, volume and diameter together. From the view point of practice (phytoextraction efficiency), root biomass is the main index indicating plant tolerance to Cd. In fact, too many experiments were focused on root biomass production and seldom on root morphology (Wisniewski and Dickson, 2003; Xie et al., 2005; Fritioff and Greger, 2006; Zheljazkov et al., 2006). Li et al. (2011) reported the effects of Zn and Cd interactions on root morphology. They found that low level of Zn addition increased the root length, surface area and volume of Zn–Cd hyperaccumulator \( Sedum \) \( alfredii \), but indicating negative impact when Zn level was high. There were some similar results with this experiment, indicating a positive relationship of plant tolerating metal with its root morphology change. In addition, some environmental factors influencing root morphology such as soil moisture (Davies and Bacon, 2003), nutrient (Zhang et al., 2003), mechanical impedance (Bengough, 2003) and even metal stress (Perriguey et al., 2008) should be considered in phytoextraction practice in order to obtain higher phytoremediation efficiency.

5. Conclusions

Through the comparative research of \( R. \) globosa and \( R. \) palustri accumulating Cd under hydroponic conditions, \( R. \) globosa showed strong tolerance, higher Cd accumulation concentration in shoot, EFs and TFs, which was further affirmed as a Cd hyperaccumulator, and \( R. \) palustri as a Cd non-hyperaccumulator. The main effects of Cd on plant root morphology were in its total root lengths, total root surface areas and total root volumes, and average root
diameter was weak, which might be part reasons of plant with strong tolerance to Cd.

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