Evaluation of the cadmium and lead phytoextraction by castor bean (*Ricinus communis* L.) in hydroponics

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Abstract. Phytoextraction has been considered as an innovative method to remove toxic metals from soil; higher biomass plants such as castor bean (*Ricinus communis* L.) have already been considered as a hyperaccumulating candidate. In the present study, castor bean was used to accumulate the cadmium and lead in hydroponic culture, and the root exudates and biomass changes were analyzed. Results demonstrated that ratios of aerial biomass/ root biomass (AW/RW) in treatments declined with concentrations of Cd or Pb. Optical density (OD) at 190 nm and 280 nm of root exudates observed in Cd and Pb treatments were lower than the control. In single Cd or Pb treatments, bioconcentration factors (BCF) of Cd or Pb increased with time and decreased with concentrations, the highest BCFs appeared in Cd5 (14.36) and Pb50 (6.48), respectively. Cd-BCF or Pb-BCF showed positive correlations with AW/RW ratios and OD values, and they were negative correlated with Cd and Pb concentration. Results in this study may supply useful information for phytoremediation of soil contaminated with cadmium and lead in situ.

1. Introduction

Heavy metals form the main group of inorganic contaminants and the recovery of sites contaminated with such compounds is one of the major challenges for environmental institutions [1]. Traditional solutions such as disposal of contaminated soil in landfills account for a large proportion of the remediation operations at present. However, some of these remediation methods currently in use will probably lose economic favour and public acceptance in the near future [2],[3]. Recent development in phytoextraction technology marks significant progress in this field. It makes use of plants to remove pollutants or to render them harmless [4],[5]. It has been suggested that higher biomass plants can be used such as castor bean [6]. Cindy et al. [7] reported that Cd²⁺ and Pb²⁺ in soil could be phytoextracted by castor bean associated with *Pseudomonas putida*. Murillo et al. [8] found that castor bean could enrich significantly more arsenic, cadmium, copper and zinc in spill-affected soil than the control. Guo et al. [9] considered that castor bean seedlings had high ability of enrichment to Cu, Pb, and their roots were main organs of enrichment. Recently, many articles have addressed that castor bean could be used as a candidate in phytoremediation of Cd and Pb, for its strong tolerance to Cd and Pb [10] and ability of accumulation of them [11].

The root exudates of hyperaccumulating plants in response to heavy metal exposure are crucial for the successful implementation of phytoremediation technologies [12] Studies showed that root exudates could mobilize soil Cd and promote the Cd uptake by wheat plant (*Triticum aestivum* L.) [13]; and the
root exudates could be applied to enhance the accumulation of Cd and Cu in *Echinochloa crus-galli* [12]. In addition, evidences demonstrated that artificial cultures, such as sand culture, could make root exudates collected without interference from other organic matters, and responses of roots exudates to heavy metals could be analysed accurately [14], [15].

The aim of our study was to evaluate the characteristics of Cd and Pb phytoextraction by castor bean through the growth and root exudates changes in culture. Meanwhile, relationships among bioaccumulation, biomass and root exudates were discussed.

2. Materials and methods

2.1. Experiment design

The heavy metal salts (reagent grade) (CdCl$_2$•2.5H$_2$O and Pb(NO$_3$)$_2$•H$_2$O) were separately diluted in deionized water, then solutions were added into culture and homogenized respectively. The Cd and Pb concentrations designed were as follows, (i) control; (ii) Cd 5 mg•L$^{-1}$, 10 mg•L$^{-1}$, 50 mg•L$^{-1}$ (the following as Cd5, Cd10, Cd50, respectively); (iii) Pb 50 mg•L$^{-1}$, 100 mg•L$^{-1}$, 300 mg•L$^{-1}$ (the following as Pb50, Pb100, Pb300, respectively). Controls and treatments were in triplicates for analysis.

2.2. Seed and artificial culture preparation

Seeds of castor bean (*Ricinus communis* L.) obtained from Shenyang Agricultural University were surface sterilized by immersion in 20% v/v commercial bleach and shaken at 144 r/min on an orbital shaker (Beijing) in sterile distilled water for 6 hours. Then they were sown onto stainless plate with aseptic gauze in incubator, the temperature and moisture were kept on 28 ºC and 60%. When the sterile seedlings reached approximately 10 cm, seedlings were transferred to each sterilized apparatus with sand.

The quartz/feldspar sand was first washed with concentrated HCl (32%), and rinsed several times with deionized water to eliminate residual acid, then sand was completely dried after heat-sterilizing (120ºC for 60 min). 4.5 kg of sand was added into an individual apparatus.

2.3. Collection of root exudates

The collection of castor bean root exudates was adapted from Yoshitomi and Shann [15]. The central portion of this setup was a glass chamber, including the solid rooting media of acid washed quartz sand. The seedlings were sealed around the stem with a sterile 9:1 lanolin: paraffin wax mixture, maintaining aseptic conditions around the roots and in the remainder of the system. The sealed apparatuses were arranged randomly in a greenhouse under 14 h light cycles (110 µmol•m$^{-2}$•s$^{-1}$) and in a temperature range of 18-28 ºC. A sterile 1/4 strength modified Hoagland and Arnon [16] nutrient solution was used to irrigate the root zone in the first week, and then nutrient solution was changed to full Hoagland’s solution. Root exudates were collected every 15 d during experimental period, and analyzed for optical density at 190 nm and 280 nm (UV 2401PC, Shimadzu).

2.4. Analysis of biomass and contents of cadmium and lead

Every 15 days, plants samples were harvested by clipping the shoot at the culture level. The roots and aerials were washed in dilute detergent solution, followed by several rinses in distilled water. All plant parts were dried in an oven at 70 ºC for 72 h, and the dry weights were recorded by electronic balance (the limit is 0.1 mg). Parts of plants including roots and aerials were digested, and the digestion was accomplished using an electric hot plate (Beijing) at 105 ºC for 30 min with 10 ml of concentrated HNO3 (trace pure). Subsequently, the sample volume was adjusted to 20 ml with double deionized water and all sample extracts were analyzed using a flame atomic absorption spectroscopy (Spectra AA220, Varian).

Two bioconcentration factors, as defined in Eqs below and computed from the treatments concentrations, will be used to discuss the results from this study.

$$BCF \ (bioconcentration \ factors) = \frac{C\ plants}{C\ culture}$$
All the data were subjected to the analysis of variance (ANOVA) and linear correlations were quantified using Pearson’s correlation coefficients. Statistical analyses were performed using SPSS statistical software (SPSS Inc., Chicago, IL). Differences at the P < 0.05 level were considered to be statistically significant.

3. Results

3.1. Ratios of aerials biomass/ roots biomass
AW/RW ratios in single Cd or Pb treatments declined with concentrations of metals (Figure 1), the highest value was observed in Pb50 treatment at the 60th day (12.60) ($P < 0.05$). In the Pb50 treatments, the AW/RW ratios increased with time and the ratios showed decreased with concentrations of lead.

![Figure 1](image-url)

**Figure 1.** The biomass ratios of aerials and roots in treatments (AW/RW: aerials biomass / roots biomass; bars: standard deviation)

3.2. Optical density (OD) of roots exudates
OD values at 190 nm in Cd and Pb treatments were lower than the control (Table 1). In the single Cd or Pb treatments, ODs decreased with the increment of concentrations at the end of experiment. According to OD values at 280 nm in Cd or Pb treatments, OD values displayed the same trend with those at 190 nm, and the highest was 1.31 in Pb50 ($P < 0.05$).

|         | 190nm | 15day | 30day | 45day | 60day |
|---------|-------|-------|-------|-------|-------|
| Cd5     | 1.25  | 1.69  | 2.82  | 3.18  |
| Cd10    | 1.70  | 1.83  | 2.02  | 2.59  |
| Cd50    | 1.75  | 1.98  | 2.11  | 2.20  |
| Pb50    | 1.87  | 2.92  | 3.29  | 3.43  |
| Pb100   | 1.95  | 1.80  | 2.12  | 2.34  |
| Pb300   | 2.50  | 1.64  | 1.79  | 2.05  |

|         | 280nm | 15day | 30day | 45day | 60day |
|---------|-------|-------|-------|-------|-------|
| Cd5     | 0.67  | 0.88  | 0.98  | 0.93  |
| Cd10    | 0.63  | 0.73  | 0.73  | 0.77  |
| Cd50    | 0.55  | 0.62  | 0.61  | 0.72  |
| Pb50    | 0.76  | 1.03  | 1.35  | 1.31  |
| Pb100   | 0.82  | 0.95  | 1.01  | 1.20  |
Pb300  0.75  0.73  0.66  0.98
CK      3.64  3.89  4.24  4.40
Note: means in the same column followed by the same letter are statistically not
different at the 5% probability level (n=3); CK: control.

3.3. BCF values of Cd and Pb in treatments
As a whole, Cd-BCFs increased with time, decreased with concentrations in Cd treatments (Figure 2),
and the highest BCF appeared in Cd5 treatment at the 60th day (14.36) (P < 0.05). Data showed that
the highest Pb-BCF reached 6.48 in Pb50 treatment (P < 0.05) in single Pb treatments. And the
BCFs in Pb treatments were lower than those in Cd treatment almost.

3.4. Correlations between BCF and AW/RW, OD and Cd/Pb concentrations
BCFs in Cd and Pb treatment had positive correlations with AW/RW ratios and OD values (190 nm
and 280 nm) (P < 0.01), while they were negatively correlated with concentrations of Cd and Pb (r=-
0.766, P < 0.01; r=-0.800, P < 0.01, respectively) in single Cd or Pb treatments (Table. 2).

Table 2 Correlation coefficients between BCFs and AW/RW, OD values, Cd/Pb concentrations in Cd
or Pb treatments (n=54)

| BCF   | AW/RW   | OD-190nm | OD-280nm | C-Cd   | C-Pb   |
|-------|---------|----------|----------|--------|--------|
| Cd    | 0.712(**)| 0.812(**)| 0.728(**) | -0.766(**)| -      |
| Pb    | 0.944(**)| 0.645(**)| 0.894(**) | -      | -0.800(**) |

Note: AW/RW: aerials biomass / roots biomass;
** Correlation is significant at the 0.01 level (2-tailed);
* Correlation is significant at the 0.05 level (2-tailed).

4. Discussion
The growth condition of plants and the tolerance of plants to toxic metals could use ratios of aerials
biomass/roots biomass to reflect [17]. And the ratio changes might be resulted from the inhibition of
photosynthetic CO2 fixation and disturbance of the Calvin cycle due to cadmium or lead [18],[19]. Toxic
metals ions disturbed respiratory carbohydrate metabolism in plant cells, probably by substituting
irreversibly for another micronutrient in critical enzymes. It also might inhibit the formation of
chlorophyll by interfering with protochlorophyllide reduction and the synthesis of aminoevulinic acid
[20],[21],[22]. On the other hand, specific concentrations of phytotoxic metals might play a positive role in
the growth of plants [23]. Lu and He [24] presented that low concentration of Cd appeared to have little
damage to plants, even improved the growth condition of some plants such as castor bean and
sunflower. Besides, Liu and Wang [25] found that low concentration of Cu (≤ 80 mg·L⁻¹) could promote AW/RW ratios of wheat, and high Cu concentration (> 80 mg·L⁻¹) inhibited germination and seedling growth of wheat. This may be concerned with characteristics of plants and heavy metals.

The optical density monitored in our experiment increased with time, which was coincided with the study of Wang et al. [13], who considered that the production of amino acids and proteins in roots exudates was an accumulative process with time. Moreover, the presence of Cd and Pb in cultures exhibited different effects on OD values. Pinto et al. [26] studied the exudation patterns of sorghum and maize with cadmium existence, results showed that sorghum enhanced malate exudation over the entire range of applied Cd in the uptake solutions, maize increased mainly citrate; at the same time, a significant decrease in the bioavailable Cd was found due to the increase of Cd organic complexation. In addition, Liu and Wang [25] found that the combined effect of Cd and Pb could inhibit the length and amount of exudates of wheat root.

The BCF values of castor bean in this experiment showed different correlation with AW/RW ratios and concentrations of metals. Cd and Pb could disturb the psychological processes of plants inevitably. Paczkowska et al. [27] considered that the interaction between enzymes and cadmium or lead might result that metals posed deleterious effects on much of the biochemical machinery required for cell survival. Besides, it is more likely that accumulation may be a process of sequestering heavy metals in a less toxic form. In general, Cd has more toxic to plants than Pb, the damage to plants made by Cd may affect the growth of plants and tolerance to heavy metals much more than Pb in our experiment. Also, the concentration of heavy metals is an important factor to the phytoextraction. Higher concentration may increase the content of heavy metals in tissue compartments of plants, meanwhile, it could make damage to the growth of castor bean, and decreased accumulation of metals conversely. Lu and He [24] reported that Ricinus communis L. could bioaccumulate Cd in soil at concentrations ranged from 10 mg·kg⁻¹ to 400 mg·kg⁻¹. Growth of ricinus began to be slow and inhibited at concentrations beyond 40 mg·kg⁻¹. Bioaccumulation reached the maximum (4460.3 mg·kg⁻¹) at the concentration of 360 mg·kg⁻¹ but not 400 mg·kg⁻¹, it meant that higher concentration affected the metabolism of castor bean greatly, which weaken the accumulation ability.

5. Conclusions

Castor bean is a good candidate for phytoremediation of soil contaminated with heavy metals for its high biomass. When exposed to Cd and Pb in culture, the growth and roots exudates were changed. Dissimilar relationships among bioaccumulation of Cd and Pb, AW/RW ratios and OD values of root exudates might be due to physiological characteristics of ricinius and category or concentrations of metals. Mechanisms of uptake by castor bean and the changes of exudates when exposed to Cd and Pb still require further study.

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