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Comparison of chelates for enhancing *Ricinus communis* L. phytoremediation of Cd and Pb contaminated soil

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ABSTRACT

We studied chelate effects on castor bean (*Ricinus communis* L.) growth. These effects included Cd and Pb accumulation in plant tissues and the chemical behavior of Cd and Pb in the plant rhizosphere and non-rhizosphere. Tests were conducted in a glasshouse using the rhizobag method. Two castor bean cultivars (Zibo-3 and Zibo-9) were grown in soil contaminated with 3.53 mg/kg Cd and 274 mg/kg Pb. The soil was treated with citric acid (CA), ethylenediamine disuccinic acid (EDDS) or ethylenediamine tetraacetic acid (EDTA) (5 mmol/kg). EDDS-treated soil produced 28.8% and 59.4% greater biomass for Zibo-3 and Zibo-9 respectively. In contrast, CA and EDTA inhibited the growth of the two cultivars. Zibo-9 had greater tolerance than Zibo-3 to chelate toxicity. Based on Cd and Pb plant uptake, EDDS could substitute for EDTA for phytoremediation of Cd in soil. EDTA was the most effective of the three chelates for Pb phytoremediation but it is less suitable for field use due to toxicology environmental persistence. Acid extractable Cd and Pb in the rhizosphere or reducible Cd and Pb in the non-rhizosphere of soil were the main influences on Cd and Pb accumulation in castor bean.

1. Introduction

Human activities can release heavy metals such as Cd and Pb into the soil environment. Heavy metal contamination of soil is a global problem. Cd and Pb in soil are dangerous to human health because of their lengthy residual and potential entry into the food chain (Mühlbachová, 2011). It is essential to remediate Cd and Pb contaminated soil to reduce the risk of Cd and Pb human exposure via consumption of contaminated produce.

Phytoremediation can be used to remove heavy metals from polluted soil or to render them harmless. This is a cost-effective and environmentally friendly technology (Shi and Cai, 2009). Successful phytoremediation requires a suitable plant for use on the toxicant. Castor bean (*Ricinus communis* L.), a C3 plant of the Euphorbiaceae, has great potential for phytoremediation of Cd or Pb contaminated soil (Bauddh and Singh, 2012; Huang et al., 2011; Liu et al., 2008; Shi and Cai, 2009; Zhang et al., 2014a, 2015). Castor bean is also an important energy crop for biofuel or biodiesel production but inedible for humans and animals. It grows on marginal or infertile lands that are usually unsuitable for other crops (Berman et al., 2011). Castor bean can be cultivated to provide phytoremediation and also for bioenergy production, making it a highly valuable, renewable resource (Olivares et al., 2013).

Chelate enhanced phytoremediation is an effective approach for the removal of heavy metals from soils by plants (Romkens et al., 2002). **Citric acid (CA), Ethylenedinitrilotetraacetic acid (EDTA), S,S-ethylenediaminedisuccinic acid (EDDS)** have been studied for their potential to mobilize heavy metals in soil and to increase the accumulation of heavy metals in plants (Luo et al., 2005; Mohtadi et al., 2013). EDTA is an effective and commonly used metal chelate to enhance metal concentration in the soil solution and metal accumulation in plants (Cutright et al., 2010; do Nascimento et al., 2006; Meers et al., 2005). However, the application of EDTA in soil may result in contamination and increases the risk of groundwater pollution, due to its poor degradability (Saifullah et al., 2009). Therefore, EDTA is inappropriate for commercial field application because of unacceptable toxicity and environmental persistence (Saifullah et al., 2009). More rapidly degradable alternatives, such as EDDS and CA, have been examined for enhancing phytoremediation under field conditions.

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EDDS, a biodegradable chelate, increases heavy metal accumulation and uptake in several plant species (Luo et al., 2006; Wang et al., 2009; Zhao et al., 2010). CA, a low molecular weight organic acid, occurs in the cell vacuoles of photosynthetic plant tissues and is also exuded by plant roots into the soil. It has been suggested as an alternative to synthetic chelates for enhancing phytoremediation (Callahan et al., 2006; Evangelou et al., 2006).

In this study, we estimated the efficiency of EDTA, EDDS and CA, applied to castor bean, to mobilize the heavy metals in a contaminated alkali soil. We evaluated (1) the effects of chelates on growth, heavy metal accumulation, and uptake in two castor bean cultivars, (2) cultivar effects and chelate effects on heavy metals species in the rhizosphere and non-rhizosphere, and (3) the influences of metal bioavailability in the rhizosphere and the non-rhizosphere on metal accumulation in castor bean.

2. Materials and methods

2.1. Castor bean cultivars and soil preparation

Seeds of castor bean Zibo-3 and Zibo-9 were obtained from the Zibo Academy of Agricultural Sciences, Zibo, Shandong province, China.

The soil used was collected from a depth of 0–20 cm from contaminated farmland in Jiyuan, Henan Province, China. The soil was air dried and sieved through a 1-cm sieve. Chemical properties of the soil were as follows: total nitrogen 0.89 g/kg, total phosphorus 0.51 g/kg, available phosphorus 8.97 mg/kg, total potassium 18.79 g/kg, available potassium 128.05 mg/kg, organic matter 16.98 g/kg, Cd 3.53 mg/kg, Pb 274.16 mg/kg, Zn 116.27 mg/kg, Cu 47.34 mg/kg, As 23.76 mg/kg, Ni 38.69 mg/kg, Cr 85.87 mg/kg and pH 7.7. The Cd concentration and Pb concentration were much higher than 0.8 mg/kg and 80 mg/kg limits respectively (pH > 7.5), according to Grade II of the Chinese environmental quality standard for soils (GB 15,618-2008).

2.2. Experimental design

A rhizobag experiment was conducted in a greenhouse from 24 Sept. to 30 Nov. 2013. The dimensions of the rhizobag which was made of 300 mesh nylon mesh were 100 mm × 150 mm (diameter × height) filled with 0.4 kg air-dried soil, which was the rhizosphere treatment. The non-rhizosphere treatment was 1.1 kg air-dried soil per pot. Four treatments were used: **CK (Control Check)**, CA, EDDS, and EDTA. Three seeds with the same size of the two castor bean cultivars were sown directly into the soil in pots on 24 Sept. The deionized water was added appropriately to per tray whenever the pot was dried up, to keep the soil moist. In the process of castor cultivation, the weeds were removed. Thirty days after sowing, the seedlings were thinned to one per pot. The chelates (5 mmol/kg) were added after growth for 30 d. The plants were harvested for analysis on 30 Nov.

2.3. Soil and plant sample preparation and chemical analysis

Each harvested plant was divided into roots and shoots. Individual plant parts (roots and shoots) were washed with tap water to remove soil particles, and rinsed three times with deionized water. All plant parts were oven dried at 110 °C for 30 min, and then dried at 70 °C for 96 h to constant weight. Roots and shoots were separately ground in a mill, and then digested with concentrated HNO_3 in flasks on an electric heating plate at 80 °C. The temperature was then increased to, and maintained at, 110 °C, until the solution became clear (Alexander et al., 2006). The sample volume was adjusted to 25 mL with ultrapure water. Standard reference material for bush twigs and leaves (GBW07603: GSV-2) was used to monitor the Cd recovery from the plant samples (recovery: 90 ± 10%).

The soil samples from the rhizosphere (R) and non-rhizosphere (NR) were homogenized separately and air-dried before the extraction of heavy metal speciation. Cd and Pb fractionation was based on the optimized Bureau Community of Reference (BCR) sequential extraction protocol, and the Cd or Pb speciation was divided into acid extractable-metal, reducible-metal, oxidizable-metal, and residue-metal (Rauret et al., 1999).

Inductively coupled plasma optical emission spectrometry (ICP-OES) was selected for analyzing the Cd and Pb concentrations in plant and soil samples. Atomic absorption spectrophotometry (AAS) is another alternate method for determining the content of Cd and Pb. Compared with AAS, the proposed method decreases chemical interference, and simultaneously determines the Cd and Pb content. The Cd and Pb concentrations in plant and soil samples were measured by inductively coupled plasma optical emission spectrometry (ICP-OES) (Optima 5300 DV; Perkin-Elmer, Norwalk, CT, USA). Using the proposed method, the detection limit of Cd is 0.005 mg/L, and the detection limit of Pd is 0.01 mg/L.

2.4. Tolerance index, bioconcentration factor and translocation factor

The tolerance index (TI) was defined as the ratio of plant biomass after chelate treatment to that of the control group biomass. The **bioconcentration factor (BCF)** was defined as the ratio of Cd or Pb in the shoot or root of a plant to the soil concentration. The **translocation factor (TF)** was described as the ratio of Cd or Pb in the shoot to that in the root. The indexes are defined as follows:

$$TI = \frac{W_{chelate}}{W_{control}}$$

where $W_{chelate}$ (g) and $W_{control}$ (g) represent the biomass after chelate treatment and the biomass of the control group, respectively.

$$BCF = \frac{C_{tissue}}{C_{medium}}$$

where, C_{tissue} (mg/kg) and C_{medium} (mg/kg) represent the Cd or Pb concentration in the shoot or root and the Cd or Pb concentration in the soil, respectively.

$$TF = \frac{C_{shoot}}{C_{root}}$$

where C_{shoot} (mg/kg) and C_{root} (mg/kg) represent the Cd or Pb concentration in the shoot and the Cd concentration in the root, respectively.

2.5. Statistical analysis

Data were analyzed using ANOVA and regression analysis with SPSS 16.0 software (SPSS, Chicago, IL, USA). There were four replications for all measurements. Data are presented as mean ± SE, and compared using Duncan's test. A p value less than 0.05 indicated a significant difference.

3. Results

3.1. Biomass of two castor bean cultivars

Compared with CK treatments, the dry biomass of Zibo-3 decreased by 33.5% and 20.3% and that of Zibo-9 decreased by 13.7%