The Potentiality of *Crassocephalum crepidioides* for Phytoremediation of Cd Contaminated Soil under the Application of EDTA

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Abstract: Aiming to identify measures to improve the cadmium (Cd) extraction efficiency of *Crassocephalum crepidioides* on Cd contaminated soil, a pot experiment was conducted to investigate plant growth and Cd accumulation in *Crassocephalum crepidioides* enhanced by ethylenediaminetetraacetic acid (EDTA) application. The results revealed that plant growth was inhibited and biomass decreased while treated with EDTA. The application of EDTA activated Cd in soil, thus increasing the Cd concentration of leaf, stem and root of *Crassocephalum crepidioides* by 10%–91%, 13%–98%, 20%–93%, respectively. According to the Cd extraction amount by the plant, potential phytoremediation capability of *Crassocephalum crepidioides* in Cd contaminated soils would be enhanced effectively with application of 4 mmol·kg⁻¹ EDTA, however, the environmental risks should be considered synthetically when using. This study can provide a basis for the remediation of Cd contaminated soil.

1. Introduction
In recent years, heavy metal pollution of soils has become a global environmental concern, which seriously threatens agricultural production, ecosystem safety and human health [1]. The absorption and accumulation of excessive heavy metals in plants will affect the normal physiological function of plants, and heavy metals will enter animals and human body through food chains, and seriously endangers human health [2]. The Bulletin for National Survey of Soil Contamination released that 16.1% of soil sites surveyed in China have heavy metals concentrations higher than the Chinese Environmental Quality Standard for soils. Furthermore, the pollution rate of cadmium (Cd) has reached 7.0%, ranking first among heavy metals. Cd has become the most prominent heavy metal pollutants due to its high toxicity, strong migration, easy to be absorbed and accumulated by plants [3]. With the frequent occurrence of Cd pollution incidents in recent years, increasing attention has been paid to the remediation of Cd contaminated soil.

Conventional technologies for the remediation of heavy metal polluted soil such as soil excavation, fixation, landfiling or soil leaching mainly involve physical and chemical methods. Although these methods can bring rapid effects, most of them are expensive, need intensive works, generate secondary pollution and can damage soil [4]. In comparison with traditional techniques, phytoremediation has been proposed as an environmentally friendly and low-cost remediation strategy, since it is a technology that used plants to mitigate environmental problems without excavating the contaminating material and dispose of it elsewhere, thus reducing exposure risks for cleanup personnel or secondary contamination in transport [5].

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Recently, the screening and application of Cd hyperaccumulators has been intensively conducted worldwide, and increasing numbers of Cd hyperaccumulators have been identified and gradually applied in the remediation of Cd contaminated soil [6]. However, many of them usually grow slowly and have a low biomass, which greatly limits their remediation efficiency [7]. Recent studies have shown that chelating agents can be used as adjuvants to promote plant uptake of heavy metals in contaminated soil. However, the properties of chelating agents also have toxic effects on plants and soil, which may lead to a decrease in plant biomass and a decrease in the accumulation of heavy metals in plants [8]. Therefore, it is the key to study the concentration of chelating agent which is suitable for plant growth and promotes its absorption of heavy metals.

*Crassocephalum crepidioides*, an annual herb Compositae plant with fast growth and spread rates, occur widely throughout tropical and subtropical China. Previous study has shown that *Crassocephalum crepidioides* can be regarded as Cd-hyperaccumulator [9]. Can adding chelating agent further promote the extraction amount of Cd from *Crassocephalum crepidioides*? Therefore, in the present study, a pot experiment of Cd contaminated soil was conducted and the growth condition as well as Cd accumulation in *Crassocephalum crepidioides* were analyzed after the application different concentrations of EDTA. The results of this study could provide the optimal application concentration of EDTA to improve the remediation efficiency of *Crassocephalum crepidioides* in Cd contaminated soil.

2. Materials and Methods

2.1 Experimental materials

The soil used in the pot experiment was artificially contaminated with Cd. The natural soil came from 0-20 cm topsoil (Cd concentration was 0.25 mg/kg) in the suburb of Guiyang. About 4.0 kg portions of the soil were transferred to plastic pots (30 cm in diameter and 25 cm in height). Cd was added at a concentration of 10 mg·kg⁻¹ dry soil as an aqueous solution of CdCl₂·2.5H₂O. The test plant *Crassocephalum crepidioides* seedlings (2~5 cm in height) were collected from the natural wasteland in the same non-contaminated area, then washed and transplanted in a greenhouse for pre-cultivation for 2 weeks.

2.2 Experimental methods

After the first pair of healthy tender leaves appeared, the seedlings were thinned to four plants per pot and grown for 3 months. Each pot was watered twice a week and the moisture level of the soil was maintained at 60~70% WHC. The application concentration of EDTA was 0, 1 mmol·kg⁻¹, 2 mmol·kg⁻¹, 4 mmol·kg⁻¹, 6 mmol·kg⁻¹, which were labeled as CK, T1, T2, T3, T4, respectively. EDTA was added twice, half a month before and half a month after transplanting of *Crassocephalum crepidioides*. Each treatment was performed in three replicates.

At the harvest, plants and rhizosphere soil samples were collected, and the individual plant height were measured. The plant samples were separated into different parts (e.g., leaf, stem, and root) and washed thoroughly with tap water followed by distilled water to remove adhering soil particles. The dry weights of the samples were recorded after treating with high-temperature desiccation under 105 °C for 30 min and drying at 65 °C for 3 days to gain a constant weight. Subsequently, each part was milled to a fine powder prior for chemical analysis.

The plant samples were digested with a 4:1 ratio of concentrated HNO₃ to HClO₄. The residuals were re-dissolved by HNO₃ (2%) and diluted with distilled water. The available Cd was extracted with a 0.05 mol·L⁻¹ DTPA solution, a 0.01 mol·L⁻¹ Calcium chloride solution, and a 0.1 mol·L⁻¹ triethanolamine solution. Cd concentrations were determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 5300DV, PerkinElmer, US). Certified reference materials (GSV-7) obtained from the Center of National Standard Reference Materials of China, as well as blank samples, were included in each batch of analyses for quality assurance procedures. Good agreement was obtained between our method and certified values. All samples were analyzed in duplicate and the analytical precision was accepted when the relative standard deviation was within 5%.
2.3 Data processing
The experimental data were processed and plotted using Excel 2013, and the single-factor analysis of variance of the relevant data was performed by SPSS 22.0.

3. Results and analysis

3.1 Effects of EDTA on the growth of Crassocephalum crepidioides
The growth parameters of plants including the average shoot height, dry weights of aboveground and roots in each treatment are shown in Table 1. It could be seen that the shoot height and biomass yield of plants decreased with the increase of EDTA application. Relative to the CK, none of the three growth parameters showed significant differences under the T1 treatment (P > 0.05), the T2 treatment provided significant biomass inhibition (P < 0.05). After exposure to the T4 treatment, the shoot height, aboveground weights and roots weights of test plants were reduced by 45%, 54% and 55%, compared with the values for the control. Under T4 treatment, the studied plants showed obvious toxicity symptoms including chlorosis, reddish veins, petioles and curled leaves. Evangelou et al. found that 1.5 mmol·kg⁻¹ EDTA promoted the growth of tobacco, while tobacco growth was inhibited only when the addition amount is greater than 6.25 mmol·kg⁻¹ [10]. The results of Zhang showed that 2.5 mmol·kg⁻¹ EDTA had no significant effect on aboveground biomass of cotton [11]. However, Liu et al. stated that the same amount of EDTA could significantly inhibit the growth of Amaranthus retroflexus [12]. Collectively, the effect of EDTA on plant growth is related to the amount of EDTA and plant species.

Table 1 Growth parameters of Crassocephalum crepidioides

| Treatment | Shoot height (cm) | Aboveground weight (g/pot) | Root weight (g/pot) |
|-----------|------------------|---------------------------|-------------------|
| CK        | 35.5±2.1a        | 40.71±2.42a               | 25.37±1.78a       |
| T1        | 33.9±2.3a        | 37.49±2.51ab              | 23.02±1.60a       |
| T2        | 32.0±1.7a        | 33.32±2.39bc              | 17.56±1.56b       |
| T3        | 24.7±1.5b        | 29.16±2.49bc              | 15.17±1.35bc      |
| T4        | 19.5±1.5c        | 18.71±2.07d               | 11.56±1.12c       |

3.2 Effects of EDTA on Cd concentrations in Crassocephalum crepidioides
The Cd concentrations in the different parts of the Crassocephalum crepidioides under different EDTA treatments are shown in Fig.1. Generally, the concentrations of Cd in various parts of the test plants exhibited a linear increase in response to an increasing amount of EDTA. Except for T1 treatment, other treatments significantly increased the Cd content of each part of studied plants (P < 0.05). The highest concentrations of Cd were found in the leaf, stem and root grown in T4 treatment soil, which were 1.91, 1.98 and 1.93 times those of the control, respectively, which obviously indicated that EDTA promoted the absorption of Cd in Crassocephalum crepidioides.

Fig.1 Cd concentration in organs of Crassocephalum crepidioides
3.3 Effects of EDTA on the available Cd content in soil

Generally, the available heavy metal refers to the heavy metals in soil that can be absorbed and utilized by organisms and have an impact on biological activity. After heavy metal ions enter the soil, most of them are adsorbed, complexed and precipitated with inorganic and organic matter, forming carbonate, phosphate, iron manganese oxide bound state, organic matter sulfide bound state and other forms. Only a small part of them exist in water-soluble state and ion-exchange state. The latter are bioavailable and could be directly used by organisms and effectively affect the metabolic activity of soil microorganisms [13]. Therefore, the bioavailability of heavy metals directly affected the efficiency of phytoremediation of heavy metal contaminated soil [14]. The content of available Cd in the soil under different EDTA treatments is shown in Figure 2. As the concentration of EDTA increased, the available Cd also increased. This is mainly since EDTA has a strong complexing effect on heavy metals such as Pb and Cd. Thus, part of the Cd originally fixed on the soil is activated by inertsness, which improves the mobility of Cd in the soil and promotes the absorption of Cd by plants. In addition, the metal complexes are easy to migrate to the aboveground with plants transpiration, thus increasing the content of heavy metals in plants [15].

![Fig.2 Concentration of available Cd of soils affected by EDTA application](image1)

3.4 Cd extraction amount in various parts of Crassocephalum crepidioides

By combining the weighted mean concentrations of Cd and dry weight in different tissues, the Cd extraction amount by the test plant was calculated under the EDTA application treatments (Figure 3). In general, the extraction amount of Cd in shoot was 3.4-4.3 times of that in root. Under T1 and T2 treatments, the amount of Cd uptake in organs of the studied plants increased compared to the control, while the increase was not significant (p<0.05). After exposure to the T3 treatment, the extraction amount of Cd by the test plants was significantly higher than that of the control, increasing by 24%. However, under the T4 treatment, the biomass of the studied plants was significantly inhibited, and the extraction amount of Cd by plants was significantly lower than that of the control (p<0.05).

![Fig.3 Amount of Cd uptake in organs of Crassocephalum crepidioides](image2)
4. Discussion

There are different views on the effect of EDTA on the absorption of elements in soil. Some people believe that EDTA forms a stable complex with cations after it is applied to soil, which reduces the activity of metal ions in soil solution, thus reducing metal toxicity and plant absorption. Another view is that EDTA promotes the formation of metal complexes in soil, increases the solubility and mobility of metals, and thus improving the possibility of being absorbed by plants [15]. In the present study, EDTA increased the content of available Cd in soil and promoted the absorption of Cd by plants. However, with the increase of EDTA concentration, although the content of Cd in the different organs of *Crassocephalum crepidioides* increased, the growth of the plant was inhibited, and the biomass decreased. On the one hand, EDTA has a certain toxic effect on plant growth. On the other hand, Cd in soil was activated by EDTA, which caused the accumulation of Cd in the body exceed a certain extent of burden and inhibits cell division, thereby affecting the growth of plants, and causing toxic symptoms [16]. Usually, to evaluate the phytoremediation efficiency of heavy metals, two factors are mainly considered, heavy metal content and biomass. Considering the biomass and Cd content in plants, 4 mmol·kg⁻¹ EDTA application can significantly increase the amount of Cd extracted by *Crassocephalum crepidioides*, improve the efficiency of phytoremediation, and shorten the remediation period.

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