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Potential of *Leersia hexandra* Swartz for phytoextraction of Cr from soil[☆]

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ABSTRACT

Research on the phytoextraction of Cr from contaminated soils has been scarce, because very few plant species have been reported to accumulate Cr to high concentrations in their aerial parts. In this study, a Cr-hyperaccumulator, *Leersia hexandra* was investigated for its potential to remove Cr from the soil in a series of pot experiments. The results showed that *L. hexandra* had a high extraction capacity for chromium in soil, with the highest Cr concentration in shoot of 1844 mg/kg. Model calculation based on the negative linear relationship between the bioconcentration factors (BCF) and the concentrations of soil Cr indicated that phytoremediation using *L. hexandra* was feasible when soil was only moderately contaminated with Cr. Three sequential harvests did not significantly alter Cr accumulation and shoot biomass ($p > 0.05$), indicating that sequential harvest might be an available and convenient way to achieve the decontamination of Cr-contaminated soils by *L. hexandra*. On average, fertilization increased the shoot biomass by 45% and the total amount of extracted-Cr by 26%, suggesting that fertilization is able to enhance Cr phytoextraction of *L. hexandra*. Although EDTA increased the concentrations of Cr in shoots by 1.4 times, it also resulted in low plant biomass, thereby decreasing the amount of Cr accumulation.

1. Introduction

Chromium can be both beneficial and toxic to animals and humans depending on its oxidation state and concentration [1]. Trivalent Cr is required in trace amounts for sugar and lipid metabolism in humans [2], whereas hexavalent chromium is a very toxic, powerful epithelial irritant and an established human carcinogen by the International Agency for Research on Cancer and the World Health Organization [3,4].

On the other hand, chromium is broadly employed in several industrial processes including smelting, leather tanning, electroplating and mining. Due to its wide industrial use, chromium is considered a serious environmental pollutant [5]. According to one estimate, in the world, about 3×10^4, 1.42×10^5 and 8.96×10^5 tons of chromium escape annually into the atmosphere, water and soil, respectively [6]. China is a major chromate producing country where the discharge amount of chromium slag is estimated at 3.5×10^5–4.2×10^5 tons per year. A great deal of chromium slag was piled in open-air sites, posing potential human health and ecological risks [7]. In view of the seriousness of Cr pollution, considerable efforts have been made to develop suitable methods for the clean up of Cr-contaminated soils and waters.

Phytoextraction is an emerging technology that can be considered for cleaning up of Cr and other metal contaminated sites because of its cost effectiveness, aesthetic advantages, and long term applicability. There are numerous successful examples of plants being used to extract contaminant from environments containing cadmium, copper, lead, nickel, arsenic and selenium [8,9]. However, relatively few works were conducted on phytoextraction of Cr compared to other heavy metals. This is largely due to the fact that very few plants are able to accumulate chromium in their aerial parts. Chromium levels in the foliar portions of plants are normally <1 mg/kg over a wide range of soil Cr concentrations [10,11]. At present, there are only several species, such as *Ambrosia artemisiifolia* [12], *Convolvulus arvensis* [13], *Dicoma niccolifera* [14], *Sutera fodina* [15], *Spartina argentinensis* [16] and *Brassica juncea* [17] that have been reported to be able to accumulate more than 1000 mg/kg of Cr in their aerial parts. Therefore, it is important to exploit new useful extractors of Cr to develop the groundwork for the successful phytoremediation of Cr-contaminated soils.

Leersia hexandra has been found to be a Cr hyperaccumulator with an extraordinary accumulation capacity for both trivalent Cr and hexavalent Cr [18]. Moreover, this species often grows rapidly and densely, and easily adapts to artificial cultivation [19]. All of these properties indicate that *L. hexandra* is a good candidate for phytoextraction of Cr-contaminated soil. In this work, a

Table 1
The properties of the soil used in the experiments.

pH		6.2
Cation exchange capacity (CEC)	cmol/kg	11.9
Soil organic matter	%	3.1
Available N	mg/kg	150
Available P	mg/kg	9.5
Available K	mg/kg	97.8
Total Cr	mg/kg	27.8
Texture		Sandy clay loam
Clay	%	23.4
Silt	%	3.4
Sand	%	73.2

pot culture experiment was conducted in green house to study Cr phytoextraction capability of *L. hexandra*. In addition, the effects of sequential harvest, fertilizer and ethylenediamine tetraacetate (EDTA) application on the Cr bioaccumulation in this species were also investigated. The aims of this paper are to assess the potential of *L. hexandra* for phytoextraction of Cr from contaminated soil and to explore the possible approaches to enhance the Cr phytoextraction with this species

2. Materials and methods

2.1. Plant and soil materials

Seedlings of *L. hexandra* were collected from a paddy filed in Guilin, China. Chromium concentrations in the shoots of *L. hexandra* grown in this site were 9.17–41.5 mg/kg. The seedlings were washed with redistilled water three times and cultured in a plastic box filled with half-strength Hoagland's nutrient solution which consisted of 5 mmol/L Ca(NO$_3$)$_2$, 5 mmol/L KNO$_3$, 2 mmol/L MgSO$_4$, 1 mmol/L KH$_2$PO$_4$, 0.1 mmol/L EDTA-Fe, 46 mmol/L H$_3$BO$_3$, 9.1 mmol/L MnCl$_2$, 0.32 mmol/L CuSO$_4$, 0.76 mmol/L ZnSO$_4$, and 0.5 mmol/L H$_2$MoO$_4$. After a week, seedlings with little biomass were used for the pot experiments. All plants were grown under controlled environmental conditions with a 14 h photoperiod, a 25 °C/18 °C day/night temperature regime, and 65% relative humidity.

The soil used for plant culture in this study was collected from 0 to 30 cm surface layer of the farms in Guilin Academy of Agricultural Sciences. The collected soil was air-dried at room temperature, sieved through 2-mm sieves. Some physical and chemical properties of the soil were measured with routine analytical methods [20], and listed in Table 1. For the pot experiments with *L. hexandra*, 4 kg of air dried and sieved soil was filled in 20-cm diameter round plastic pots.

2.2. Phytoextraction experiment

To assess the efficiency of Cr extracted by *L. hexandra*, the phytoextraction experiment included the control treatment (no additional Cr), and treatments with 80, 130, 180, 230, 330, 430 and 480 mg/kg Cr applied as CrCl$_3$. The soil was thoroughly mixed with chromium salts before filling into the pots and equilibrated for 2 weeks. During these 2 weeks the soil passed two wet–dry cycles to achieve an ageing of the metals in the soil. Ten seedlings of *L. hexandra* with uniform growth were planted into the pot containing soil. Each treatment had three replicates. The plants were watered with 500 mL of distilled water daily in the morning. After 60 days of growth, the shoots of plants were cut 1-cm above soil with a scissors and used for biomass and Cr concentration measurement. Due to the short growing season, we did not expect to observe measurable differences in Cr concentrations in the soil; hence, soil samples were not collected at harvest.

2.3. Sequential harvest experiment

The procedure of sequential harvest experiment was similar to that of the phytoextraction experiment; however, Cr was added in four levels: 0, 100, 200 and 300 mg/kg. Each treatment was performed in triplicates. The above-ground parts of the plants were cut off as described above after 60, 120 and 180 days of Cr treatment. The underground rhizomes and roots were left intact to allow for the re-growth of shoots the following season.

2.4. Fertilization experiment

To evaluate the effect of fertilization on the Cr accumulation of *L. hexandra*, a trial was done by the phytoextraction experiment procedure. The Cr introduced in soil was the same as that of sequential harvest experiment. One hundred milliliters of liquid fertilizer that contained 0.75 mmol/L K$_2$SO$_4$, 0.25 mmol/L KH$_2$PO$_4$, 0.1 mmol/L KCl and 2.0 mmol/L Ca(NO$_3$)$_2$ were applied to each pot every 20 days. The plants without fertilization were used as control.

2.5. EDTA amendment experiment

The EDTA amendment experiment was also similar to phytoextraction experiment. There were four treatments for plants: (1) 0.1 mmol/kg EDTA, (2) 0.1 mmol/kg EDTA + 100 mg/kg CrCl$_3$, (3) 0.1 mmol/kg EDTA + 200 mg/kg CrCl$_3$, and (4) 0.1 mmol/kg EDTA + 300 mg/kg CrCl$_3$. The plants without EDTA application were used as control. After 60 days of treatment, the shoots of the plants were harvested to determine biomass and Cr concentration.

2.6. Biomass and Cr concentration measurement

The harvested plants were rinsed three times in deionized water, and oven dried at 70 °C for 72 h to determine the biomass (dry weight, DW). The dried plant tissues were ground using an agate mortar to pass a 40-mesh screen. The triturated plant tissues (about 0.5 g) were digested with a mixture of HNO$_3$ and HClO$_4$ (5:3, v:v) in a block heater [21]. After cooling, the extracts were diluted up to 50 mL by 0.2% HNO$_3$. Cr concentrations of the extracts were determined by flame atomic absorption spectrophotometer (PE-AA700). The measured values of chromium were checked using certified standard reference material (GBW08501, peach leaves). The recovery of Cr in all cases was 92–106%.

2.7. Phytoextraction efficiency assessment

The dependence of Cr in soil on shoot Cr concentration, or bioconcentration factor (BCF) of shoots, was described by unary linear regression model. The rate of Cr phytoextraction by one crop was calculated by the following equation [22]:

$$R = \frac{C_p \times B}{C_s \times M} \times 100$$

or

$$R = \frac{\text{BCF} \times B}{M} \times 100$$

where R is the percentage of soil Cr removed by one crop, B is the shoot biomass, C_p is the shoot Cr concentration, C_s is the soil Cr concentration, M is the soil mass in the rooting zone, and BCF (bioconcentration factor) is the ratio of the shoot Cr concentration and the soil Cr concentration.
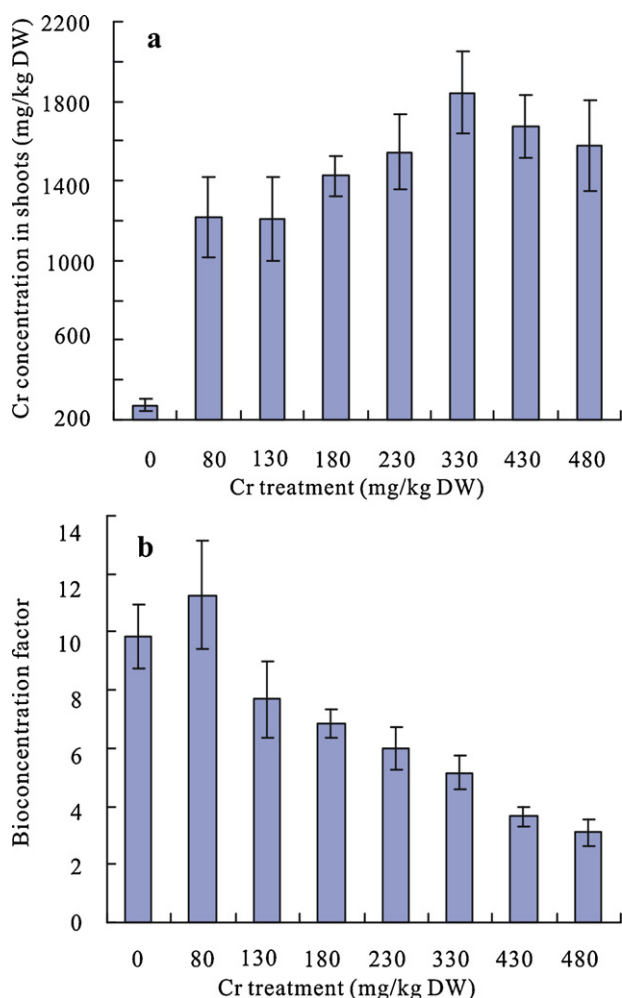
Fig. 1. Relationship between Cr concentration in shoot and the concentration of Cr in soil (a); and relationship between BCF for Cr and soil Cr concentration (b).

2.8. Statistical analysis

Each Cr concentration and biomass was performed in triplicates ($n = 3$). Two-way analysis of variance (ANOVA) was used to test the significance of differences among means of the biomass and shoot Cr concentration in sequential harvest experiment, fertilization experiment and EDTA amendment experiment. When $p < 0.05$, post-hoc test was done (with 95.0% confidence level) to find out which means were significantly different. This test is based on Fisher's least significant difference (LSD) procedure.

3. Results

3.1. Cr phytoextraction assessment

The concentration of Cr in the shoots of L. hexandra with different soil Cr treatment is shown in Fig. 1a. The concentration of Cr in the shoots of L. hexandra increased during soil Cr treatment below 330 mg/kg and peaked at 1844 mg/kg. However, the concentration of Cr in shoots fell when the soil Cr treatment increased from 430 to 480 mg/kg. Therefore, the correlation between shoot Cr concentration and soil Cr concentration ($r^2 = 0.59$) was less significant than that between BCF and soil Cr concentration (Fig. 1b). In the range of soil Cr concentration in this experiment, the BCF of L. hexandra decreased with the increase in soil Cr concentration. The relationship between the BCF and soil Cr concentration could be described by a linear model ($y = -0.0169x + 11.201$, $r^2 = 0.87$).
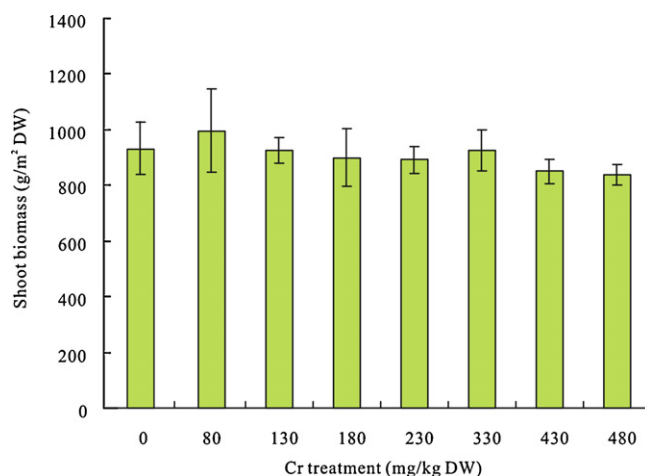

Fig. 2. Effects of soil Cr concentration on shoot biomass of L. hexandra.

Besides the BCF, the efficiency of phytoextraction bears on the biomass production. Due to the generation of modular and individual, it is difficult to determine the individual biomass of L. hexandra. In the present work, the harvestable biomass of L. hexandra was expressed by the dry weight per unit area (biomass per pot divide the area of the pot). In the range of concentration of soil Cr in the experiment, the shoot biomass of L. hexandra varied from 838.3 g/m^2 to 996.7 g/m^2 (Fig. 2), which was in agreement with a field survey in contaminated site [23]. Although the low levels of soil Cr (108 mg/kg) seemed to enhance the biomass production of L. hexandra, there were no statistical differences in the harvestable biomass among the plants exposed to the different soil Cr ($p > 0.05$).

Due to the better correlation between BCF and soil Cr, the calculation of the rate of Cr phytoextraction based on BCF and biomass was more reasonable. Since the biomass of shoot was not significantly different in all the Cr-spiked soil, the average harvestable biomass in pot experiment of 904 g/m^2 was used to calculate the biomass yields of L. hexandra (about 9 t/ha). It was also assume that metal pollution occurred only in the active rooting zone, i.e., top 20-cm soil layer, which gave a total soil mass of 2600 t/ha (assuming a soil bulk density of 1.3 t/m^3). For an initial concentration of soil Cr of 200 mg/kg, it would take 11 crops of L. hexandra to reduce soil Cr to meet the China Environmental Quality Standard for Soils (GB15618-1995, Grade II for soil pH <6.5: Cr < 150 mg/kg, indicating a pollution warning threshold). If the soil is more contaminated with an initial Cr concentration of 300 mg/kg, it would require 25 crops of L. hexandra to reduce soil Cr to 150 mg/kg.

3.2. Effect of sequential harvest on the Cr phytoextraction

Sequential harvests did not influence the growth of L. hexandra. As observed in Table 2, the harvested biomass of L. hexandra was not significantly different among the three harvests with the

Table 2
Effects of successive harvest on shoot biomass of L. hexandra (g/pot).

	Spiked Cr in soil (mg/kg)			
	0	100	200	300
First harvest	30.0 ± 2.8	27.4 ± 3.7	30.1 ± 6.7	28.5 ± 2.8
Second harvest	28.7 ± 2.4	29.0 ± 1.1	26.7 ± 0.7	30.4 ± 3.3
Third harvest	28.7 ± 4.3	27.8 ± 4.3	28.0 ± 1.2	27.6 ± 2.8
	F values of two-way ANOVA			
Harvest	0.28 ns			
Spiked Cr	0.19 ns			
Harvest × spiked Cr	0.45 ns			

Results are means ± SD, $n = 3$, ns: not significant.

Table 3
Effects of successive harvest on Cr concentrations in shoot of *L. hexandra*.

	Spiked Cr in soil (mg/kg)			
	0	100	200	300
First harvest	$273 \pm 31a$	$1244 \pm 176b$	$1576 \pm 165c$	$1877 \pm 278d$
Second harvest	$158 \pm 18a$	$1205 \pm 211b$	$1572 \pm 172c$	$1973 \pm 220d$
Third harvest	$162 \pm 25a$	$1336 \pm 173b$	$1567 \pm 45c$	$1848 \pm 178d$
	F values of two-way ANOVA			
Harvest	0.03 ns			
Spiked Cr	181.61**			
Harvest × spiked Cr	0.48 ns			

Results are means ± SD, *n* = 3; ns: not significant. Different letters in the same row indicate that differences are statistically significant (LSD, *p* < 0.05).
** *p* < 0.01.

same growth period (60 days). Two-way ANOVA analysis showed spiked Cr, harvest and their interaction did not significantly affect the harvestable biomass of *L. hexandra*. Although Cr concentrations in harvestable parts were significantly different among the different Cr soil treatments, they were not significantly different among the three harvests (Table 3), indicating sequential harvest in a year did not change the Cr bioaccumulation capability of *L. hexandra*. Since there were not significantly differences in biomass and Cr accumulation among the three harvests, sequential harvests might not decrease Cr phytoextraction efficiency of *L. hexandra*.

3.3. Effect of fertilization on the Cr phytoextraction

Fig. 3a showed that the application of liquid fertilizer effectively enhanced the biomass production of *L. hexandra*. A significant difference was found in the shoot biomass between fertilized and non-fertilized plant (*p* < 0.01), but no significant differences were found among the plants treated by different Cr levels (Table 4). Fertilization increased the shoot biomass by 44.8%, 45.0%, 44.8% and 45.2% at 0, 100, 200, and 300 mg/kg Cr treatment, respectively.

Although fertilization stimulated growth of plant, it decreased the Cr concentration in shoots (Fig. 3b). At 200 mg/kg and 300 mg/kg Cr treatment, fertilization led to a statistically reduction of shoot Cr concentration (*p* < 0.05). Two-way ANOVA analysis showed soil Cr treatment significantly increased Cr concentration in shoots (*p* < 0.01), whereas fertilization significantly decreased Cr concentration in shoots (*p* < 0.05).

Because the efficiency of phytoextraction is associated with both shoot biomass and Cr concentration in shoots, it can be evaluated by the amount of Cr extracted by the shoots (product of shoot biomass and Cr concentration in shoots). As observed in Fig. 3c, Cr amount in extracted by the shoots was increased by fertilization in all the soils with or without Cr addition. Due to the significantly enhancement of Cr amount in shoots (Table 4), fertilization exerted a positive effect on Cr phytoextraction of *L. hexandra*.
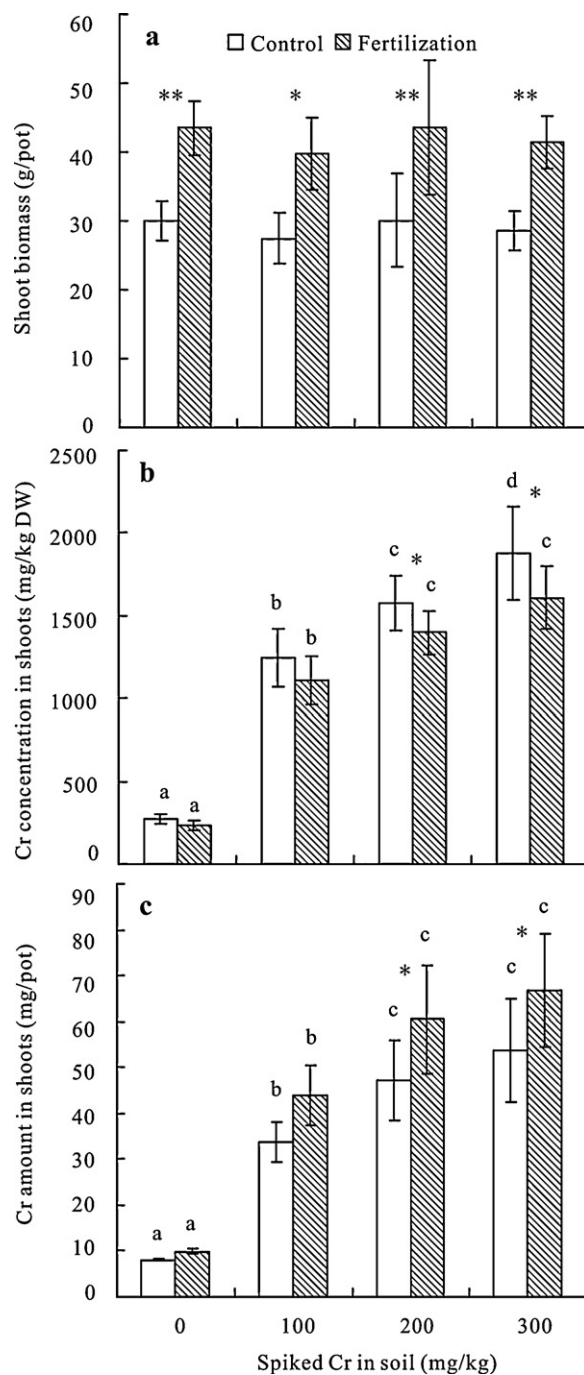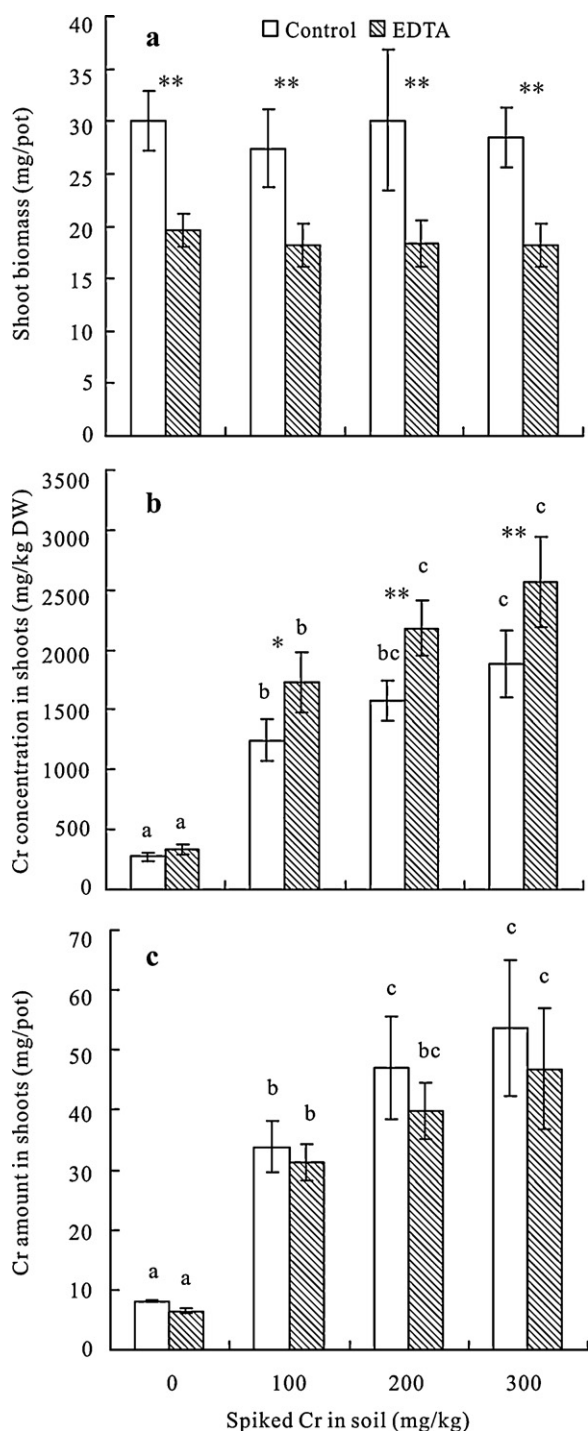
Fig. 3. Effects of fertilization on shoot biomass (a), shoot Cr concentration (b) and Cr amount removed by *L. hexandra* (c). One asterisk and two asterisks denote that there are statistically differences between the fertilized and non-fertilized treatments at 0.05 and 0.01 significance levels, respectively. Different letters indicate that there are significant differences between the soil Cr treatments (*p* < 0.05).

Table 4
Two-way ANOVA *F* values of fertilization, spiked Cr and their interaction.

	ANOVA *F* values		
	Shoot biomass	Shoot Cr	Cr amount in shoots
Fertilization	35.81**	5.58*	7.90*
Spiked Cr	0.51 ns	96.06**	44.80**
Fertilization × spiked Cr	0.02 ns	0.49 ns	0.63 ns

ns: no significant.
* *p* < 0.05.
** *p* < 0.01.

3.4. Effect of EDTA on the Cr phytoextraction

The application of EDTA decreased the biomass of *L. hexandra* (Fig. 4a). In Cr-spiked soil of 0, 100, 200, and 300 mg/kg, EDTA decreased the shoot biomass by 34.4%, 33.5%, 38.9% and 36.3%, respectively, compared to the control (no EDTA). Two-way ANOVA analysis indicated EDTA significantly decreased the shoot biomass of *L. hexandra* (*p* < 0.01), while soil Cr treatment did not affect the biomass production (Table 5).

Fig. 4. Effects of EDTA on shoot biomass (a), shoot Cr concentration (b) and Cr amount removed by *L. hexandra* (c). One asterisk and two asterisks denote that there are statistically differences between the EDTA and non-EDTA treatments at 0.05 and 0.01 significance levels, respectively. Different letters indicate that there are significant differences between the soil Cr treatments ($p < 0.05$).

Table 5
Two-way ANOVA *F* values of EDTA, spiked Cr and their interaction.

	ANOVA *F* values		
	Shoot biomass	Shoot Cr	Cr amount in shoots
EDTA	57.00**	25.24**	0.11 ns
Spiked Cr	0.43 ns	83.69**	0.03 ns
EDTA × spiked Cr	0.14 ns	2.34 ns	0.84 ns

ns: no significant.
** $p < 0.01$.

The effect of EDTA on the Cr concentration in shoots is shown in Fig. 4b. EDTA application increased the Cr concentration in shoots. For example, addition of EDTA raised the Cr concentration in shoots by 1.4-fold in comparison with the control at 200 mg/kg Cr soil treatment. Two-way ANOVA analysis revealed EDTA significantly increased the shoot Cr concentration (Table 5), especially at 200 mg/kg and 300 mg/kg Cr treatments (Fig. 4b, $p < 0.01$).

Due to the opposing effects of EDTA on biomass and shoot Cr concentrations, the amount of Cr extracted by plants was also used to assess the effect of EDTA on the efficiency of Cr phytoextraction. As showed in Fig. 4c, regardless of Cr concentration added to soil, the amount of Cr extracted by plants treated with EDTA were all lower than those without EDTA. There were no statistical differences between the amounts of Cr extracted in treatments with and without EDTA addition (Table 5), indicating 0.1 mmol/kg EDTA did not enhance the Cr phytoextraction of *L. hexandra*.

4. Discussion

The effectiveness of Cr phytoextraction depended on the plant's ability to accumulate Cr, harvestable biomass and the number of crops that might be needed to decontaminate soils. The present study showed that the Cr concentrations in the shoots of *L. hexandra* exceeded 1000 mg/kg, the critical concentration for a Cr-hyperaccumulator [15]. The high Cr concentration in shoots means that the prospects for using *L. hexandra* as phytoremediator on chromium-contaminated sites are better than most plants reported previously [24–27]. Although *Spartina argentinensis* [16] and Pusa Jai Kisan genotype of *B. juncea* [17] showed higher shoot Cr concentration and BCF, it is difficult to compare these results with those of this study, due to the different culture conditions and growth times. Schnoor [28] suggested that any plant useful for phytoremediation should be vigorously growing, easily harvestable and should exhibit a biomass of more than 3 tons DW/ha/year. Our study showed that *L. hexandra* followed this suggestion. According to the calculation, the shoot dry weight production of this plant reached 9 tons DW/ha after only 60 days of growth. Due to the better correlation between BCF and Cr in soil, the linear relationship between BCF and soil Cr was used to calculate the number of crops. In this calculation model, it would take 11 and 25 crops to achieve the 150 mg/kg soil Cr target with the initial Cr concentrations of 200 and 300 mg/kg, respectively. This result compares favorably to experiments performed with non-accumulation genotype of *B. juncea*, which required more than 600 crops to reduce soil Cr from 300 mg/kg to 150 mg/kg [29]. The Cr phytoextraction in this study was assessed on the basis of the artificial contaminated soil under greenhouse conditions which were different from the field conditions; hereby future field studies are necessary to evaluate real phytoextraction efficiency under natural conditions.

As mentioned above, to remove Cr from soil, it was necessary to harvest the plants many times. However, would the sequential crops decrease the biomass yield or Cr accumulation in *L. hexandra*? In the present work, three successive harvests did not reduce the biomass yield in the aerial parts of *L. hexandra* (Table 2). *L. hexandra* belongs to Gramineae and has a high regeneration capacity and rapid growth rate. It was observed that *L. hexandra* grew rapidly in the early growth period, whereas beyond 60 days, the biomass of the plants added very slowly [30]. In this case, harvesting after 60 days was best for the biomass yield of *L. hexandra*. Moreover, there were not statistical differences in the Cr concentration in the aerial parts among the three harvests (Table 3), indicating sequential harvests might not affect Cr accumulation capacity of *L. hexandra*. A similar result was found by Li et al. [31] who reported that sequential harvests did not decrease the arsenic uptake rates by *P. vittata*. *L. hexandra* is a perennial plant and can be harvested

several times during its growth period. Therefore, the enhancement of Cr phytoextraction with *L. hexandra* was feasible by means of increasing the harvest numbers. Taking into account climate conditions in southern China, 4 harvests per year could be expected for the practical application of *L. hexandra*. According to our calculations, to decrease soil Cr concentrations from 200 and 300 mg/kg to 150 mg/kg, *L. hexandra* needs 2.75 and 6.25 years, respectively. Robinson et al. [32] suggested that any period of phytoextraction exceeding 10 years would be economically inefficient. Taking this into account, the results of our study indicate that when soils are moderately contaminated by Cr, phytoextraction of *L. hexandra* is a useful technique. For highly contaminated soils (such as 1000 mg/kg Cr), the phytoextraction of *L. hexandra* is too slow.

Due to the long period of phytoextraction, further improvements are necessary to make phytoextraction a feasible option for restoration of Cr-contaminated territories. As an important way for increasing yield in agricultural production, fertilization also can be used to enhance phytoremediation [33]. Barrutia et al. [34] reported that fertilization greatly stimulated growth of *Rumex acetosa* L. and increased the effectiveness of its phytoextraction. A similar phenomenon was also observed in the present study where fertilization increased the shoot biomass of *L. hexandra* by an average of 45%. Although application of liquid fertilizer resulted in a decline of shoot Cr concentration, which could attribute to a dilution effect resulting from increased dry weight of the shoots [35], it effectively enhanced the amount of Cr removed by *L. hexandra* (Table 5). According to the estimation of this study, fertilization increased the efficiency of Cr phytoextraction with *L. hexandra* by 26%. Accordingly, it only required 19 crops to reduce soil Cr from 300 mg/kg to 150 mg/kg when fertilizer was applied. Although fertilization may increase the cost of phytoextraction by about 4%, it should be considered for the enhancement of Cr phytoextraction with *L. hexandra*. In addition, it was reported that fertilization could increase the bioavailability of metals [36] and metal uptake by plants [37,38]. Therefore, optimizing fertilization for enhanced Cr uptake by *L. hexandra*, and the possible savings of the cost of phytoextraction, should be further investigated.

Several studies have documented that chelating agents can be used to increase metal mobility, thereby enhancing phytoextraction [39–41]. Blaylock et al. [42] and Wu et al. [43] suggested that EDTA is most efficient at increasing the solubility of heavy metals in soils, thus increasing the concentration of heavy metals in plant shoot tissues. Athalye et al. [44] reported that EDTA enhanced Cr accumulation in plants. In the present work, EDTA increased shoot Cr concentration by an average of 33.8%, which demonstrated that EDTA could enhance the uptake and accumulation of Cr in *L. hexandra*. However, the application of EDTA also resulted in severe biomass loss. As a result, the total amount of Cr removed by *L. hexandra* with EDTA application was not statistically different from the control. Therefore, the application of EDTA did not assist the Cr phytoextraction of *L. hexandra*. The biomass reduced by EDTA might be ascribed to the phytotoxicity of EDTA [29,40]. Compared with other studies [41,42,44], the dose of EDTA in this study is moderate; therefore, *L. hexandra* is relatively sensitive to the toxicity of EDTA. Furthermore, EDTA is a synthetic chelator and can form metal complexes with high stability that are slowly degraded and relatively biologically stable, even under conditions favorable to biodegradation [45]. Due to the phytotoxicity and environmental risk of EDTA, adding EDTA was not an appropriate approach to enhance phytoextraction of *L. hexandra*.

5. Conclusion

The high accumulation of Cr and biomass production indicated that *L. hexandra* has a great potential for Cr phytoextraction. The

model calculation based on the relationship between BCF and soil Cr concentration suggested that phytoextraction using *L. hexandra* is feasible when soil is only moderately contaminated. However, for heavily contaminated soils with Cr concentrations in the orders of thousands mg/kg, phytoextraction using *L. hexandra* would not be feasible due to the too long period. Three sequential harvests did not affect the shoot Cr accumulation and biomass production, indicating that more harvests might enhance Cr phytoextraction of *L. hexandra*. Fertilization highly stimulated the biomass yield of *L. hexandra* and thus increased the phytoextraction efficiency of Cr. The application of EDTA decreased both biomass yield and the total amount of Cr extracted by *L. hexandra*, which demonstrated that Cr phytoextraction cannot be improved by adding EDTA.

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