Bull Environ Contam Toxicol (2009) 82:358–362
DOI 10.1007/s00128-008-9587-2

Bioaccumulation and Chemical Form of Chromium in *Leersia hexandra* Swartz

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Received: 21 December 2007 / Accepted: 8 October 2008 / Published online: 25 October 2008

Abstract The chromium bioaccumulation ability of *Leersia hexandra* was assessed and the chromium distribution in the deferent chemical forms in plant tissues was determined. The hydroponic experimental results indicated that the maximum chromium concentration in the dry leaf matter of *Leersia hexandra* reached 4302 mg kg^{-1}. Chromium treatment could significantly increase the proportions of oxalic integrated chromium in leaves and residue chromium in roots, which might be related to the high resistance and bioaccumulation capacity for chromium in *Leersia hexandra*.

Keywords *Leersia hexandra* · Cr · Bioaccumulation · Chemical form

A number of plant species have the unusual ability of accumulating metals such as zinc, nickel, copper and arsenic to very high concentrations in leaves and stems. Approximately more than 400 of so-called "metal hyperaccumulators" are currently known (Baker and Brooks 1989; Reeves 2003). They are potential tools for phytoremediation, a new technology based on the use of plants to remove metals from contaminated sites (Baker et al. 1994). *Leersia hexandra*, a new chromium hyperaccumulator found in China, could accumulate up to 5608 mg kg^{-1} Cr in its leaves and was suggested to be potentially used in clean-up chromium in metal contaminated soil or wastewater (Zhang et al. 2007). However, it is unclear why *L. hexandra* can accumulate such high levels of Cr and how it tolerates Cr. Uncovering Cr resistance mechanism in this hyperaccumulating plant is essential to understand Cr hyperaccumulation and the evolution of this unique capacity.

Very few studies have an attempted to identify the chemical form of Cr in plants. Skeffington et al. (1976) used solvent extraction and high voltage paper electrophoresis to determine the chemical forms of Cr in plant tissue. Lytle et al. (1998) and Zayed et al. (1998) used high-energy X-ray absorption spectroscopy (XAS) to determine Cr chemical species in tissue of several vegetable crops and wetland plant species. In this study, a sequential extraction procedure proposed by Yang et al. (1995) was carried out to separate Cr in the plant tissues into six different chemical forms: (1) inorganic Cr giving priority to nitrate/nitrite, chloride, and aminophenol chromium, (2) water-soluble Cr of organic acid, (3) Pectates and protein integrated Cr, (4) undissolved chromium phosphate, (5) chromium oxalic, (6) residual fraction. The purpose of this study was to assess the Cr bioaccumulation ability of *L. hexandra* and determine the Cr distribution in the deferent chemical forms in plant tissues.

Materials and Methods

Seedlings of *L. hexandra* were collected from a paddy field in Guilin, China. Chromium concentrations in the tissue of *L. hexandra* grown in this site were 9.17–41.5 mg kg^{-1}. The seedlings were washed with redistilled water for three times and placed in 15 cm diameter round plastic pots

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filled with three liters half strength Hoagland's nutrient solution in a greenhouse (12 h photoperiod; 25°C day/20°C night, relative humidity 70%–75%). After 15d, Cr treatment was conducted. Cr solution (as $CrCl_3$) was added to the pots in four levels: 0, 5, 30, 60 mg L^{-1}. Each treatment had three replicates, 25–30 plants per replicate. The solutions were renewed every three days to maintain the chromium concentration and species during the 60d culture period.

Determination of Cr chemical forms was carried out using the method of Yang et al. (1995). Chromium in different chemical form was extracted in the order of the extraction solutions listed below: (1) 80% ethanol, extracting inorganic Cr giving priority to nitrate/nitrite, chloride, and aminophenol chromium (F1); (2) Distilled water (d-H_2O), extracting water-soluble Cr of organic acid (F2); (3) 1 M NaCl, extracting Pectates and protein integrated Cr (F3); (4) 2% HAC, extracting undissolved chromium phosphate (F4); (5) 0.6 M HCl, extracting chromium oxalic (F5).

The fresh plant tissues were homogenized in extraction solution with a mortar and a pestle, diluted at the ratio of 1:100 (w/v) and shaked for 22 h at 25°C. The homogenate centrifuged at 5000*g* for 10 min, obtaining the first supernatant solution in a flask bottle. The sedimentation was re-suspended twice in extraction solution and shaked for 2 h at 25°C, centrifuged at 5000*g* for 10 min, and then pooled the supernatant of the three suspending and centrifuge steps for each of the five extraction solutions. Each of the pooled supernatant solution and the residue (F6) were evaporated on an electric-plate at 70°C to constant weight, then digested with a mixture of HNO_3 and $HClO_4$ (5:3, v:v). The concentrations of Cr in each fraction were determined by flame atomic absorption spectrophotometer (PE-AA700).

Sixty days after Cr treatment, the plants were harvested and washed with ultrapure water for three replicates. The washed plants were separated into roots, stems and leaves. They were first dried at 105°C for 30 min, and then at 70°C for 48 h to constant weight. The biomass (dry weight, DW) was determined. Furthermore, the dried plant tissues were ground with 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) that was heated on an oven. After cooling, the extracts were diluted up to 50 mL 0.2% HNO_3. Chromium concentrations of the extract were determined by AAS.

One-way ANOVA was used to test the significance of differences among biomass and proportion of Cr in different chemical forms. Means of plant biomass and proportion of different Cr chemical forms in plant tissues were compared with least significant difference method (LSD).

Results and Discussion

Root, stem, leaf and total biomass of *L. hexandra* decreased with the increasing Cr concentration in nutrient solution, except for the leaf biomass with 5 mg L^{-1} Cr treatment (Table 1). Although the Cr treatments decreased the biomass of roots, stems and leaves, there were no significant differences among all the Cr-treated plants and control ($p > 0.05$). At lower Cr concentration treatments ($\leq$30 mg L^{-1}), the biomass of roots, stems, leaves and total were only decreased by 8.2%, 11.6%, 21.5% and 13.2% respectively. Even at highest Cr concentration treatment (60 mg L^{-1}), the reduction of root, stem, leaf and total biomass was not significant ($p > 0.05$).

The Cr concentrations in roots, stems and leaves of *L. Hexandra* cultivated in the nutrient solution containing different Cr concentration are listed in Table 2. A great bioaccumulation capacity for Cr was observed in the leaves, stems and roots of *L. Hexandra*. The maximum Cr concentrations in the leaves, stems and roots were 5430 mg kg^{-1}, 1956 mg $kg^{-1}$and 40599 mg kg^{-1} respectively. When the Cr concentration in nutrient solution was high ($\geq$30 mg L^{-1}), Cr concentrations in leaves were higher than 1000 mg kg^{-1}, the minimum Cr concentration for a Cr-hyperaccumulator. However, the translocation factors (ratio of Cr concentrations in leaves to those in roots) were decreased with the increasing of Cr concentration in nutrient solution. In comparison with the control plants, the translocation factor in the plants exposed to 60 mg L^{-1} Cr deceased by 91.5%.

The Cr distribution in the different chemical forms was significantly different between the control and the Cr-treated plants (Tables 3, 4). For control plants, the Cr form extracted by 1 M NaCl (F3) was predominant in the roots, accounting for 71.40% of the total Cr amount, followed by F6 (14.72%) and F4 (13.88%), while the forms extracted by 80% ethanol (F1), d-H_2O (F2) and 0.6 M HCl (F3) were not detected. However, in the roots with 60 mg L^{-1} treatment, the residue Cr (F6) occupied the largest proportion of the total Cr (90.40%), followed by F5 (6.29%), and F1 had the lowest Cr. Moreover, Cr treatment obviously increased the proportion of the oxalic integrated

Table 1 Biomass of *L. hexandra* with different Cr treatment

Treatment (mg L^{-1})	Biomass (g pot^{-1}, dry weight)			
	Root	Stem	Leaf	Total
0	9.17 ± 1.33	8.82 ± 1.88	8.92 ± 3.08	26.91 ± 4.95
5	8.42 ± 2.39	7.87 ± 2.13	9.05 ± 3.05	25.34 ± 7.13
30	8.55 ± 3.29	7.79 ± 1.63	7.01 ± 1.54	23.35 ± 6.41
60	6.96 ± 1.47	5.50 ± 0.51	5.57 ± 0.60	18.04 ± 0.83

Results are means ± SD, n = 3

Table 2 Cr concentration in plant tissue of *L. hexandra* with different Cr treatment

Treatment (mg L^{-1})	Cr concentration (mg kg^{-1} DW)			Leaf/root
	Root	Stem	Leaf	
0	34.38 ± 7.07	23.49 ± 1.66	36.66 ± 7.06	1.07 ± 0.07
5	993.4 ± 395.9	335.7 ± 214.1	891.4 ± 68.82	0.98 ± 0.33
30	20253 ± 1999	930.4 ± 226.8	4302 ± 978.8	0.21 ± 0.03
60	33048 ± 7201	1486 ± 443.3	2932 ± 581.9	0.09 ± 0.01

Results are means ± SD, n = 3

Table 3 Cr concentration of different chemical forms in *L. hexandra* with different Cr treatment

Treatment (mg L^{-1})	Tissue	Cr concentration (mg kg^{-1} FW)							Recoveries (%)
		F1	F2	F3	F4	F5	F6	Total	
0	Root	nd	nd	4.23 ± 0.82	0.82 ± 0.45	nd	0.86 ± 0.21	5.91 ± 0.50	110.3
	Stem	nd	nd	2.06 ± 0.37	1.19 ± 0.44	0.74 ± 0.13	0.94 ± 0.44	4.93 ± 1.15	88.0
	Leaf	nd	0.64 ± 0.17	2.23 ± 0.12	1.06 ± 0.11	0.48 ± 0.17	0.83 ± 0.30	5.26 ± 0.20	94.6
5	Root	nd	2.12 ± 0.95	11.31 ± 2.28	8.91 ± 1.17	19.60 ± 3.18	104.87 ± 5.17	146.8 ± 9.29	94.9
	Stem	nd	4.01 ± 1.29	3.49 ± 0.40	7.43 ± 0.57	28.97 ± 2.45	23.91 ± 2.42	67.81 ± 1.69	84.6
	Leaf	nd	10.17 ± 2.51	19.24 ± 2.03	15.33 ± 3.34	41.14 ± 0.15	48.60 ± 10.49	134.5 ± 23.5	99.5
30	Root	nd	22.78 ± 6.41	12.37 ± 1.29	60.14 ± 43.92	255.7 ± 21.3	2606 ± 770	2957 ± 830	93.7
	Stem	nd	12.42 ± 2.07	6.77 ± 0.79	21.77 ± 4.09	85.5 ± 9.2	108.3 ± 25.9	234.8 ± 29.5	105.7
	Leaf	nd	16.42 ± 7.43	14.96 ± 2.62	58.77 ± 3.89	205.3 ± 37.6	318.2 ± 58.5	613.7 ± 106.5	94.1
60	Root	5.19 ± 1.35	68.24 ± 20.68	15.42 ± 2.80	84.92 ± 21.20	329.5 ± 75.1	4708 ± 808	5211 ± 919	101.2
	Stem	1.25 ± 0.48	13.93 ± 4.30	9.52 ± 0.89	22.58 ± 7.20	130.8 ± 68.2	126.4 ± 76.4	304.5 ± 156.4	85.8
	Leaf	0.51 ± 0.23	19.25 ± 1.43	11.76 ± 0.90	38.04 ± 4.60	148.7 ± 5.6	220.8 ± 38.9	439.0 ± 43.6	98.8

Results are means ± SD, n = 3; nd = no detect

Table 4 Proportion of different Cr chemical forms in *L. hexandra* with different Cr treatment

Treatment (mg L^{-1})	Tissue	Percentage (%)					
		F1	F2	F3	F4	F5	F6
0	Root	–	–	71.40 ± 11.18a	13.88 ± 7.36b	–	14.72 ± 4.10f
	Stem	–	–	42.20 ± 3.24b	23.74 ± 3.45a	15.55 ± 4.75d	18.51 ± 4.50f
	Leaf	–	12.21 ± 2.73a	42.62 ± 3.92b	20.18 ± 2.36a	9.19 ± 2.93ef	15.80 ± 5.57f
5	Root	–	1.48 ± 0.76e	7.67 ± 1.16d	6.06 ± 0.64d	13.31 ± 1.53de	71.49 ± 1.61b
	Stem	–	5.91 ± 1.87bc	5.14 ± 0.53de	10.96 ± 0.98bc	42.74 ± 3.73a	35.25 ± 3.2e
	Leaf	–	7.54 ± 1.01b	14.53 ± 2.25c	11.38 ± 1.28bc	30.60 ± 0.36c	35.94 ± 1.77e
30	Root	–	0.77 ± 0.08e	0.43 ± 0.07e	1.93 ± 1.28e	8.95 ± 1.72ef	87.91 ± 1.29a
	Stem	–	5.30 ± 0.70c	2.89 ± 0.18de	9.22 ± 0.64 cd	36.89 ± 6.61b	45.70 ± 6.11cd
	Leaf	–	2.61 ± 0.77de	2.44 ± 0.01de	9.70 ± 1.08bcd	33.43 ± 1.82bc	51.82 ± 1.39c
60	Root	0.10 ± 0.02b	1.30 ± 0.27e	0.30 ± 0.01e	1.62 ± 0.28e	6.29 ± 0.32f	90.40 ± 0.72a
	Stem	0.50 ± 0.37b	5.04 ± 1.71c	3.62 ± 1.50de	7.95 ± 1.57cd	42.97 ± 1.29a	39.92 ± 5.66de
	Leaf	0.12 ± 0.07a	4.40 ± 0.40cd	2.69 ± 0.17de	8.65 ± 0.22cd	34.11 ± 3.86bc	50.03 ± 4.20c

Results are means ± SD, n = 3. Values followed by same letters are not significantly different at $p < 0.05$, according to least significant difference method (LSD)

Cr (F5) in leaves and stems, and proportion of residue Cr (F6) in roots, but significantly decreased the proportion of NaCl-extracted Cr (F3) ($p < 0.05$). For instance, application of 60 mg L^{-1} Cr increased the proportion of F5 by 3.7-folds in leaves and by 2.8-folds in stems, and increased the proportion of F6 by 6.1-folds in roots.

Plants ideal for phytoremediation should possess multiple traits. They must be fast growing, have high biomass, deep roots, be easy to harvest and should resistant and accumulate a range of heavy metals in their aerial and harvestable parts (Clemens et al. 2002; Wei et al. 2005). In this study, under experiment conditions, the aboveground

biomass (the sum of dry stems and leaves) of *L. hexandra* did not significantly decreased compared with the control when they are growing in medium contaminated by chromium seriously (Table 1). Although there were some extent restricted Cr movement from roots to leaves, the Cr accumulation in leaves of *L. hexandra* was great higher than 1000 mg kg^{-1}, the critical concentration standards for a Cr-hyperaccumulator suggested by Baker and Brooks (1989). Moreover, this species can grow rapidly and densely in Cr-contaminated medium, and easily adapts to artificial cultivation. These results and the field data previously reported (Zhang et al. 2006), corroborate that *L. hexandra* is a suitable candidate for the reclamation of Cr contaminated soil and water.

Very few researchers have reported resistance mechanisms of chromium in plants. Root sequestration was suggested to be an important mechanism of heavy metal resistance of plants (Tang et al. 1999; Liu et al. 2004). In the present work, the reduction of translocation factors by Cr supply indicated that the roots of *L. hexandra* could accumulate substantial amounts of Cr and restrict them transport to stems and leaves, accordingly protect the stems and leaves from phytotoxicity of higher concentration of Cr. Moreover, the most accumulations of Cr in root were observed in residue fraction (F6), which indicated that the high Cr concentration accumulated in roots might be bound to the low bioavailability forms. In addition, a high proportion of the oxalic integrated Cr was found in the leaves of *L. hexandra* with Cr treatment, which was similar to the finding of Lytle et al. (1998), who suggested a large portion of Cr in leaves of *E. crassipes* might be bound to oxalate ligands. Previous research indicated organic acids were important metal chelators in hyperaccumulator and played an important role in metal accumulation and detoxification in plants. Krämer et al. (2000) reported that about 28% of Ni was coordinated by citrate in the leaves of *T. goesingense* and mainly located in vacuole. Tolra et al. (1996) found that there was a positive correlation between the soluble Zn concentration in shoots of *T. careulescens* and the concentrations of malic acid and oxalic acid. Oxalate is a strong dicarboxylic acid, and acts as a metal chelator. Its role in resistance of buckwheat to Al toxicity was well demonstrated (Ma et al. 1997). The presented research suggested that the high oxalic integrated Cr content in leaves might be related to the Cr resistance in *L. hexandra*.

In conclusion, *L. hexandra* had a great resistance and accumulation capacity for Cr. The aboveground biomasses of *L. hexandra* were not significantly reduced when the polluted levels in medium are high enough to make the contents of heavy metals absorbed by plants reaching the critical concentration standards what hyperaccumulators should accumulate. Therefore, this species has the potential to be used for the in situ phytoremediation of Cr-contaminated soil and water. The sequestration of root, along with the high concentration of residual Cr in roots and oxalic integrated Cr in leaves, may be related to Cr resistance and accumulation in *L. hexandra*.

Acknowledgements The authors thank the financial supports from the Scientific Research and Technological Development Project of Guangxi (GuiKeGong 0816003-1-1), the Natural Science Foundation of China (No.40663002, 20665003) and the Provincial Natural Science Foundation of Guangxi (GuiKeNeng 0701K005, GuiKeZi 0728222). Financial assistance for this research was also provided through the Program to Sponsor Teams for Innovation in the Construction of Talent Highlands in Guangxi (GuiKeRen 2007-71, 2004217).

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