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Phytoremediation of Zn- and Cr-Contaminated Soil Using Two Promising Energy Grasses

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Abstract The outstanding biological performance and non-food utilization of bioenergy grass possibly make it to be the best candidate for phytoremediation of heavy metal-contaminated soil, but evidence is limited. In this study, we conducted pot experiments to quantify the performance of two promising energy grasses, *Arundo donax* and *Miscanthus sacchariflorus*, in the phytoremediation of Zn- and Cr-contaminated soil. The results showed that (1) the biomass and root length of the two grasses were firstly increased and then kept stable or slightly decreased with increasing soil Zn/Cr concentration, implying that the two grasses had strong tolerance to Zn/Cr contamination; (2) the Zn/Cr concentration in the grass roots was two to seven times of that in the shoots, while both of them were positively correlated with the Zn/Cr concentration in soil; (3) the total accumulation of Zn/Cr in the grass (shoots + roots) was firstly determined by their concentration in the shoots and secondly determined by the shoots' biomass, indicating that most of the Zn/Cr could be removed from contaminated soil by harvesting the aboveground parts; (4) the accumulating amount of the two grasses for Zn were 17.5 and 12.1 mg plant^{-1}, respectively; while the accumulating amount for Cr were 3.9 and 2.9 mg plant^{-1}, respectively. Taken together, the two energy grasses had strong tolerance and high accumulating ability for Zn/Cr, and therefore, they are promising candidates for the phytoremediation of Zn-/Cr-contaminated soil.

Keywords *Arundo donax* · *Miscanthus sacchariflorus* · Heavy metal contamination · Soil contamination · Soil remediation · Hyperaccumulator

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1 Introduction

Soil contamination by heavy metals (e.g., Zn, Cu, Pb, Ni, Cr, Cd, and As), mainly caused by the fast development of metal smelting industry, inappropriate disposal of sewage sludge, and intensive human activities, is becoming serious environment problems all over the world (Garbuio et al. 2012; Guo et al. 2006). It has been reported that the high concentration of heavy metals decreased the activity of soil enzymes (Wang et al. 2007) and the diversity of soil microbes (Giller et al. 2009) and finally caused the deterioration of soil quality (Wu et al. 2010). Moreover, heavy metals affected the physiological processes of some plants and subsequently led to their abnormal growth and decrease in biomass (Schützendübel and Polle 2002). Most importantly, heavy metals possibly transported from soil to plant, then accumulated in the body of animals or human beings through food chains, and finally caused heavy metal poisoning (Järup 2003; Liu et al. 2013). Nowadays, many efforts and several technologies have been used for the remediation of soil contaminated by heavy metals (Shi et al. 2009; Ouhadi et al. 2010; Gu et al. 2013). Martin and Ruby (2004) reviewed seven in situ remediation technologies for soil contamination and subsequently concluded that each technology has both strengths and weaknesses for addressing particular situation. Generally, physical and chemical remediation technologies are usually expensive and are only applicable for a small area, and sometimes, chemical methods possibly produce secondary pollution due to the addition of chemical materials (Okieimen 2011). Compared to physical and chemical technologies, biological remediation technologies are generally cost-effective and environment-friendly, and certainly they are commonly regarded as the most promising remediation technologies for soil contamination by heavy metals (Kavamura and Esposito 2010; Ali et al. 2013).

Phytoremediation, defined as the use of plants to clean pollutants from environment or make them harmless by David E Salt et al. (1995), is a kind of clean, cost-effective, and green remediation technologies (Flathman and Lanza 1998). Phytoremediation technologies were fast developed in the past two decades and recently are widely used in the remediation of contaminated soil (Chaudhry et al. 1998), especially in large fields where other remediation technologies are expensive or not practicable (Garbisu and Alkorta 2003). However, the effectiveness and efficiency of phytoremediation technologies are largely dependent on the physiological characteristics of the selected plants and the kind of pollutants (Megateli et al. 2009). Many studies confirmed that the cleanup ability of different plants for different heavy metals is greatly different (Citterio et al. 2003; Murakami and Ae 2009). Thus, it is most important to find specific plants for a specific heavy metal in the application of phytoremediation technologies (Sarma 2011). Researchers have screened out a number of special plants called hyperaccumulators (Kramer 2010), which do not only grow well in heavy metals contaminated soil but also accumulate extraordinary heavy metals in their harvestable parts. It has been reported that *Arabidopsis halleri* (Kashem et al. 2010), *Thlaspi caerulescens* (Pence et al. 2000), *Potentilla Griffithii* Hook (Qiu et al. 2006), and *Sedum alfredii* (Yang et al. 2004) are hyperaccumulators for Zn, while *Leersia hexandra* Swartz (Zhang et al. 2006) and *Spartina argentinensis* (Redondo-Gómez et al. 2011) are hyperaccumulators for Cr. However, most of these hyperaccumulators only have small biomass and require regular and precise management practices (Gleba et al. 1999). Moreover, there is no suitable utilization for these hyperaccumulators, which enrich a large amount of heavy metals and easily release them to surrounding environment again. Therefore, energy plants/grasses could be promising candidates of hyperaccumulator because they usually have large biomass, strong stress resistance, and non-food ways of utilization (directly burned or converted into bioenergy) (Lewandowski et al. 2003; Rascio and Navari-Izzo 2011).

A. donax and *M. sacchariflorus* are two promising perennial energy grasses due to their fast growth rate (Fan et al. 2010), very high biomass (30–40 t ha^{-2}) (Mantineo et al. 2009), strong stress resistance (e.g., cold, drought, salt, and barren) (Lin et al. 2013; Zeng et al. 2013), and high conversion rate into ethanol (Scordia et al. 2012). Their outstanding biological performance and non-food utilization possibly make them to be the best candidates for the phytoremediation of soil contaminated by heavy metals, but evidence is limited at present. On the other hand, Zn and Cr are two common types of heavy metals in soil, and their contamination is widely distributed all over the world due to the leakage from mining and refining activities and intensive use in galvanization industries (Shanker et al. 2005). Owing to the above reasons, the pot experiments were conducted in this study to evaluate the tolerance and accumulating ability of *A. donax* and *M. sacchariflorous* for heavy metals Zn and Cr and finally to quantify their performance in the phytoremediation of Zn- and Cr-contaminated soil.

2 Materials and Methods

2.1 Experimental Design

Two heavy metals (Zn and Cr) and two grasses (*A. donax* and *M. sacchariflorus*) were considered in this study. Five levels of heavy metal concentration (Zn—0, 250, 500, 1,000, and 2,000 mg kg^{-1}; Cr—0, 125, 250, 500, and 1,000 mg kg^{-1}) were set according to the Environmental Quality Standard for Soils of China, GB15618-1995 (National Environmental Protection Agency of China, 1995). There were totally 20 treatments (two grasses×two heavy metals×five levels of heavy metal concentration), and each treatment had four replications.

2.2 Plant and Soil

The rhizomes of *A. donax* L. and *M. sacchariflorus* (Maxim.) Benth. were collected from the grass garden. The soil came from top 20 cm of local farmland (116° 26' E, 40° 10' N and 50 m in altitude) in the north of Beijing. The collected soil was firstly air-dried and then sieved through 2-mm screen. The physical and chemical properties of the soil were listed as follows: silt loam texture (30 % sand, 55 % silt, and 15 % clay), pH=7.2, soil organic matter=21.3 g kg^{-1}, total nitrogen= 1.5 g kg^{-1}, total phosphorus=1.1 g kg^{-1}, alkali-hydrolyzed nitrogen=97.7 mg kg^{-1}, and available phosphorus=11.2 mg kg^{-1}. The background values of Zn and Cr in the used soil were 88.3 and 67.5 mg kg^{-1}, respectively, which was not included in the Zn/Cr concentration in the experimental design.

According to the experimental design, 72 pots with 45-cm diameter and 33-cm height were prepared in the greenhouse. Each pot was filled with 8 kg of air-dried soil and evenly mixed with the designed amount of Zn/Cr (the amount of soil multiplied by the designed concentration of Zn/Cr). The Zn and Cr in soil were supplied with the form of $ZnCl_2$ and K_2CrO_4, respectively. After about 5 days, each prepared grass containing two buds, defined as two plants in the experiment, was planted in the middle of each pot. Necessary management practices, including sufficient irrigation (to keep soil water content at about 80 % of the field capacity) and weed control, were regularly performed during the experiments.

2.3 Sampling and Measurement

After 60 days, the aboveground (shoots) and belowground parts (roots) of the two grasses were completely harvested by hands, respectively, for each pot. The aboveground parts of the grasses were cut into segments by scissors. The belowground parts of the grasses was firstly separated from soil by screens and then washed out with running water. All the roots were scanned with an ordinary scanner and analyzed with the root analysis system (WinRHIZO, Regent Instruments Inc. in Canada) to obtain the total root length.

The third, fourth, and fifth leaves of each grass were collected to measure the physiological indices listed as follows: (1) Chlorophyll content—about 0.5 g of samples were cut into pieces and immersed into the acetone-ethanol mixtures (1:1, *v*/*v*) for 24 h in a dark environment; then, the chlorophyll content was measured with the spectrophotometry (DR5000, HACH in USA) at 646- and 663-nm wavelength according to Wellburn (1994). (2) Malondialdehyde (MDA) content—at first, about 1 g of samples was cut into pieces and put into 10 mL of trichloroacetic acid and then centrifuged for 10 min at 4,000 r min^{-1}; after that, 2 mL of supernatant was sampled, and 2 mL of thiobarbituric acid (0.6 %) was added; next, the mixtures were boiled for 15 min and quickly cooled down to room temperature; finally, the MDA content was measured with the spectrophotometry at 450-, 532-, and 600-nm wavelength according to Hodges et al. (1999).

About 2 g of shoots and roots of the grasses in each pot were sampled, and then digested in the mixed acid (HNO_3/$HClO_4$=5:1, *v*/*v*) with the procedure of LY/T1270-1999 (National Standard of China, 1999). The concentration of Zn and Cr in the samples was measured with the flame atomic absorption spectrophotometer (AA-6300C, Shimadzu in Japan).

At last, the grass shoots and roots were dried at 105 °C for 0.5 h and then at 80 °C for 24 h to obtain the aboveground and belowground biomass, respectively.

2.4 Data Analysis

The experimental data were analyzed based on the descriptive statistics in SPSS 20, and the final results of each treatment came from the mean values of the four replications and expressed as mean ± standard error. The differences between the treatments were statistically evaluated at 5 % probability level by the three-way ANOVA and the multiple comparisons tests (LSD). The correlation and regression analysis were also conducted in SPSS 20. The representation and graphical fits of experimental data were obtained using OriginPro 9.0.

3 Results

3.1 Effects of Zn and Cr on Grass Biomass

From the appearance (e.g., height, leaf area, leaf color) of the two grasses, we observed no obvious symptoms of heavy metal toxicity, implying that the two grasses grew normally in the Zn-/Cr-contaminated soil during the experiment. This result was confirmed by the biomass of the two grasses listed in Table 1.

Table 1 showed that the biomass of *A. donax* linearly (r=0.99, p=0.01) increased from 71.7 to 104.5 g plant^{-1} and then decreased to 74.4 g plant^{-1} with increasing soil Zn concentration, whereas the biomass of *M. sacchariflorus* increased from 28.3 to 47.9 g plant^{-1} and then linearly (r=−0.96, p=0.04) decreased to 24.1 g plant^{-1} with increasing soil Zn concentration. However, the differences in the grass biomass among the Zn treatments were insignificant (F=0.81, p=0.57 for *A. donax*; F=0.98, p=0.49 for *M. sacchariflorus*). Similarly, the biomass of the two grasses slightly increased at first and then sharply decreased with increasing soil Cr concentration. Table 1 showed that the differences in the biomass of *A. donax* among the Cr treatments were insignificant (F=0.60, p=0.68), while the differences in the biomass of *M. sacchariflorus* among the Cr treatments were significant (F=4.53, p=0.04).

The biomass given in Table 1 consistently showed that the presence of Zn/Cr in soil increased the biomass of the two grasses at a low concentration range (less than 1,000 mg kg^{-1} for Zn; less than 500 mg kg^{-1} for Cr) as well sharply decreased their biomass at a high concentration range. Meanwhile, the biomass of *A. donax* was greatly higher (2.3 times) than that of *M. sacchariflorus* in all the treatments, but the differences between the two grasses were insignificant (F<14.62, p>0.06). In addition, the biomass of the two grasses in the Zn treatments was a little higher (1.3 times) than that in the Cr treatments, but these differences were also insignificant (F<4.05, p>0.18).

3.2 Effects of Zn and Cr on Grass Root Length

In Fig. 1, each plant of the two grasses produced at least 150-m roots, showing that the growth rate of the two grasses was very fast. Like the biomass in Table 1, the root length of the two grasses was firstly increased and then decreased with increasing Zn/Cr concentration in soil (Fig. 1), but the changes were not remarkable. The longest root length of the two grasses in the Zn-contaminated soil reached 307.7 and 367.2 m plant^{-1}, respectively. Correspondingly, the root length of the two grasses in the Cr-contaminated soil reached 179.2 and 378.4 m plant^{-1}, respectively. The differences in the root length among the treatments were insignificant (F=3.75, p>0.09) with only an exception for *A. donax* in the Zn-contaminated soil (F=16.47, p=0.002). Figure 1 also gave the result that the root length of the two grasses in the Zn treatments was a little higher (about 10 %) than that in the Cr treatments, but the differences were insignificant (F<15.59, p>0.06).

3.3 Effects of Zn and Cr on the Physiological Index of Grass

Table 2 showed that the chlorophyll content in the leaves of the two grasses was generally slightly decreased with increasing soil Zn/Cr concentration, whereas the MDA content was generally slightly increased with increasing soil Zn/Cr concentration. However, there were no significant differences (F<1.31, p>0.38) in the chlorophyll and MDA content among the different treatments except for *A. donax* in the Cr treatments. In Table 2, it was shown that the chlorophyll content of *A. donax* was significantly (F=2.82, p=0.04) decreased and correspondingly the MDA content was significantly (F=3.65, p=0.02) increased when the soil Cr concentration exceeded 1,000 mg kg^{-1}. The results of the chlorophyll and MDA content listed in Table 2 indicated that the two grasses had strong tolerance ability to soil Zn/Cr contamination, but their physiological activities and processes would be possibly inhibited if the heavy metal concentration (especially for Cr) in soil exceeded the threshold values. For example, the physiological activities (represented by the chlorophyll and MDA content) of *A. donax* was inhibited by the high concentration of Cr (more than 1,000 mg kg^{-1}).

3.4 Zn and Cr Concentration in Grass

The Zn/Cr concentration in the two grasses was linearly increased with increasing Zn/Cr concentration in soil (Zn—r>0.91, p<0.03; Cr—r>0.89, p<0.04), indicating that the Zn/Cr concentration in the grass and the soil was closely correlated with each other (Fig. 2). In Fig. 2, the Zn/Cr concentration in the shoots and roots among the treatments was significantly different (Zn—F=3.88, p<0.001 and F=60.29, p<0.001 for the two grasses; Cr—F=14.55, p=0.005 and F=10.79, p=0.011 for the two grasses). However, the significant differences in the Zn concentration almost existed between every two treatments, whereas the significant differences in the Cr concentration, especially in the shoots, only existed between the treatments of high and low soil Cr concentration (cutoff point was 500 mg kg^{-1}). In other words, the increase

Table 1 The biomass of *Arundo donax* and *Miscanthus sacchariflorus* after 60 days of growth in the Zn-/Cr-contaminated soil

Heavy metal	Concentration (mg kg^{-1})	Biomass of *Arundo donax* (g plant^{-1})			Biomass of *Miscanthus sacchariflorus* (g plant^{-1})		
		Shoots	Roots	Total	Shoots	Roots	Total
Zn	0	67.9±11.1a*	3.8±0.9a	71.7±12.0a	22.3±8.5a	6.0±1.8a	28.3±10.2a
	250	71.8±6.0b	3.1±0.6b	75.0±6.6a	37.6±13.9a	10.3±3.3a	47.9±17.1a
	500	82.7±12.3bc	3.6±1.3b	86.4±13.6a	36.9±8.6a	9.9±3.7a	46.8±12.3a
	1,000	97.2±22.8c	7.3±1.7b	104.5±24.0a	35.9±7.7a	8.3±0.9a	44.2±8.6a
	2,000	69.5±21.2c	4.9±1.3b	74.4±14.1a	19.2±3.2a	4.8±0.6a	24.1±3.8a
Cr	0	67.9±11.1a	3.8±0.9a	71.7±12.0a	22.3±8.5b	6.0±1.8a	28.3±10.2b
	125	68.8±11.3a	4.3±0.5a	73.0±11.8a	34.7±6.4ab	8.7±1.1a	44.9±4.2ab
	250	76.7±5.9a	4.0±0.5a	80.7±6.3a	35.6±3.2ab	9.3±0.9a	43.4±7.4ab
	500	74.3±13.1a	6.0±0.8a	80.3±12.3a	46.6±4.8a	11.1±3.2a	57.7±1.5a
	1,000	55.5±16.8a	3.6±1.0a	59.2±12.9a	21.2±3.9b	4.4±0.4a	25.6±3.4b

*Different letters in the same column indicate that there were significant differences at 5 % probability level

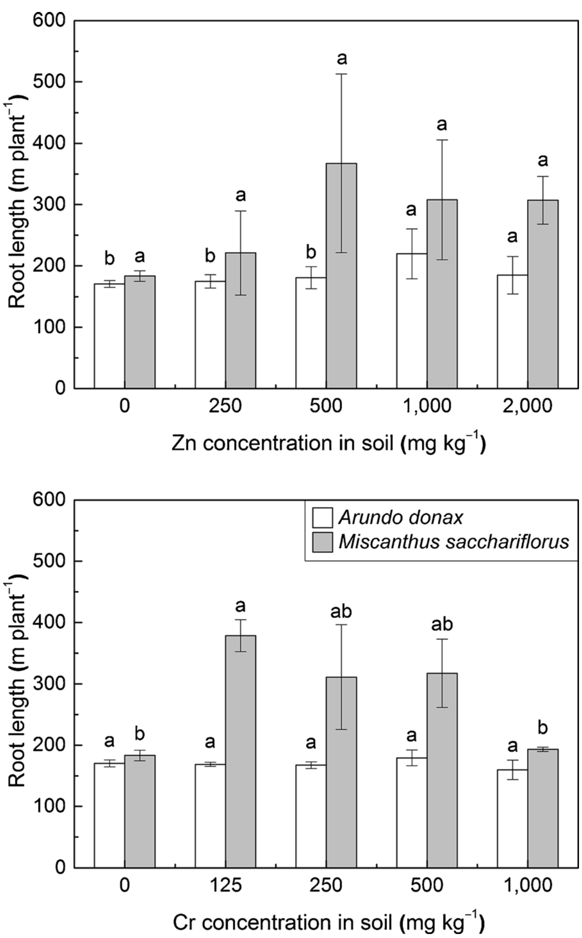

Fig. 1 The root length of *Arundo donax* and *Miscanthus sacchariflorus* after 60 days of growth in the Zn-/Cr-contaminated soil. *Different letters* on the bar for same grass indicate that there were significant differences at 5 % probability

of Zn concentration in soil always resulted in a significant increase of Zn concentration in grass, while the increase of Cr concentration in soil only resulted in a significant increase of Cr concentration in grass at a high concentration range (more than 500 mg kg^{-1}).

In addition, the Zn/Cr concentration in the grass roots was great, higher than that in the grass shoots. The Zn concentration in the roots of the two grasses was averagely 3.5 and 2.8 times of that in the shoots, respectively, whereas the Cr concentration in the roots was averagely 4.3 and 1.8 times of that in the shoots, respectively. The highest Zn concentration in the two grasses reached 541.3 and 1,105.1 mg kg^{-1}, respectively, whereas the highest Cr concentration reached 348.1 and 159.4 mg kg^{-1}, respectively.

Furthermore, the Zn concentration in *A. donax* was lower (63 and 77 % in the shoots and roots, respectively) than that in *M. sacchariflorus* (insignificant, $F<11.76$, $p>0.08$), whereas the Cr concentration in *A. donax* was higher (1.2 and 2.4 times in the shoots and roots, respectively) than that in *M. sacchariflorus* (insignificant, $F<18.78$, $p>0.05$). Lastly, we found that the concentration of Zn in the grasses was greatly higher (1.6 and 3.3 times for the two grasses) than the concentration of Cr in the grasses at the same soil Zn/Cr concentrations (significant, $F>23.5$, $p<0.04$).

3.5 Accumulation of Zn and Cr in Grass

The accumulation of Zn/Cr in the shoots and roots had a similar trend with the increase of Zn/Cr concentration in

Table 2 The physiological indices of *Arundo donax* and *Miscanthus sacchariflorus* after 60 days of growth in Zn-/Cr-contaminated soil

Heavy metal	Concentration (mg kg^{-1})	*Arundo donax*		*Miscanthus sacchariflorus*	
		Chl (a + b) (mg g^{-1})	MDA (μmol g^{-1})	Chl (a + b) (mg g^{-1})	MDA (μmol g^{-1})
Zn	0	2.11±0.18a*	15.91±1.10a	2.81±0.60a	29.69±1.14a
	250	2.22±0.27a	16.20±0.37a	2.83±0.62a	29.02±1.91a
	500	2.00±0.13a	15.70±1.41a	2.63±0.34a	31.00±0.22a
	1,000	2.00±0.14a	17.51±2.47a	2.72±0.19a	33.14±2.13a
	2,000	2.07±0.13a	17.11±0.70a	2.72±0.25a	31.85±0.69a
Cr	0	2.11±0.18a	15.91±1.10b	2.81±0.60a	29.69±1.14a
	125	1.79±0.22a	16.73±0.18ab	2.60±0.13a	30.21±1.40a
	250	1.70±0.06a	16.69±1.39ab	2.65±0.63a	29.49±2.24a
	500	1.75±0.12a	18.79±0.13ab	2.73±0.22a	30.91±0.87a
	1,000	1.49±0.13b	19.58±0.39a	2.61±0.27a	32.02±1.53a

*Different letters in the same column indicate that there were significant differences at 5 % probability level

soil, which showed that the amount of Zn/Cr accumulation in the grasses firstly increased and then kept stable or decreased with increasing Zn/Cr in soil (Fig. 3). For example, at first, the accumulation of Zn in *A. donax* linearly increased from 1.6 to 17.5 mg and then decreased to 14.0 mg, while the accumulation of Cr in *A. donax* firstly increased from 2.5 to 3.5 mg and then kept stable with increasing soil Cr concentration from 250 to 1,000 mg kg^{-1}. Figure 3 also showed that the Zn accumulation in the 1,000-mg kg^{-1} treatment was significantly higher than that in the other treatments (F> 9.71, p<0.01), whereas there were no significant differences in the Cr accumulation among the treatments (F< 3.61, p>0.10).

Moreover, Fig. 3 gave the result that the amount of Zn/Cr accumulation in the shoots was much greater than that in the roots (insignificant, F<14.77, p>0.06), indicating that the shoots uptook and stored more Zn/Cr

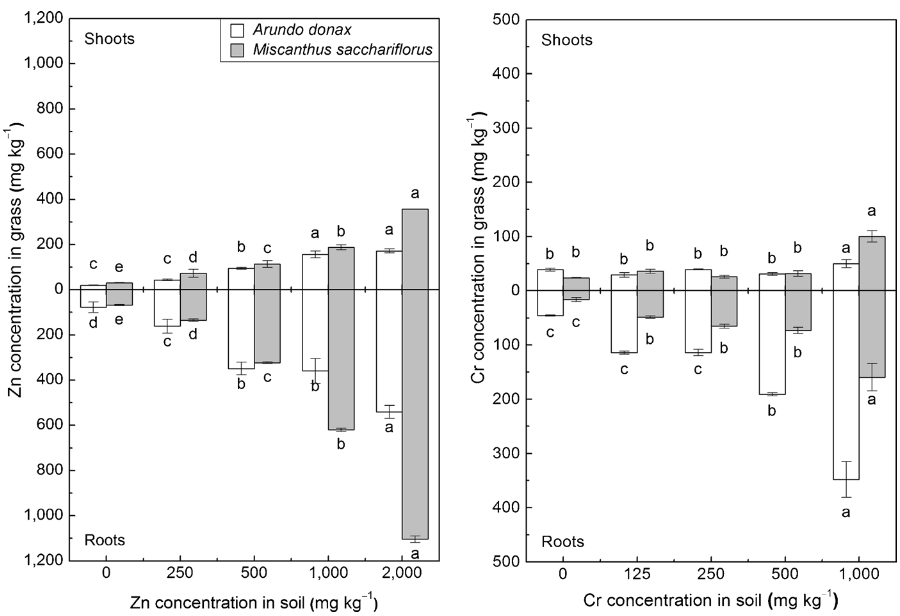

Fig. 2 The Zn/Cr concentration in *Arundo donax* and *Miscanthus sacchariflorus* after 60 days of growth in the Zn-/Cr-contaminated soil. *Different letters* on the bar for same grass indicate that there were significant differences at 5 % probability level

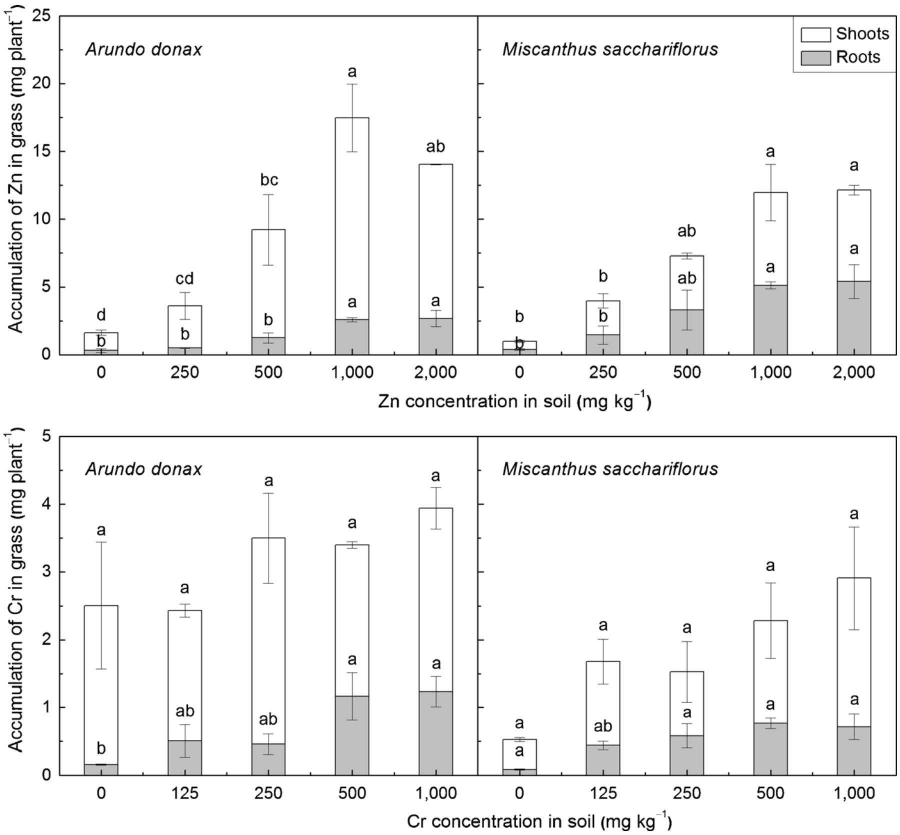

Fig. 3 The accumulation of Zn/Cr in *Arundo donax* and *Miscanthus sacchariflorus* after 60 days of growth in the Zn-/Cr-contaminated soil. *Different letters* on the bar for same grass indicate that there were significant differences at 5 % probability level

compared to the roots. In Fig. 3, the amount of Zn accumulation in the shoots was averagely 5.5 (up to 7.9) and 1.5 (up to 2.6) times of that in the roots for *A. donax* and *M. sacchariflorus*, respectively, whereas the amount of Cr accumulation in the shoots was averagely 6.4 (up to 17.3) and 3.0 (up to 5.2) times of that in the roots for the two grasses, respectively. In addition, most of the Zn/Cr accumulated in the shoots rather than the roots. In Fig. 3, the Zn accumulation in the shoots of the two grasses averagely accounted for 84 and 58 % of the total amount of Zn accumulation in the grasses (shoots + roots), while the Cr accumulation in the shoots of the two grasses averagely accounted for 79 and 72 % of the total amount of Cr accumulation in the grasses.

The Zn/Cr accumulation in *A. donax* was little higher than that in *M. sacchariflorus* (insignificant, $F<4.65$, $p>0.16$). The accumulation of Zn and Cr in *A. donax* was averagely 1.3 (up to 2.3) and 2.5 (up to 7.1) times of that in *M. sacchariflorus*, respectively. The highest Zn accumulation in the two grasses was 17.5 and 12.1 mg plant^{-1}, respectively, whereas the highest Cr accumulation was 3.9 and 2.9 mg plant^{-1}, respectively. Additionally, the Zn accumulation in the two grasses was 1.9 (up to 4.9) and 2.7 (up to 4.8) times higher than the Cr accumulation in the two grasses, but their differences were insignificant in most cases ($F<14.29$, $p>0.06$).

As a whole, the total amount of the added Zn/Cr were more than 1,000 mg for each pot, while the total amount of Zn/Cr accumulated by the two grasses in each pot was less than 35 mg. The recovery rate of the added Zn/Cr by the two grasses was less than 1 %, and it generally decreased with increasing Zn/Cr concentration in soil.

3.6 Correlation and Regression Analysis

Table 3 showed that the total Zn/Cr accumulation in the grasses was significantly correlated with the Zn/Cr concentration in the shoots, Zn/Cr concentration in the roots, and Zn/Cr concentration in the soil ($r>0.645$, $p<0.044$), respectively, with only an exception for Cr in *A. donax*. The amount of Zn/Cr in the grasses was closely correlated with their concentration in the grasses or the soil rather than the grass biomass.

The order of regression coefficient given in Table 4 demonstrated that the amount of Zn/Cr accumulation was significantly determined by the Zn/Cr concentration in the shoots at first (regression coefficient >0.81, $F>7.36$, $p<0.001$) and secondly by the biomass of the shoots (regression coefficient >0.42, $F>4.28$, $p<0.008$) with only an exception for Cr in *M. sacchariflorus*. The Zn/Cr concentration and the biomass of the roots had no significant influence on the amount of Zn/Cr accumulation in the grasses. Table 4 confirmed that the shoots of the two grasses made greater contribution to their ability in Zn/Cr accumulation compared to the roots.

4 Discussion

Biological parameters of plants, including height, aboveground and belowground biomass, root characteristics, chlorophyll, and MDA content, are usually regarded as the most important aspects in the evaluation of their performance in phytoremediation (Zhao et al. 2003). From this study, we found that the biomass and root length of *A. donax* and *M. sacchariflorus* were slightly decreased or even increased with the increase of Zn/Cr concentration in soil (Table 1 and Fig. 1), while the chlorophyll content in the two grasses did not significantly decrease and the MDA content did not significantly increase with the increase of Zn/Cr concentration in soil (Table 2). These results maybe caused firstly by the large biomass and perennial characteristics of the two grasses and secondly by the type of heavy metals, especially for Zn, which could improve plant growth at a low concentration. The results implied that the two grasses have strong tolerance ability to the high concentration of Zn (2,000 mg kg^{-1}) and Cr (500 mg kg^{-1}) contamination, which are much higher than the others plants. For example, Kausar et al. (2012) reported that the tolerance ability of *A. donax* for Zn was 900 mg kg^{-1}; An et al. (2006) found that the tolerance ability of *Pteris vittata* L. for Zn was 2,000 mg kg^{-1}; Han et al. (2004) gave a result that the tolerance ability of *Brassica juncea* for Cr was 100 mg kg^{-1}; Dong et al. (2007) recorded that the tolerance ability of *Typha angustifolia* L. for Cr was 42 mg kg^{-1}.

This study showed that the concentration of Zn/Cr in the two grasses (especially in the roots) was closely correlated with their concentration in the soil (Fig. 2), which is reasonable because the heavy metals were uptook by grass roots from soil and subsequently transported to shoots. In this study, the highest Zn and Cr concentration in the two grasses was 1,105.1 and 348.1 mg kg^{-1} (in the roots), respectively, which is below the criterion of the hyperaccumulator for Zn (10,000 mg kg^{-1}) and Cr (1,000 mg kg^{-1}) (Verbruggen et al. 2009). However, the ability of the two grasses to absorb and store Zn/Cr may be underestimated in this study due to the following two reasons. Firstly, the Zn/Cr concentration in the grasses may be greatly increased if the soil Zn/Cr concentration was higher in the experimental design because this study confirmed that the Zn/Cr concentration in grasses was linearly increased with their concentration in soil. Secondly, the growth of the grasses and their performance maybe largely restricted by the experimental conditions in this study, including limited soil in the pots and the short-term growth times (60 days). In spite of that, we still believed that *A. donax* and *M. sacchariflorus* are two promising candidates for the phytoremediation of Zn-/Cr-contaminated soil due to their large biomass (100 times more than the others plants) (Hu et al. 2009; Yang et al. 2004), non-food ways of utilization (avoiding secondary pollution and food safety risk problems) (Rascio and Navari-Izzo 2011), and high accumulating ability (Fig. 3).

Moreover, this study showed that the Zn/Cr concentration in the roots was greatly higher than that in the shoots, which was up to seven times in some treatments. This result agrees well with Guo and Miao (2010), who reported that the concentration of As, Cd, and Pb in the roots of *A. donax* was obviously higher than that in the shoots. The similar results were also obtained from other plants or other heavy metals (Brown et al. 1995; Shahandeh and Hossner 2000; Danh et al. 2009). Salt and Rauser (1995) described that heavy metals entered into roots by bounding to particles through metal-chelating molecules around rhizosphere. Once heavy metals entered into roots, it was either stored in roots or transferred to shoots. In some particular situations, heavy metals preferred to accumulate in roots and not to transfer to shoots, which maybe a special mechanism to protect plant from heavy metal toxicity (David E Salt et al. 1998).

From this study, we concluded that the amount of Zn/Cr accumulation in the grasses firstly determined by

Table 3 The Pearson correlation coefficient between the Zn/Cr accumulation in the grasses (shoots and roots) and the related factors

Grass	Heavy metal	Biomass of shoots	Biomass of roots	Heavy metal concentration in shoots	Heavy metal concentration in roots	Heavy metal concentration in soil
Arundo donax	Zn	0.571	0.823**	0.905**	0.763*	0.746*
	Cr	0.268	0.443	0.455	0.645*	0.498
Miscanthus sacchariflorus	Zn	0.193	0.067	0.776**	0.851**	0.827**
	Cr	0.258	−0.13	0.784**	0.759*	0.793**

*The Pearson correlation coefficient is significant at 5 % probability level

**The Pearson correlation coefficient is significant at 1 % probability level

their concentration in the shoots and secondly determined by the biomass of the shoots (Table 3). As we expected before, the large biomass of the two grasses and their strong tolerance to heavy metal contamination made a great contribution to their ability in heavy metal accumulation. In this study, the amount of Zn accumulation in each plant of the two grasses reached up to 17.5 and 12.1 mg, respectively, while the amount of Cr accumulation in each plant of the two grasses reached up to 3.9 and 2.9 mg, respectively. According to the references, the accumulating ability of the two grasses for Zn/Cr is higher than most of the other plants. For example, Ghosh and Singh (2005) recorded that the accumulation of Cr reached 0.15 mg in *Ipomoea carnea* and 0.03 mg in *B. juncea*; Murakami and Ae (2009) reported that the accumulation of Zn reached 3.7 mg in soybean, 1.7 mg in rice, and 1.6 mg in maize. However, He et al. (2010) found that the accumulation of Zn in *Orychophragmus violaceus* reached up to 179 mg, which is far away from our results. Moreover, the aboveground parts of the grasses accumulated more Zn/Cr compared to their belowground parts, indicating that most of the Zn/Cr in soil could be removed through harvesting their aboveground parts. This is exactly the most promising advantage of phytoremediation technologies compared to engineering technologies.

In this study, *A. donax* had larger biomass, higher concentration of Zn/Cr, and more accumulation of Zn/Cr than *M. sacchariflorus* at the same situations, implying that *A. donax* performed much better than *M. sacchariflorus* in the phytoremediation of Zn/Cr contamination. Additionally, we found that the two grasses had higher tolerance and better performance in Zn-contaminated soil compared to Cr-contaminated soil, especially at a low concentration range. To our knowledge, this result is reasonable because Zn is an essential element for plants (improving plant growth at low concentration) and the only metal represented in the six enzyme classes (Broadley et al. 2007), whereas Cr has no such effects on plant growth (Shanker et al. 2005).

5 Conclusions

The pot experiments were conducted in this study to quantify the performance of *A. donax* and *M. sacchariflorus* in the phytoremediation of Zn-/Cr-

Table 4 The regression coefficient among the Zn/Cr accumulation in the grasses (shoots and roots) and the related factors

Grass	Heavy metal	Biomass of shoots	Biomass of roots	Heavy metal concentration in shoots	Heavy metal concentration in roots
Arundo donax	Zn	0.420**	−0.022	0.907*	−0.074
	Cr	0.500**	0.392*	0.812**	0.258
Miscanthus sacchariflorus	Zn	0.799	−0.382	0.073	0.862
	Cr	0.592**	0.042	0.886*	0.179

*The regression coefficient is significant at 5 % probability level

**The regression coefficient is significant at 1 % probability level contaminated soil through their tolerance and accumulating ability at a high concentration range of 0–2,000 mg kg^{-1}. The results showed that (1) the biomass and root length of the two grasses were firstly increased and then kept stable or slightly decreased with increasing soil Zn/Cr concentration, implying that the two grasses had strong tolerance to Zn/Cr contamination; (2) the Zn/Cr concentration in the grass roots was two to seven times of that in the shoots, and both of them were significantly positively correlated with the Zn/Cr concentration in soil ($r>0.89$, $p<0.04$); (3) the accumulation of Zn/Cr in the grass (shoots + roots) was firstly determined by their concentration in the shoots and then determined by the shoot biomass, indicating that most of the Zn/Cr could be removed from contaminated soil by harvesting the aboveground parts; (4) the accumulating amount of the two grasses for Zn were 17.5 and 12.1 mg plant^{-1}, respectively, while the accumulating amounts for Cr were 3.9 and 2.9 mg plant^{-1}, respectively. We concluded that both *A. donax* and *M. sacchariflorus* had high tolerance and accumulating ability for Zn and Cr, and therefore, they are promising candidates for the phytoremediation of Zn- and Cr-contaminated soil.

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