Water Air Soil Pollut (2007) 180:131–139
DOI 10.1007/s11270-006-9256-1

Phytoextraction of Metal-Contaminated Soil by *Sedum alfredii* H: Effects of Chelator and Co-planting

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Received: 3 May 2006 / Accepted: 31 July 2006 / Published online: 3 November 2006

Abstract Phytoextraction is a promising technology that uses hyperaccumulating plants to remove inorganic contaminants, primarily heavy metals, from soils and waters. A field experiment was conducted to evaluate impacts of a mixture of chelators (MC) upon the growth and phytoextraction of heavy metals by the hyperaccumulator *Sedum alfredii* Hance in a co-planting system in a paddy soil that was historically irrigated with Pb and Zn contaminated mining waste-waters. The co-planting system used in this study was comprised of a Zn- and Cd-hyperaccumulator (*S. alfredii*) and a low-accumulating crop (*Zea mays*). Results showed that yields of *S. alfredii* were significantly increased with the addition of the MC and by co-planting with *Z. mays*. Our study further revealed that concentrations of Zn, Pb, and Cd in the corn grains of *Z. mays* conform to the Chinese hygiene standards for animal feeds and in the other parts of *Z. mays* conform to the Chinese organic fertilizer standards. The uptake of Zn, Cd, and Pb by *S. alfredii* was significantly increased with the addition of MC. The uptake of Zn by *S. alfredii* was also significantly enhanced by co-planting with *Z. mays*, but the interaction between MC and co-planting was not significant, meaning the effects of the two types of treatments should be additive. When the MC was applied to the co-planting system in the soil contaminated with Zn, Cd, and Pb, the highest phytoextraction rates were observed. This study suggested that the use of the hyperaccumulator *S. alfredii* and the low-accumulating crop *Z. mays* in the co-planting system with the addition of the MC was a more promising approach than the use of a single hyperaccumulator with the assistance of EDTA (ethylenediaminetetraacetic acid). This approach not only enhances the phytoextraction rates of the heavy metals but also simultaneously allows agricultural practices with safe feed products in the metal-contaminated soils.

Keywords co-cropping · mixed chelators · metals · phytoremediation · *S. alfredii* · *Zea mays*

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1 Introduction

Heavy metals are introduced into the environment naturally through the weathering of parent materials as well as from a variety of human activities. Certain heavy metals such as Cu and Zn are essential micronutrients required for the growth of organisms.

Table I Tolerance limit of heavy metals according to Chinese standards of foods, feeds and organic fertilizers (mg kg^{-1})[a]

	Foods		Feeds		Organic fertilizers	
	Standard value	Standard code	Standard value	Standard code	Standard value	Standard code
Cd	0.2	GB15201-1994	0.5	GB13078-2001	3	NY 525-2002
Pb	0.4	GB14935-1994	5.0		100	
Cu	10	GB15199-1994	NP[b]		NP[b]	
Zn	50	GB13106-1991	NP		NP	

[a] Food standards from CAPM (1998) and feed standards from Luo and Jiang (2003).

[b] *NP* No promulgation.

Other heavy metals such as Pb and Cd are not required for growth and have been considered to be noxious to human health and aquatic life. All heavy metals exert toxic effects at high concentrations, including those that are essential micronutrients. Heavy metal contamination in soils and waters has created a social health risk and is an issue of increasing environmental concern. Such contamination is often caused by human activities including mining, smelting, electroplating, and other industrial processes that have metal residues in their wastes.

Many remediation techniques have been employed to address the rising number of heavy metal contaminated sites. Most of the traditional methods such as soil washing/flushing, vitrification, electrokinetics, incineration and landfilling are extremely expensive (Mulligan, Yong, & Gibbs, 2001; Pulford & Watson, 2003). A promising and relatively new technology for remediation of heavy metal contaminated sites is phytoextraction. Several studies dealing with metal hyperaccumulator plants concluded that phytoextraction of heavy metals was a feasible remedial technology for decontamination of metal-contaminated soils (Baker, McGrath, Sidoli, & Reeves, 1994; Brown, Chaney, & Angle, 1995; Liao, Chen, Xie, & Xiao, 2004; Ma et al., 2001; McGrath, Zhao, & Lombi, 2002; Salt, Blaylock, & Kumar, 1995; Schwartz, Echevarria, & Morel, 2003). The success of phytoextraction depends on several factors, including the concentration of soil contaminants, soil metal bioavailability for root uptake, and plant capability to intercept, absorb, and accumulate metals in plant tissues (Chen, Lin, Luo, & He, 2003; Ernst, 1996). Ultimately, the success of phytoextraction depends on interactions among soils, metals, and plants. Several studies have documented that chelating agents such as ethylenediaminetetraacetic acid (EDTA) and citric acid can be used to increase metal mobility, thereby enhancing phytoextraction (Chen & Cutright, 2001; Chen et al., 2003; Cunningham, Berti, & Huang, 1993; Elless & Blaylock, 2000; Huang, Chen, Berti, & Cuningham, 1997; Wu, Luo, Xing, & Christie, 2004). However, EDTA is relatively expensive and can cause soil and groundwater contamination, and thereby a more ‘green’ enhancing chelator, the mixture of chelator (MC), has been exploited in recent years (Wu, Deng, & Long, 2004). The MC is comprised of monosodium glutamate waste liquid (MGWL), citric acid and EDTA at a mole ratio of 1:10:2. Advantages for using the MC are that this compound is less expensive, causes lower metal leaching from the soils into the underlying groundwater than that of EDTA.

Recently, a co-planting system (i.e., the growth of a metal hyperaccumulator plant associated with a low metal accumulating crop) was introduced simultaneously to phytoextract heavy metals as well as to practice an agricultural production for the contaminated sewage sludge (Wu, Samake, Mo, & Morel, 2002). Results show that this co-planting system can effectively remove the heavy metals from the sewage sludge by the hyperaccumulator plant while the harvested products from the agricultural crop meet the Chinese standards (Table I) for animal feeds (Liu, Wu, & Banks, 2005). However, a comprehensive literature search reveals that little study has been devoted to applying such a co-planting system in field experiments, nor to combining chelators with polyculture systems for further enhancement of phytoextraction of heavy metals. Therefore, the goal of this study is to test the feasibility of the removal efficiency of heavy metals from the contaminated soils using a

Table II Selected properties of the soil used in this study

pH	T–N ($g\ kg^{-1}$)	T–P ($g\ kg^{-1}$)	T–K ($g\ kg^{-1}$)	OM ($g\ kg^{-1}$)	CEC ($cmol\ kg^{-1}$)	DTPA-extractable Metals ($mg\ kg^{-1}$)		
						Zn	Cd	Pb
6.62	3.03	0.961	12.5	5.53	18.4	121	0.990	268

Total Zn ($mg\ kg^{-1}$)				Total Cd ($mg\ kg^{-1}$)				Total Pb ($mg\ kg^{-1}$)			
Block 1	Block 2	Block 3	Mean	Block 1	Block 2	Block 3	Mean	Block 1	Block 2	Block 3	Mean
774	572	532	626 (250)	2.39	2.07	1.85	2.04 (0.3)	912	716	690	762 (300)

The value in ( ) was the standard value of the heavy metal for the soils with pH from 6.5 to 7.5 for agricultural use according to the Chinese National Standard GB15618–1995.

co-planting system associated with the addition of the MC. Our specific objectives were to: (1) assess the ability of a MC for enhancement of phytoextraction of heavy metals under field conditions; (2) estimate the effects of a co-cropping system on the growth of *Sedum alfredii* and *Zea mays*, the extraction and accumulation of heavy metals by *S. alfredii*, and the production of safe feeds by *Z. mays* in a contaminated field site; and (3) evaluate the feasibility of combining a co-planting system and with the MC for remediation of heavy metal contaminated soils.

2 Materials and Methods

2.1 Site description

The field plot experiments were conducted on a paddy soil located at the Lechang Lead and Zinc Mining Site (25°07′ N; 113°22′ E) in Lechang District in Northern Guangdong Province, China. This site has a long history of surface irrigation with the Pb and Zn mining wastewaters. Lechang situates in a sub-tropical area with an average annual rainfall of

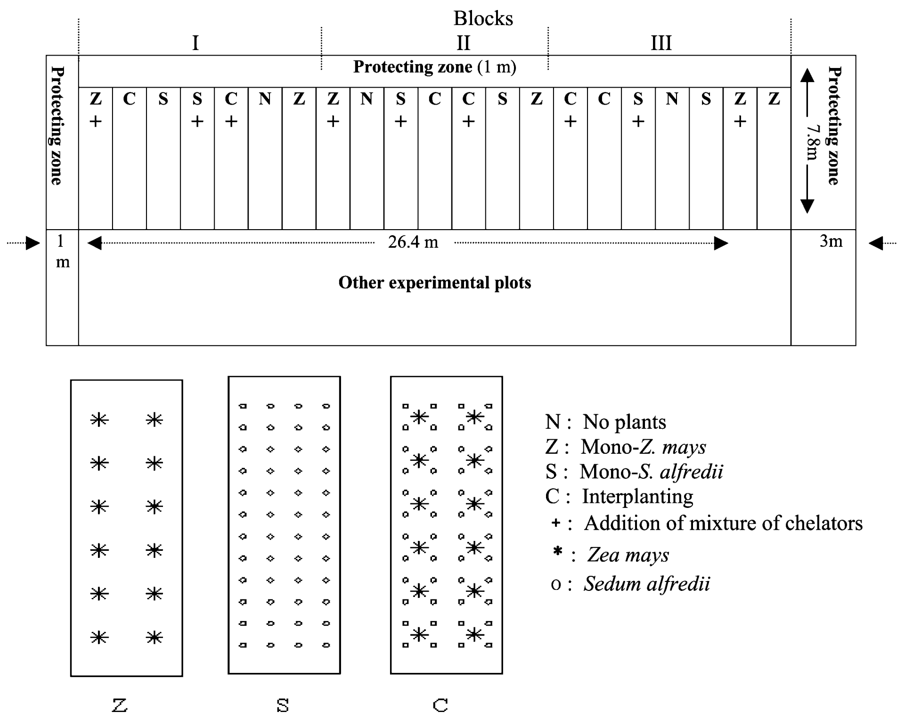

Figure 1 A schematic diagram showing the layout of the plots (*top*) and the planting pattern (*bottom*) for the two plant species in monoculture and in poly-culture planting treatments (*from left to right*). Note that only one half of the planting pattern plot (*in length*) is shown here.

Table III Comparison of heavy metal analysis results between our samples and the standard reference samples (SRS) (mg/kg)

	SRS No. 1		SRS No. 2	
	Measured Value	Standard Value	Measured Value	Standard Value
Zn	65.3 (63.1~66.3)*	65.0	27.5 (26.3~28.0)	26.3
Pb	24.3 (19.6~24.6)	26.0	3.07 (2.54~3.56)	3.10
Cd	0.12 (0.115~0.130)	0.14	0.22 (0.215~0.225)	0.22

1,500 mm and a mean annual temperature of 19.6°C. Metal concentrations and the selected soil properties are given in Table II. According to the Chinese national standards of soils (GB15618–1995), this soil is contaminated with Cd, Pb and Zn. The total metal contents vary among field blocks and they are normally higher for the blocks near than far from the irrigation ditch with the following order: block 1> block 2>block 3 (Figure 1).

2.2 Materials and chemical chelators

Two plant species, namely *S. alfredii* Hance and *Z. mays*, were selected in our experiments. *S. alfredii* was obtained from an old Pb and Zn mining area in Zhejiang Province, China. It is a new Zn hyper-accumulator native to China (Yang, Long, & Ni, 2002) and has an ability to hyperaccumulate not only Zn but also Cd (Yang, Long, Ye, & Calvert, 2004; Ye, Yang, He, & Long, 2003). *S. alfredii* was propagated before transplanting in our experiments. *Z. mays* (var. Yunshi-5) was a low metal-accumulating cultivar with low concentrations of heavy metals in its grains (Samake, Wu, Mo, & Morel, 2003). The chelator used in our experiments was a mixture of chelator (MC) that comprised of monosodium glutamate waste liquid (MGWL), citric acid and EDTA at a mole ratio of 1:10:2 (Wu et al., 2004). The citric acid and EDTA were the AR grade chemical reagents and MGWL was purchased from Guangzhou Glutamate Factory with a residual monosodium glutamate concentration of 0.8 mol l^{-1}, pH of 6.3, and chemical oxygen demand of 41,668 mg l^{-1}.

2.3 Treatments and experimental design

The planting treatments were as follows: Control (CK), *Z. mays* (*Z. mays*), *S. alfredii* Hance (*S. alfredii*), Co-crop of *Z. mays* and *S. alfredii* Hance (Co-crop), *Z. mays* amended with the MC (*Z. mays*+MC), *S. alfredii* Hance amended with MC (*S. alfredii*+MC), Co-crop amended with MC (Co-crop+MC). There were a total of seven treatments and each with triplicates during the experiments.

In December 2003, 21 field plots of 1.2 m×6.8 m were set up with at least 1 m protecting zone all around the experimental plots and there was a ditch in 0.10 m width between two plots (Figure 1). There are a total of seven plots in each block and each plot is randomly distributed within a block. Eighty-eight healthy and equal-size *S. alfredii* were planted per plot at four columns and 21 rows and 22 plants of *Z. mays* per plot were added in 2 columns × 11 rows in March 2004 (Figure 1). Twenty days before harvest, the MC was supplied to the amended treatments (*Z. mays*+MC, *S. alfredii*+MC, Co-crop+MC) at a rate of 5 mmol MC kg^{-1} soil. The MC was dissolved by water and sprinkled to soil surface by means of watering pot.

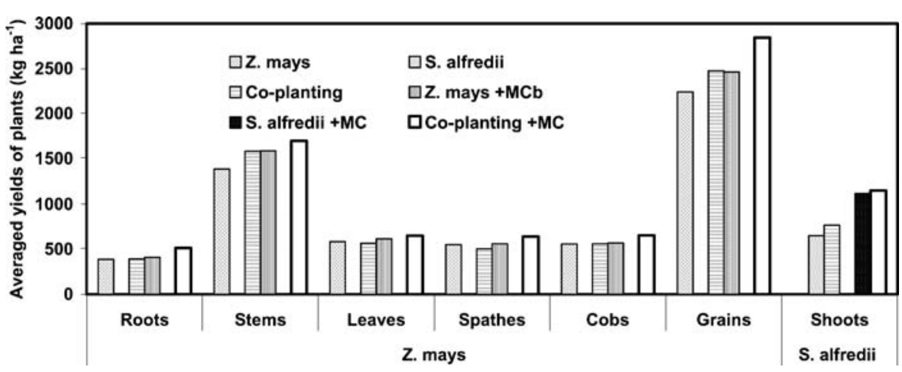

Figure 2 Averaged yields of plant parts for *Z. mays* and *S. alfredii* among different treatments.

Table IV Concentrations of Zn, Cd and Pb in the tissues of *Z. mays* under different planting treatments (mg kg^{-1} DW)

	Z. mays	Interplanting	*Z. mays* + MC[b]	Co-planting + MC
Zn				
Grains	25.7±1.35[a]	29.9±5.53	29. 6±4.96	28.6±3.31
Cobs	29.5±4.24	48.1±18.7	53.2±21.2	40.9±10.8
Spathes	78.7±18.5	135±74.6	133±84.0	97.2±36.5
Leaves	53.7±9.42	106±64.1	107±60.4	72.1±28.7
Stems	140±35.9	214±119	188±88.7	143±62.4
Roots	94.7±10.6	154±70.1	124±45.7	99.6±21.6
Cd				
Grains	0.325±0.131	0.202±0.034	0.201±0.046	0.286±0.140
Cobs	0.185±0.097	0.239±0.095	0.193±0.082	0.251±0.099
Spathes	0.866±0.141	0.947±0.185	0.868±0.214	0.969±0.169
Leaves	0.841±0.151	1.09±0.286	1.23±0.503	1.26±0.124
Stems	1.39±0.152	1.39±0.325	1.27±0.413	1.80±0.401
Roots	1.88±0.167	1.97±0.059	2.13±0.240	2.27±0.092
Pb				
Grains	0.220±0.022	0.443±0.238	0.406±0.103	0.318±0.058
Cobs	0.507±0.020	1.21±0.738	1.24±0.376	0.859±0.173
Spathes	4.60±0.629	7.71±2.79	7.50±1.04	7.31±1.28
Leaves	8.13±2.47	10.9±3.79	14.0±3.19	11.4±1.09
Stems	6.42±0.745	10.8±3.61	8.55±2.07	8.84±0.453
Roots	35.1±4.75	48.4±14. 9	42.4±2.69	30.1±2.78

[a] The values in the table were Mean±STD. No significant difference was observed between treatments at $\alpha = 0.05$ level according to ANOVA.

[b] *MC* Mixture of chelators.

During the period of plant growth, all the plots received inorganic fertilization with a compound fertilizer (N: P_2O_5: K_2O=15:15:15) at a rate of 150 kg ha^{-1} monthly. Normal maintenance of the plants was conducted until they were harvested on June 22, 2004.

2.4 Sample collection and analysis

Soil samples from the topsoil (0–20 cm depth) were collected from each plot in December 2003 before planting and following the harvest in June 2004. Key soil properties of the soil used for this study are shown in Table II. At harvest, three *Z. mays* and five *S. alfredii* were collected from each plot and each plant species was composite to one mixed plant sample. *Z. mays* was divided into roots, stems, leaves, spathes, cobs and grains, whereas *S. alfredii* was cut to collect only shoots. The plant parts were dried at 70°C for 76 h to obtain the dry weights. The oven-dried materials were finely ground in an agate mill.

Soil pH was measured with a soil to water ratio of 1:2.5. Samples were digested with HF–$HClO_4$–HNO_3 for total heavy metal content (Lu, 2000). Plant samples were incinerated to smokeless on hotplate and dry destructed in muffle furnace at 500°C for 5.5 h, the dry ash was then dissolved by 2 ml 1:1 (v/v) HCl and was transferred and adjusted to a final volume of 25 ml for total metal analysis. Concentrations of Zn, Cd and Pb in solutions were determined with the Atomic Absorption Spectrometry (Hitachi Z-5300). The plant metal analysis was conducted according to Chinese standard methods (CAPM, 1998) and calibrated using National Standard Reference Samples (SRS) obtained from the Ministry of Agriculture of China with a reasonable agreement (Table III).

Means and standard deviations were calculated with triplicates using Microsoft Excel. Statistical analysis was performed with SAS (r) Proprietary Software Release 8.1 (SAS Institute Inc., Cary, NC, USA) (Hong & Hou, 2004). The effects from interplanting, MC addition, blocks and interactions between co-planting and MC were statistically evaluated using ANOVA.

3 Results and Discussion

3.1 Yields of *Z. mays* and *S. alfredii*

There were no visible symptoms of heavy metal toxicity to the two plants. Yields of stems, leaves, spathes, cobs, grains and roots of *Z. mays* (Figure 2) showed no significant differences (at α=0.05) among the treatments. However, the co-planting and co-planting + MC treatment have both resulted in a significant (at α=0.05) increase in biomass of the shoots of *S. alfredii*. Figure 2 further reveals that among all of the treatments, the highest yields of the plants were found in the interplating + MC treatment and the yields for *S. alfredii* and for corn grains of *Z. mays* were about 1,403 (1,145) and 3,487 kg ha^{-1} (2,845 g plot^{-1}), respectively. This might be due to the additional supply of nutrients by the MC. In

Table V Zn, Cd and Pb uptakes by plant aerial parts under the different planting treatments (mg plot^{-1})

	Z. mays	*S. alfredi.*	Total
Zn			
Z. mays	395±145[c]		395±145
S. alfredii		3831±1810	3831±1810
Interplanting	563±224	4836±2075	5399±2286
Z. mays + MC[a]	600±270		600±270
S. alfredii + MC		6432±2124	6432±2124
Co-planting + MC	506±164	7544±1802	8050±1966
ANOVA significance level			
Co-planting effect	0.5918	0.0245	0.0002
MC effect	0.2999	0.0003	0.0471
Interactions[b]	0.0919	0.8852	0.3390
Block effect	0.0004	<0.0001	0.0019
Cd			
Z. mays	4.43±0.708		4.43±0.707
S. alfredii.		33.9±13.2	33.9±13.2
Co-planting	4.54±0.509	37.8±14.8	42.3±15.2
Z. mays + MC	4.75±1.270		4.75±1.27
S. alfredii. + MC		50.1±13.6	50.1±13.6
Co-planting + MC	6.85±1.494	57.1±12.1	64.0±13.1
ANOVA significance level			
Co-planting effect	0.2484	0.1561	0.0524
MC effect	0.1772	0.0019	0.0001
Interactions	0.2917	0.6642	0.3350
Block effect	0.1135	0.0001	0.0038
Pb			
Z. mays	30.3±1.83		30.3±1.83
S. alfredii.		19.9±0.728	19.9±0.728
Co-planting	35.0±0.519	22.3±0.039	57.3±0.480
Z. mays + MC	39.4±2.71		39.4±2.71
S. alfredii. + MC		35.5±0.157	35.5±0.157
Co-planting + MC	44.2±7.03	30.5±1.78	74.7±13.6
ANOVA significance level			
Co-planting effect	0.2678	0.3262	0.0403
MC effect	0.2063	0.0131	0.0329
Interactions	0.1930	0.1459	0.5731
Block effect	0.0978	0.6399	0.0081

[a] *MC* Mixture of chelators

[b] Interactions between co-planting and MC

[c] The values were Mean±STD.

addition, *S. alfredii* is a shade-requiring plant and was benefited by the shade provided by *Z. mays*.

3.2 Concentrations of heavy metals in plants

Concentrations of the three heavy metals, i.e., Zn, Cd and Pb, showed no significant differences in tissues of *Z. mays* among the planting treatments (Table IV). According to the Tolerance Limit of Heavy Metal for Food and Feed of China (CAPM, 1998; Luo & Jiang, 2003), the concentrations of Cd and Pb in the corn grains of *Z. mays* were below the standard limit for animal feeds (Table IV). Note that the standard limit for Zn has not been established because the animal feeds need a rather high content of this trace element. The content of Zn from the corn grains of *Z. mays* produced in our experiments is within the recommended range for animal feeds (45–80 mg/kg) (Coic & Coppenet, 1989). This indicated that corn grains were safe as feed for animal nutrition. Our study further reveals that the spathes, leaves, stems and roots of *Z. mays* were also safe as organic manure according to the Organic Fertilizer Standard of Agricultural Ministry of China (NY 525–2002) (Table I). One reason to choose the low metal-accumulating *Z. mays* in phytoextraction of heavy metal contaminated soils is that this plant species has large biomass and can be an energy crop in producing biofuel with its corn grains (Altm, Cetinkava, & Yucesu, 2001) to avoid heavy metals entering the food chain.

3.3 Total removal of heavy metals in different treatments

Phytoextraction efficiency is related to both plant metal concentrations and dry matter yields. Table V shows the uptakes of Zn, Cd and Pb by plant aerial parts in different treatments. In the case of monocultures, uptakes of Zn and Cd by *S. alfredii* were nearly 10-fold higher than those by *Z. mays*, whereas the opposite was true for Pb. That is, the uptake of Pb by *Z. mays* was about 1.5 times greater than that of *S. alfredii.* With the addition of the MC, the uptakes of Zn, Cd and Pb by *S. alfredii* were enhanced by 65.71%, 47.93% and 78.49%, respectively, and were all statistically significant. Our results also disclose that the interactions between the co-planting and MC treatments were always insignificant, indicating the effects of the two types of treatments should be additive and the two techniques (i.e., the co-planting and the use of the MC) were suitable to be combined together. The total uptakes of Zn and Cd in the co-crop treatments were evidently higher than those for mono-maize or mono-*S. alfredii*. When the MC was applied to the co-crop system, the best result of heavy metal phytoextraction was achieved. Under these conditions, the overall accumulations of Zn, Cd and Pb in the two plants were, respectively, 9.87 (8,050), 0.08 (63.96) and 0.09 kg ha^{-1} (74.70 mg plot^{-1}) in one harvest when these plants were grown on the metal-contaminated paddy soil that has the initial

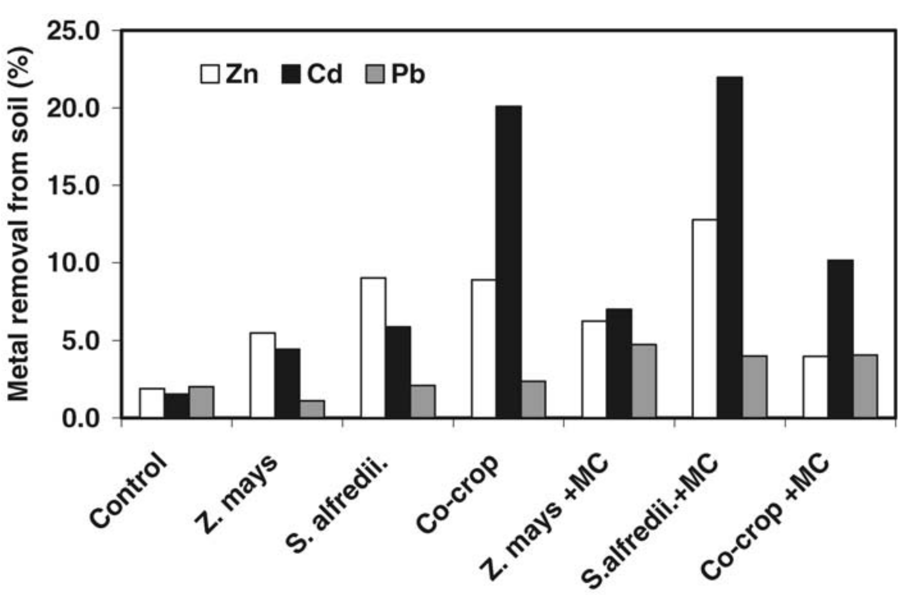

Figure 3 Removal of heavy metals from the soil among different treatments.

levels of total Zn, Cd and Pb at the concentrations of 700, 2 and 750 mg kg^{-1} soil, respectively. Yang et al. (2002) reported that *S. alfredii* is perennial plant and could propagate for 3- to 4-fold in a year if the environmental conditions were favorable for its growth and *Z. mays* could also be grown three times a year in South China. Furthermore, increasing the density of hyperaccumulator in a plot and the application of fertilization could ameliorate phytoextraction (Sun, Ni, Yang, & Ding, 2003; Ye et al., 2003). Therefore, the co-cropping system with the addition of the MC has great potentials for use on phytoremediation of Cd and Zn on the large-scale contaminated soils in South China and hastens significantly the natural attenuation of Cd and Zn in agricultural lands.

3.4 Removal of heavy metals from soil

Removal of heavy metals from the soil among different planting treatments at the end of the experiments is shown in Figure 3. This figure shows that the removal of Zn and Cd from the soil were highest for the *S. alfredii* + MC treatment followed by the co-crop treatment. More than 9% and 13% of the soil Zn and Cd were, respectively, removed by these two treatments. Figure 3 further disclose that removal of the soil heavy metals was not highest for the co-crop + MC treatment although this treatment had shown a highest phytoextraction rate in soil heavy metals. We attributed this discrepancy to the sampling error and/or unevenly leaching of soil heavy metals.

4 Conclusions

Field experiments were performed to investigate the effects of a mixture of chelator (MC) on the growth and phytoextraction efficiency of the heavy metals by the hyperaccumulator *S. alfredii* in a co-cropping system in a paddy soil that has a previous history of irrigation with Pb and Zn contaminated mining wastewaters. The co-cropping system used in this study comprised of a Zn- and Cd-hyperaccumulator (*S. alfredii*) and a low-accumulating crop (*Z. mays*). Based upon the present study, we concluded that the growth of *S. alfredii* and *Z. mays* was a suitable co-planting system for simultaneously phytoextraction of heavy metals and production of safe animal feeds and organic fertilizers. The addition of the MC, combining with the co-cropping system, can significantly enhance the removal of heavy metals from the contaminated soil. The combination of the co-planting system with the MC not only can additively enhance the removal of heavy metals from the contaminated soils but also can produce corn grains for animal feeds simultaneously and thereby allows farmers to continue their agricultural activities, and to minimize the cost of phytoremediation and the contamination risk to underground water by leaching. A continued agricultural production with safe feeds and materials in the contaminated soils is important, considering the limited arable lands available in China and years needed for phytoremediating metal-contaminated soils. Further studies are warranted to optimize the combination ratios of the two plants, to assess *in situ* environmental risk of the MC to groundwater resources and to elucidate the interaction mechanisms between the two plants.

Acknowledgments This work is supported by National High-Technology Project of China (863 Project no: 2001-AA-640501-3), French–Chinese Programme de Recherche Avancee (PRA E-03-02) and Natural Science Foundation of China (40571141).

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