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Removal of metals by sorghum plants from contaminated land

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Abstract

The growth of high biomass crops facilitated by optimal of agronomic practices has been considered as an alternative to phytoremediation of soils contaminated by heavy metals. A field trial was carried out to evaluate the phytoextraction efficiency of heavy metals by three varieties of sweet sorghum (*Sorghum biocolor* L.), a high biomass energy plant. Ethylene diamine tetraacetate (EDTA), ammonium nitrate (NH_4NO_3) and ammonium sulphate ($(NH_4)_2SO_4$) were tested for their abilities to enhance the removal of heavy metals Pb, Cd, Zn, and Cu by sweet sorghum from a contaminated agricultural soil. Sorghum plants always achieved the greatest removal of Pb by leaves and the greatest removal of Cd, Zn and Cu by stems. There was no significant difference among the Keller, Rio and Mray varieties of sweet sorghums in accumulating heavy metals. EDTA treatment was more efficient than ammonium nitrate and ammonium sulphate in promoting Pb accumulation in sweet sorghum from the contaminated agricultural soil. The application of ammonium nitrate and ammonium sulphate increased the accumulation of both Zn and Cd in roots of sorghum plants. Results from this study suggest that cropping of sorghum plants facilitated by agronomic practices may be a sustainable technique for partial decontamination of heavy metal contaminated soils.

Key words: *Sorghum biocolor*; field trial; phytoextraction

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Introduction

Heavy metal pollution in soil systems usually is related to human activities such as industrialization, applications of fertilizers and pesticides, generation of energy and production of fuels, mining and metallurgical processes, waste disposal etc. A variety of technologies has been developed to remediate heavy metal contaminated soils, and phytoextraction offers an economical alternative remediation approach, in which plants are used to extract metals from soils and the extracted metals are translocated to the harvestable parts of the plants. For heavy metal contaminated agricultural lands, selection of appropriate metal tolerant crop plants to remove the metals and to harvest the valuable farm produce synchronously can be a key element of a new strategy for land management. Many studies have indicated that certain varieties of high-biomass crops displayed heavy metal tolerance, such as Indian mustard (*Brassica juncea*), oat (*Avena sativa*), maize (*Zea mays*), barley (*Hordeum vulgare*), sunflower (*Helianthus annuus*) and ryegrass (*Lolium perenne*) (Salt *et al*., 1998; Shen *et al*., 2002; Meers *et al*., 2005; Komárek *et al*., 2007). Moreover, fast growing willows (*Salix viminalis*), poplars (*Populus* sp.) are excellent producers of biomass, they are also characterized by array of characteristics that make these species promising for phytoremediation application (Vervaeke *et al*., 2003).

In the phytoextraction process, the integration of specially selected high biomass crops with improved plant husbandry and innovative soil management practices is a promising alternative strategy to achieve high biomass and metal accumulation rates from contaminated soil (Nowack *et al*., 2006; Evangelou *et al*., 2007). Many chemical amendments, such as EDTA, HEDTA, NTA (nitrilotriacetic acid) and organic acids, have been used in pot and field experiments to enhance extraction rates of heavy metals and to achieve higher phytoextraction efficiency (Blaylock *et al*., 1997; Huang *et al*., 1997; Kayser *et al*., 2000; Ke *et al*., 2006; Wang *et al*., 2006; Wu *et al*., 2006). There is much evidence confirming that EDTA is one of the most efficient chelating agents in enhancing Pb phytoavailability in soil and subsequent uptake and translocation in shoots after treating for a few days (Chen and Cutright, 2001; Shen *et al*., 2002). However, in some cases *in situ* application of some chelating agents may pose a high potential risk of underground water pollution during extended period of time. As an alternative, it has been reported that the application of ammonium to soil could promote the phytoavailability of heavy metals from the contaminated soil (Lasa *et al*., 2000; Xiong and Lu, 2002; Zaccheo *et al*., 2006), suggesting that the accumulations of Cd and Zn could be enhanced by inorganic agents like elemental sulfur or ammonium sulfate (Villarroel

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et al., 1993; Zhuang *et al*., 2005). The application of plant nitrogen nutrition was also exploited to enhance the efficiency of phytoextraction (Salt *et al*., 1998; Schmidt, 2003). However, a majority of studies on phytoremediation was based on pot experiments and hydroponic culture, and only a few reports actually intended to evaluate the phytoextraction potential of hyperaccumulators or high biomass crops under field conditions (Hammer and Keller, 2003; McGrath *et al*., 2006; Zhuang *et al*., 2007). It should be especially pointed out that only a few attempts have been made to evaluate the possibility of metal removal in response to modifications of agronomic practices (Marchiol *et al*., 2007).

One advantage of using crops for phytoextraction is the possibility of obtaining some economic returns during the process. Sweet sorghum (*Sorghum bicolor*) is a hardy, C_4 grass that is widely used as a forage crop (Buxton *et al*., 1998; Unger, 2001), and it is considered as a great promising energy plant, due to its fast-growing and high biomass production. Cereal grains or edible parts were perfectly suited for livestock and their use for stock fodder would contribute to the profitability of using phytoremediation under the experimental conditions. For example, in the US, some sweet sorghum varieties such as Rio, Mray and Keller have been bred to produce ethanol. Some studies showed that sweet sorghum have a ability to accumulate metal elements (Kumawat and Dubey, 1991; An, 2004; Marchiol *et al*., 2007). The sorghum plants could be cropped each year and the biomass could be removed from the area. It should be an environmentally friendly and sustainable technique. Therefore, a field experiment was conducted here: (1) to compare the phytoextraction efficiency of three varieties of *S. bicolor*, Keller, Mray and Rio; (2) to determine the effects of application of $(NH_4)_2SO_4$, NH_4NO_3 and EDTA on heavy metal extraction efficiency of sorghum plant.

1 Materials and methods

1.1 Study site

The field plot experiments were conducted on a paddy soil located at the Lechang lead and zinc mining site (24°40′N and 113°20′E) in Lechang City in northern Guangdong Province, China. Lechang situates in a subtropical area with an average annual rainfall of 1522 mm and a mean annual temperature of 19.6°C. This field site has been contaminated by atmospheric emissions and surface irrigation with the Pb and Zn mining wastewaters since the 1950s. Metal concentrations and the selected soil properties are showed in Table 1. According to the Chinese National Standards of Soils (GB15618-1995), this soil is seriously contaminated with Cd, Pb and Zn, as described in previous studies (Zhuang *et al*., 2005).

1.2 Experimental design

The experimental site consisted of 48 subplots (2 m × 3 m each). The cropland was ploughed to a 25-cm depth prior to seeding. The field trial was arranged in a split plot design, with two factors (varieties and amendments) and four replications. The tested plants were three varieties of sweet sorghum (*S. bicolor*), including Keller, Mray and Rio. Seeds of three varieties of sorghum plant were sowed at the density of 300 seeds/m^2 in the field plots. After germination for 10 d, the sweet sorghum seedlings were about 15 cm height and transplanted in the designated field plot at a density of 12 plants/m^2. During the experiment period, field plots were watered and weeds removed manually when necessary, and no pesticide were used. The four treatments were performed including: control, NH_4NO_3, $(NH_4)_2SO_4$ and ethylene diamine tetraacetate (Na_2-EDTA solution). The NH_4NO_3, $(NH_4)_2SO_4$ and EDTA treatments were applied at a rate of 10, 10 and 6 mmol/kg soil (calculated using a soil depth of 20 cm and a soil density of 1.3 g/cm), respectively, whereas the control treatment received only water. The ammonium salt (NH_4NO_3 and $(NH_4)_2SO_4$) and EDTA (Na_2-EDTA solution) treatment were applied one month and one week before harvesting the sorghum plants, respectively.

Table 1 Chemical properties of the contaminated soil (mean ± SD, n = 4, dry weight (dw))

Parameter	Value
pH (1:2 H_2O m/V)	6.8 ± 0.41
OM (organic matter, g/kg)	5.4 ± 0.18
CEC (cation exchange capacity, cmol/kg)	17.1 ± 1.9
Total N (g/kg)	3.1 ± 0.8
Total P (g/kg)	0.9 ± 0.3
Total K (g/kg)	11.8 ± 2.1
Total Pb (mg/kg)	938 ± 24.4 (300)[a]
Total Cd (mg/kg)	4.9 ± 1.8 (0.3)
Total Zn (mg/kg)	834 ± 59.1 (250)
Total Cu (mg/kg)	47.2 ± 3.6 (100)
DTPA(diethylenetriaminepentaacetic acid) -extractable Pb (mg/kg)	203 ± 7.3
DTPA-extractable Cd (mg/kg)	1.02 ± 0.7
DTPA-extractable Zn (mg/kg)	118 ± 9.5
DTPA-extractable Cu (mg/kg)	6.7 ± 1.9

[a] The values in brackets are the standard values of heavy metals for soils intended for agricultural uses with pH ranging from 6.5 to 7.5 based on Chinese National Standard GB15618-1995.

1.3 Chemical analysis

After 120 d cultivation, sweet sorghum plants were harvested and the numbers of plants per subplots recorded and weighed. The biomass was partitioned into root, stem, leaves, and grain, and then taken to the laboratory. Plants at five locations were randomly sampled and bulked together to form a composite sample. Soil samples were air-dried and ground to pass a 0.5-mm sieve. Soil pH value was measured in suspension using a ratio 1:2 (W/V) of soil and deionized water. Soil extractable heavy metals (Pb, Cd, Zn and Cu) were extracted with a diethylenetriaminepentaacetic acid (DTPA) solution by adding 0.005 mol/L DTPA, 0.01 mol/L $CaCl_2$, 0.1 mol/L triethanolamine (TEA) buffered at pH 7.3 to give a 1:2 ratio (W/V) soil:solution (Lindsay and Norvell, 1978). Soil samples (0.5 g) were digested with a mixture of HNO_3, HCl and $HClO_4$ at a ratio of 3:1:1 ($V/V/V$). Soil total and extractable heavy metal concentrations were analyzed by flame atomic absorption spectrometry (AAS, GBC932AA, Australia). All these fractions were washed with distilled water and dried at 75°C until constant weight. Plant samples (0.5 g) of finely ground tissue were digested with a mixture of concentrated HNO_3 (16 mol/L) and $HClO_4$ (16 mol/L). The concentrations of Pb, Cd, Zn and Cu were determined by AAS. Quality control for the analyses for plants and soils included the use of standard reference materials (GBW 07602 and 07406) and the inclusion of blanks in digestion batches.

1.4 Statistical analysis

All statistical analyses were performed using the SPSS 11.5 package. Differences in heavy metal concentrations among different varieties of sorghum were detected using split-plot ANOVA, followed by multiple comparisons using the least significant difference (LSD) tests. A significance level of $p = 0.05$ was used throughout the study.

2 Results

2.1 Plant biomass

Table 2 presents average dry weight (dw) biomass production of sorghum plants with different treatment. After grown in the contaminated agricultural soil for 120 d, there was no visible symptom of heavy metal toxicity to their varieties (Keller, Mray and Rio) of sorghum plants. The trends of average height in different varieties were in the descending order of Keller > Mray > Rio. The average biomass production of three varieties of sorghum plant was 25.8, 24.8 and 18.7 tons/ha for Keller, Mray and Rio, respectively, in control treatment. Ammonium treatment enhanced the biomass yields of all tested plants, although there was no difference among all treatments. Treatment with EDTA resulted in significantly decreased the average biomass of Keller and Mray. The application of NH_4NO_3 and $(NH_4)_2SO_4$ increased the biomass of Keller and Mray by 8.9% and 14.3%, respectively. However, the growth of EDTA-fed plants was reduced as compared to that of ammonium-fed plants. This may be attributed to the toxicity of free EDTA to the plants.

2.2 Metal accumulation in plant tissues

Figure 1 shows the accumulation of Pb, Cd, Zn and Cu in tissues with or without the soil amendment. There was no significant difference in Pb concentrations accumulated in the Keller, Mray and Rio cultivars. The highest Pb concentrations in leaves ranged from 37.3 to 48.2 mg/kg, which were nearly 10-fold higher than the concentrations (approximately 4 mg/kg) in stems. The Cd concentrations in stems and leaves of sorghum plant grown in control plots varied between 1.16 and 2.31 mg/kg, with the highest concentration in Rio and the lowest in Mray. In the control treatment, Mray and Rio accumulated the highest Zn in leaves, at 67.1 and 80.9 mg/kg, respectively. The highest concentrations of Cu were found in grains, and the lowest concentrations were in stems. Lead concentrations in roots of Keller, Mray and Rio were 64.7, 49.6 and 96.2 mg/kg, respectively, which increased by more than 2-folds as a

Table 2 Averaged yields of sorghum plants under different treatments

Variety	Height (cm)	Averaged yield (tons/ha, dw)			
		Control	NH_4NO_3	$(NH_4)_2SO_4$	EDTA
Keller	278 ± 9.4 a	25.8 ± 1.96 a	28.1 ± 2.87 a	29.5 ± 3.21 a	21.7 ± 2.86 b
Mray	263 ± 6.8 a	24.8 ± 2.81 a	27.9 ± 3.01 a	27.3 ± 4.03 a	21.2 ± 3.42 ab
Rio	196 ± 8.2 b	18.7 ± 2.33 a	20.9 ± 1.92 a	21.1 ± 3.24 a	16.5 ± 3.29 a

Data with different letters in the same row indicate a significant difference at $p < 0.05$ according to the least significant difference (LSD) test.
Data are presented in mean ± SD ($n = 4$).

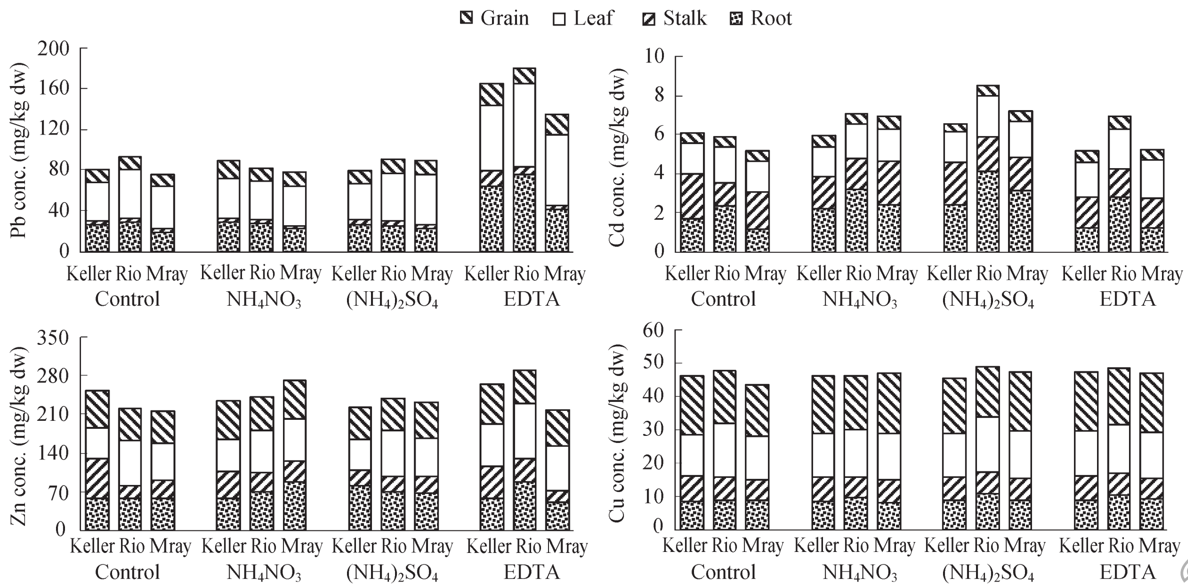

Fig. 1 Average concentrations of Pb, Cd, Zn and Cu of three sorghum varieties grown in the contaminated soil for 120 d.

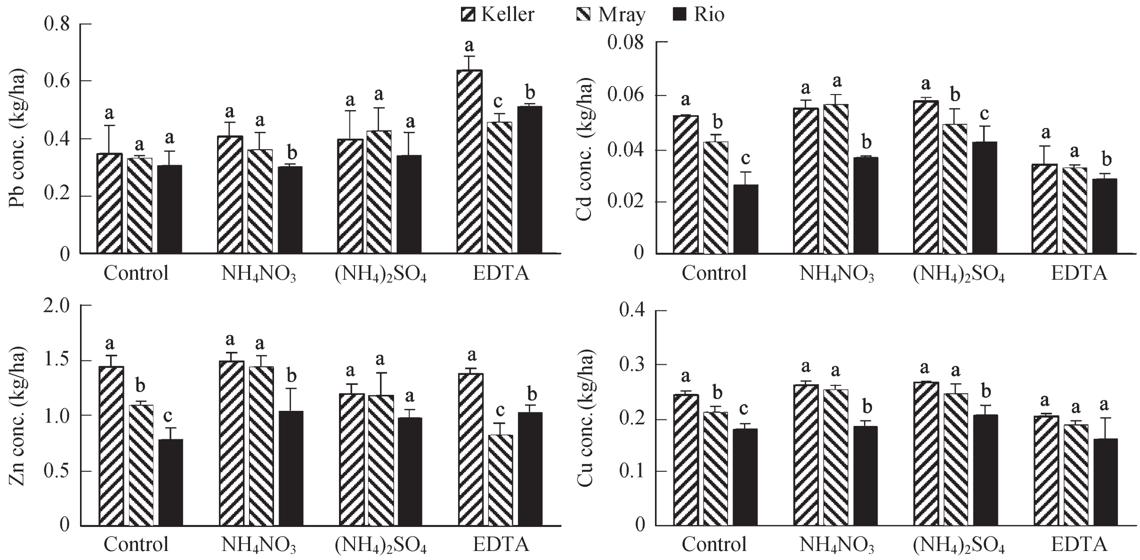

Fig. 2 Metal removals in different fractions of three sorghum varieties growing in contaminated field for 120 d.

result of the EDTA application. As compared with the control, the application of NH_4NO_3 and $(NH_4)_2SO_4$ did not affect the concentrations of Pb in different tissues of three sorghum varieties. The concentrations of Cd in roots were particularly enhanced with NH_4NO_3 and $(NH_4)_2SO_4$ application. Cadmium accumulation in roots of Rio reached 4.15 mg/kg with $(NH_4)_2SO_4$ application.

2.3 Removal of heavy metals in sorghum plant

The Pb, Cd, Zn and Cu removal by plant from contaminated soil are listed in Fig. 2. Among the three sorghum varieties, Keller had the highest removals of Zn, Cu and Cd from metal-contaminated soil. The total removals of heavy metals extracted by Keller were 0.35 kg/ha for Pb, 0.052 kg/ha for Cd, 1.44 kg/ha for Zn, and 0.24 kg/ha for Cu with a single cropping grown in agricultural field for 120 d without any treatment. Compared to the control, the application of EDTA significantly ($p < 0.01$) increased the Pb removal for Keller, Mray and Rio, with values up to 0.64, 0.46, and 0.51 kg/ha, respectively. The removal of Zn with NH_4NO_3 treatment was 1.49 kg/ha for Keller, 1.44 kg/ha for Mray and 1.04 kg/ha for Rio. Our results also suggest that soil fertility management will be important for commercial phytoextraction. A single cropping of Keller could remove Cd 0.055, 0.057, and 0.034 kg/ha from the contaminated soil with NH_4NO_3, $(NH_4)_2SO_4$ and EDTA treatment, respectively.

3 Discussion

Phytoextraction is a long-term remediation effort, requiring many cropping cycles to decontaminate metal pollutants to acceptable levels. Metal extraction performance depends on the potential biomass production and the concentration in shoots. One advantage of sweet sorghum is its high biomass production (up to 25.8 tons/ha under normal conditions), which is much higher than numerous corps such as sunflower and maize. The fundamental traits of plants useful for phytoremediation, summarized by Chaney *et al*. (2004), should be expressed in a wide range of environmental conditions to transform phytoremediation from a natural phenomenon to a sustainable technology for soil clean-up. Biomass crops' tolerance to trace metals are studied following the approach of assisted phytoextraction, in which plants have to be managed with practices to enhance the element bioavailability and the plant uptake (Meers *et al*., 2005). On the other hand, sorghum plant has great commercial value to produce ethanol and paper making. Beyond the research that deals with metal uptake, transport and tolerance of heavy metals in plants, the planning of the extensive field applications used in the agronomic management of sorghum plant may be an alternative strategy in the development of phytoextraction technology.

The addition of NH_4NO_3 and $(NH_4)_2SO_4$ to soil had no effect on increasing Zn and Cu accumulation for three sorghum plant varieties. These results are in line with the view that the effect of ammonium fertilization on phytoextraction is especially tested for enhanced Zn and Cd accumulation because these heavy metals can easily be solubilized at those pH values that are predominant in agricultural soils, whereas the solubilization of Pb and Cu occurs at lower pH values (Schmidt, 2003). It is well known that the pH of the soil is one of the most important factors governing elemental accumulation by plants (Brown *et al*., 1994). Zaccheo *et al*. (2006) found that soils amended with $(NH_4)_2SO_4$ and $(NH_4)_2S_2O_3$ decreased soil pH by about 2 units and the metal availability was increased in the soil solution. Fertilizers with high content of NH_4^+ can decrease soil pH, leading to an increase in plant uptake of Cd (Willaert and Verloo, 1992) and, thereby, ammonium nutrition may play an important role in root-mediated acidification of the rhizo-soil. Furthermore, it has also been reported that the application of ammonium to soil significantly enhanced Cs accumulation in plant (Lasat *et al*., 1998). The results in this present study indicated that the increment in Cd accumulation with NH_4NO_3 and $(NH_4)_2SO_4$ treatments was higher than that

Table 3 Comparison of the removals of Pb, Cd, Zn and Cu in several agricultural crops

Species	Biomass (tons/ha)	Pb (kg/ha)	Cd (kg/ha)	Zn (kg/ha)	Cu (kg/ha)	Reference
Sorghum (*S. bicolor*)	25.8 (dw)	0.35	0.052	1.44	0.24	Present study
Sorghum (*S. bicolor*)	22.1 (dw)	0.38	0.006	1.22	0.64	Marchiol *et al*., 2007
Sunflower (*H. annuus*)	–	0.091	0.002	0.41	0.12	Marchiol *et al*., 2007
B. juncea	7.3 (dw)	–	0.007	0.89	0.15	Keller *et al*., 2003
Nicotiana tabacum	12.6 (dw)	–	0.042	1.83	0.47	Keller *et al*., 2003
Sunflower (*H. annuus*)	24 (dw)	0.016	–	2.14	–	Madejón *et al*., 2003
Medicago sativa	45.9 (ww)	0.115	0.013	0.438	0.124	Ciura *et al*., 2005
Maize (*Zea mays*)	92.7 (ww)	0.042	0.0093	0.45	0.096	Ciura *et al*., 2005
Barley (*H. vulgare*)	5.84 (dw)	0.035	0.014	1.07	0.057	Soriano *et al*., 2003

ww: wet weight. –: no data.

observed by Villarroel *et al*. (1993), who reported that Cd uptake was increased by 50% following the application of NH_4^+. Soils fertilized with NH_3 and repeatedly sown with corn accumulated lower Cd and Zn than did soils under crop rotation (Basta and Tabatabai, 1992). Schmidt (2003) suggested that since nitrogen fertilization is a common agricultural measure, the use of ammonium sulfate can be considered as a low-cost strategy, but one which only minimally enhances phytoextraction.

It has been reported that EDTA was one of the most effective desorbing agents in enhancing the shoot concentration of Pb (Blaylock *et al*., 1997; Huang *et al*., 1997; Shen *et al*., 2002). The application of EDTA significantly ($p < 0.01$) increased the Pb concentration in the roots and leaves of three varieties of sorghum plants (Fig. 1). However, EDTA as soil amendment showed no significant effect on Cd, Zn and Cu accumulations for the three varieties of sweet sorghum plants grown in the contaminated soil. The result in this present study is consistent with those obtained in previous investigations (Lai and Chen, 2004; McGrath *et al*., 2006), showing that EDTA could not enhance Cd, Zn and Cu accumulation in plants. This may be due to the specific nature of a chemical amendment regarding the plant and metal species involved, and may be subject to the interactions and subsequent inhibitory effects when multiple heavy metals are present. In our previous study, we found that EDTA is more efficient in increasing Pb accumulation in *viola* and *Rumex* K-1 than Zn and Cd, while metal-accumulation of *V. zizanioides* had no remarkable response to the application of EDTA (Zhuang *et al*., 2005, 2007). Nevertheless, some concerns have been expressed regarding the leaching of metal-chelating agents to groundwater, posing potential risks over extended periods of time. Several reports have indicated the possible threat of groundwater contamination (Nowack *et al*., 2006), which has made some scientists and legislators frown on utilizing EDTA to enhance phytoextraction.

The development of large-scale phytoextraction techniques could consider agricultural crop species as bioaccumulators of heavy metals, in fact, some of them can accumulate heavy metals while producing high biomass in response to established agricultural management (Ebbs and Kochian, 1998). This approach has been followed by Keller *et al*. (2003) and Ciura *et al*. (2005) on maize, by Madejón *et al*. (2003) on sunflower, and by Soriano and Fereres (2003) on Barley. The results from the present study showed that compared to above-mentioned crops, sorghum plants could extract certain metals from the contaminated soil (Table 3). We calculated the potential removals of Pb and Zn for sorghum plant to be 0.35 and 1.44 kg/ha, respectively. These values were based on the metal concentration and biomass production of Keller observed in the field experiment. These results are similar to that found by Marchiol *et al*. (2007) who calculated values of 0.38 kg/ha for Pb and 1.22 kg/ha for Zn in an alkaline, industrial polluted soil. Comparing the amount of Pb extracted from soils by agricultural crops (Table 3), none of the plant species has a potential for phytoextraction of Pb contaminated site. Compared to sorghum plant, sunflower could extract significantly greater amount of Zn with the value up to 2.14 kg/ha, when the roots were also considered in the calculations (Madejón *et al*., 2003). Even when grown in the contaminated soil, sorghum plant can extract more than 0.05 kg/ha of Cd in a single crop, which is higher compared to others crops showed in Table 3. Keller *et al*. (2003) estimated that *Nicotiana tabacum* with 12.6 tons/ha biomass could extract 1.83 kg/ha of Zn, 0.47 kg/ha of Cu and 0.042 kg/ha of Cd. In conclusion, the Keller cultivar may be a more efficient alternative in removing heavy metals from soils among the three varieties of sorghum plant. sorghum being a solar energy-efficient plant has a great potential in phytoremediation of toxic metals.

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