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Effects of ethylenediaminetetraacetic acid and ammonium sulfate on Pb and Cr distribution in *Kochia scoparia* from compost

S. L. Zhao · X. J. Shang · L. A. Duo

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Abstract In a municipal solid waste (MSW) compost field, Kochia scoparia, an easy-to-grow weed plant, gradually invaded the experiment site and became the dominant species after 4 years' succession. Ethylenediaminetetraacetic acid (EDTA) solution at five rates 0, 25, 50 mmol L^{-1} , 25 mmol L^{-1} + 1 g L^{-1} ammonium sulfate $(NH_4)_2SO_4$, and 50 mmol $L^{-1} + 1 \text{ g } L^{-1} (NH_4)_2SO_4$ was added to the tested plant root medium. The effects of EDTA and (NH₄)₂SO₄ on Pb and Cr distribution in K. scoparia were investigated. Results suggested that plant biomass increased greatly with height, showing an "inversion pyramid" pattern in spatial structure. At the level of 50 mmol L^{-1} EDTA, single additions and combined additions with (NH₄)₂SO₄ increased Pb and Cr concentrations in plant shoots at different heights. Lead and Cr uptakes increased toward the top of the shoot. Combined application of 50 mmol L^{-1} EDTA and $(NH_4)_2SO_4$ increased Pb uptakes by 21.6, 19.2, 111.3, 124.3, and 154.0 % in 0-30, 30-60, 60-90, 90-120, and over 120 cm spatial shoots, respectively, as compared to those of controls. The increment for Cr uptake was 244.5, 281.7, 100.0, 77.2, and 187.4 %. The relationship between Pb and Cr concentrations in plant shoots and spatial height was found to be positively linear and statistically significant at 1 % level at 50 mmol L^{-1} EDTA alone and 25 mmol L^{-1} EDTA together with (NH₄)₂SO₄. Results presented here indicated that K. scoparia had potential in removal of Pb

S. L. Zhao · X. J. Shang · L. A. Duo Tianjin Key Laboratory of Animal and Plant Resistance, Tianjin 300387, People's Republic of China

S. L. Zhao · X. J. Shang · L. A. Duo (⊠) College of Life Sciences, Tianjin Normal University, Tianjin 300387, People's Republic of China e-mail: duolian_tjnu@163.com and Cr from MSW compost with the combined application of EDTA and $(NH_4)_2SO_4$.

Keywords Heavy metals · *Kochia scoparia* · Municipal solid waste compost · Phytoextraction · Spatial distribution

Introduction

With the economic, industrial, and agricultural development, environmental pollution has become an increasingly serious issue in the developing countries (Mittal et al. 2008; Gupta et al. 2007a). Many scientists have contributed to the control of environmental pollution (Jain et al. 2003; Gupta et al. 2007b; Mittal et al. 2009, 2010). Heavy metal contamination is one of the important environmental problems (Colak et al. 2011; Gupta et al. 2013a). This is attributed to non-biodegradability, the potential toxicity, and the high persistence of heavy metals in the environment. Various human activities, including mining and metal treatment operations, industrial and agricultural activities, and vehicle emissions, lead to an accelerated release of various metals into the environment, posing a potential hazard to human health and ecosystems (Adriano 2001). Many efforts have been made to remove toxic metals from contaminated environment (Olgun and Atar 2012; Gupta et al. 2011a, b, 2013b).

Due to rise in population and urban development, the amount of municipal solid waste (MSW) is rapidly increasing (Ogundiran and Afolabi 2008). As the most populous country, China faces the urgent problem of MSW disposal (Zhang et al. 2010). Composting MSW is seen as the effective method of reducing MSW in large quantity, diverting organic wastes from landfill while producing a stable humus-like material suitable for agricultural purposes (Moldes et al. 2007). Application of composted



municipal waste in agricultural soils may improve the soil aggregation and structure, increase water-holding capacity, cation-exchange capacity, and buffer capacity, provide organic matter and micronutrients, and further improve crop yield and quality (Soumaré et al. 2003; Cherif et al. 2009). In spite of the beneficial effects of MSW compost, the presence of toxic metals in compost causes the greatest concern about its adverse environmental impacts as a result of excessive application to agricultural soils, especially in developing countries (Achiba et al. 2009; Businelli et al. 2009; Ayari et al. 2010).

Phytoextraction, which uses green plants to remove toxic metals from contaminated soils, has emerged as an environmentally safe, relatively cheap, and technically applicable strategy for the remediation of heavy-metalcontaminated sites (Garbisu and Alkorta 2001; Quartacci et al. 2006; Laidlaw et al. 2012). The natural phytoextraction technique utilizes hyperaccumulator plants that have exceptionally high capacity to accumulate heavy metals. However, the phytoextration efficiency of hyperaccumulators is generally conceived as too slow due to the limitations of their low biomass production, slow growth rate, and high metal specificity (Mulligan et al. 2001; Puschenreiter et al. 2001; Lesage et al. 2005). Another option for phytoextraction technology is to grow high biomass non-hyperaccumulator plants (Zhang et al. 2009) and to optimize metal removal by the application of chemical amendments, including chelators, soil acidifiers, etc. (Alkorta et al. 2004; Saifullah et al. 2010). Of the chelators tested, EDTA was found to be the most effective one to increase the mobility and bioavailability of heavy metals in the soil, and has been widely used in phytoextraction process (Huang et al. 1997; Mosekiemang and Dikinya 2012). As a weak extractant, $(NH_4)_2SO_4$ mainly extracts the water-soluble metals and the exchangeable fraction of metals, and it has been popularly and widely used as a soil fertilizer. Soil pH is a key factor influencing heavy metal availability for root uptake (Dijkshoorn et al. 1983; Zeng et al. 2011). In acidic soil, H⁺ competition for soil-binding sites stimulates metal desorption from binding sites (Alkorta et al. 2004). Studies have found that lowering soil pH decreases heavy metal adsorption and thus increases their concentrations in the aqueous phase (Harter 1983; Salt et al. 1995). In soil, metal uptake into roots occurs from the solution except Hg. As an acidifying fertilizer, application of $(NH_4)_2SO_4$ could decrease soil pH and enhance the availability of heavy metals in the soil, thereby promoting plant to absorb heavy metals from the soil solution. This has been confirmed by many researchers (Eriksson 1990; Lou et al. 2005; Wang et al. 2008). Metal availability in the soil may be increased by maintaining a moderately acid pH through the application of ammonium fertilizers (Salt et al. 1998; Alkorta et al. 2004).



Wild plant species usually own inherent properties such as strong tolerance to various adverse environments including heavy metal stress. It was demonstrated that some wild plants had the potential for phytoremediation of metal-contaminated soils (Wei et al. 2008; Bareen and Tahira 2011). The advantages of applying weeds to phytoremediation are the large biomass' the adaptability of the environment, and the early growing season. Kochia scoparia is known as a wild fodder plant with the characteristics of high adaptability, strong reproductive capacity, fast growth, and large biomass with plant height over 1.5 m. Under favorable conditions, it grows robustly. However, K. scoparia has not been used as test plant for phytoextraction of heavy metals. Little is known about its phytoextraction efficiency and heavy metal distribution in it. In the present study, a field experiment was conducted at northern campus of Tianjin Normal University from April 20, 2004 to Sep. 30, 2009. The main objectives were to investigate Pb and Cr distributions in K. scoparia, to evaluate the effectiveness of EDTA combined with (NH₄)₂SO₄ as mobilizing agents of heavy metals in MSW compost, and to evaluate the potential of wild plant with high annual biomass (K. scoparia) for phytoextraction of heavy metals (lead and chromium).

Materials and methods

Chemicals

All chemicals used in the experiment were of analytical purity purchased from Tianjin chemical reagent supply company, China. A stock solution of EDTA was prepared in 1 mol L^{-1} using ethylenediaminetetraacetic acid disodium salt and diluted to 25 and 50 mmol L^{-1} . As for EDTA combined with (NH₄)₂SO₄ treatments, 1 g ammonium sulfate was added to 1 L EDTA solution of 25 or 50 mmol L^{-1} .

MSW compost

MSW compost samples were supplied by Xiaodian Compost Plant of Tianjin, China, after sufficient composting process. Compost pH was measured by glass electrodes in 1:2.5 water extracts. Organic matter content was determined by the method of $K_2Cr_2O_7$ – H_2SO_4 oxidation, and 0.1 g compost sample was mixed with H_2SO_4 – $K_2Cr_2O_7$ solution and then heated at 170–180 °C. The solution was kept boiling for 5 min. After cooling, the residual $K_2Cr_2O_7$ was titrated by FeSO₄ standard solution with *O*-phenanthroline hydrate as an indicator. Organic matter content was calculated based on the amount of $K_2Cr_2O_7$ consumed. Total nitrogen content of compost sample was analyzed

Table 1 Some properties of the MSW compost

рН	Organic matter (%)	Total N (%)	Total P (%)	Total K (%)	Ca (g kg ⁻¹)	Fe	Mg	Pb (mg kg ⁻¹	Cr ¹)
7.62	22.0	0.57	0.34	1.21	30.6	20.0	5.78	172	89.9

using Kjeldahl digestion (Bremner and Mulvaney 1982). Compost samples were wet-digested with a mixture of aqua regia and $HClO_4$. A flame atomic absorption spectrophotometer (TAS-990, purchased from Beijing Purkinje General Instrument Co., Ltd) was employed to analyze the digests for concentrations of P, K, Ca, Fe, Mg, and heavy metals (Pb and Cr). Some properties of the MSW compost used in this study were shown in Table 1.

Experimental design

The experiment site was located at northern campus of Tianjin Normal University, China (117°2′E and 39°13′N). It is characterized by a warm and semi-humid monsoon climate with an annual average temperature of 12.3 °C and an annual average rainfall of 615 mm. The soil at the sample plot is sandy loam. MSW compost was spread as plant medium on the experimental site to a thickness of 20 cm. After full press, the compost became compact and flat. The experiment site area was 200 m².

Kochia scoparia, an easy-to-grow plant with high biomass production, is a dominant weed in local agricultural ecosystem, and it is also a wild fodder for animal production. It gradually invaded the experiment site from outer environment. After 4 years' succession, *K. scoparia* became the dominant species in the experimental site with plant density 3 plants m⁻².

EDTA disodium salt was dissolved in deionized water to obtain five treatments: EDTA single (0, 25, 50 mmol L⁻¹) and EDTA (25, 50 mmol L⁻¹) combined with 1 g L⁻¹ (NH₄)₂SO₄; 0 mmol L⁻¹ EDTA was used as the control. For convenience, five treatments were expressed as CK, E25, E50, E25+N, and E50+N, respectively. In Sep. 10, 2009, 15 *K. scoparias* with identical growth state were selected as experimental materials. One liter of EDTA solution at above five rates was watered to the nearest root area (10 × 10 cm²) of the corresponding plant as slowly as possible to ensure that the solution completely seeped into the compost medium around the plant, entering into available root zone area. Each treatment was replicated three times.

Plant sampling and analysis

The tested plants were uprooted carefully 20 days after EDTA and $(NH_4)_2SO_4$ application. After having harvested, the plants were washed with deionized water and separated

into roots and shoots. Then, the aerial shoot parts were divided into five sections (0-30, 30-60, 60-90, 90-120, and over 120 cm) from lower stems (near medium surface) to upper spike. The detached plant parts were dried at 80 °C to a constant weight and weighed.

Dried plant samples were ground and wet-digested with a mixture of concentrated HNO₃ (16 mol L^{-1}) and HClO₄ (12 mol L^{-1}) at the ratio of 5:1 (v/v). The concentrations of Pb and Cr in the digested samples were determined by AAS.

Statistical analysis

The data presented in this paper were means of triplicates with standard deviations. All the data were tested by oneway analysis of variance (ANOVA), and means were compared by Duncan's multiple range test at significance level 5 % using the SPSS 12.0 statistical package. Nonlinear regression analysis was performed on plant biomass and heavy metal spatial pattern using Microsoft Excel 2003.

Results and discussion

Plant biomass

Kochia scoparia did not show any toxicity symptoms such as wilting and necrosis, suggesting its high tolerance to heavy metals and EDTA. No toxicity symptoms were also observed by Chen and Cutright (2001) on sunflower and by Zaier et al. (2010) on Brassica napus. The vertical distribution of plant biomass is reported in Table 2. The shoot weights were significantly higher than root weights. The aerial biomass increased greatly with plant height. The maximum biomass appeared in upper part with height over 120 cm, followed by 90-120 cm height, and the minimum biomass occurred in roots. Shoot biomass with height over 120 cm accounts for 33.1 % of the total weight of the plant, while root biomass only occupies 8.0 %. The results suggested that there were significant differences in the vertical distribution of plant biomass, and the plant biomass showed an "inversion pyramid" pattern in vertical spatial structure. K. scoparia was subjected to extract heavy metals from contaminated medium because of its high above-ground biomass. Figure 1 showed nonlinear regression simulation between spatial pattern of plant biomass



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Parts of plant	Roots	Shoots									
Plant height (cm)		0–30	30-60	60–90	90-120	>120					
Dried weight (g plant ⁻¹)	$10.9 \pm 2.5^{\rm e}$	15.5 ± 3.4^{d}	14.3 ± 2.1^{d}	$20.9 \pm 4.1^{\circ}$	29.5 ± 4.7^{b}	45.0 ± 6.3^{a}					
Percentage in total plant (%)	8.0	11.4	10.5	15.4	21.7	33.1					

 Table 2 Spatial pattern of plant biomass

Data are means of three observations \pm SD. Values with the same letter are not significantly different between plant shoots with different height (P < 0.05)



Fig. 1 Nonlinear regression simulation between plant biomass and height

and plant height. The correlation coefficient was 0.9928 and statistically significant at 1 % level.

Pb and Cr concentrations and partitioning in plant shoots

It is well known that heavy metal uptake into roots mainly takes place from the aqueous phase; yet strong binding to soil particles or precipitation hinders soil metals soluble and available for plant uptake. Synthetic chelating agents like EDTA have been shown to enhance the solubility of heavy metals in soil and to increase metal uptake in plants (Bareen and Tahira 2010). This is mainly due to the formation of metal-chelate complex capable of being translocated easily from root to shoot. An increase in Pb, Zn, and Cd concentrations by 104.6, 3.2, and 2.3 times in the above-ground plant biomass has been observed by Grčman et al. (2001) at a single dose of 10 mmol kg⁻¹ EDTA addition. Blaylock et al. (1997) confirmed that EDTA effectively prevented cell wall retention of heavy metal and not only influenced heavy metal uptake but also enhanced heavy metal translocation in the plant. Dede et al. (2012)



found that applications of EDTA resulted in a considerable increase in copper and lead concentrations in *Brassica juncea* from sewage sludge.

In the present study, single EDTA additions and combined additions with $(NH_4)_2SO_4$ at higher dose increased Pb and Cr concentrations in *K. scoparia* shoots at different heights (Fig. 2). It seems that high concentrations of EDTA cause desorption of some heavy metals that are more strongly bound to the soil particles. Compared with control plants, EDTA addition increased Pb concentration in high spatial shoots, especially at higher tested level. Pb concentrations peaked at 50 mmol L⁻¹ EDTA combined with $(NH_4)_2SO_4$ in shoots of 60–90, 90–120, and over 120 cm, respectively, which were all two times more than those of controls.

Chromium concentration in shoots of K. scoparia was enhanced by EDTA addition at higher dose. $(NH_4)_2SO_4$ was more effective in assisting Cr accumulation in plant shoots. As shown in Fig. 2, a combined addition of 25 mmol L^{-1} EDTA and (NH₄)₂SO₄ increased Cr concentrations in five aerial parts from lower part to upper part by 28, 81.4, 47.6, 42.7, and 82.9 %, respectively, as compared to corresponding parts treated with 25 mmol L^{-1} EDTA alone. Chromium concentrations peaked at 50 mmol L^{-1} EDTA combined with $(NH_4)_2SO_4$ in shoots of 0-30, 30-60, 60-90, and 90-120 cm, respectively, and increased by 152.5, 107.1, 47.2, and 39 % when compared with 50 mmol L⁻ EDTA single treatment. Soil pH plays a key role in influencing the availability of heavy metals for root uptake. A lowering of pH often leads to a significant increase in solubility of metals in the soil and their availability for plant uptake. The acidifying effect of NH₄⁺-containing fertilizers can be due to nitrification of NH_4^+ and exchange of NH_4^+ for H⁺ in the uptake process of fertilizer of N (Eriksson 1990), leading to the release of H^+ .

Though EDTA addition increased Pb and Cr concentrations in plant shoots, the effect was not prominent. The application of chelating agents in field experiments has not been widely demonstrated. The findings observed by Kayser et al. (2000) have shown that there were much higher metal tissue concentrations under laboratory greenhouse conditions as compared to the natural conditions in the field.



Fig. 2 The spatial distribution of Pb and Cr concentrations in plant shoots. Bars represent standard deviation



Fig. 3 The spatial distribution of Pb and Cr uptakes in plant shoots. Bars represent standard deviation

Pb and Cr uptake and partitioning in plant shoots

It is well known that high heavy metal concentration and high biomass production of plant are of vital importance for successful phytoextraction (Roger et al. 2000; Saifullah et al. 2009). When considering the phytoextraction potential of a given species, the amount of heavy metal removed from soil is more important than heavy metal concentration. *K. scoparia*, a wild fodder plant with height over 1.5 m, could produce high above-ground biomass, suggesting its potential ability for phytoextraction of heavy metals.

Results of our study indicated that Pb uptake increased toward the top of the shoot, peaked at shoots over 120 cm for five different EDTA treatments (Fig. 3). Lead uptakes increased 1.3, 2.4, 5.7, 7.1, and 3.9 times from the lowest to highest shoot height for E0, E25, E50, E25+N, and E50+N treatments, respectively. Relative to control, 50 mmol L⁻¹EDTA increased Pb uptake in plant shoots over 30 cm height. Combined addition of 50 mmol L⁻¹ EDTA and $(NH_4)_2SO_4$ increased Pb uptake in five aerial parts from lower part to upper part by 21.6, 19.2, 111.3, 124.3, and 154.0 %, respectively, as compared to those of controls. Many factors affect Pb transport and accumulation in plant-soil systems, such as soil pH, soil redox potential, cation-exchange capacity, and fertilizer application, etc. Mobilization of Pb is highly increased by inorganic fertilizers containing NH_4^+ even at low pH (Tu et al. 2000; Schmidt 2003). Similarly, the application of nitrogen amendments effectively increased Pb accumulation by two



Fig. 4 The correlation of Pb and Cr concentrations in spatial plant shoots and height (1–5 represents plant shoots with height 0–30, 30–60, 60–90, 90–120, and over 120 cm, respectively)



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crops (Lin et al. 2010). However, the effect of $(NH_4)_2SO_4$ on enhancing Pb uptake by *K. scoparia* was not prominent.

Relative to controls, EDTA single addition at 50 mmol L^{-1} increased Cr uptake by 36.3, 84.2, 35.8, 27.0, and 199.5 % in five aerial parts from lower part to part, respectively. Combined upper addition of 50 mmol L^{-1} EDTA and $(NH_4)_2SO_4$ increased Cr uptakes in five aerial parts by 244.5, 281.7, 100.0, 77.2, and 187.4 %, respectively, when compared with controls. A significant effect of EDTA was observed by Saifullah et al. (2009) at lower doses in Cr uptake into wild plant-Suaeda fruticosa and its translocation into the leaf. Ammonium sulfate had a prominent effect on assisting EDTA enhancement of Cr uptake. This can be explained from two aspects: (1) Ammonium sulfate addition decreased soil pH through nitrification and ion exchange and thus increased metal solubility in soil and availability to plants; (2) as a N fertilizer, ammonium sulfate promoted plant growth, leading to an increase in plant biomass and thus metal uptake (Zhao et al. 2013).

Spatial simulation of Pb and Cr concentrations in plant shoots

Figure 4 shows the correlation of Pb and Cr concentrations in plant aerial parts and height. There was a significant correlation between Pb and Cr concentrations in plant shoots and height at all the treatments. Under EDTA single addition at 50 mmol L^{-1} and EDTA combined addition at 25 mmol L^{-1} with $(NH_4)_2SO_4$, the relationship between Pb and Cr concentrations and plant height was found to be positively linear and statistically significant at 1 % level. The correlation coefficients of Pb at above two treatments were 0.9881 and 0.9779, respectively, and thev were 0.9502 and 0.9961 for Cr. The positive correlation indicated that the 50 mmol L^{-1} EDTA single addition and 25 mmol L^{-1} EDTA combined addition with $(NH_4)_2SO_4$ enhanced Pb and Cr translocation from lower part to upper part of shoots.

Conclusion

*Kochia sco*paria, the indigenous fast growing wild plant with high biomass production, has the potential of metal phytoextraction from MSW compost under mobilizing agent application. The partitioning pattern of metals in shoots showed that Pb and Cr uptakes increased toward the top of the shoot; 50 mmol L^{-1} EDTA combined addition with (NH₄)₂SO₄ enhanced Pb, Cr uptake in each part of plant shoots due to the formation of a metal–chelate complex which was capable of being translocated easily from root to shoot. Considering adverse effect of EDTA application such as the leaching risk of heavy metals, research on the application of other chelates is needed to be carried out.

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