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Assisted phytoremediation of Cd-contaminated soil using poplar rooted cuttings

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Abstract. To investigate the effect of amended substrates on cadmium uptake by one-year old poplar rooted cuttings a pot culture was carried out. Pots were filled with three substrates. Four treatments of Cd supply including were organized. The results showed that higher biomass productions in substrates A and B compare to substrate C, led to an increase total Cd uptake two times more than that in substrate C, at 150 mg kg^{-1} concentration. Meanwhile maximum total uptake occurred in substrate B at 100 mg kg^{-1} concentration. Using synthetic chelators such as ethylenediaminetetraacetic acid in order to achieve high removal rate led to increased environmental impacts while they are not expected when such environmental friendly approaches are applied.

Keywords: soil, cadmium, ethylenediaminetetraacetic acid, phytoextraction, substrate

INTRODUCTION

Contamination of soils with heavy metals is an important environmental problem all over the world (Assareh *et al*., 2008). Heavy metals have a considerable toxicity for microorganisms, plants, humans and animals (Fotakis and Timbrell, 2006). Moreover, degradation processes do not have any effect on heavy metals therefore these chemicals stay in the environment, although the bioavailability of them can alter significantly as a result of their interactions with the various soil components (Doumett *et al*., 2008). Removal of this persistent pollutant is necessary but very difficult. Using physiochemical technologies to remediate large volumes of such soils would be very expensive. Phytoremediation is an emerging technology that aims to extract or inactivate metals in soils. The technology has attracted attention for the low cost of implementation and environmental benefits. In addition, phytoremediation is likely to be more acceptable to the public than other traditional methods (Evangelou *et al*., 2007a,b; Wei *et al*., 2008, Włodarczyk *et al*., 2012).

In order for phytoremediation to be effective, it is necessary that plants accumulate high quantity of heavy metals, tolerate soil contamination, and also produce a great deal of biomass in contamination conditions (McGrath *et al*., 2002). Recently, research in the field of metal phytoremediation has concentrated on hyperaccumulators. These are plants with a highly abnormal potential to accumulate metals (Reeves and Baker, 2000). However, these plants are slow-growing and produce low biomass as well; moreover, hyperaccumulators generally accumulate only one specific element and have a limited root system. These limitations make them impractical for the remediation of sites with deep contamination and/or contamination caused by more than one metal (Begonia *et al*., 2005; Luo *et al*., 2005). Selection of woody species that are metal resistant, high-depth rooted and fast-growing, and that have the ability to grow on nutrient-poor soil, can be a suitable alternative to clean up sites with heavy metal contaminated soil (Pulford and Watson, 2003).

Low solubility of metals and the sorption of them to soil particle surfaces can reduce the efficiency of phytoextraction. Many researchers have been concentrated on addition of natural and/or chemical chelating agents to increase uptake of heavy metals from soil and to reach high removal rates (Munn *et al*., 2008; Pastor *et al*., 2007; Quartacci *et al*., 2007). However, chelators have negative effects including elevated toxicity to plants and soil microorganisms and their potential risk of leaching to ground water (Evangelou *et al*., 2007b). Therefore, in order to induce phytoextraction, applying environmental friendly approaches is more beneficial (Wei *et al*., 2010). Improvement of growth conditions for plant especially by amendment of substrate can strengthen phytoextraction through increasing plant biomass. Addition of organic matter is a common method for increasing plant biomass.

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In this research, cocopeat and peatmoss will be added to the soil to investigate the effect on cadmium accumulation by poplar rooted cuttings (*Populus alba* L.) a fast-growing and high-biomass producer plant with high transpiration rate and low ecological requirements that make it practical for remediation of contaminated sites.

MATERIALS AND METHODS

The unpolluted soil used in this study was collected from depth of 0 to 30 cm from the campus of Agriculture and Natural Resources of University of Tehran. The soil was air dried and passed through 2 mm sieve and mixed uniformly. The results of chemical and physical properties of the soil are presented in Table 1.

Preparation of rooted cuttings was carried out in Masir-e-Sabz nursery, Karaj, Iran. Uniform-in-size cuttings (length (25±3 cm), diameter (8±1 mm) and number of bud (8)) were taken from single *Populus alba* (L.) tree were rooted and planted in the nursery from January 2009 to February 2010. 72 homogenously and uniform-in-size rooted cuttings were selected for the pot experiment.

To prepare substrates, loam soil, cocopeat and peatmoss were used. Pots were filled with three substrates mentioned below: A – loam soil and cocopeat were mixed in 1:1 (v/v) ratio (P50%), B – loam soil, peatmoss were mixed in 1:1 (v/v) ratio and C – loam soil. Four treatments of Cd contamination including 0 mg kg^{-1} (the control, no external Cd), 50, 100, and 150 mg kg^{-1} (were spiked as $CdCl_2$ $2.5H_2O$) were organized. Plastic pots with 35 cm diameter and 45 cm height were filled with contaminated airdried A, B and C substrates which were passed through a 4 mm sieve and then equilibrated for one month. During fifteenth of February 2010, rooted cuttings were transplanted in to the pots. All treatments were replicated three times. Loss of water was made up daily using tap water (no detection of Cd). To avoid nutrient limitation, fertilizers were added to each pot. The plants were harvested at the end of September 2010.

The harvested plants were washed with tap water and deionized water to remove soil and then separated into leaves, shoot and root. The samples were dried at 70°C in an oven to constant mass. The dried plant samples were ground in a stainless steel mill to pass through a 20-mesh sieve. The milled samples were digested using a digesdahl apparatus with concentrated H_2SO_4 and H_2O_2 (Vicentim and Ferraz, 2007). To determine Cd content, the solution was analyzed in ICP-OES equipment.

The experiments were arranged in a factorial experimental design with completely randomized basic design. Data were processed using SAS software. Statistical differences between treatments were tested by two-ANOVA followed by HSD test to separate level means. Results were considered significant at $p<0.01$.

RESULTS AND DISCUSSION

Analysis of variance revealed significant differences between the main effects ($p<0.01$ and $p<0.05$) for the studied attributes (Cd supplies and substrates). It would show that there are significant differences among root, shoot and leaf growing responses as well as root, shoot and leaf Cd content in different treatments (Cd supplies and amendments) (Table 2). Non-significant interactions indicated that the rates of the trait changes are constant between the factors levels.

T a b l e 1. Physical and chemical characteristics of agricultural soil used in this study before adding cadmium

Parameter	Quantity	Parameter	Quantity
Clay (%)	24	Total nitrogen (%)	0.08
Silt (%)	35	Available phosphate (mg kg^{-1})	18
Sand (%)	41	Available potassium (mg kg^{-1})	232
Field capacity (%)	26	Fe (mg kg^{-1})*	5.10
pH	7.5	Cu (mg kg^{-1})*	4.00
EC (dS m^{-1})	4.42	Zn (mg kg^{-1})*	1.01
$CaCO_3$ (%)	8.1	Mn (mg kg^{-1})*	7.90
C_{org} (%)	0.86	Cd (mg kg^{-1})*	0.13
CEC (C mol kg^{-1})	25	Pb (mg kg^{-1})*	1.94
SO_4 (meq l^{-1})	37.20		

*DTPA - extractable.

Table 2. Mean squares of studied traits

Source of variation	df	Mean square						Total uptake
		Biomass			Concentration			
		leaf	root	shoot	leaf	root	shoot	
Cd conc.	3	**	**	**	*	**	**	**
Substrates	2	**	**	**	*	**	*	**
Cd conc. × subst.	6	ns	ns	ns	ns	ns	ns	**
CV	-	13.9	14.74	11.00	17.67	21.58	22.96	24.36

*, ** – significant at a probability level $p < 0.05$, 0.01, respectively; ns – not significant.

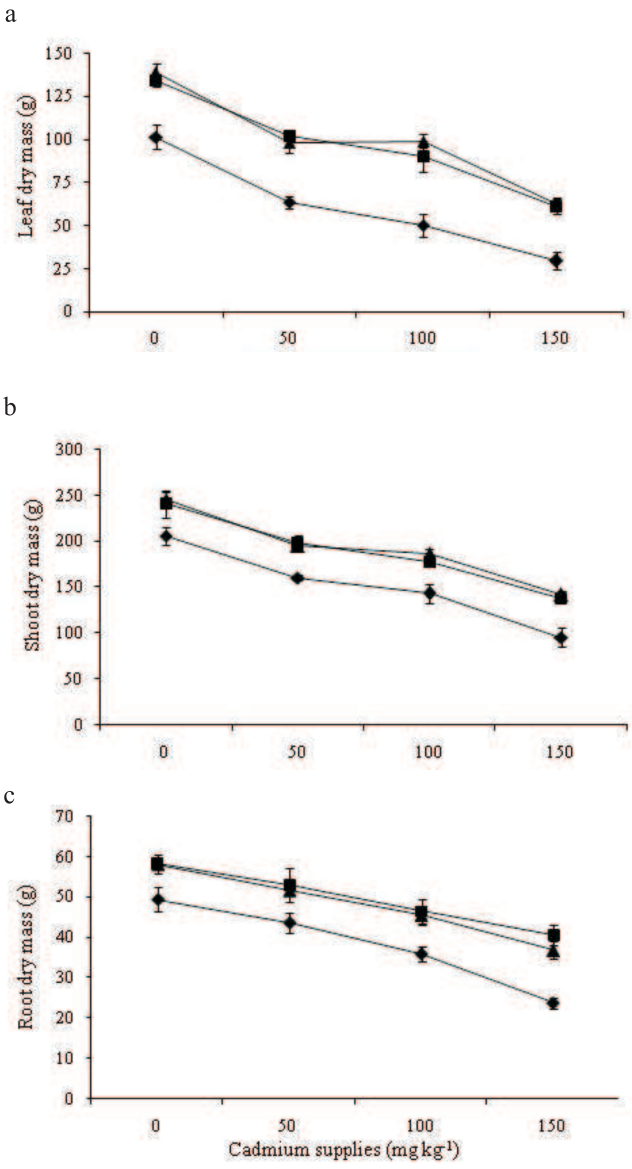

Fig. 1. Poplar: a – leaf, b – shoot, c – root biomass (means±SD) in respond to different cadmium supplies in different substrates (A■ , B▲ , C◆).

The results of poplar biomass responses are presented in Fig. 1. In all three substrates, rooted cuttings showed a decreased trend in the leaf, shoot and root biomass with increasing Cd concentration. Maximum decrease in biomass production was observed in leaf biomass in comparison with root and shoot biomass in all three substrates (Fig. 1a). Poplar showed maximum amounts of leaf, shoot and root biomass in substrates B and A, at 0 mg kg^{-1} Cd supply. Minimum amounts of biomass elements for poplar were occurred in substrate C and in cadmium supply with 150 mg kg^{-1} ($p<0.01$) (Fig. 1). Alternatively, only Cd supplies with 50 and 150 mg kg^{-1} significantly ($p<0.01$) decreased leaf and shoot biomass of poplar, whereas Cd supply with 100 mg kg^{-1} had insignificantly ($p<0.01$) effects on poplar leaf and shoot biomass in substrate B.

In all three substrates, the similar order (root >shoot >leaf) of Cd concentration was observed in *Populus alba* (L.) rooted cuttings (Fig. 2). However, Cd concentrations in leaf, shoot and root were increased with the increase of 100 mg kg^{-1} cadmium supply ($p<0.01$). Substrate B caused maximum Cd concentration in leaves at 100 mg kg^{-1} (Fig. 2a). Increasing Cd supplies with 50 and100 and 150 mg kg^{-1}, increased Cd concentration in roots of poplar ($p<0.01$). Maximum root accumulation of Cd (135 mg kg^{-1}) was observed in substrate B, at 150 mg kg^{-1} Cd supply (Fig. 2c). The tendency of Cd concentration in shoots was similar to roots with the only exception in case of substrate C, at 150 mg kg^{-1} Cd supply which showed a statistically significant decrease (Fig. 2b). Maximum amount of shoot Cd content (75 mg kg^{-1}) was found in substrate B, at 150 mg kg^{-1} Cd supply.

Total Cd uptake was increased across different treatments in terms of applying substrates in plants in similar levels of Cd ($p<0.01$). However, As shown in Table 3, in all three substrates, increasing Cd supplies with 50 and 100 mg kg^{-1}, increased total Cd uptake plants ($p<0.01$) while a decreased trend was observed between 100 and 150 mg kg^{-1} Cd supplies. In the same Cd supplies, substrates B and C caused maximum and minimum Cd uptake respectively ($p<0.01$). Substrate B caused maximum total uptake of Cd (20952.14 µg plant^{-1}) in Cd supply with 100 mg kg^{-1} (Table 4).

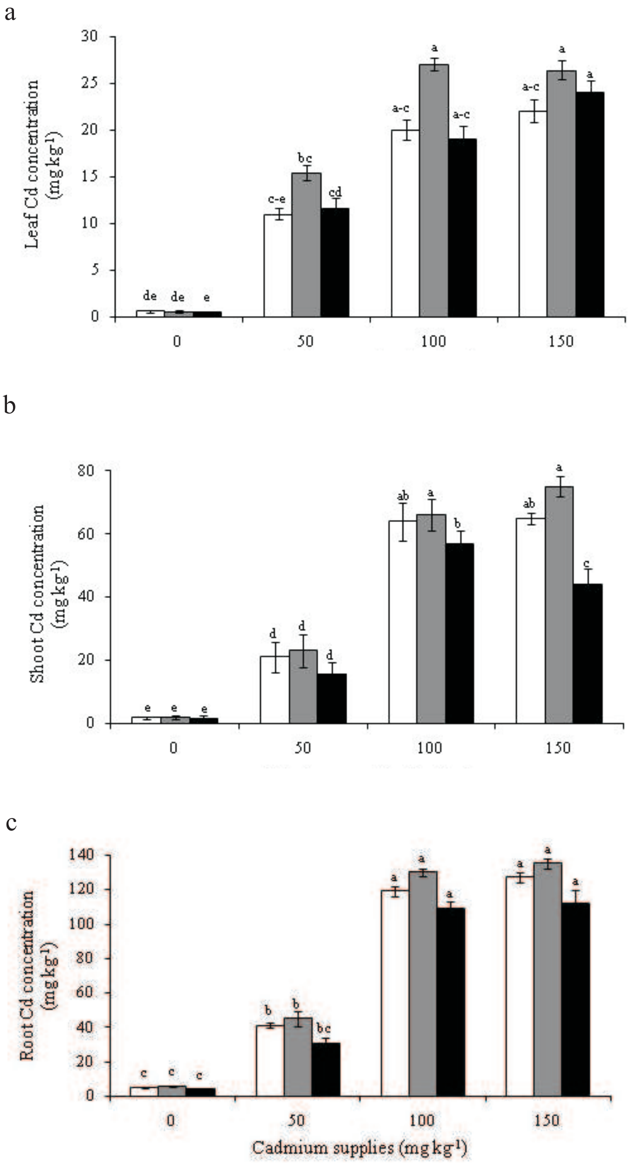

Fig. 2. Concentrations of Cd in: a – leaf, b – shoot, c – root of *Populus alba* (L.) rooted cuttings responded to different Cd supplies in substrates A (☐), B (▨), and C (■). Error bars show SD, n=3.

Heavy metals are released in the environment by power generating stations, heating systems, waste incinerators, metal working industries, and many other sources. Their accumulation in soils can become dangerous to all kinds of organisms, including plants (Gichner *et al*., 2006). This accumulation in arable soils is more serious, since these toxic elements can be taken up by plants and transferred to human (Chehregani *et al*., 2009). In the present experiment, the addition to soil of cocopeat and peatmoss was intentionally designed to prove the hypothesis that application of these materials to substrate would assist plant growth and Cd uptake in two poplars (species with fast growth rate and deep root system) in the same time. One year old rooted cuttings were planted in prepared substrates for one growing season. This short-term exposure was nonetheless enough to reach early evidence of positive effects on leaf functioning and structure and biomass partitioning (Sebastiani *et al*., 2004). Substrates (B and A), despite this short-term exposure, did exert positive effects on growth responses in both poplar clones compare to control substrate (C). Substrate B, at 150 mg kg^{-1} Cd supply, caused 54% and 33% increase in leaf and shoot biomass production compared to substrate C, respectively (Fig. 1a, b). Maximum difference among root biomass production (43%) was observed in substrate A at 150 mg kg^{-1} Cd supply compared to substrate C (Fig. 1c). In most cases, the differences between effects of B and A substrates on growth responses were not significant ($p<0.01$) (Table 3). Such increases in growth responses might be because that organic matter would increase cation exchange capacity (CEC) (Lin and Chen, 1998) and aeration as well as improve structure (Martínez-Fernández and Walker, 2011) in the substrates.

The results showed that, in all three substrates, there was no statistically significant difference among the root Cd concentrations in similar levels of Cd supply, whereas in some cases, a significant difference among shoot Cd concentrations as well as leaf Cd concentrations was observed (Fig. 2). However, Wei *et al*. (2010) reported that bioavailability of heavy metal in soil can be decreased by the organic amendments. It could be related to side effects of organic matter in soil acidification. On the other hand, Lin and Chen (1998) stated that metal concentrations and CEC in sediments were positively correlated with organic matter content.

T a b l e 3. Total Cd uptake (µg plant^{-1}) (means ± SD) by poplars responded to different Cd supplies in different substrates

Subatrates	Cd supply (mg kg^{-1})			
	0	50	100	150
A	802.33 ± 14(k)	7506.50 ± 30(h)	18729.88 ± 49(b)	15510.22 ± 32(d)
B	905.05± 11(j)	8374.68 ± 22(f)	20952.14 ± 55(a)	17295.77 ± 54(c)
C	559.84± 16(l)	4578.48 ± 24(i)	13168.28± 51(e)	7598.92 ± 24(g)

Different letters show the significant differences among the treatments ($p< 0.01$, $n = 3$).

Phytoextraction efficiency is related to both plant metal concentration and biomass production. (Wu *et al.*, 2004). Hyperaccumulators produce low biomass, therefore, to improve phytoextraction efficiency, using fast-grow and deep-root plants is more practical compare to hyperaccumulators (Pulford and Watson, 2003). Zaleśny *et al.* (2007) found that there is a great potential for remediation of pollutants using poplar. Total Cd uptake in treatment 150 mg kg^{-1} Cd supply was twice as high in substrate B and A compare to substrate C while this amount was not observed in other treatments of Cd supply (Table 3). In all three substrates, specifically, there were two trends in the performance of phytoextraction by plants. First, with increasing Cd supply to 100 mg kg^{-1}, an increased trend was observed. Second, the productivity of phytoextraction was decreased in Cd supply with 150 mg kg^{-1}.

The results suggest that application of substrates can significantly enhance the Cd uptake capacities of poplar even under the conditions of Cd pollutant levels. This might be because that amendments improved plant biomass production and maintaining a threshold level of Cd content (Jalloh *et al.*, 2009). Generally, the results demonstrated that used substrates showed a great potential in enhancement of Cd uptake by poplars.

CONCLUSIONS

1. The results of this research work indicated that, at least in some cases, poplars grown in amended substrates have a higher efficiency in remediating contaminated soils than do some slow-growing hyperaccumulators.

2. In most cases, Cd concentrations in leaf, shoot and root were increased with the increase of Cd supply.

3. Applying substrates caused increase in biomass production and finally enhanced Cd uptake by poplar rooted cuttings.

4. Although using complexing agents such as EDTA increase metal accumulation in plants considerably, but they have toxic effects on plants and soil microorganisms, whereas applying substrates can be considered as a promising and environmental friendly alternative for phytoremediation of Cd polluted soil due to their conditions that prepare to poplar growth and not having any outcome for the environment.

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