The Effect of Leaf Litter on Phytoextraction of Cr(VI) by Two Salicaceae Species from Contaminated Soils

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Abstract

Contamination of the agricultural land by the toxic chromium is considered as a worldwide hazard that has accelerated dramatically since the beginning of industrial revolution. phytoextraction as an alternative treatment has shown great potential for remediation of heavy metal-contaminated soils. The present work is an attempt to assess the ability of *Populus alba* L. and *Populus nigra* L. for the phytoextraction of Cr(VI) from a contaminated soil and leaf litter usefulness for increase the phytoextraction efficiency. To prepare amended substrate, loam soil and leaf litter were mixed in 1:1 (v/v) ratio (P50%). The substrate were contaminated with 50, 100 and 150 mg kg^{-1} of Cr(VI) [were spiked as potassium dichromate ($K_2Cr_2O_7$)]. Homogenously and uniform-in-size rooted cuttings of *P. alba* L. and *P. nigra* L. were transplanted in contaminated substrates. At the end of growing season, the amounts of Cr accumulation in leaves, shoots and roots were measured. The results indicated that, in all treatments, Cr(VI) was accumulated in roots, shoots and leaves of seedlings. Amended substrate was able to enhance the total uptake of Cr by plants ($P<0.01$). This study thus suggested that the leaf litter due to its conditions that prepare to poplar root system and not having any outcome for the environment could be exploited for remediation of chromium from chromium contaminated sites.

Key words: Chromium, *Populus alba* L., *Populus nigra* L., Potassium dichromate, Total uptake

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1. Introduction

Anthropogenic activities such as metallurgical (steel, ferro- and nonferrous alloys), refractories (chrome and chrome-magnesite), and chemical (pigments, electroplating, tanning and other) have led to the widespread contamination that Cr shows in the environment (Kotas and Stasicka, 2000). Chromium occurs in nature as trivalent [Cr(III)] being an essential element in human and animal physiology and as hexavalent [Cr(VI)] which is a mobile contaminant entering the cells, extremely toxic and carcinogen (Mandiwana et al., 2007 and ATSDR, 2002). Totally, Cr is not considered as an essential element for plant nutrition (Mallick et al., 2010). phytoextraction can be defined as the combined use of plants, soil amendments and agronomic practices to remove contaminant from contaminated sites or decrease their toxicity (Salt et al., 1998 and Brunetti et al., 2012). At present, a great deal of information accumulated concerning plants' ability of accumulation and responses of functional systems to various heavy metals (Maine et al., 2004).

On the other hand, 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 (Pastor et al., 2007 and Munn et al., 2008). However, chemicals have negative effects including elevated toxicity to plants and soil microorganisms and their potential risk of leaching to ground water (Evangelou et al., 2007). A promising strategy has been presented to be the application of soil amendments which are able to improve phytoextraction efficiency by increasing metal availability in the soil (Brunetti et al., 2012). In this research, we investigated the phytoextraction potential of *P. alba* L and *P. nigra* L. one-year old cuttings, as two high-biomass producer species from salicaceae, as well as tested the hypothesis that in presence of leaf litter phytoextraction efficiency of these two species may alter.

2. Research Methodology

The unpolluted soil was air dried and its physiochemical properties were measured through standard methods (Table 1).

Parameter	Quantity	Parameter	Quantity
Soil texture	Loam	Total nitrogen (%)	0.076
Clay (%)	24	Available phosphate (mg kg^{-1})	18
Silt (%)	35	Available Potassium (mg kg^{-1})	232
Sand (%)	41	Field Capacity (F.C)	26
pH	7.5	Cu (mg kg^{-1})*	4.002
EC(dS m^{-1})	4.42	Zn (mg kg^{-1})*	1.01
$CaCO_3$%	8.1	Mn (mg kg^{-1})*	7.854
OC%	0.86	Cd (mg kg^{-1})*	0.13
CEC(Cmolkg^{-1})	25	Pb (mg kg^{-1})*	1.94
So_4 (meq L^{-1})	37.20	Fe (mg kg^{-1})*	5.1

* DTPA-Extractable

Table 1. Physical and chemical properties of soil before adding Cr (VI)

Rooted cuttings were prepared in Masir-e-Sabz nursery, Karaj, Iran. Uniform-in-size cuttings [length (25±3 cm), diameter (8±1mm) and number of bud (8)] were taken from two single *P. alba* L. and *P. nigra* L. trees. The cuttings were rooted and planted in the nursery from January 2009 to February 2010. 48 homogenously and uniform-in-size rooted cuttings were selected for the pot experiment. Loam soil (as control) and mixture of loam soil and leaf litter in 1:1 (v/v) ratio (P50%) were organized as substrates. The physiochemical properties of leaf litter are presented in Table 2.

Parameter	Litter
Salt content	1.2 g kg^{-1}
Content of organic matter (%)	38
pH	6.2
K_2O	78 %
P_2O_5	23.2 mg kg^{-1}
N	0.74 %
Cr (VI) Content	Not detected

Table 2. Chemical properties of leaf litter

Four treatments of Cr contamination including 0 mg kg^{-1} (the control, no external Cr), 50, 100, and 150 mg kg^{-1} (were spiked as $K_2Cr_2O_7$) were applied. During fifteenth of February 2010, rooted cuttings were transplanted in to the pots and 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 samples were ground in a stainless steel mill and digested using a digesdahl apparatus with concentrated H_2SO_4 and H_2O_2 (Vicentim and Ferraz, 2007). To determine Cr content, of the extraction, it was analyzed using ICP-OES equipment.

All treatments were replicated three times. The whole experiment was set up in the completely randomized factorial design. Two-way ANOVA was used to confirm statistical differences between treatments and followed by honestly significant difference (HSD) to separate level means. Results were considered significant at $p<0.01$.

3. Results and Analysis

3.1 Biomass

Biomass growth of the two poplars grown in different treatments (including contaminated and amended substrates) after planting for 6 months later are listed in Table 3. There are significant differences among root shoot and leaf biomass production in different treatments (Cr supplies and amendment) ($p< 0.01$). In both substrates, rooted cuttings showed a decreased trend in the biomass elements (leaf, shoot and root) with increasing Cr concentration. Poplar seedlings showed maximum amounts of leaf, shoot and root biomass production in amended substrate (soil + leaf litter), at 0 mg kg^{-1} Cr supply. *P. nigra* L. produced greater amounts of biomass elements than *P. alba* L. In the same pollution treatment, applying leaf litter led to significant increase in leaf and shoot production between two species whereas this difference was not significant in absence of leaf litter ($p< 0.01$) (Table 3). In agreement with these findings, Alizadeh et al. (2012) stated that addition of cocopeat and peatmoss (two kinds of substrates) to soil, caused higher biomass production by *P. alba* L. Increases in biomass production might be because that organic matter would increase cation exchange capacity (CEC) (Lin and Chen, 1998) and aeration as well as improve the substrate structure (Martínez-Fernández and Walker, 2011).

Species	Substrate	Cr supply (mg kg^{-1})	Leaf biomass (g DW)	Shoot biomass (g DW)	Root biomass (g DW)
P. alba	soil	0.0	101.5± 6 e-g	205.66 ± 5e	49.4 ± 7c-e
		50.0	72.96 ± 2.7 g-i	169.3 ± 19fg	45.7 ± 4.1ef
		100.0	41.66 ± 5 i	125.5 ± 8h	40.4 ± 6f
		150.0	39.7 ± 3 i	104.9 ± 15i	28.4 ± 1.3h
	soil + leaf litter	0.0	152.7 ± 14 bc	253.3 ± 25c	61.4 ± 7ab
		50.0	110.8 ± 12 d-f	204.2 ± 23e	58.4 ± 10.3b
		100.0	84.3 ± 11 f-h	170.1 ± 17f	54.7 ± 6.4b-d
		150.0	76.8 ± 9.5 f-h	153.6 ± 13g	46.8 ± 4.9d-f
P. nigra	soil	0.0	123.3 ± 9.1c-e	233.23 ± 27d	59.3 ± 5.5b
		50.0	103.6 ± 7 e-g	200.2 ± 8.3e	49.4 ± 6c-e
		100.0	69.1 ± 6.1 hi	155.8 ± 10.6fg	44 ± 4.3e-g
		150.0	66.4 ± 6.2 hi	133.4 ± 13.2h	36.4 ± 5.1gh
	soil + leaf litter	0.0	191.7± 15.4 a	299.7 ± 8.3a	70.9 ± 6.9a
		50.0	174.7 ± 14 ab	271.3 ± 29b	62.5 ± 10.3ab
		100.0	140.6.7 ± 15.5 cd	226.3 ± 23d	57.6 ± 6bc
		150.0	132.2 ± 17 c-e	205 ± 24e	55.5 ± 4.2bc

Table 3. Biomass production of *P. alba* L. and *P. nigra* L. rooted cuttings exposed to different Cr concentrations during a 6 months period. Values are mean ± SD of three replications. Values in each column followed by the same letter are not significantly different at $p< 0.01$ (Tukey's Multiple Range Test).

3.2 Accumulation

Heavy metal 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 both substrates, the similar order (root >shoot>leaf) of Cr concentration was observed in *P. alba* L. and *P.nigra* L. A similar order for Cd accumulation (root > shoot> leaf) was found in *P. alba* L. seedlings (Alizadeh et al., 2012). January et al. (2008) reported higher Cr accumulation in *Helianthus annuus* below-ground parts. Higher Cr accumulation in root might be due to accumulation of Cr in vacuoles of root cells (Shah et al., 2001). However, Cr concentrations in shoot and root were increased with the increase of 150 mg kg^{-1} Cr supply, whereas leaf Cr concentration was increased with the 100 mg kg^{-1} Cr supply. Maximum leaf, shoot and root Cr concentration were observed in amended substrate at 100, 150 and 150 mg kg^{-1} Cr supply (Figs 1-3). In most cases, applying leaf litter led to increase in Cr concentration in plant tissues. The majority of observations indicated that, in the same cases, Cr concentrations in *P. alba* L. tissues were higher than that in *P. nigra* L. (Figs 1-3).

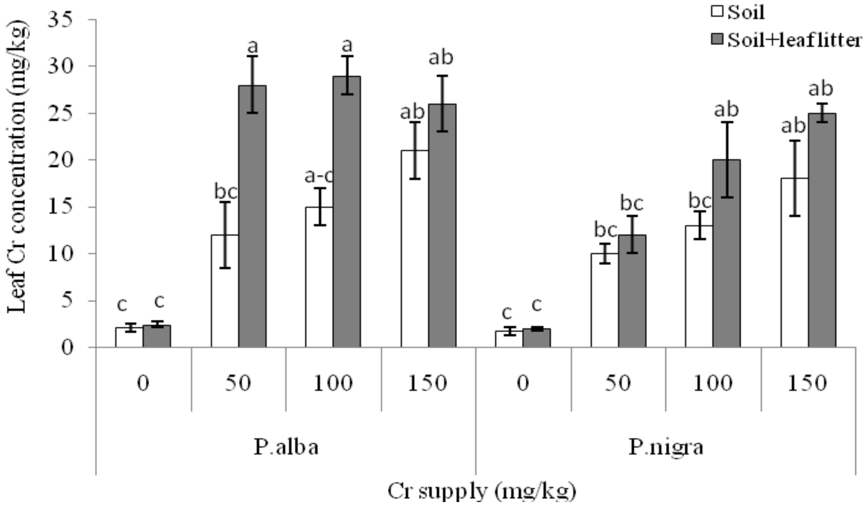

Fig. 1 Chromium concentration in leaves of *P. alba* L. *and P. nigra* L. rooted cuttings responded to different Cr supplies in two substrates. Error bars show SD, n=3. The different letters show significant difference among treatments (p< 0.01) (Tukey's Multiple Range Test).

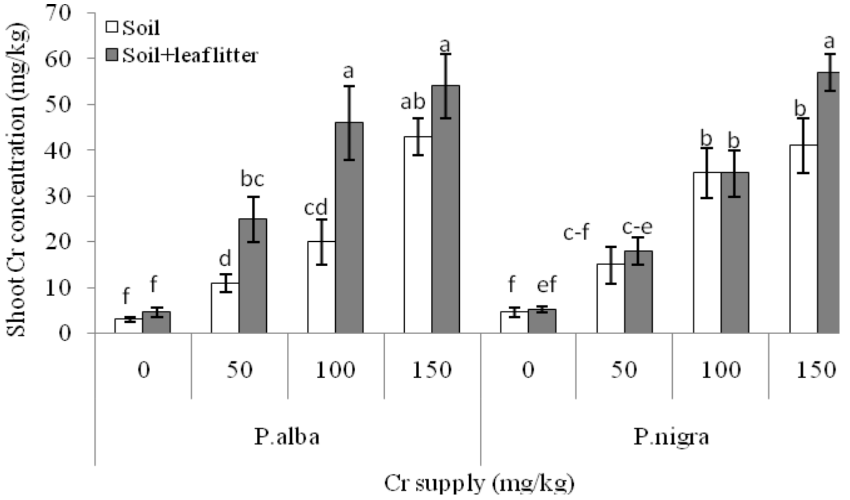

Fig. 2 Chromium concentration in shoots of *Populus alba* L. *and Populus nigra* L. rooted cuttings responded to different Cr supplies in two substrates. Error bars show SD, n=3. The different letters show significant difference among treatments (p< 0.01) (Tukey's Multiple Range Test).

Two prerequisite are necessary for effectiveness of phytoremediation. First, plants which are utilized for phytoremediation purposes accumulate high quantity of heavy metals. The second prerequisite is that those plants tolerate soil contamination, and also produce a great deal of biomass in contamination conditions (McGrath et al., 2002). Therefore, total uptake is considered as a good parameter to evaluate phytoremediation efficiency. Considering high biomass production of woody species, they are suitable alternatives to clean up heavy metal-contaminated soil. The total uptake of Cr for each treatment is presented in Table 4. Cr total uptake by poplar increased with the increase of Cr concentration in soil. Maximum total uptake was observed at 150 mg kg^{-1} Cr supply in amended substrate by *P. nigra* L. Higher total uptake by *P. nigra* L. might be due to its higher biomass production (Table 4). Alizadeh et al. (2012) stated that applying organic matter (cocopeat and peatmoss) led to increase in Cd total uptake two times more than control (soil without amendment).

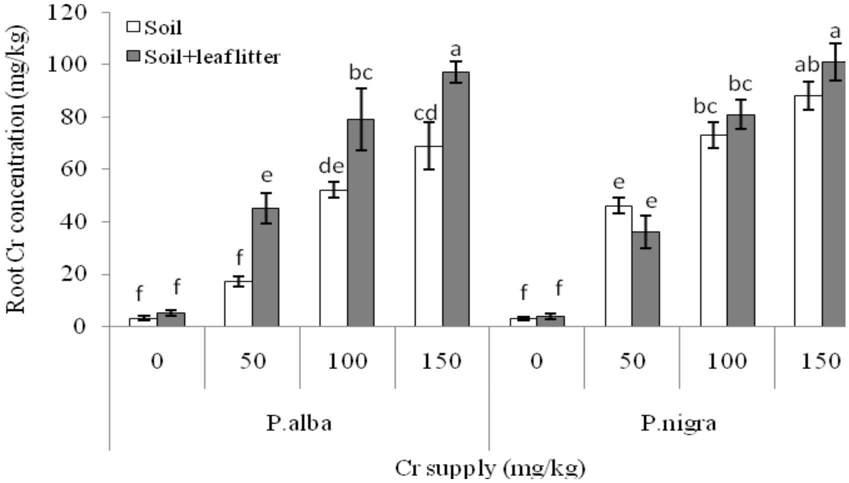

Fig. 3 Chromium concentration in roots of *Populus alba* L. *and Populus nigra* L. rooted cuttings responded to different Cr supplies in two substrates. Error bars show SD, n=3. The different letters show significant difference among treatments (p< 0.01) (Tukey's Multiple Range Test).

Species	Substrate	Cr supply mg kg^{-1}			
		0	50	100	150
P. alba L.	Soil	1003.1h	3484.7gh	5225.5fg	7264.5d-f
	Soil + leaf litter	1834.3gh	10872.2c	14850.8b	14870.7b
P. nigra L.	Soil	1454.7h	6310.4ef	9575cde	9879.3cd
	Soil + leaf litter	2237.4gh	9234.6c-e	15403.3b	20625.3a

Table 4. Total Cr uptake µg (means ± SD) by poplars responded to different Cr supplies in different substrates. The different letters show significant difference among treatments (p< 0.01) (Tukey's Multiple Range Test).

4. Conclusions

Heavy metals are released in the environment by many sources. In the present experiment, the addition of leaf litter to soil was intentionally designed to prove the hypothesis that application leaf litter to substrate would assist plant growth and Cr uptake in two poplars in the same time. We concluded that, although using complexing agents such as EDTA increase metal accumulation in plants considerably, but they have harmful effects on plants and soil microorganisms, whereas applying substrates can be considered as a promising and environmental friendly alternative for phytoremediation of Cr polluted soil due to their conditions that prepare to poplar growth and not having any outcome for the environment.

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