Reclamation of Soil Contaminated by Chromium(VI) Using *P.nigra* L.

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Paper Reference Number: 07-90-8050
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Abstract

Nowadays, heavy metals constitute a global environmental treat. Plants could be utilized to remediate metal contamination by removing metal from contaminated soil. Through a pot experiment, the bioaccumulation of Cr (VI) in *Populus nigra* L. one-year old rooted cuttings and the effect of this heavy metal on plant growth were assessed. Three concentrations of Cr contamination including 50 mg kg^{-1}, 100 mg kg^{-1} and 150 mg kg^{-1} [were spiked as potassium dichromate ($K_2Cr_2O_7$)] and one as uncontaminated soil (the control, no external Cr) were organized. The pots were filled with the contaminated soil and the prepared cuttings transplanted into the pots. At the end of growing season, the amounts of Cr accumulation in leaves, shoots, roots and growth responses were measured. The results indicated that Cr concentrations in leaf, shoot and root were increased with the increase of Cr supply and the order root >shoot >leaf of Cr concentration was observed. Considering Cr total uptake, it can be concluded that, *P.nigra* L. is a suitable species for phytoremediation of Cr-contaminated soils.

Key words: Bioaccumulation, Heavy metal, Phytoremediation, *Populus nigra* L., Uptake

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1. Introduction

Contamination of soils with heavy metals is considered as an important hazard facing our environment today. Since hexavalent Cr is a powerful oxidizing agent, Cr (VI) contamination of the environment is a matter of concern. Naturally there are many Chromium oxidation states, ranging from Cr (II) to Cr (VI) but mostly exists as Cr (III) and Cr (VI). These states are more stable in the common pH range of agricultural soils (Sinha et al., 2009). Cr (VI) is the major chromium species used in industrial processes, such as iron and steel production, chrome plating, leather tanning, wood processing, and so on (Economou-Eliopoulos et al., 2012).

Many industries release their untreated effluents into open land because of the high cost of treatment and water scarcity for dilution and inadequate sewage treatment facilities (Sundaramoorthy et al., 2010). Phytoremediation, as an emerging technology that aims to extract or inactivate metals in soils using plants, has attracted attention for the low cost of implementation and environmental benefits. Moreover, it is likely to be more acceptable to the public than other traditional methods (Alizadeh et al., 2012 and Wlodarczyk et al., 2012). To improve phytoremediation efficiency, plants which are utilized, should produce a great deal of biomass in contaminated conditions as well as accumulate high quantity of heavy metals (McGrath et al., 2002). Selection of appropriate woody species that fulfill these parameters is critical in the successful application of phytoremediation techniques to clean up sites with heavy metal contaminated soil. Several properties such as high-biomass production, high-transpiration rate and low ecological requirements make *Populus nigra* L. practical for remediation of contaminated sites. In this research, through a pot experiment, chromium accumulation by *Populus nigra* L. one-year old rooted cuttings was assessed.

2. Research Methodology

2.1 Experimental setup and design

The unpolluted soil was collected from depth of 0 to 30 cm from the campus of Agriculture and Natural Resources of University of Tehran. The physiochemical properties of the soil are presented in Table 1. Plastic pots with 35 cm diameter and 45 cm height were filled with the air-dried soil and treated with 0, 50, 100 and 150 ppm of potassium dichromate ($K_2Cr_2O_7$)] and then equilibrated for one month. During fifteenth of February 2010, one-year old rooted cuttings, produced in Masir-e-Sabz nursery, were transplanted into the pots. The experiment was conducted in three replicates and harvested at the end of September 2010.

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)

2.2 Growth responses and biomass production

At the end of growing season, stem diameter (to the nearest 0.01 mm) as well as stem height (to the nearest 1.0 cm) were measured. Height was measured from ground level to the base of the apical bud on the terminal shoot. To reduce experimental error associated with stump swell, stem and diameters was measured at 10 cm above the soil surface. Total tree leaf area (TTLA) was estimated according to the following equation: TTLA = (area of subsampled leaves/dry mass of subsampled leaves) × total tree leaf dry biomass (Zalesny et al. 2007). 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 and the dry weights were recorded.

2.3 Chemical analysis

After dry weight determination, the oven-dried tissue samples were ground and digested in H_2SO_4 (98%) and H_2O_2 using a digesdahl apparatus (Vicentim and Ferraz, 2007). The Cr content was determined using ICP-OES equipment.

2.4 Statistical analysis

The experiment was performed in a completely randomized design. To determine the variability of the data and validity of the results, all the absolute values were subjected to a two-way ANOVA and to confirm the significant difference between treatments, Tukey's range test was calculated.

3. Results and Analysis

3.1 Growth responses and biomass production of P. nigra L. to different Cr supply levels

Cr is a non-essential element for plant nutrition. In plants, chromium toxicity includes alterations in growth and development, may affect total dry matter production and yield (Shanker et al., 2005). Table 2 shows the effect of Cr on growth responses and yield performance of *P. nigra* L. plants after 6 months of metal exposure. All studied growth responses of *P. nigra* L. to Cr supply levels followed a decreased trend. Poplar showed maximum and minimum diameter at 0 and 150 mg kg^{-1} Cr levels respectively. The effect of Cr on the height of *P. nigra* L. was more pronounced than that of diameter and significant differences among all Cr levels was observed. Sharma and Sharma (1993) found that the plant height reduced significantly in wheat cv. UP 2003 grown in sand with 0.5M sodium dichromate. The reduced plant height might be mainly because of the reduction in root growth and consequent less nutrients and water transport to the above-ground parts of the plant.

The effect of Cr on TTLA was not significant between 0 and 50 mg kg^{-1} as well as 100 and 150 mg kg^{-1} ($p< 0.01$). At the highest Cr concentration (150 mg kg^{-1}), the TTLA was decreased by 44% compared to the control (0 mg kg^{-1} Cr treatment) (Table 2). The TTLA is a component of length, breadth and number of leaves, which reflects in the photosynthesis process. Steady reduce in TTLA at higher Cr supply levels may be attributed either to reduction in the number of cells in the leaves stunted by Stalinization or reduction in cell size (Sundaramoorthy et al., 2010). Another factor responsible for reduction in TTLA by chromium treatment is the variation in size of areoles (Purohit et al., 2003).

When Cr concentration was 50 mg kg^{-1}, the leaf biomasses were not significantly different to those of the control plants whereas there were significant differences among the shoot biomasses of all Cr treatments ($p< 0.01$). With the increase of Cr contamination, the root biomass decreased significantly. The maximum decrease (39% compared to control) was observed at the highest Cr concentration. The minimum leaf, shoot and root biomass production were observed at 150 mg kg^{-1} Cr treatment (Table 2). In agreement with these observations, Arduini et al. (2006) stated that Cr decreased the dry weight of culms, leaves (green + dead) and rhizomes, compared to control plants. The reduction in biomass might be due to disturbed carbohydrate and nitrogen metabolisms and decrease in protein synthesis or low photosynthetic reactions under Cr contamination levels (Hanus and Tomas, 1993).

3.2 Removal of chromium from contaminated soil by plants

Analysis of Cr content in *P. nigra* L. revealed a higher metal accumulation in leaves, shoots and roots with increase of Cr contamination. The differences among all cases were at $p< 0.01$. The maximum Cr concentrations in the leaf shoot and root matter were 18, 41 and 88 mg kg^{-1} (Table 3). The increase of Cr content in plant tissues might be due to the enhancement of Cr bioavailability in soil. A similar order for Cd accumulation (root > shoot> leaf) was found in poplar seedlings (Alizadeh et al., 2012). January et al. (2008) reported higher Cr accumulation in *Helianthus annuus* below-ground parts.

Cr (mg kg^{-1})	diameter cm	Height m	TTLA m^2	Leaf biomass g DW	Shoot biomass g DW	Root biomass g DW
0	14 ±3a	2.56±0.3a	2.16±0.21a	123.3±19a	233.2±30a	59.3±7a
50	11.7±2ab	2.38±0.22b	1.83±0.11a	103.6±20a	200.2±13b	49.4±3bc
100	10.5±1b	2.03±0.14c	1.25±0.2b	69.1±9.3b	155.8±21c	44±11c
150	9.1±1.2b	1.93±0.32d	1.21±0.36b	66.4±12.2b	133.4±11d	36.5±3.1d

Table 2: Growth responses and biomass production of *P. nigra* L. plants 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).

Cr (mg kg^{-1})	Cr concentration (mg kg^{-1})		
	Leaves	Shoots	Roots
0	1.71±0.1d	4.6±0.4d	2.9±0.7d
50	10±1.5c	15±0.8c	46±11c
100	13±2.1b	35±12b	73±18b
150	18±4a	41±8a	88±9a

Table 3: Cr content in *P. nigra* L. tissues 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).

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). Therefore, total uptake is considered as a good parameter to evaluate phytoremediation efficiency. Woody species, regarding to their high biomass production, are suitable alternatives to clean up heavy metal-contaminated soil. Fig. 1 presents the total uptake of Cr by plants which grew in the artificially contaminated soils. 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.

Translocation factors (TF), the ratio of metal concentration in the shoots to the roots, calculated to evaluate plant ability to translocate the metals from roots to the harvestable aerial part (Fellet et al., 2007). The Cr translocation factors for each treatment are shown in Fig 2. The highest TF was recorded at 0 mg kg^{-1} Cr supply level. The low transport of Cr from below-ground to above-ground parts observed here was similar to earlier observations on cauliflower and cabbage (Zayed et al., 1998) and Indian mustard (Han et al., 2004). Plants accumulate Cr in the vacuoles of root cell which could be a natural strategy for decreasing metal toxicity (Sinha et al., 2009).

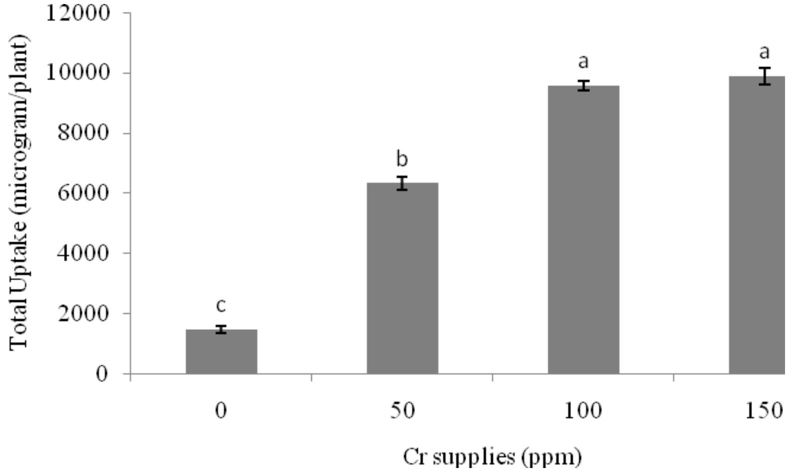

Fig. 1 Chromium total uptake by plants

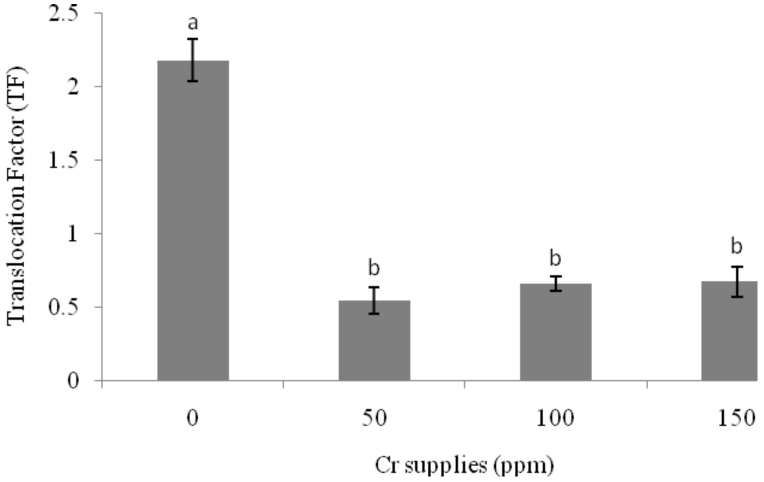

Fig. 2 Chromium Translocation factor

4. Conclusions

The results of the present study indicated that the chromium contaminated soils are phytoremediated by *P. nigra* L. seedlings. It can be concluded that the phytoremediation technology can be utilized wherever possible in order to clean up the Cr contaminated soil medium.

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