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[Research]

Growth and accumulation responses of *Populus nigra* L. exposed to hexavalent chromium excess

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ABSTRACT

Phytoremediation of heavy metals is employed as a technological approach for a non-destructive remediation of contaminated soils. One of the most recently studied tree species used in phytoremediation applications are poplars. In this study, the one-year old rooted seedlings of *Populus nigra* L. used to decontaminate hexavalent chromium-contaminated soils. Five treatments of Cr (VI) supply (were spiked as potassium dichromate) including 0 (control, no external Cr = T_0), 50 (T_{50}), 100 (T_{100}), 125 (T_{125}) and 150 mg.kg^{-1} (T_{150}) were employed. It was found that this species not only can reduce large amounts of Cr (VI) in the soil, but also can uptake and accumulate this element in its organs. Both mentioned mechanisms were found to be dose-dependent. A significant linear regression was observed between the all biomass parameters except root diameter ($p \leq 0.05$) as well as Cr accumulation in plant tissues ($p \leq 0.01$) and chromium concentration in the soil. These findings suggest that this species is suitable for Cr (VI) biomonitoring programs of Cr environmental contamination.

Key words: Soil, *P. nigra* L, Hexavalent chromium, Phytoremediation, Biomonitoring, Potassium dichromate.

INTRODUCTION

Heavy metals, toxic elements, organic pollutants and radionuclides are serious contaminants to the environment and harmful to human health (Stoláriková *et al*. 2012).

The concentrations of many trace metals have raised dramatically in and around the industrial cities and may pose a threat to the human health (Khosropour *et al*. 2013) which may be aggravated by their long-term persistence in the environment (Rahmanian *et al*. 2012).

Chromium is a heavy metal which release into the environment through various industrial activities and has become a serious concern to biologists over the past decade. Chromium and its compounds have multifarious industrial applications. Operations such as smelting, tanning, electroplating, and mining activities are the causes of raising chromium contamination in water and soil (Aldrich *et al*. 2003).

Trivalent Cr (III) and hexavalent Cr (VI) species are the major stable chemical forms of Cr (Arduini *et al*. 2006), although there are various other valence states which are unstable and short-lived in biological systems. Hexavalent Cr (VI) is considered as the most toxic form of Cr, which usually occurs associated with oxygen as dichromate ($Cr_2O_7^{2-}$) or chromate (CrO_4^{2-}) oxyanions. Trivalent Cr (III) is less mobile, less toxic and is mainly found bound to organic matter in soil and aquatic environments (Becquer *et al*. 2003). This trace element is observed in all phases of the environment, including air, water and soil (Zayad & Terry 2003).

Because of the potential toxicity and high persistence of heavy metals, the remediation of contaminated soils is one of the most difficult responsibilities for environmental engineering. Some *'ex situ'* and *'in situ'* techniques have been developed to remove heavy metals from contaminated soils (Wu *et al*. 2004). However, some specific plant species are capable of growing on contaminated soils and accumulate significant levels of specific metals (Solhi *et al*. 2005). This process, which is named "phytoremediation", is one of the 'in situ' techniques and has become an important environmental method for cleaning up contaminated sites since late 1990s (Wei *et al*. 2006). Low-cost implementation and environmental benefits are the advantages of this technology. In addition, phytoremediation seems to be more acceptable to the public than other traditional methods (Evangelou *et al*. 2007; Wei *et al*. 2008; Alizadeh *et al*. 2012).

Efficiency of phytoremediation depends on 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). Woody species with characteristics such as metal resistant, high-depth rooted, fast-growing and being able to grow on nutrient-poor soil, can be a suitable alternative to clean up sites with heavy metal contaminated soil (Pulford & Watson 2003; Alizadeh *et al*. 2012).

The *Salicaceae* family includes three genera, i.e. poplar (*Populus*), chosenia (*Chosenia*) and willow (Salix), with over 300 known species (Drzewiecka *et al*. 2012). *Populus nigra* L. -a cultivar of Populus genera- has shown great potential in phytoremediation of metal contaminated sites and, in biomass production, as a source of renewable energy.

On the other hand, Cr in contrast to other heavy metals like cadmium, lead and mercury has received little attention by plant scientists. Its intricate electronic chemistry has been a key hurdle in unraveling its toxicity mechanism in plants (Shanker *et al*. 2005). This study was performed to investigate if popular (*P. nigra*) can remove Cr from the soil via active transport systems or not.

MATERIALS AND METHODS

The soil used for this experiment was taken from the experimental area of depth of 0 to 30 cm from the campus of Agriculture and Natural Resources in University of Tehran, Iran.

To determine the soil chemical and physical characteristics, it was air-dried and passed through 2-mm sieve and mixed uniformly.

The results of soil analysis are presented in Table 1.

Table 1. Physical and chemical characteristics of agricultural soil used in this study before adding Cr (VI).

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	Cr(VI) (mg kg^{-1})*	0.09
CEC(Cmolkg^{-1})	25	Cr (III)(mg kg^{-1})*	0.44
So_4 (meq L^{-1})	37.20	Fe (mg kg^{-1})*	5.1

* DTPA-Extractable

Preparation of cuttings was done at Masir-e-Sabz nursery, Karaj in the north of Iran. Cuttings [length (25 ± 3 cm), diameter (8 ± 1 mm) and number of bud (8)] were taken from a mature *P. nigra* parent tree. All cuttings were obtained from a single tree. They were rooted and planted at the nursery for one year. Homogenously and uniform-in-size plants were selected for the pot experiment.

To prepare substrates, polyethylene pots (with 35 cm diameter and 45 cm height) were filled with 20 kg homogeneously-mixed dried soil. Five treatments of Cr (VI) contamination including 0 (control, no external Cr = T_0), 50

(T_{50}), 100 (T_{100}), 125 (T_{125}) and 150 mg.kg^{-1} (T_{150}) (were spiked as potassium dichromate) were employed and then the substrates were equilibrated for one month.

One-year old rooted seedlings were transplanted in to the pots on February 15, 2010. The plants were irrigated with adequate water (no detection of Cr) on alternate days. Fertilizers were added to each pot with respect to the soil analysis results (Table 1). At the end of September 2010, plants were harvested to observe biomass, yield and chemical parameters. Stem height and root length as well as stem and root diameters were measured using a meter rod to the nearest 1.0 cm and 0.01 mm respectively. 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).

Furthermore, the seedlings were washed with deionized water and separated into root, shoot, and leaf portions and then placed in a 70 °C oven for 2 days to determine dry mass. The dried samples were then ground in a stainless steel mill and passed through a 20-mesh sieve. The milled samples were digested using a digesdahl apparatus with concentrated H2SO4 and H_2O_2 (Vicentim & Ferraz 2007). Thereafter, the digested samples were diluted to a 1:5 (sample: deionized water) ratio for ICP analysis. A Perkin-Elmer Optima model 4300DV ICP-OES was used to determine the Cr (VI) content in the samples. The ICP-OES was equipped with a Meinhard nebulizer, and the analyses were performed under the following conditions: sample flow rate of 1.75ml. Min-1; gas flow rate of 15ml. h^{-1}; and RF power of 1500W. A calibration correlation coefficient of 0.98 or better was obtained for all analyses.

The experiments were organized in a completely randomized basic design. The treatments were replicated five times. Data were processed by means of SAS statistical software. Statistical differences between treatments were tested by One-Way ANOVA followed by HSD test to separate mean levels. All the expressed values were presented as mean ± S.D (standard deviation) of the five replicates. Results were considered significant at $p \leq 0.01$.

RESULTS

For all analyzed traits simultaneously, the MANOVA analysis was used to assess the differentiating effect of the chromium addition level. The hypothesis of no differences between treatments was rejected ($p \leq 0.0000$), so a One-Way ANOVA test was performed for each traits separately.

Biomass parameters showed a decreased tendency for successive levels of Cr(VI) addition in the soil (Table 2). At the end of growing season, for all biomass parameters, maximum relative increase was observed in control, while minimum was found at the highest Cr (VI) concentration. Among all biomass parameters, the highest inhibition rate was observed for TTLA (42% of TTLA of the control plant), and the smallest for root length (68% of the control plant), each time for the highest Cr (VI) concentration (T_{150}).

The result of the dry mass production assessment indicated the negative effect of hexavalent chromium on plant growth. Mean values of dry mass responses were determined in *P. nigra* L., 7 months after planting, as shown in Fig. 1. Dry mass of the root, shoot and leaves showed a significant reduction with increase in Cr (VI) level ($p \leq 0.01$).

Maximum decrease was observed in dry mass of shoot (51% of dry mass of the control) in comparison with hose of root (65% of dry mass of the control) and leaves (59% of dry mass of the control plant), each time for the highest Cr (VI) concentration (T_{150}).

The order, root >shoot >leaf Cr (VI) concentration was observed in *P. nigra* (L.) rooted seedlings (Fig. 2). Chromium accumulation in *P. nigra* L. depended significantly ($p \leq 0.01$) on the plant organ and the level of Cr (VI) in addition to the soil. The highest Cr concentration was found in the roots of poplar grown in the T_{150} and the variation among the five treatments was significant ($p \leq 0.01$). The lowest Cr concentration was associated with the leaves. A remarkable decrease in the shoot Cr (VI) concentration was observed between T_{50} and T_{100} ($p \leq 0.01$).

A regression analysis was performed to assess the dependency of measured traits on the hexavalent chromium concentrations. The regression analysis revealed a significant dependence of Cr(VI) accumulation in *Populus* organs on its concentration: $R^2 = 0.9921, 0.9823$, and 0.8911 for leaves, roots and shoots, respectively, indicating strong Cr(VI) sorption by the plant organs with the increase of its concentration in the soil (Table 3).

Table 2. *P. nigra* L. biomass parameters responded to different Cr concentrations.

Cr(VI) cons. (mg kg^{-1})	**Biomass parameters**				
	Root length (cm)	**Root diameter (mm)**	**Stem height (cm)**	**Stem diameter (mm)**	**TTLA (m^2)**
0	30 ± 7 a	11 ± 1.12 a	251 ± 29 a	13 ± 2.3 a	2.170 ± 0.20 a
50	31 ± 3 a	9 ± 0.73 ab	213 ± 14 b	10.8 ± 1 ab	1.58 ± 0.31 b
100	24.5 ± 4.2 ab	8.3 ± 0.31 bc	166 ± 9 c	9.7 ± 0.5 bc	1.25 ± 0.14 c
125	22 ± 1.5 b	6.89 ± 1 c	145 ± 21 c	8.5 ± 1.1 bc	1.02 ± 0.17 d
150	20.4 ± 3.3 b	6.3 ± 0.042 c	139 ± 12 c	8 ± 0.7c	0.91 ± 0.23 d

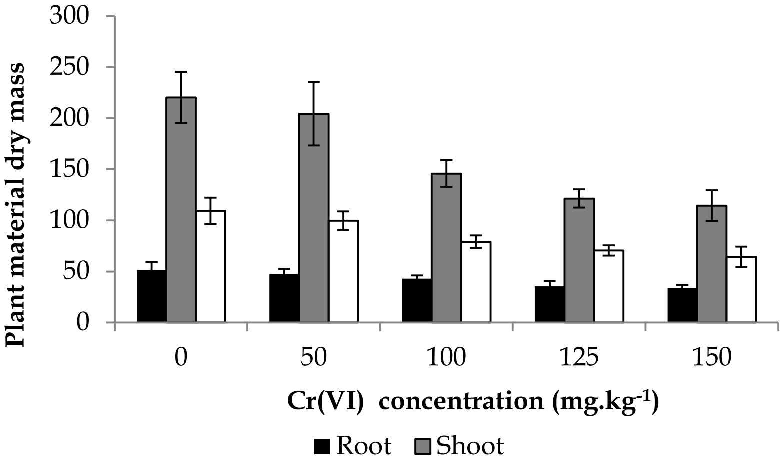

Fig. 1. Measured total dry mass production by *P. nigra* L. exposed to different Cr (VI) level. The exposure period was 7 months. The values are the mean of five replicates for samples. Vertical bars represent standard deviation.

Table 3. Analysis of significance for linear regression indicating relationships between biomass parameters and accumulation responses of *P. nigra* L. to chromium (dependent variables) and chromium concentration in the soil (independent variable) at $\alpha = 0.05$. Root diameter regression was not significant ($p \leq 0.05$) and is not presented here.

Dependent variables (*Y*)	**Linear regression analysis (cr contamination level as an independent variable *X*)**		
	***R*^{2}**	***p***	**Regression equation**
Biomass parameters			
Root length	0.6004	0.0451	$Y = 6.50 - 2.21\ X$
Stem height	0.6812	0.0199	$Y = 7.71 - 1.81\ X$
Stem diameter	0.6004	0.0451	$Y = 6.50 - 2.21\ X$
TTLA	0.9597	0.0002	$Y = 183.03 – 16.83\ X$
Cr accumulation in *P. nigra* L.			
Root	0.9823	0.0000	$Y = -0.59 + 5.01\ X$
Shoot	0.8911	0.0012	$Y = 0.17 + 1.15\ X$
Leaves	0.9921	0.0000	$Y = 0.12 + 1.23\ X$

Fig. 3 shows the results of total Cr (VI) uptake by poplars. Total Cr (VI) uptake was significantly increased across different Cr (VI) -contaminated treatments ($p \leq 0.01$). However, as shown in Fig. 3, the highest Cr (VI) uptake was observed at T_{150} (10559.92 µg plant^{-1}), and lowest at control (1430.8 µg plant^{-1}). Maximum and minimum increase in total uptake were occurred between T_0 and T_{50} as well as T_{125} and T_{150} respectively.

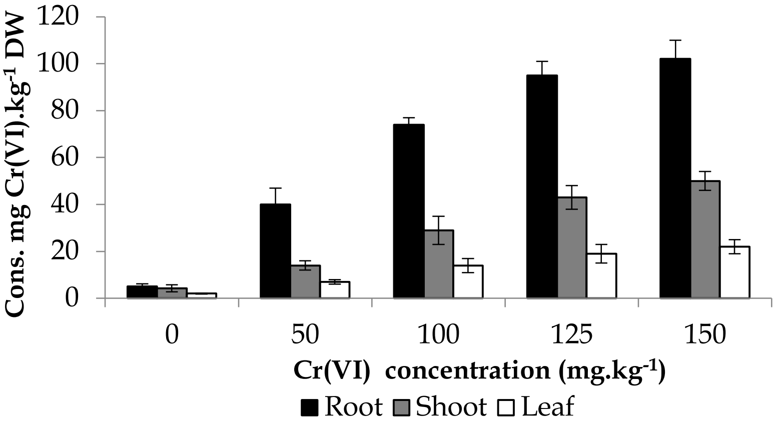

Fig. 2. Measured total Cr (VI) concentration (mg kg^{-1}) in plant organ of *P. nigra* L. exposed to different Cr (VI). The exposure period was 7 months. The values are the mean of five replicates for samples. Vertical bars represent standard deviation.

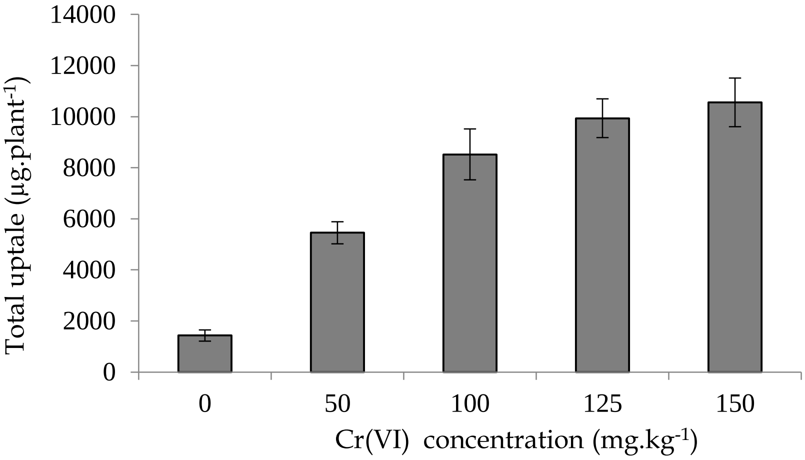

Fig. 3. Measured total Cr (VI) uptake (µg plant^{-1}) by poplars exposed to different Cr (VI) contents. The exposure period was 7 months. The values are the mean of five replicates for samples. Vertical bars represent standard deviation.

DISCUSSION

The accumulation of heavy metals in arable soils could be a great danger to all kinds of organisms, especially humans (Chehregani *et al.* 2009; Alizadeh *et al.* 2012). Since plants can withstand the presence of these toxic metals in the soils by employing divers species-specific mechanisms to avoid and tolerate (Yan & Ye 2009), phytoremediation has become into attention. In the present study, one-year old rooted seedlings of *P. nigra* L. (species with fast growth rate and deep root system) were exposed to hexavalent chromium in the soil, subjecting them to stress diversified levels of metal supply. When poplars were grown in the Cr (VI)-contaminated soils, biomass parameters declined with an increase of the hexavalent chromium strength in the soil (Table 2). Although remarkable reductions in various biomass parameters were observed, the effect was more pronounced with high Cr (VI) concentrations, which inhibit growth in comparison with control.

As demonstrated, increase in Cr (VI) concentration resulted in decreased root length. This may be due to the extending of cell cycle or inhibiting of root cell division/root elongation in the roots, thereby inhibiting root growth, while direct contact of roots with Cr(VI) in the soil, lead to a collapse and its subsequent inability to absorb water from the soil (Barcelo *et al*. 1986). Rout *et al*. (1997) reported that there were adverse effects of Cr on stem height. The same results were obtained in present study as the stem height was reduced with increased Cr (VI) concentration (Table 2). Cr (VI) transportation to the aerial part of the plant can have a direct effect on cellular metabolism, which may contribute to the height reduction. A significant relationship was found for all biomass parameters except for root diameter ($p \leq 0.05$) (Table 3). The same results were obtained the experiment conducted by Drzewiecka *et al*. (2012) and Sundaramoorthy *et al.* (2010).

Dry mass production was severely affected by Cr (VI) concentration and it was noticeable that the dry mass production responses were better in control than that under Cr (VI) stress ($p \leq 0.01$). Referring to Fig. 1, we note that there are greater potential of dry mass reduction at higher concentration of Cr (VI). Paiva *et al.* (2000) reported a significant reduction in dry weight of shoot and root with increase in heavy metals level. Such reduction, at high levels of heavy metals, becomes more damaging; therefore, plants show greater variation in morphological and physiological characters which subsequently affect mass production. Probably this is due to the reason that toxicity of heavy metals significantly decreases root vitality, preventing plant from absorbing inorganic nutrients and leading to inhibited plant growth (Shu *et al*. 1997).

As shown in Fig. 2, at each contamination level, maximum and minimum amounts of Cr (VI) accumulation was occurred in the root and leaves respectively. In a study on temperate trees, Pulford *et al*. (2001) reported that Cr was poorly taken up into the aerial tissues but was held predominantly in the root. According to Drzewiecka *et al*. (2012), there were a significant relationship between Cr content in the soil and its accumulation in plant organs. The same results were obtained in the present experiment ($R^2 = 0.9823$, 0.8911 and 0.9921 for root, shoot and leaves respectively).

The results of Cr (VI) uptake demonstrated a seven-time increase in Cr (VI) uptake by plants at T_{150} in comparison with control. Cr (VI) uptake into plant tissue was positively correlated with its contents in the soil ($p \leq 0.01$) (Fig. 3). A similar tendency was found by Arduini *et al*. (2006) who reported that Cr uptake by the whole *miscanthus* plant decreases at concentrations higher than 150 mg.kg^{-1}.

In our study, *P. nigra* seedlings were cultivated in soils contaminated with different hexavalent chromium concentrations. It can be assumed that this species exhibits sufficient resistance to Cr (VI) ions. The findings of the present study showed that the main values for employing *P. nigra* seedlings as phtoremediators on Cr-contaminated sites being to monitor and rehabilitate a degraded soil.

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پاسخ‌های رشد و انباشت توسط .*Populus nigra* L در معرض آلودگی کروم (VI)

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چکیده

گیاه‌پالایی فلزات سنگین راهکاری غیرمخرب برای پاک‌سازی خاک‌های آلوده محسوب می‌شود. یکی از گونه‌های درختی مورد استفاده در گیاه‌پالایی صنوبرها بوده که در سال‌های اخیر بیشترین مطالعه در مورد آنها صورت گرفته است. در این مطالعه، نهال‌های ریشه‌دار یکساله *P. nigra*. به منظور پاک‌سازی خاک‌های آلوده به کروم (VI) مورد استفاده قرار گرفتند. ۵ تیمار آلودگی کروم (VI) (به صورت دی‌کرومات پتاسیم)، شامل صفر، ۵۰، ۱۰۰، ۱۲۵ و ۱۵۰ میلی‌گرم بر کیلوگرم در نظر گرفته شد. نتایج نشان داد که این گونه نه تنها قادر است باعث کاهش مقادیر بالایی از کروم (VI) خاک شود، بلکه می‌تواند این عنصر را در بافت‌ها و اندام‌های خود جذب و انباشت نماید. این دو مکانیسم ذکر شده بستگی به غلظت کروم در خاک دارد. رابطه رگرسیونی خطی معنی‌داری بین کلیه پارامترهای زی‌توده بجز قطر ریشه ($p \leq 0.05$) و همچنین انباشت کروم در بافت‌های گیاه ($p \leq 0.01$) با غلظت کروم خاک مشاهده شد. یافته‌های این تحقیق نشان داد که این *P. nigra*. گونه مناسبی برای پایش زیستی کروم (VI) در مناطق آلوده به این فلز سنگین است.

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