

Ailanthus Altissima and *Phragmites Australis* for chromium removal from a contaminated soil

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Abstract The comparative effectiveness for hexavalent chromium removal from irrigation water, using two selected plant species (*Phragmites australis* and *Ailanthus altissima*) planted in soil contaminated with hexavalent chromium, has been studied in the present work. Total chromium removal from water was ranging from 55 % (*Phragmites*) to 61 % (*Ailanthus*). After 360 days, the contaminated soil dropped from 70 (initial) to 36 and 41 mg Cr/kg (dry soil), for *Phragmites* and *Ailanthus*, respectively. *Phragmites* accumulated the highest amount of chromium in the roots (1910 mg Cr/kg_(dry tissue)), compared with 358 mg Cr/kg_(dry tissue) for *Ailanthus* roots. Most of chromium was found in trivalent form in all plant tissues. *Ailanthus* had the lowest affinity for Cr^{VI} reduction in the root tissues. *Phragmites* indicated the highest chromium translocation potential, from roots to stems. Both plant species showed good potentialities to be used in phytoremediation installations for chromium removal.

Keywords Drainage water · Leaves · Phytoremediation · Roots · Stems · Toxicity

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Introduction

Hexavalent chromium is toxic to many plants (Shanker et al. 2005), aquatic animals (Velma et al. 2009), and microorganisms (Petrilli and De Flora 1977). Contrarily to Cr^{VI}, Cr^{III} is considered a nutrient in humans, being necessary for metabolism (Agency for Toxic Substances and Disease Registry 2000) and is generally not harmful. In plants, particularly crops, Cr at low concentrations (0.05–1 mg L⁻¹) was found to promote growth and increase yield, but it is not considered essential to plants (Paiva et al. 2009; Peralta-Videoa et al. 2009).

As in aquatic environment, once in the soil or sediment, Cr undergoes a variety of transformations, such as oxidation, reduction, sorption, precipitation, and dissolution (Kimbrough et al. 1999). The oxidants present in the soil (e.g., dissolved oxygen and MnO₂) can oxidize Cr^{III} to Cr^{VI} (Fendorf and Zasoski 1992).

Phytoremediation has shown a good efficacy for the remediation of heavy metal-contaminated soils and groundwater. However, the lack of knowledge regarding metals uptake/translocation mechanisms, enhancement amendments, as well as external effects on phytoremediation hindered its full-scale application (Dheeba and Sampathkumar 2012; Xu and Jaffé 2006). Recent studies reported on heavy metals with specific reference to chromium adsorption by using *Ailanthus altissima* and *Phragmites australis* (Jain et al. 2011; Ranieri 2012; Ranieri and Young 2012; Ranieri et al. 2013a).

EU has raised particular concern for chromium contamination of soils and groundwaters, with special focus on chromium release from leather tanning activities. Chromium is mainly encountered in the environment in two oxidation stages, i.e., Cr^{III} and Cr^{VI}.

Different research have been carried out on the phytoremediation and phytoextraction properties of selected

plant species (Gatti 2008; Van Nevel et al. 2007; Vervaeke et al. 2003).

Results have indicated that plant species have various capacities in removing and accumulating heavy metals. Thus, some macrophytes species are able to accumulate relatively higher quantities of heavy metals, such as *A. altissima* (Gatti 2008) and common reed (*P. australis*) (Fibbi et al. 2012; Gikas et al. 2013; Ranieri et al. 2011, 2013a, b, c). *A. altissima* is considered as a fast-growing and contamination-resistant plant species. The applicability of *A. altissima* for metals phytoextraction has been documented in recent literature (Fulekar et al. 2009; Gatti 2008).

The paper illustrates the performances of *A. altissima* and *P. australis* in chromium removal and tolerance, through the analysis and investigation of the chromium retention in the vegetal tissues: roots, stems, and leaves.

To achieve the above target, *in vivo* tests were carried out using two individual plant species for assessing the following issues:

- Determination of Cr^{VI} removal in the irrigation water, using the selected macrophytes.
- Assessment of chromium tolerance of *P. australis* and *A. altissima*.
- Assessment of chromium content within the various tissues of each plant species.

Materials and methods

Two sets of two pots, consisting of one contaminated and one control, were implanted with the selected species (*P. australis*, and *A. altissima*). Plants in control and non-control pots had initially the same size. Four 2 l pots were filled with clay soil and used throughout the laboratory experiments. Two of the cited soils were contaminated by saturation with 1 M of potassium dichromate, while the others were used as control. As cited previously, these species are known for their metals phytoremediation potentiality, especially in constructed wetlands systems (Galletti et al. 2010; Ranieri 2003; Windham et al. 2003).

The experience was carried out in a greenhouse at 20 °C and at an average relative humidity of 60 %. Pots were irrigated for 360 days, with tap water containing a concentration of 10 mg Cr^{VI}/L, at continuous flow rate of 0.2 L/min, using a peristaltic pump.

Cr^{VI} and total chromium were determined in soils, effluent water, and plant specimens, collected every 10 days during experiments, according to the following protocol: total chromium on solid samples was determined according to Standard Methods (APHA 2005), after acid digestion (1 M HNO₃/H₂O₂, microwave), followed by ICP-OES (Perkin Elmer,

Optima 3000, USA) analysis of the liquid extracts. Cr^{VI} was preliminarily concentrated in digested sample by elution onto a strong anion resin and determined according to the procedure described by Jardine et al. (Jardine et al. 1999).

Harvested plant tissues were clipped and the aboveground biomass was separated into stems, leaves (including leaf sheaths), and flowers. Belowground biomass (roots) was dug out from the sampled area. Then, all the parts were washed using deionized water to remove debris (Ranieri and Young 2012; Wolf 1982). Plant samples (roots, stems, and leaves) were dried at 90 °C for 24 h. After grinding, samples were digested with 0.5 M HNO₃ and then analyzed for their metals content using atomic absorption spectroscopy (Varian SpectrAA 880 coupled to GTA-110 CTZ graphite furnace) according to Standard Methods (APHA 2005).

All results are presented as milligram of chromium per kilogram of biomass dry weight (dw). For all measurements, standard quality assurance and quality control (QC) measures were implemented. QC samples consisted of triplicate samples and spiked samples Standard material SPS (SPS 2002). The MINEQL+ software was used for the estimation of chromium speciation in the soil/water system. The MINEQL+ software was used for the estimation of chromium speciation in the soil/water system (Westall et al. 1990).

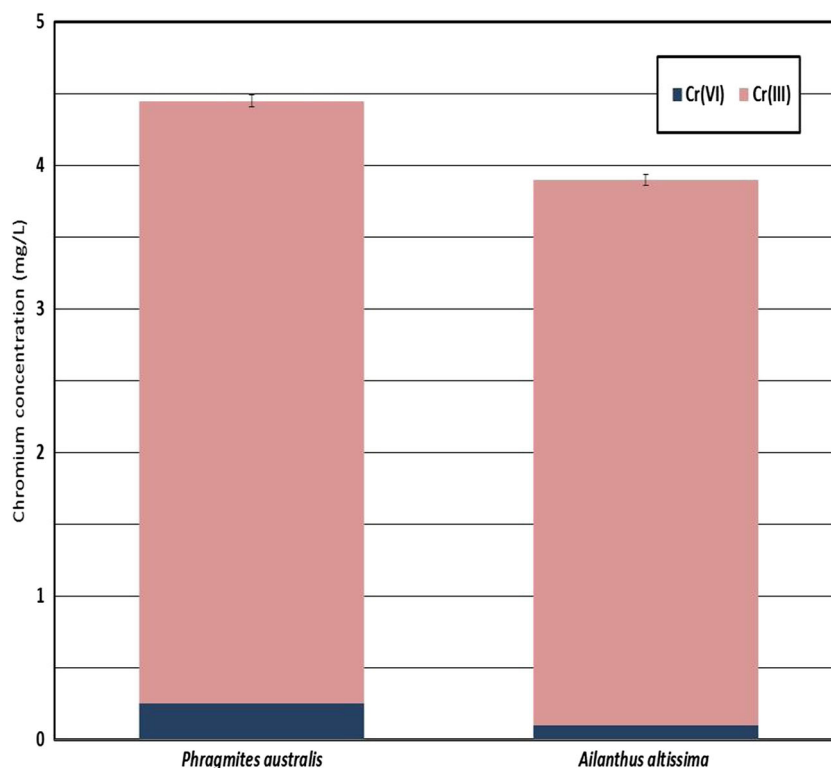
Results and discussion

Chromium concentration in drainage water

As mentioned, all pots (apart from the controls) were irrigated with chromium-contaminated synthetic wastewater (10 mg Cr^{VI}/L) to verify general plant tolerance (phytotoxicity) by assessing the maximum tolerable concentrations for each plant species. Irrigation was carried out by continuous flowing of the synthetic water for 360 days.

Total and hexavalent chromium in the drainage water for all plant species, after 360 days of irrigation, are shown in Fig. 1. Tests were carried out on five replicated samples, and it was found that the removal of total chromium concentrations in drainage water have ranged from 55 % (*Phragmites*) to 61 % (*Ailanthus*). Moreover, the evapotranspiration rate was not monitored; so, the removal in mass basis can be considered higher than those cited. Both plant species presented clear signs of toxicity to chromium species, expressed as growth reduction, after first harvest, at the 15th day. From Fig. 1, it is shown that trivalent chromium was the predominant species, as most of the hexavalent chromium, present in the irrigation water, was reduced to trivalent during irrigation (US 1998). Conversion exceeded 90 %, and, on these premises, we can reasonably assume that in drainage water the prevailing soil contaminant is trivalent chromium.

Fig. 1 Total and hexavalent chromium concentrations in the drainage water after 360 days of irrigation of *A. altissima* and *P. australis* pots, with tap water containing 10 mg/L of Cr^{VI}



Chromium concentration and speciation in plant tissues

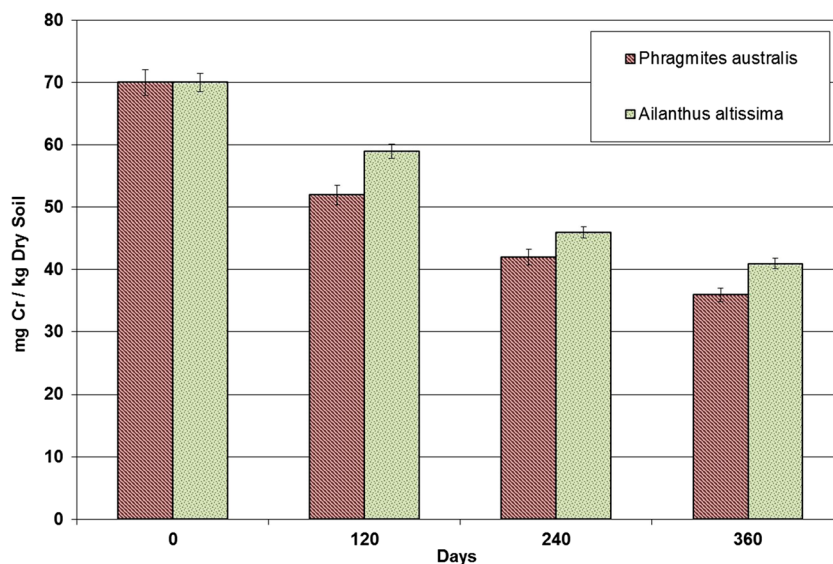
Total and hexavalent chromium concentrations in soil, along time, for all plant species, are shown in Figs. 2 and 3, respectively. A steady decrease of total chromium concentrations in the soil was monitored for all species, after 360 days of growth. Specifically, chromium content of the contaminated soil dropped from the initial 70 to 36 and 41 mg Cr/kg dry soil, for *P. australis* and *A. altissima*, with the latter being marginally the most effective (51 % reduction of total chromium content after 360 days) (Fig. 2). Based on our determination, a faster initial reduction of chromium content is observed for both species (Fig. 3). Particularly for *Phragmites*, a slight increase of the hexavalent chromium concentration in the soil is observed during the last 120 days. Referring to *Ailanthus*, after a slight drop of the initial Cr^{VI} concentration to 3.1 mg Cr^{VI}/kg dry soil, hexavalent chromium concentration in the soil increases up to 4.3 Cr^{VI}/kg dry soil (Fig. 3).

During the first 10 days, plant growth was pretty normal for all plant species with a sensible later, however, slow down, as compared to the control. Preliminary findings (Bragato et al. 2006) indicated that non-arboreal species (such as *Phragmites*) adsorbed Cr^{VI} in the early growing stage, till reaching a critical concentration in the plant tissues, followed by inhibition at full plant development, where minimal further Cr^{VI} adsorption from the contaminated wastewater was observed. This will be discussed in the following paragraph.

On the basis of the simulations carried out using the MINEQL+ software (Westall et al. 1990), it is evidenced that, in acidic environments, the more soluble Cr^{VI} species showed higher bioavailability as compared to Cr^{III} (Bartlett and Kimble 1976; Bartlett and James 1979). It is known that solubility of Cr^{III} compounds is strongly influenced by pH, thus decreasing drastically at pH >4.5 and increasing at pH >8.5, where highly stable organic complexes are formed. These latter compounds show higher affinity toward plant roots (Palmer and Wittbrodt, 1991).

Total chromium and Cr^{VI} concentrations were measured in the roots, shoots, and stems of all plant species at the end of the experimental period (day 360), while the same values at day 0 were negligible. The above values are summarized in Table 1. Table 1 shows that the highest total chromium concentration for all plant species is in the roots, with higher value for *Phragmites* (1910 mg Cr/kg_(dry tissue)), while *Ailanthus* accumulated significantly lower amounts of total chromium (358 mg Cr/kg_(dry tissue)). On the other hand, the concentration of Cr^{VI} in the plant root tissues is the greatest for *Ailanthus* (154 mg Cr^{VI}/kg_(dry tissue)), followed by *Phragmites*, with a concentration of 92 mg Cr^{VI}/kg_(dry tissue). The lowest total chromium concentration was measured in the stems of *Ailanthus* (4 mg Cr/kg_(dry tissue)), while similar trend was monitored for hexavalent chromium with concentration in *Ailanthus* stem equal to 0.2 mg Cr^{VI}/kg_(dry tissue). Similar value for hexavalent chromium was observed in *Ailanthus* leaves

Fig. 2 Total chromium concentration in the pot soil, as a function of time, monitored in the pots of *A. altissima* and *P. australis*



(0.3 mg Cr^{VI}/kg_(dry tissue)); however, in the same leaves was monitored significantly higher total chromium concentration (29 mg Cr/kg_(dry tissue)). The greatest total chromium and hexavalent chromium concentrations in the aboveground plant tissues was measured in *Phragmites* stems (579 mg Cr/kg_(dry tissue)) and leaves (62 mg Cr^{VI}/kg_(dry tissue)), respectively, indicating that *Phragmites* shows a high rate of translocation from roots to stems. Finally, the greatest total chromium concentration in the leaves tissues was measured for *Phragmites* (53 mg Cr/kg_(dry tissue)).

Previous investigations referring to *Phragmites* showed that both trivalent and hexavalent chromium were retained principally at root level, rather than in leaves (Shewry and Peterson 1974; Cary et al. 1977).

Our findings suggest that in particular, *P. australis* has indicated high affinity in chromium retaining, exhibiting the highest values in all plant tissues (Ranieri et al. 2013a, b, c). On the other hand, *A. altissima* species has confirmed its strong tendency for chromium translocation to leaves, especially in trivalent form. In conclusion, we can affirm that *Ailanthus* has lower affinity and capacity to accumulate chromium in all tissues compared to *Phragmites*, but it also appeared having the higher translocation rate (Ranieri and Gikas 2014).

Evaluation of Cr phytotoxicity

Growth of plants roots rate was monitored continuously throughout the experiment of the irrigation with chromium-

Fig. 3 Hexavalent chromium concentration in the pot soil, as a function of time, monitored in the pots of *A. altissima* and *P. australis*

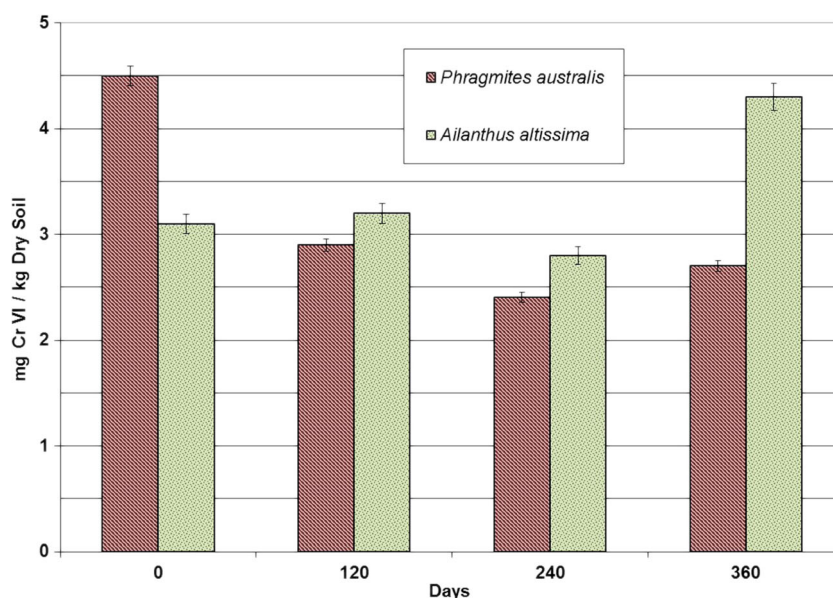


Table 1 Total chromium, Cr^{III}, and Cr^{VI} concentrations in the vegetal tissues (roots, stems and leaves) for *A. altissima*, and *P. australis*, after 360 days of irrigation with Cr^{VI}-contaminated water

<i>P. australis</i>	Roots mgCr/kg _(dry tissue)	Stems mgCr/kg _(dry tissue)	Leaves mgCr/kg _(dry tissue)
Total chromium	1910.3 (±61)	578.7 (±15)	53.2 (±2)
Chromium III	1818.3 (±52)	516.3 (±12)	49.4 (±2)
Chromium VI	92.0 (±5)	62.4 (±5)	3.8 (±1)
<i>A. altissima</i>	Roots mgCr/kg _(dry tissue)	Stems mgCr/kg _(dry tissue)	Leaves mgCr/kg _(dry tissue)
Total chromium	358.4(±10)	4.0(±0.5)	28.9(±1)
Chromium III	204.4(±12)	3.8(±0.5)	28.6(±1)
Chromium VI	154.0(±12)	0.2(±0.1)	0.3(±0.1)

contaminated water, as a function of growth time. Figure 4 shows these experimental data. Root harvesting was performed every 60 days.

The growth rate of root for *Phragmites* and *Ailanthus* was respectively ranging from 1.9 to 3.9 mm/day and from 0.8 to 3.5 mm/day showing a slowdown roots growth of approximately 20 % as average. *Ailanthus* seems to have the highest toxic effect for roots growth, as roots growth rate dropped by approximately 57 % at the end of the experiment. Because of revealed toxicity of chromium in all species, it is assumed that chemical speciation plays a significant role and chromium in vegetal tissues is initially adsorbed as Cr^{VI} and later reduced to the trivalent state in the plant tissues (Yu et al. 2008).

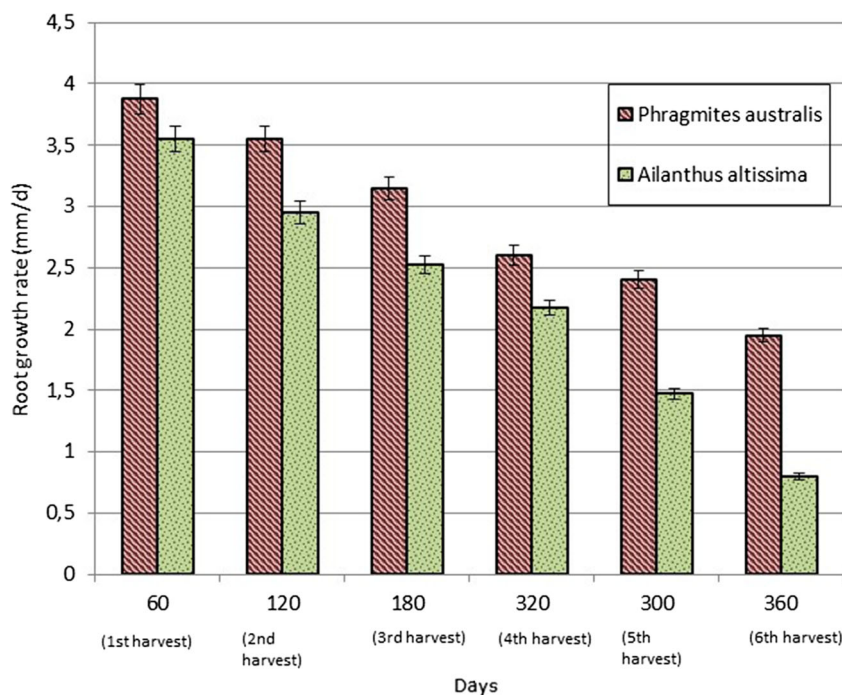
This decrease in plant height could be due to the reduced root growth and consequent decreased nutrients and water transport to the higher parts of the plant. Moreover, Cr transport to the aerial part of the plant can directly impact cellular

metabolism of shoots contributing to the reduction in plant height.

Chromium had a more evident negative effect on plant growth in *A. altissima*, than *P. australis*, which could be characterized by a more efficient control of the oxidative stress induced by Cr than *Ailanthus*. Decrease in root growth in presence of Cr^{VI} can be explained by inhibition of root cell division and/or elongation, which might have occurred as a result of tissue collapse and consequent incapacity of the roots to absorb water and nutrients from the medium (Barceló et al. 1985) combined with extension of cell cycle (Gatti, 2008; Sundaramoorthy et al. 2010).

With regard to the toxic effect to the plants, based on the present findings, it may be concluded that chromium, and particularly Cr^{VI}, acts principally at the root system and an intense growth inhibition has been showed (Banks et al. 2006).

Fig. 4 Root growth rate in *A. altissima*, and *P. australis* in the pots



Conclusions

The affinity for hexavalent chromium reduction and removal from irrigation water (contaminated with 10 mg Cr^{VI}/L) has been assessed for two heavy metal-tolerant plant species: *P. australis* and *A. altissima*. Based on the findings, the following conclusions may be drawn:

- Total chromium removal in the drainage water ranged from 55 % (*Phragmites*) to 61 % (*Ailanthus*).
- More than 90 % of total chromium in the drainage water was present as Cr^{III}, for both plant species (the irrigation water contained Cr^{VI} species only).
- After 360 days of irrigation, the chromium content of the contaminated soil dropped from 70 (initial) to 36 and 41 mg Cr/kg dry soil, respectively for *Phragmites* and *Ailanthus*.
- The roots of both plant species accumulated the larger amount of chromium, as compared to the aboveground parts of the plants.
- *P. australis* showed higher chromium translocation affinity from roots to stems.

Both plant species showed good potentialities to be used in large-scale phytoremediation installations for chromium removal from contaminated soils and groundwater.

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