Phytoextraction of Cr(VI) from soil using *Portulaca oleracea*

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Abstract

Cr(VI) represents an environmental challenge in both soils and waters as it is soluble and bioavailable over a wide range of pH. In previous investigations, *Portulaca oleracea* (a plant local to the UAE) demonstrated particular ability for the phytoextraction of Cr(VI) from calcareous soils of the UAE. In this publication, the results of the evaluation of *P. oleracea* phytoextraction of Cr(VI) from UAE soil at higher concentrations are reported. *P. oleracea* was exposed to nine different concentrations of Cr(VI) in soil from 0 to 400 mg kg^{-1}. The uptake of Cr(VI) increased as its concentration in soil increased between 50 and 400 mg kg^{-1}, with the most efficient removal in the range from 150 to 200 mg kg^{-1}. The total chromium concentrations exceeded 4600 mg kg^{-1} in roots and 1400 mg kg^{-1} in stems, confirming the role of *P. oleracea* as an effective Cr(VI) accumulator. More than 95% of the accumulated Cr(VI) was reduced to the less toxic Cr(III) within the plant.

*Keywords***:** Phytoremediation; phytoextraction; hexavalent chromium; Cr(VI); *Portulaca oleracea*; UAE soil.

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1. Introduction

Some metal ions may have adverse effects on human health above certain concentrations or doses of uptake, and therefore metal contamination of the food chain deserves special attention (Ali, Khan, and Sajad 2013). Chromium, one of the more problematic metals, occurs in several oxidation states, but mainly as cationic Cr^{3+} and as hexavalent chromium [Cr(VI)] in the anions $Cr_2O_7^{2-}$ and CrO_4^{2-} (Wyszkowski and Radziemska 2013). Hexavalent chromium is strongly oxidative and toxic to bacteria, plants, and animals and therefore, as an established carcinogen, the levels of Cr(VI) are regulated specifically in air and water. The origin of Cr(VI) is mostly anthropogenic, for example, from chrome plating and the production and corrosion of steel alloys. Cr(VI) is very soluble and thus bioavailable (Oliveira 2012) in soils unless reduced to Cr(III) by organic matter or humus.

In arid subtropical climates such as that in the UAE, the soil is mostly sandy with some calcium carbonate and contains less than 0.5% humus, so there is little loss of Cr(VI) by reaction or aqueous run off; under such conditions, Cr(VI) contamination may be a serious problem. In such a situation, phytoextraction can remediate the land inexpensively and with low environmental impact (Alyazouri et al. 2014) The earliest studies on the interaction of Cr(VI) with plants were on chromium uptake as a problem of crop and plant contamination, so they mostly involved food crops such as wheat, barley and oat (Shewry and Peterson 1974; Skeffington, Shewry, and Peterson 1976; McGrath 1982). More recent studies have investigated non crop plants in order to identify Cr(VI) accumulators. Hydroponic systems were used in most of these studies because of the ease of experimental design (Arteaga et al. 2000; Zhang et al. 2007; Diwan, Ahmad, and Iqbal 2012; Duarte Silva, and Caçador 2012). In other studies, natural or synthetic polluted soils

were used in studying the phytoextraction of Cr(VI) (Prakash 1995; Shahandeh and Hossner 2000; Dong et al. 2007; Adki, Jadhav, and Bapat, 2012).

The most efficient species undergoing phytoaccumulation are often described as hyperaccumulators. The term was introduced to describe the accumulation of nickel at over 1000 mg kg^{-1} in dry leaf tissue by the tree *Sebertia acuminata* (Jaffré et al., 1976), although it has since been used without a consistent definition (van den Ent et al., 2013). For Cr(VI), the grass *Spartina argentinensis* has been reported to accumulate Cr at up to 1500 mg kg^{-1} (Redondo-Gómez et al. 2011), *Leersia hexandra* has shown accumulation in leaves up to 600 mg kg^{-1} (Zhang et al., 2007) and *Nopalea cochenillifera* is reported to accumulate Cr in leaves up to 700 mg kg^{-1} (Adki, Jadhav, and Bapat, 2013). In addition to high uptake of the metal ion, a useful hyperaccumulator plant must also show a high growth rate under the environmental conditions including pH and salinity.

Species which may grow in arid conditions are *Prosopis spp* (Aldrich et al. 2003) and *Convolvulvus arvensis* (Gardea-Torresdey et al. 2004); they have been studied on agar based nutrient at pH5.3 and pH5.8, whereas for arid climatic conditions soils tend to be alkaline. This is significant as Cr(VI) at low pH is predominantly in the highly oxidizing (E^{e} = 1.38V) $Cr_2O_7^{2-}$ form rather than the less oxidising (E^{e} = -0.11V) CrO_4^{-} form predominant at pH8. (Pourbaix, 1973)

For studies on accumulator plants, the bioaccumulation factor (BAF) and the translocation factor (TF) should be taken into consideration. The BAF is the ratio of the concentration of a metal in dry roots (mg kg^{-1} dry weight) versus the concentration of the metal in dry soil or wastewater (mg kg^{-1}) (Kim, 2003; Gonzaga et al., 2006; Ali, Khan, and Sajad 2013,). The TF is the ratio of

concentration of metal in dry shoots to its concentration in dry roots. Values of more than 1.0 for both BAF and TF are an indication of a promising accumulator plant (Gonzaga, Santos, and Ma 2006; Cao 2008).

Portulaca oleracea is a succulent plant which thrives very well in the climatic conditions of the UAE. Phytoextraction of a low concentration of Cr(VI) (50 $mg.kg^{-1}$) using this plant was investigated in a previous study (Alyazouri et al., 2014), which showed that for small concentrations of Cr(VI), *P. oleracea* was the best among 22 plants investigated. In this study, a more comprehensive evaluation of this potential accumulator was carried out in order to:

1- study the phytoextraction at higher concentrations of Cr(VI),
2- investigate the speciation of Cr inside the plant tissues,
3- determine the ideal range of Cr(VI) in soil that may be phytoextracted using *P. oleracea* and the phytotoxic limit of Cr(VI) for this plant in soil, and
4- calculate the total amounts of Cr removed at different concentrations of Cr(VI) in soil.

2. Materials and methods

2.1 Chemicals and reagents

A standard solution (1000 mg kg^{-1}) of chromium (III) (Fluka Chemicals, Gillingham, UK) was used for the preparation of calibration standards for inductively-coupled plasma optical emission spectroscopy (ICP-OES). Nitric acid (ACS Reagent ≥ 90.0%) for wet acidic digestion was purchased from the same company. Reagent grade sodium carbonate, sodium hydroxide and magnesium chloride (Panreac Química, Barcelona, Spain) were used for alkaline digestion of soil and plant samples in order to determine Cr(VI). Reagent grade sodium chromate (Panreac Química) was used for irrigation solutions.

2.2 Instruments and equipment

An ICP-OES instrument(Sequential Liberty AX, Varian, Mulgrave, Victoria, Australia) was used for measuring total chromium concentrations in soil and plant tissues, with the conditions recommended by the manufacturer. A UV-Visible spectrometer (HI 93723, Hanna Instruments, Leighton, Buzzard, Bedfordshire, UK) was used to determine Cr(VI) in plants and soils. A pH Meter, (PerpHecT Basic Benchtop Model Orion 320, Thermo-Orion, Loughborough, UK) was used for measuring the pH of soil and irrigation solutions.

2.3 Field Experiments

Forty pots of 85% (v/v) of normal clean soil from Ajman desert in the United Arab Emirates [extremely calcareous (FAO classification), (Abu-Zeid, Baghdady, and El-Etr, 2001, Omar et al., 2003). total carbonate = 42% wt/wt, less than 0.5% humus, and pH7.9] (and 15% (v/v) of potting soil (Blumenerde, from Syker Agrarberatungs- und Handels GmbH, Skye, Germany, containing 70% organic matter) were prepared. The total mass of soil in each pot was 1500 ± 100 g. In each pot, five stems of *P. oleracea* were propagated as cuttings and irrigated with deionised water. Plants were grown at the Ajman municipality nursery in a partially sunny area (35 % shaded nursery, 65% of light intensity to pass through the net). The mean daily maximum temperature is 41°C, the mean at night temperature is 25°C and the mean relative humidity is 54%.

Using a stock solution of 10 g kg^{-1} Cr(VI) as Na_2CrO_4 in deionised water, nine solutions were prepared with at concentration intervals of 50 mg kg^{-1} from 0 to 400 mg kg^{-1} Cr(VI). The volume of each solution was 5 litres and the pH of each solution was adjusted to 8.0 ± 0.1.

Once there was considerable vegetation in each pot (usually 60 days of growth) 27 pots were chosen with the best vegetation. Pots were irrigated with each of the nine concentrations of

Cr(VI) in triplicate. After 10 doses of 150 mL of irrigation by the pollutant solutions, the plants were harvested, washed by deionised water and divided into; leaves, stems, and roots.

2.4 Determination of chromium species

Samples of plants were dried in an oven at 65 °C for 48 hours, then weighed and ground using a mortar and pestle. The samples were analysed in two ways: (i) nitric acid digestion to determine the total chromium using ICP-OES and (ii) alkaline digestion to determine Cr(VI) in each sample using EPA method 3060A (United States Environmental Protection Agency, 1996). The extracted Cr(VI) was reacted with 1,5-diphenylcarbazide (ACS Reagent, Sigma-Aldrich, Gillingham, UK) in the presence of sulfuric acid and analysed using a UV- visible spectrometer at a wavelength of 540 nm. Cr(III) was calculated by subtraction of Cr(VI) from total Cr in roots, leaves and stems. Composite soil samples were taken from each pot, dried, sieved, digested and three replicates from each sample were analysed for chromium (VI) and total chromium.

2.5 Statistical analysis

SPSS software (Version 15, SPSS UK Ltd., Woking, Surrey) was used for statistical analysis. Microsoft Excel (Microsoft UK, Reading, Berkshire) was used for the preparation of the graphs and for simple statistical operations. Results are reported in tables and graphs as with 95% confidence intervals calculated using Student's t test. Analysis of variance (ANOVA - post hoc by a Tukey test) between the means is used to identify if there are significant differences between means.

3. Results and Discussion

3.1 Concentration and speciation of chromium in plant tissues

The concentrations of total chromium, Cr(VI) and Cr(III), in the leaves, stems and roots of *P. oleracea* were determined over a range 50 to 350 mg kg^{-1} Cr(VI) concentrations in the irrigation solution and are plotted in Figures 1 to 3. In the leaves, as the concentration of Cr(VI) increased in the irrigation solution, the total concentration of Cr concentration increased from 100 mg kg^{-1} to 1100 mg kg^{-1} and the Cr(VI) concentration increased from 3 to 30 mg kg^{-1}. The total Cr in dry stems increased from 70 mg kg^{-1} to 1400 mg kg^{-1} and Cr(VI) concentration ranged from 2 to 40 mg kg^{-1} in dry stems of the plant. In the dry roots, total Cr concentration increased from 400 mg kg^{-1} to 4600 mg kg^{-1} and the Cr(VI) concentration ranged from 30 to 140 mg kg^{-1}.

For each 100 mg kg^{-1} increase in concentration of Cr(VI) in the irrigation solution, the concentration of total Cr in leaves, stems and roots was shown to increase significantly ($p<0.01$ using ANOVA Post hoc test "Tukey") and plots of the concentrations showed reasonable linear fits. (Figure 4.)

The observation that the uptake of chromium by plants increases as the introduced Cr(VI) increases agrees with some previous investigators who studied the uptake of Cr by other plants such as barley seedlings (Shewry and Peterson, 1974), *Cynodon dactylon* (Sampanpanish et al., 2006), *Leersia hexandra* (Zhang et al., 2007), and *Typha angustinfolia* (Dong et al., 2007).. The results of these studies differ from the results reported in another study (Gardea-Torresdey et al., 2004) which introduced Cr(VI) to *Convolvulus arvensis* in three concentrations 80, 40, and 20 mg kg^{-1} and found that the highest uptake by roots was observed at the lowest concentration of Cr(VI) (20 mg kg^{-1}). The roots of *C. arvensis* began to be severely affected by Cr(VI) at concentrations higher than 20 mg kg^{-1} so the uptake of Cr(VI) decreased at the higher concentrations.

For *P. oleracea*, the maximum concentrations of total chromium measured in leaves of 1100 mg $kg^{-1}$and in stems of 1400 mg kg^{-1} exceed the target of the 1000 mg $kg^{-1}$of pollutant in both cases indicating the potential of the plant to be a hyperaccumulator for Cr(VI). When comparing *P. oleracea* with the other accumulators of Cr(VI) from soil such as *Leptospermum scoparium*, *Typha angustinfolia*, *Zea mays*, and *Leersia hexandra* , it can be concluded that *P. oleracea* may be the best among them in achieving maximum concentration of chromium in roots and in being the second for chromium in shoots (Table 1). The percentage of Cr in the reduced Cr(III) form was above 92% in roots and above 96% in stems and leaves. Thus almost all of the Cr(VI) that was accumulated by *P. oleracea* was reduced to less toxic Cr(III) inside the plant tissues.

Soil analysis after harvesting showed that Cr(VI) was still the major species in soil. This confirms that most of chromium was absorbed as Cr(VI) then was reduced with a high efficiency in plant tissues (Table 2). The results indicate that most of Cr(VI) was reduced in the roots before reaching shoots and this may explain the degradation of roots at the high concentration of 400 mg kg^{-1} of Cr(VI) as compared to stems and shoots. These results are in agreement with those reported elsewhere for other plants and as expected from the chemistry of chromate. (Shanker and Pathmanabhan, 2004; Shanker et al., 2005; Zhang et al., 2007)

3.2 Bioaccumulation Factors and Translocation Factors

The calculated bioaccumulation factors (Table 3) were around the value of 10 for concentrations of 50 to 250 mg kg^{-1} of Cr(VI) in irrigation solution indicating that *P. oleracea* accumulated Cr(VI) in roots in a constant ratio within this range of Cr(VI) concentration. Bioaccumulation factors increased as the concentration of Cr(VI) increased over 250 mg kg^{-1} to

reach 15 at 350 mg kg^{-1} of Cr(VI). Bioaccumulation factor values of 10 to 15 confirm the potential of *P. oleracea* as an efficient accumulator for Cr (VI).

Translocation factors (Table 3) ranged from 0.24 to 0.35 for leaves and from 0.18 to 0.46 for stems in agreement with reported translocation factors for Cr using other plants that did not reach the value of 1.0 (Zhang et al., 2007; Buendia-Gonzalez et al., 2010) . The low translocation factors of Cr observed in *P. oleracea* are likely to be due to the stress of highly oxidative Cr(VI) species which would cause severe damage to plant tissues, especially roots.

3.3 Plant growth and total removed chromium

No significant difference was observed between means of roots' dry weight up to 150 mg kg^{-1} of Cr(VI) and in that of shoots up to 100 mg kg^{-1} ($p>0.05$) (Figure 5). This shows that plants were not affected significantly by Cr(VI) at these concentrations. A significant decrease in dry weight of plants was observed at levels of irrigation solution higher than 200 mg kg^{-1} of Cr(VI) ($p<0.01$). Phytotoxicity symptoms were also noticed since the leaves became yellow and inflated at concentrations higher than 200 mg kg^{-1}. It was observed that the roots of the plants exposed to 400 mg kg^{-1} of Cr(VI) were degraded. This significant decline is an indication of the severe stress caused by Cr(VI) that oxidizes the bioorganic materials especially the protein of the cells. Several previous studies have indicated the reduction of other plants biomass as the concentration of Cr(VI) increases in the nutrient medium (Lytle et al., 1998; Shahandeh and Hossner, 2000; Shanker et al., 2005). When the total chromium in both roots and shoots is calculated, the total amount removed by five plants grown in one pot was increased from 0.90 ± 0.15 mg for plants irrigated with 50 mg kg^{-1} to 3.00 ± 0.34 mg at 200 mg kg^{-1} , then it declined as the chromium in irrigation solution was increased above 200 mg kg^{-1} (Figure 6).

4. Conclusions

P. oleracea has been shown to be a very promising accumulator species for the phytoextraction of Cr(VI) from calcareous soil in an arid hot climate, such as that typically found in the Arabian peninsula including the UAE. Under these conditions Cr(VI) will be present as chromate, CrO_4^{2-} which is reduced in the body of the plant to Cr(III). Thus highly toxic Cr(VI) is reduced upon phytoextraction by *P. oleracea* to the less toxic Cr(III). Animals may feed on the plant safely even after the plant had extracted Cr(VI). Much of the extracted chromium remains in the roots, in the form of Cr(III). The investigation of the mechanism of the reduction of Cr(VI) to Cr(III) in the body of the phytoextractor is now under way.

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