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**REDUCTION OF HEALTH RISKS DUE TO CHROMIUM(VI) USING MESQUITE:
A POTENTIAL Cr PHYTOREMEDIATOR**

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INTRODUCTION

Hexavalent chromium [Cr(VI)] is toxic for living systems at low concentrations. Exposure to Cr(VI) may damage the lungs, liver, nervous system and kidneys and may cause skin diseases, as well as various types of cancer in mammals¹⁻³. Excess of Cr in arid and windy regions represents a health concern due to an uncontrolled contaminant movement caused by wind erosion. Current Cr removal from contaminated soil is labor consuming and soil disturbing. A relatively new technology, phytoremediation, which is the use of plants to restore heavy metal contaminated sites is an attractive alternative to conventional technologies⁴. The majority of plants that have been identified for the purpose of phytoremediation do not grow well in desert environments. However, desert plants have adapted to tolerate and thrive in harsh desert conditions. These characteristics suggest that desert plants can respond favorably to other stress-induced adaptive mechanisms such as growth in heavy metal contaminated sites.

Previous studies demonstrated that other desert plant species such as creosote bush (*Larrea tridentata*) absorb heavy metals such as copper with no apparent physiological damage. X-ray absorption spectroscopy (XAS) studies showed that copper was in the tissue of *L. tridentata* as both Cu(I) and Cu(II)⁵. In the present study, the ability of the desert plant species mesquite (*Prosopis spp.*) to uptake Cr(VI) from contaminated solid media was investigated. Mesquite plants were exposed for 26 days to 75, 80 and 125 mg L⁻¹ of Cr(VI) supplied as potassium dichromate to an agar media. Cr uptake was determined using ICP/OES and X-ray absorption spectroscopy (XAS) studies were

used to determine the coordination chemistry of chromium in the roots, stems and leaves of the plant. The results are reported herein

Materials and Methods

Plant cultivation and metal determination

Seeds from Wild Seeds Company (Tempe, AZ) were soaked overnight in distilled water, washed and treated with Captan® to eliminate fungal contamination. A modified Hoagland's nutrient solution (pH 5.3 ± 0.2) was used for the experiments at half strength as described by Peralta *et al.*⁶. $K_2Cr_2O_7$ was used as the chromium(VI) source. During preparation, aliquots of 75, 80 and 125 mL of a 1000 mg L^{-1} solution of Cr(VI) were added to three separate aliquots of nutrient solution, whose volume was adjusted to one liter. The nutrient solutions containing the $K_2Cr_2O_7$ were then autoclaved. Bacto-Agar™ was added at 0.48 % w/v. The seeds were placed directly on the growing medium under a laminar flow hood and set under an 18/6-hour light/dark cycle. The temperatures were set to $25^\circ\text{C}/18^\circ\text{C}$ at day and night, respectively. Plants were harvested after 26 days, washed in 0.01 M HCl for 5 seconds, rinsed in deionized water and separated into roots, stems and leaf.

The plant samples were set in a 70°C oven for 2 days to dry. The dried samples were digested by using 10 ml of trace pure nitric acid in a Perkin Elmer Multiwave® microwave oven, following the pre-programmed EPA method 3051⁷. After digestion, the chromium concentration in plant digested samples was determined by using an inductively coupled plasma optical emission spectrometer (ICP/OES).

XAS sample preparation

Samples for XAS studies were immersed in liquid nitrogen for 25 minutes and then freeze-dried in a Labconco Freeze Dry System (Freezone 4.5™) (Labconco Corporation, Kansas City, MO). After lyophilization, the samples were ground and packed into 1.0 mm sample plates with Mylar tape windows and taken to Stanford Synchrotron Radiation Laboratories (SSRL) for X-ray absorption spectroscopy analyses. These analyses were performed in order to determine the oxidation state, the interatomic distance and number of nearest neighbor atoms to the chromium atoms in the different parts of the mesquite samples and the agar⁸. The beamline operating conditions were a beam current of 60-100 mA and energy of 3 GeV. All samples were run using a helium cryostat at a temperature of about 15 K to reduce Debye-Waller effects that occur from thermal disorder in samples. Fluorescence spectra of the chromium laden mesquite samples were taken using a 30-element Canberra germanium detector. On the other hand, transmission measurements were recorded for the model compounds chromium(III) sulfide, chromium(III) acetate, chromium(III) phosphate, chromium(III) nitrate and potassium dichromate using helium filled ionization chambers. The model compounds were diluted in boron nitride to give a change of one absorption unit across the edge. A silicon (220, at ϕ 90) double crystal monochromator with an entrance slit of 1 mm was used for all measurements.

XAS data analysis

The data were analyzed using the WinXAS software⁹. The samples were calibrated against the chromium edge energy using 1st and 2nd degree derivatives of the foil edge energy (5.989 keV). The sample spectra were then background corrected using a 1 degree polynomial on the pre-edge region and a spline of 7 knots on the post-edge region. The XANES region was then extracted from the entire spectra, from 5.95 keV to 6.20 keV. This was then fitted using a LC-XANES (linear combination XANES) fitting of 5000 iterations of the chromium model compounds. The entire XAS spectra were then converted into K space for the extraction of the EXAFS spectra. The spectra were converted into k space (or wave vector space). The resulting EXAFS spectra were then k weighted to 2, between 2 and 12.2 Å⁻¹ and subsequently Fourier transformed using a modified Hanning window over the first and last 10% of the EXAFS. The fitting of the EXAFS was performed using least squared fitting of the Fourier filtered spectra, using FEFF V 8.00 an *ab initio* multiple-scattering code¹⁰. The FEFF input files were created using the ATOMS software from crystallographic data of the model compounds¹¹. From the *ab initio* calculations of FEFF, the number of coordinating atoms, the nearest neighboring atoms and the Debye-Waller factors were calculated.

Results and Discussion

Chromium uptake

The results of Cr uptake by mesquite plants exposed to 75 and 125 mg L⁻¹ of Cr(VI) are shown in Table 1. As one can see in this table, mesquite plant roots supplied with 75 mg L⁻¹ of Cr(VI) concentrated 7636 mg Cr kg⁻¹ DW, but those grown with 125 mg L⁻¹ of Cr(VI) accumulated 10984 mg Cr kg⁻¹ DW. At stem level, the Cr concentration found in plants exposed to 125 mg L⁻¹ of Cr(VI) (2263 mg kg⁻¹) were 2.5 times higher than the concentration found in plants exposed to 75 mg L⁻¹ of Cr(VI) (889 mg kg⁻¹). However, chromium concentration in the leaf of plants exposed to 125 mg L⁻¹ of Cr(VI) (991.5 mg Cr kg⁻¹ DW) was more than 3 times higher than the Cr found in the leaf of plants exposed to 75 mg L⁻¹ of Cr(VI) (322.8 mg Cr kg⁻¹ DW). The amounts of Cr found in mesquite plants are higher than those found by Chatterjee and Chatterjee in cauliflower plant supplied with Cr sulfate at 0.5 mM¹². Furthermore, the Cr concentrations found in leaves of mesquite plants supplied with 125 mg L⁻¹ of Cr(VI) indicate that mesquite can be considered as a potential Cr hyperaccumulator desert plant.

Table 1. Cr uptake by mesquite plants supplied with Cr(VI) at 75 mg L⁻¹ and 125 mg L⁻¹ for 26 days after sprouting with ± 95% confidence interval. Values are mg Cr kg⁻¹ dry weight.

Plant Portion	Control	75 mg L⁻¹	125 mg L⁻¹
Leaf	0.03 ± 0.0	322.8 ± 69.1	991.5 ± 114.9
Stem	0.0 ± 0.0	888.9 ± 137.1	2262.9 ± 81.2
Root	0.02 ± 0.0	7636.3 ± 2463.4	10983.8 ± 1641.9

XAS results

The X-ray absorption near edge spectroscopy (XANES) data for Cr(VI) and Cr(III) model compounds are shown in Figure 1. As seen in this figure, potassium dichromate [Cr(VI)] presents a pre edge peak between 5.958 and 6.000 KeV, which is characteristic for Cr(VI). On the other hand, Cr(III) model compounds do not present this characteristic.

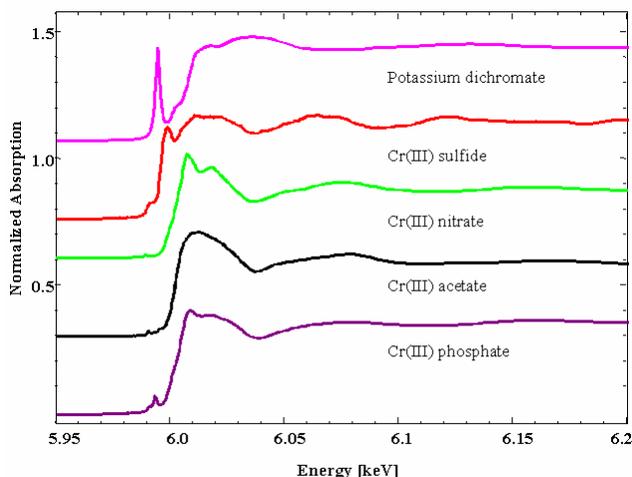


Figure 1. XANES data for the model compounds of Cr(VI) and Cr(III)

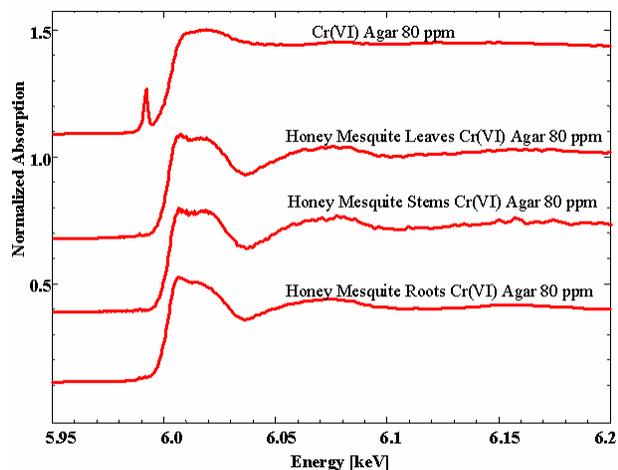


Figure 2. XANES data for the mesquite roots, stems and leaves.

The XANES data for Cr present in agar, and in mesquite roots, stems and leaves are shown in Figure 2. As seen in this figure, the pre-edge feature of the agar spectra is approximately half of the spectra of the Cr(VI) model compound (potassium dichromate), which indicate that approximately half of the Cr(VI) remained as Cr(VI) in the medium. However, the spectra obtained from all the tissues of mesquite plants resemble the spectra of Cr(III) model compounds. These results suggest that part of Cr(VI) was absorbed but reduced to the less toxic Cr(III) inside mesquite plants.

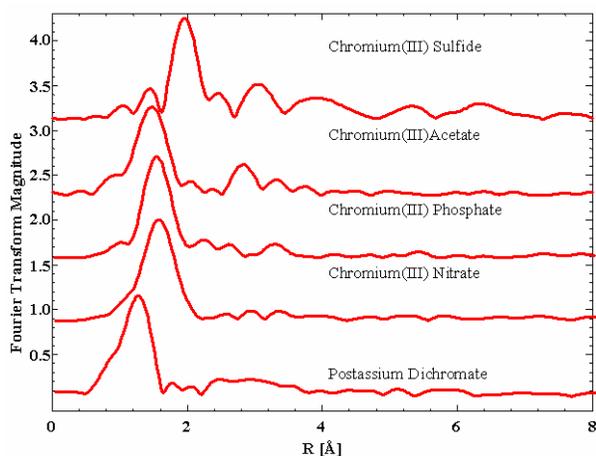


Figure 3. Fourier transformed EXAFS spectra of model compounds as potassium dichromate and the Cr(III) compounds.

The Fourier Transformed (extended X-ray absorption fine structure (EXAFS) K weighted to 2 for the model compounds are shown in Figure 3. The EXAFS of the model compounds chromium(III) acetate and chromium(III) sulfide show an interatomic bonding distance of 1.96 Å and 2.38 Å, respectively, near to the absorbing chromium atom. This indicates a geometrical arrangement of an octahedron or a distorted octahedron¹³. The Fourier transformed EXAFS data for the agar samples are shown in Figure 4. The spectra of the agar sample show Cr present in two different oxidation states. However, the interatomic distance of the mesquite spectra shown in Figure 4 are very similar to those corresponding to Cr(III) acetate. These results corroborate that all the chromium present in the plant tissues is Cr(III).

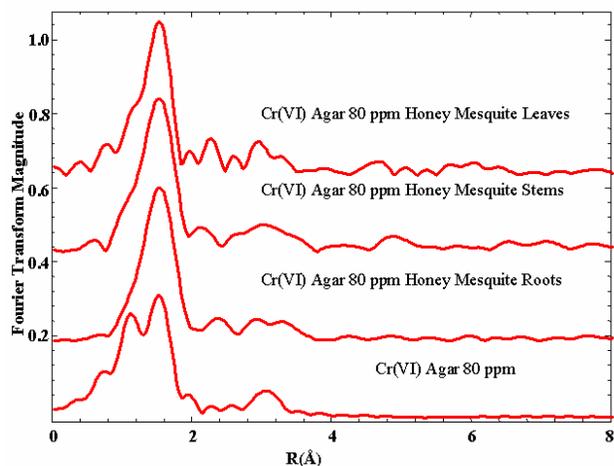


Figure 4. Fourier transformed EXAFS spectra of the agar, roots, stems, and leaves of mesquite grown with 80 mg L⁻¹ of Cr(VI)

The main conclusions of this research were that mesquite plants are able to concentrate enough Cr in the tissue of leaves to be considered as a Cr hyperaccumulator desert

plant species. In addition, this plant absorbs Cr(VI) and converts this chromium to the less toxic Cr(III).

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REFERENCES

1. Pausterbach, D.J., Sheenan, P.J., Paull, J.M., Wisser, L.M. and Finley, B.L. *J. Toxicol. Environ. Health*, 1992, **37**, 177.
2. Ringenberg, Q.S., Doll, D.C., Patterson, W.P., Perry, M.C. and Yarbrow, J.W. *Southern Medical J.*, 1988, **81**, 1132.
3. Hartwig, A. and Schwerdtle, T. *Toxicol. Lett.*, 2001, **7**, 47.
4. Chaney, R.L. In: Land Treatment of Hazardous Wastes. Ed. Parr, J.F., Marsh, P.D. and Kla, J.M., Park Ridge, NJ: Noyes Data Corp., 1983, 50.
5. Gardea-Torresdey, J.L., Arteaga, S., Tiemann, K.J., Chianelli, R., Pigitore, N. and Mackay, W. *Environ. Toxicol. Chem.*, 2001, **20**, 2572.
6. Peralta, J.R.; Gardea-Torresdey, J.L.; Tiemann, K.J.; Gomez, E.; Arteaga, S.; Rascon, E. and Parsons, J.G. *Bull. Environ. Contam. Toxicol.*, 2001, **66**, 727.
7. Kingston, H.M. and Jassie, L.B., Eds; ACS Professional Reference Book Series; Amer. Chem. Soc: Washington D.C., 1988.
8. Penner-Haln, J. E. *Coord. Chem. Rev.*, 1999, **190-192**, 1101.
9. Ressler, T. *J. Synchrotron Radiat.*, 1998, **5**, pp. 118.
10. Ankudinov, A.L., Ravel, B., Rehr, J.J. and Conradson, S.D.; *Phys. Rev. B.*, 1998, **58**, 7565.
11. Ravel, B. *J. Synchrotron Radiat.*, 2001, **8**, 314.
12. Chatterjee, J. and Chatterjee, C. *Environ. Pollut.*, 2000, **109**, 69.
13. Vaijpayee, P., Sharma, S.C., Tripathi, R.D., Rai, U.N. and Yunus, M. *Chemosphere*, 1999, **39**, 2159.