ENHANCED METAL-BINDING CAPACITY OF NaOH TREATED *LARREA TRIDENTATA* LEAF TISSUE

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ABSTRACT

Larrea tridentata, or creosote bush, is a common desert plant that inhabits the Chihuahuan Desert. Due to its great abundance and high growth tolerance of heavy metal-contaminated soils, it was chosen as the primary candidate for this study. Previous experiments conducted on inactivated and untreated sodium hydroxide (NaOH) *Larrea tridentata* leaf tissues showed that it effectively bound metal ions from aqueous solutions. These pure metal ion solutions consisted of copper(II), cadmium(II), nickel(II), lead(II), zinc(II), and chromium(III). In order to understand the effects of NaOH modification on the creosote bush's metal ion-binding ability, batch modification studies were conducted. Results from these modification studies showed an increase in metal binding for every metal tested. We believe that the enhanced metal-binding ability of the NaOH modified biomass of *Larrea tridentata* is due to the conversion of methyl esters to carboxylic groups. Carboxylic groups have a higher affinity to bind metal ions at pH 5 since they are deprotonated and thus promote the approach of positively charged cations. Data from these experiments suggest that NaOH modification may be a process to further improve heavy metal binding by creosote bush tissues.

Key words: *Larrea tridentata, creosote bush, metal binding, enhanced binding*

INTRODUCTION

In recent years, global pollution has become an increasing problem. Pollution of the Earth's land, water, and air by heavy metals is implicated in causing the health degradation of living organisms. Heavy metal soil contamination has been blamed for the deterioration of forest ecosystems (Allen et al., 1995). A study conducted on tree root systems showed that root elongation, root hair formation, and root biomass production were greatly inhibited by high concentrations of heavy metals (Kahle, 1993). Once in the environment, metals have been found to adversely affect human health. As much as 6400 parts per million (ppm) of lead were found to be present in the blood of children living in the vicinity of an ore smelter (Landrigan et al., 1975). This smelter was found to have emitted 1012 metric tons of lead, 508 metric tons of zinc, 11 metric tons of cadmium, and 1 metric ton of arsenic in a three-year period. Contact with low levels of lead is the cause of inhibited mental development and learning disabilities in babies and young children (Primary Drinking Water Rules, 1992). High levels of cadmium have been shown to cause kidney damage, and arsenic has proven to be a carcinogen, causing kidney, lung, and liver cancers (Weymuller, 1997). There are approximately 40,000 hazardous waste sites in the United States and 1200 of the most toxic sites are called Superfund sites and are primary targets for environmental cleanup (Weymuller, 1997). They are targeted because as many as 85% of these Superfund sites are responsible for the contamination of the nation's groundwater, a source which supplies the United States with 50% of its drinking water (Weymuller, 1997).

Therefore, new technologies are needed for the treatment of contaminated groundwaters

because previous methods employed (such as ion exchange, reverse osmosis, micro filtration, precipitation, carbon adsorption, neutralization, and flocculation) are expensive, cannot remediate waters containing low concentrations of heavy metals, and are unable to distinguish between different contaminants. One of these new technologies is phytoremediation, the use of living plants to store heavy metals into their tissues from the environment. Live plant systems, such as sunflowers, poplars, and Indian mustard plant, have been shown to effectively remove heavy metals from aqueous solutions (Dushenkov, et al., 1997; Newman, et al., 1997; Salt, et al., 1997). A study conducted on *Brassica juncea* showed that this plant was able to decrease polluted selenium soils by as much as 50% (Moffat, 1995). In contrast to using live plants, the use of inactivated or nonliving plant tissues has proven to be as effective in their ability to bind metal ions. Gardea-Torresdey and coworkers found that inactivated alfalfa and *Larrea tridentata* tissues were shown to rapidly bind heavy metals in high capacities (Gardea-Torresdey, et al., 1996a, 1996b, 1996c, 1997, 1998).

Metal uptake in plants is believed to occur through a wide variety of functional groups present on cell walls. Some of these groups include sulfhydryl, carboxyl, carbonyl, and hydroxyl groups. Previous experiments conducted on functional groups showed that by hydrolyzing *Datura innoxia* cell wall ester groups, the carboxylate content was increased and this in turn caused an increase in metal uptake (Drake, et al., 1996). These carboxyl groups have been shown to be very important for metal binding. In an experiment conducted by Gardea Torresdey et al., algal cell carboxyl groups were transformed into ester groups and a decrease in copper and aluminum uptake was observed (1990). By determining the amounts that these constituents make up on the cell wall surface and their affinity to bind metal ions, chemical modification of existing groups can lead to enhanced metal-binding abilities.

Inactivated cells of *Larrea tridentata*'s leaves were chosen for conducting sodium hydroxide (NaOH) modification studies. This shrub is one of five species belonging to the plant family Zygophyllaceae and is the only one that grows in North America. This plant is commonly found growing in soils containing high quantities of calcium carbonate and gravel (MacMahon, 1985). *Larrea tridentata* has already proven to bind aqueous heavy metals in high quantities and found to be resistant to heavy metal-contaminated soils (Gardea-Torresdey, et al., 1996a, 1997). A reason given for metal accumulation by plants is that metal accumulation acts may be a defense mechanism, thus deterring grazing animals, insects, and microbes from eating plant tissues (Moffat, 1995). *Larrea tridentata*, commonly known as creosote bush or gobernadora, contains resinous phenolic constituents, especially nordihydroguaiaretic acid, that deter grazing on its outer leaves and stems. In addition, wax esters are also present on the outer leaves and stems and play a major role in the adaptation of *L. tridentata* to the arid desert environment (Mabry et al., 1977).

The objective of this study was to determine any enhanced metal binding in sodium hydroxide

modified creosote bush leaf tissue versus unmodified leaf tissues. In order to determine differences in metal binding for unmodified and modified creosote bush biomass, batch pH and capacity experiments were performed for lead(II), nickel(II), cadmium(II), zinc(II), copper(II), chromium(III), and chromium(VI). Results of our experiments are reported herein.

MATERIALS AND METHODS

Plant Material

Larrea tridentata (creosote bush) samples were collected in a non-heavy metal-contaminated area 10 miles east of El Paso, Texas. Three plants with similar characteristics, such as height (3 ft) and maturity were removed from the site. Upon arrival at the laboratory, the shrubs were washed with deionized (DI) water and the leaves were removed, oven dried at 90° C until dry, and then ground until the biomass passed through a 100-mesh sieve.

Biomass Modification with Sodium Hydroxide

A 6.12-gram sample of dried creosote leaves was weighed and washed twice with 0.1M HCl and centrifuged at 2500 rpm. The biomass was then reacted with 50 mL of 0.1M sodium hydroxide (NaOH) for 24 hours. The sample was subsequently centrifuged and the supernantant was removed. The creosote-leaf-treated biomass was then washed with deionized water three times, followed by centrifugation each time, and then lyophilized in a Labconco freeze dryer overnight.

pH Profile Studies for Metal Binding

A 250 mg sample of creosote bush leaves (both NaOH-modified and unmodified samples) was weighed and washed twice with 0.1 M HCl to remove any soluble biomolecules or debris that might interact with metal binding. The washings were collected and dried to account for any biomass loss during washing. The biomass was then suspended in 50 mL of 0.01 M HCl (biomass concentration of 5 mg/mL), adjusted to pH of 2.0, and allowed to equilibrate. Two mL of the suspension (10 mg of biomass) were added to clean plastic test tubes. The pH was then adjusted to 3.0, 4.0, 5.0, and 6.0 by adding a solution of NaOH. At each respective pH, after equilibration, 2 mL aliquots were removed and placed in clean tubes. Individual metal solutions of 0.1 mM were prepared from the following salts, $Pb(NO_3)_2$, $Cd(NO_3)_2$, $ZnCl_2$, $Ni(NO_3)_2$, $K_2Cr_2O_7$, and $Cr(NO_3)_2$9H2O. At each pH, 2 mL of the metal solution were added to the respective biomass pellet. This was carried out in triplicate to maintain quality assurance. All the tubes were equilibrated by rocking for one hour and then centrifuged for five minutes at 2,500 rpm. The supernatants were transferred to clean test tubes where the final pHs were recorded, and metal analysis was performed by flame atomic absorption spectroscopy.

Metal-Binding Capacity Studies

Batch laboratory methods were used to determine the binding capacity of Cd(II), Cr(III), Pb(II), Ni(II), and Zn(II) to the creosote bush leaves (NaOH-treated and untreated). For these

experiments, 50 mg of biomass were washed twice with 0.1 M HCl and the washes were collected and weighed to determine any biomass loss. Two ml aliquots of the suspension were transferred to 3 tubes and then centrifuged. The supernatants were saved for further testing. Two ml of 0.3 mM metal solution at pH 5 (in 0.01 M sodium acetate) were added to each of the tubes and equilibrated by rocking for 15 minutes. After centrifugation, the supernatants were saved for analysis and again 2 ml of 0.3 mM metal solution were added. This was repeated 10 times or until the saturation point was achieved. Subsequently, the final pHs of all the supernatants were recorded. Samples were diluted as required to remain within the instrumental calibration linear range and metal concentrations were determined by flame atomic absorption.

Desorption of the Adsorbed Metal Ions

The creosote pellets loaded with the respective metal to capacity were treated with 0.1M HCl, and equilibrated for 15 minutes, and then centrifuged for five minutes at 2500 rpm. The supernatants were removed and the pellets were once again treated with 0.1M HCl and equilibrated on a rocker for 15 minutes. After centrifugation, the supernatants were analyzed for metal content using flame atomic absorption spectroscopy.

Metal Analyses Performed by Flame Atomic Absorption Spectroscopy

The metal content present in the supernatants was determined using a Perkin Elmer model 3110 Atomic Absorption Spectrometer with deuterium background subtraction. Wavelengths used for the metals under investigation were cadmium-228.8 nm, chromium-358.2 nm, copper-327.4 nm, nickel-352.5 nm, lead-283.3 nm, and zinc-213.9 nm. The analytical conditions that were followed were obtained from the manual of the Perkin Elmer model 3110 Atomic Absorption Spectrometer. In addition, a calibration curve was obtained with a correlation coefficient greater than 0.98 and the instrument's response was checked regularly with known metal standards. Each sample was read three times and the mean value and relative standard deviations were computed. Metal bound to *Larrea tridentata* leaf tissue (modified and unmodified) was calculated as the difference of the controls and the final metal present in the remaining respective supernatants.

RESULTS AND DISCUSSION

In an attempt to promote increased metal binding by the creosote biomass, the biomass was reacted with 0.1M NaOH so that ester groups might be converted into carboxylate groups. The following chemical reaction that produces carboxylate groups from methyl esters is depicted below:

$$R\text{-}COO\text{-}CH_3 + NaOH \rightarrow R\text{-}COO^- + CH_3OH + Na^+$$

The possible formed carboxylate groups tended to have a much higher metal-binding ability than methyl ester groups (mainly at pH 5). This is due to the fact that at pH 5, the carboxylate groups

are primarily deprotonated (pKas for carboxylate groups are generally between 3-4) and thus negatively charged. Consequently, the attraction of positively charged metal cations is greatly promoted at pH 5. On the other hand, pKa values for methyl esters are in the order of 12 or higher, and they are mainly undissociated at pH 5. Therefore, metal cation coordination by methyl ester groups is significantly lower than carboxylate groups (especially at pH 5). Indeed, an increase in metal binding was observed for the NaOH-modified biomass. Table 1 summarizes the percent increase in metal binding observed for the NaOH-modified biomass. The percent increases in metal binding of the NaOH-treated creosote leave biomass (as compared to the untreated biomass) for the following metals were as follows: 13% for Cd(II), 109% for Cr(III), 61% for Cu(II), 67% for Ni(II), 10% for Zn(II), and 73% for Pb(II). These findings suggest that the increase in metal binding is due to the formation of newly synthesized carboxylate groups. Thus, our hypothesis was correct since we are obtaining an enhancement in metal binding in the NaOH-modified biomass.

It can be seen from Figures 1 through 3 that for most metal ions involved (except for Cr(VI)), the metal ion binding by the NaOH modified biomass increases as the pH increases. Metal binding was optimum at pHs between 5 and 6 for Cd(II), Cr(III), Pb(II), Cu(II), Ni(II), and Zn(II). This trend is similar to the one previously shown with unmodified creosote leaf tissues (Gardea-Torresdey et al., 1997).

Figure 1 shows the pH profile obtained for Cr(VI) and Zn(II) binding for the NaOH-treated biomass. In the case of Cr(VI), relatively low levels of this metal bound to the NaOH-treated creosote leaf tissue. This behavior could be due to the fact that Cr(VI) exists as a negatively charged oxo-anion in an aqueous environment. The negatively charged oxo-anion, ($Cr2O7^{-2}$) cannot bind to the negatively charged carboxyl groups once they are deprotonated. Figures 2 through 3 depict pH profiles for the remaining metal ions. As can be seen in these figures, at low pHs, metal ion binding is decreased, but increase dramatically as the pH increases to 6 (except for cadmium). By comparing the pH profiles for metal binding by the NaOH-treated creosote biomass to the pH profiles of the untreated biomass (data not shown), it can be seen that there is only one major difference. The NaOH-modified creosote leaf tissues show a greater affinity for the metal ions and therefore higher metal-binding ability at all pH values. This increase in metal ion binding is attributed to the newly formed carboxylate groups and further corroborates the fact that methyl esters were converted to carboxylate groups.

The pH profile studies showed that metal ion binding was lower at lower pHs than at higher pHs. Thus, one could suggest that by lowering the pH, these metal ions could be removed since protons could displace the bound-metal cations. Table 2 shows the percentages of metal ions recovered from the unmodified and NaOH-modified creosote biomasses by treatment with 0.1 M HCl. From this table, it can be observed that most metal ions were recovered from the biomass (except for chromium(III)). Copper, zinc, and lead ions showed that they could be easily removed

when exposed to an acidic environment. These elements averaged about a 90% recovery rate. In addition, cadmium and nickel averaged a 60% recovery. By stripping these metal ions through the use of 0.1M HCl, the creosote biomass may be able to be used again and the recovered metal ions may be recycled.

CONCLUSIONS

An enhanced metal-binding ability of the NaOH-treated biomass of creosote leaves was observed (as compared to the untreated biomass). The following increases in metal binding by the NaOH-treated biomass were obtained: 13% for Cd(II), 109% for Cr(III), 61% for Cu(II), 67% for Ni(II), 10% for Zn(II), and 73% for Pb(II). These findings support our hypothesis that by hydrolysis of methyl esters in creosote leaves, the metal-binding ability of creosote biomass can be greatly improved. In addition, pH profile experiments for metal ion binding, by the NaOH-treated and untreated creosote leave biomass, have supported that carboxylate groups were produced by hydrolysis of methyl esters by treatment with NaOH. Our results may be implemented for the use of a new technology to remove toxic heavy metal ions from contaminated waters.

ACKNOWLEDGMENTS

The authors acknowledge the financial support from the National Institutes of Health (NIH), grant # GM 08012-25. We also acknowledge the financial support from the University of Texas at El Paso's Center for Environmental Resource Management (CERM) and from the U.S. Environmental Protection Agency (cooperative agreement CR-819849-01).

REFERENCES

Allen, H., Chin Huang, George Bailey, and Alan Bowers, 1995. Metal Speciation and Contamination of Soil, Lewis Publishers, Boca Raton, Florida, p.2.

Drake, L., S. Lin, and G.D. Rayson, 1996. Chemical Modification and Metal Binding Studies of *Datura innoxia,* Environmental Science and Technology, 30, pp. 110-114.

Dushenkov, S., D. Vasudev, Y. Kapulnik, D. Gleba., et al., 1997. Removal of Uranium from Water Using Terrestrial Plants, Environmental Science and Technology, 31, pp. 3468-3474.

Gardea-Torresdey, J.L., M.K. Becker- Hapak, J.M. Hosea, and D.W. Darnall, 1990. Modification of Algal Carboxyl Groups in Metal Binding, Environmental Science and Technology, 24, pp.137-145.

Gardea-Torresdey, J.L., L. Polette, S. Arteaga, K.J. Tiemann, J. Bibb, and J.H. Gonzalez, 1996a. Determination of the Content of Hazardous Heavy Metals on *Larrea Tridentata* Grown Around a Contaminated Area, Proceedings of the Eleventh Conf. on Hazardous Waste Research, (HSRC/WERC Joint Conference on the Environment), Edited by L.R. Erickson, D.L. Tillison, S.C. Grant, and J.P. McDonald, Albuquerque, NM, pp. 660-669.

Gardea-Torresdey, J.L., K.J. Tiemann, J.H. Gonzalez, J.A. Henning, and M.S. Townsend, 1996b. Ability of Silica-Immobilized *Medicago Sativa* (Alfalfa) to Remove Copper Ions from

Solution, J. of Haz. Mat., 48, pp. 181-190.

Gardea-Torresdey, J.L., K.J. Tiemann, J.H. Gonzalez, J.A. Henning, and M.S. Townsend, 1996c. Removal of Nickel Ions from Aqueous Solution By Biomass and Silica Immobilized Biomass of *Medicago Sativa*(Alfalfa), J. of Haz. Mat., 47 pp. 205-216.

Gardea-Torresdey, J.L., K.J. Tiemann, J.H. Gonzalez, O. Rodriguez, and G. Gamez, 1998. Phytofiltration of Hazardous Cadmium, Chromium, Lead, and Zinc Ions by Biomass of *Medicago Sativa*(Alfalfa), J. of Haz. Mat., 57, pp.29-39.

Gardea-Torresdey, A. Hernandez, K.J. Tiemann, J. Bibb, and O. Rodriguez, 1997. Adsorption of Lead, Cadmium, Zinc, and Nickel by Inactivated Cells of *Larrea Tridentata* (Creosote Bush), Proceedings of the Hazardous Substance Research Center (HSRC) & Waste-management Education and Research Consortium (WERC)1997 Joint Conference on the Environment, April 22-24, Edited by D.D. Reible, and T.J. Ward, Albuquerque, N.M., pp. 482-488.

Kahle, Henning, 1993. Response of Roots of Trees to Heavy Metals, Environmental and Experimental Botany, 33, pp. 99-119.

Landrigan, P., S. Gehlbach, B. Rosenblum, J. Shoults., et al., 1975. Epidemic Lead Absorption Near an Ore Smelter: The Role of Particulate Lead, The New England Journal of Medicine, 292, pp. 123-129.

Mabry, T.J., J.H. Hunziker, and D.R. DiFeo, Jr., 1977. Creosote Bush: Biology and Chemistry of *Larrea* in New World Deserts, The Institute of Ecology , IBP Synthesis Series 6. Library of Congress Catalog Card Number: 76:5838, p. 117.

Macmahon, J., 1985. Deserts, Alfred A. Knopf, Inc., New York, pp. 498-499.

Moffat, A.S., 1995. Plants Proving Their Worth in Toxic Metal Cleanup, Science., 269, pp. 302-303.

Newman, L., S. Strand, N. Choe, J. Duffy, et al., 1997. Uptake and Biotransformation or Trichlorethylene by Hybrid Poplars, Environmental Science and Technology, 31, pp. 1062-1067.

Primary Drinking Water Rules[Sec. 141.32(e)(20)], Federal Regulations, The Bureau of National Affairs, Inc. (1992)

Salt, D., I. Pickering, R. Prince, D. Geba., et al, 1997. Metal Accumulation by Aquacultured Seedlings of Indian Mustard, Environmental Science and Technology, 31, pp. 1636-1644.

Weymuller, Sims, 1997. PRG:Make Polluters Pay!:The Campaign to Save the Superfund Program, http://www.pirg.org/enviro/toxics/superfund/index.htm.

Table 1. Adsorption capacity for metal uptake by unmodified and modified creosote leaves.

METAL ION	mg metal / g biomass		
	Modified Biomass	Unmodified Biomass	% Increase in Binding
Cd^{2+}	15.49 + 2.73	13.65 + 6.11	13%
Cr^{3+}	8.71 + 1.15	4.17 + 2.80	109%
Cu^{2+}	10.53 + 0.94	6.56 + 1.79	61%
Ni^{2+}	5.97 + 2.17	3.58 + 1.85	67%
Zn^{2+}	33.09 + 7.62	30.22 + 6.63	10%
Pb^{2+}	40.17 + 4.64	23.21 + 3.23	73%

Table 2. Percent recovery of bound-metal ions from creosote leaves using 0.1M HCl.

Metal Ion	Modified Biomass	Unmodified Biomass
Cd^{2+}	71%	58%
Cr^{3+}	15%	17%
Cu^{2+}	93%	84%
Ni^{2+}	78%	51%
Zn^{2+}	90%	86%
Pb^{2+}	90%	100%

Figure 1. pH profile for chromium(VI) and zinc(II) binding by Creosote Bush NaOH treated Leaf Tissues. (Chromium (VI) Δ, Zinc (II) ■) .

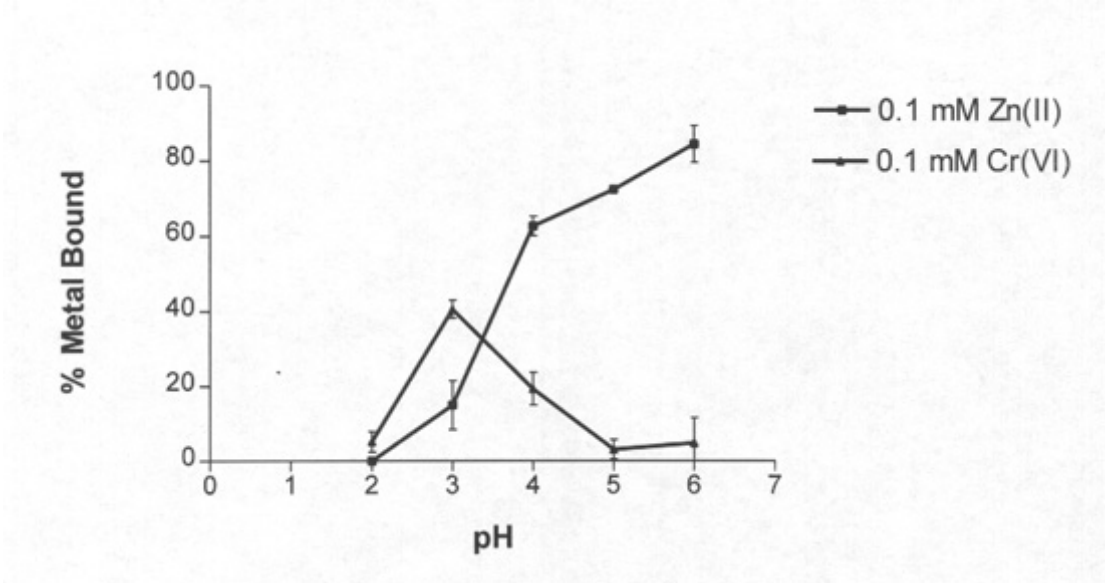

Figure 2. pH profile for lead(II), nickel(II), and cadmium(II) binding by Creosote Bush NaOH treated Leaf Tissues. (Lead (II) ■, Nickel (II) Δ, Cadmium (II) ∇).

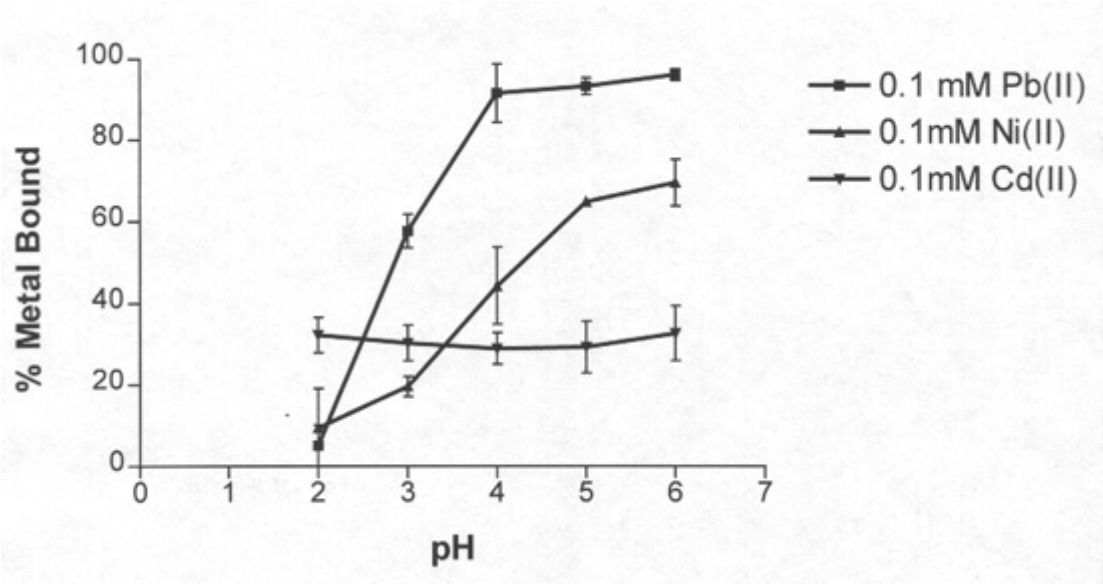

Figure 3. pH profile for chromium(III) and copper (II) binding by Creosote Bush NaOH treated Leaf Tissues. (Chromium (III) ■, Copper (II) Δ).