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Adsorption of Toxic Metal Ions From Solution by Inactivated Cells of Larrea Tridentata Creosote Bush

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ADSORPTION OF TOXIC METAL IONS FROM SOLUTION BY INACTIVATED CELLS OF LARREA TRIDENTATA (CREOSOTE BUSH)

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ABSTRACT

Larrea tridentata (creosote bush) is a plant that grows abundantly in the desert environment. This desert plant has been found naturally growing in heavy-metal contaminated soils. Previous experiments showed that the inactivated biomass of creosote bush was able to adsorb Cu(II) ions from aqueous solutions. The copper-binding capacity of the bush biomass that grows in heavy-metal uncontaminated soils was higher than the biomass that grows in heavy-metal contaminated soils. Experiments were performed to determine the ability of creosote bush biomass (grown in heavy metal uncontaminated soils) to adsorb Pb(II), Cd(II), Zn(II), Cr(III), Cr(VI), and Ni(II) ions from aqueous solutions. Batch pH profile experiments for these metal ions showed that the metal ion binding was different for every metal tested but increased as the pH was raised from 2.0 to 6.0. The metal ion uptake by the roots, stems, and leaves was quite fast. Binding capacity experiments showed a more significant binding capacity for lead(II) and chromium(III) ions and in general, the leaves bound more metal ions than the stems and roots. A great portion of the metal ions adsorbed by the creosote's roots, stems, and leaves was desorbed by treatment with 0.1 M HCl (up to 99% in some cases). Biomass of creosote bush may prove to be useful to remove and recover metal ions from contaminated waters.

Key words: *Phytoremediation, Larrea tridentata, creosote bush, heavy metal binding.*

INTRODUCTION

Due to the great amount of industrial activity throughout the last century, heavy metal contamination of the environment has become a serious problem. Many of these toxic metals enter the environment through fossil fuel combustion as well as mining and smelting processes (Carson *et al.*, 1986; Bewley, 1980; Micera and Dessi, 1988). The natural process of metal transportation between the soil and water concentrates heavy metal contamination in the environment (Runnels and Shepherd, 1992). Once in the environment, metals are difficult to remediate and can adversely impact human health. Even in low doses, metals which do not have distinguishable odor and color characteristics, still pose a major threat. Current technologies for cleaning up heavy-metal contaminated soils involve excavation and reburial in landfills. This process is expensive and usually reserved for small areas, and the metals are still not isolated from the environment. Contaminated waters are typically detoxified through the use of ion exchange and activated charcoal filters which are not only costly, but nonselective for heavy metal removal. Due to the high cost of these methods, there is a need for the development of a more cost-effective method.

Bioremediation has emerged as an inexpensive solution. Many researchers have studied the use of live microbial systems for the purpose of remediating contaminated soils and waters (Atlas, 1995; Cervantes and Gutierrez-Corona, 1994; Baker *et al*., 1994; Carvalho *et al.*, 1995; Zhang and Majidi, 1993, 1994). More recently, phytoremediation has appeared as a cost-effective technology for the removal of metals from contaminated areas (Scott, 1992; Nada Kumar *et al*., 1995a, 1995b). A few plant species have shown a remarkable resistance to heavy metals such as copper, zinc, and lead (Wu and Antonovics, 1976; Macnair, 1983; Watkins and Macnair, 1991). This ability to grow in highly contaminated areas is thought to be due to the evolution of chemical functional groups which inhibit the toxicological effects of the heavy metals (Lue-Kim and Rauser, 1986). Phytochelatins and proteins within the plant may be produced in large concentrations to bind the metals and reduce their harmful effects (Mofa, 1995). Therefore, plants that grow in contaminated areas should show a greater ability to recover heavy metals and may be a good source for naturally occurring biological compounds that have potential for contaminant remediation.

Although live biological systems work well for low concentrations, they cannot survive the high levels that are found in seriously contaminated areas and industrial effluents. The use of non-living biomaterial containing metal-binding compounds would have the advantage of not requiring care and maintenance as well as being useful in remediating areas with high levels of contaminants that would otherwise kill live systems. Lujan, as well as Gardea-Torresdey and coworkers, have shown that phytofiltration using dead or inactivated biomass is very effective for the removal and recovery of heavy metal contaminants in aqueous environments (Lujan *et al*., 1994; Gardea-Torresdey *et al*., 1996a, 1996b). Hence, a plant such as the creosote bush which has the ability to tolerate heavy metals and adverse environments might contain natural metal binding compounds and could be a possible source for an inactivated phytofiltration system.

Larrea tridentata, better known as creosote bush, is a common desert shrub that can be found growing in the Sonoran and the Chihuahuan deserts of North America. This desert plant has been found naturally growing in heavy-metal contaminated soils. Previously performed experiments in our laboratory have shown that inactivated creosote biomass is able to adsorb Cu(II) ions from aqueous solutions. When the heavy-metal tolerant plant was compared to plants grown in uncontaminated areas, the uncontaminated plant showed a higher capacity for copper

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binding. This may be due to the occupation of the chemical binding sites by previously uptaken copper ions.

The objective of this study is to report the ability of creosote bush biomass grown in uncontaminated soils to adsorb or uptake lead, cadmium, zinc, nickel, chromium(III), and chromium(VI) from aqueous solutions. In order to help understand the metal binding mechanism, batch laboratory experiments were performed to determine optimal binding pH, time dependency of binding, and binding capacity for each of the above mentioned metals. These experiments were carried out separately for the creosote bush roots, stems, and leaves.

MATERIALS AND METHODS

Larrea tridentata Collection

The sample was collected outside the city limits of El Paso, Texas, nearly 25 miles east of The University of Texas at El Paso. Soil samples were taken to confirm that the area was not laden with heavy metals. Three plants of similar characteristics (height of 3 feet) and maturity were removed from the location and washed with deionized water(DI). The roots, stems, and leaves were separated and oven dried at 90°C for four days. Next, the samples were ground using a mill and sieved to pass through a 100-mesh screen.

pH Profile Studies for Metal Binding

Batch laboratory methods were carried out as previously described (Gardea-Torresdey *et al*., 1996c). A 250 mg sample of creosote bush biomass was weighed and washed twice with 0.1M HCl to remove any soluble biomolecules or debris that might interact with the 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 0.01M HCl (biomass concentration of 5mg/mL), adjusted to pH of 2.0, and allowed to equilibrate. Two mL of the suspension (10mg of biomass) were added to clean test tubes. The pH was then adjusted to pH 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. Solutions of 0.1mM were prepared from the following salts, $Pb(NO_3)_2$, $Cd(NO_3)_2$, $ZnCl_2$, and $Ni(NO_3)_2$, $K_2Cr_2O_7$, and $Cr(NO_3)_3{\cdot}9H_2O$. At each pH, 2 mL of solution were added to the respective pH biomass pellets. 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 pH was recorded and metal analysis was performed by flame atomic absorption spectroscopy. The experiment was performed in triplicate.

Time Dependency Studies for Metal Binding

The batch laboratory time dependency studies were performed using the procedure previously reported (Gardea-Torresdey *et al*., 1996c). A 250 mg sample was weighed, washed as indicated before, and then adjusted to pH 5.0. Two mL of 0.3 mM metal solutions were added to 21 tubes, three for each time interval. The time intervals chosen for the time dependence studies were 5, 10, 15, 30, 60, 90, and 120 minutes. After the appropriate time interval passed, the samples were centrifuged and the supernatants decanted into clean tubes. This procedure was repeated for each of the metal ions being analyzed. Final pHs for all tubes were recorded and metal concentrations were determined by flame atomic absorption spectroscopy.

Metal Binding Capacity Studies

The batch laboratory methods used to determine the binding capacity of Cd(II), Cr(III), Cr(VI), Pb(II), Ni(II), and Zn(II) to the creosote biomass were performed as reported previously (Gardea-Torresdey *et al*., 1996c). For these experiments, 50 mg of biomass were washed twice with 0.1 M HCl and the washings 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 testing. Two mL of 0.3 mM metal solution was added to each of the tubes and biomass-free controls which were equilibrated by rocking for 15 minutes. After centrifugation, the supernatants were saved for analysis and again 2 mL of 0.3 mM metal solution was added. This was repeated 7 times or until the saturation point was achieved and the final pH for all supernatants was recorded. Samples were diluted as required to remain within the calibration linear range and metal concentrations were determined by flame atomic absorption spectroscopy.

Desorption of the Adsorbed Metal Ions

The pellets from binding-capacity studies with the adsorbed metal were exposed to 2 mL of 0.1 M HCl, equilibrated by rocking for 15 minutes, and then centrifuged as indicated by Gardea-Torresdey et al., (1996c). Supernatants were collected for analysis and diluted as

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required to stay within the calibration range. Pellets were once again exposed to 2 mL of 0.1 M HCl to remove any remaining metal and equilibrated by rocking for 15 minutes. After centrifugation, the supernatants were analyzed, using flame atomic absorption spectroscopy.

Metal Analyses

The metal content in all the experiments was determined by using a Perkin Elmer model 3110 Atomic Absorption Spectrometer with deuterium background subtraction. The instrument response was periodically checked with known standards. A calibration curve was obtained with a correlation coefficient of 0.98 or greater. The samples were read three times and the mean values and the relative standard deviations were computed. The following wavelengths were used for the metals studied: cadmium 228.8 nm, lead 283.3 nm, nickel 352.2 nm, zinc 213.9 nm, and chromium 359.6nm. An impact bead was utilized to improve the sensitivity, but in the case of zinc, a flow spoiler was used. The difference between the initial metal concentration and the remaining metal concentration was assumed to be bound to the biomass.

RESULTS AND DISCUSSION

Figure 1 shows the pH profile for the binding of Ni(II), Cd(II), Pb(II), Zn(II), Cr(III), and Cr(VI) to creosote bush roots. The y-axis represents the percent metal bound at each pH tested. It is apparent from this figure that the metal binding is a function of pH; as the pH increases, so does the binding. Maximum binding occurred at pHs 5 and 6. It can also be seen that lead binding, although reduced, still occurs at pH 2.0. Figure 2 represents the pH profile for the binding of Ni(II), Cd(II), Pb(II), Zn(II), Cr(III), and Cr(VI) to creosote bush stems. This pH profile is very similar to that seen for the roots, with the exception of nickel which has slightly less binding. Figure 3 shows the pH profile for the binding of Ni(II), Cd(II), Pb(II), Zn(II), Cr(III), and Cr(VI) to creosote bush leaves. It can be observed in this figure that Cr(III) has the highest binding followed by Pb(II). It is noted that the leaves have a slightly different pH profile than the stems and roots. This may be due to differences in the concentrations of metal binding compounds in the different locations within the plant. It has been reported that creosote leaves possess compounds such as nordihydroguaiaretic acid, guaiaretic acid, norisoguaiacin, 3'-demethoxyisoguaiacin, dihydroguaiaretic acid, and partially demethylated dihydroguaiaretic acid (Mabry et al., 1977). These compounds possess hydroxyl groups which could be possible metal

binding sites. In addition, the overall trend seen in pH-dependent binding suggests that the metal binding could occur through an ion exchange type of mechanism. It is believed that carboxyl groups may be responsible for some of the metal binding since carboxyl groups have pKa values between 3 and 4 (Segel, 1976). This trend in pH suggests that by reducing the pH, the bound metal ions can be desorbed.

Table 1 shows the percentage of metal bound to the creosote bush stems after different times of exposure with the 0.3 mM metal solutions at pH 5.0. It can be seen that rapid metal binding occurs within 15 minutes of exposure and remains relatively constant thereafter (with the exception of chromium). This same trend was observed for the roots and leaves as seen in Tables 2 and 3, respectively. This rapid metal binding suggests that the metal ligands might be on the cell wall surface and the metal is not being internalized by diffusion across the cell walls of the creosote bush. The relatively steady-state binding indicates that binding is not a function of time after the initial 15-minute exposure and that binding remains stable thereafter. Further studies are being performed to give us more information about the binding mechanisms for these metals with creosote biomass.

Table 4 shows the amount of Cd(II), Ni(II), Pb(II), Zn(II), Cr(III), and Cr(VI) that was adsorbed from aqueous solution as the saturation point was reached. The binding capacities for the creosote roots, stems, and leaves are given in mg of metal adsorbed per gram of biomass. It is noted that these capacities are comparable to, if not greater than, most biologic adsorbents (Lujan, et al., 1994; Gardea-Torresdey et al., 1996a-c), especially for chromium(III) and lead(II) which were as high as 39.6 mg/g and 36.4 mg/g, respectively. It can also be observed from Table 4 that in most of the experiments, the leaves had the highest capacity for metal binding. The three different biomass fractions bound very low quantities of chromium(VI). Since both the roots and stems are composed of woody material necessary for support of the plant, the leaves may have different types of metal-binding compounds compared to the stems and roots (as explained above). The major polymeric components of woody plant materials (such as those in roots and stems) are cellulose and hemicellulose. Hemicellulose contains free carboxyl groups that coordinate metal ions. Also, both compounds contain hydroxyl groups that may bind metal ions but to a lesser extent than carboxyl groups (Borman, 1990). On the other hand, the leaves

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may contain higher protein levels that will supply sulfhydryl, amino, and carboxyl groups. These groups may be responsible for the difference in metal binding by the creosote leaves

The pH profile experiments for the binding of Ni(II), Cd(II), Pb(II), Zn(II), Cr(III), and Cr(VI) to creosote bush biomass demonstrated that the adsorption of the metal ions was reduced at lower pHs. This would suggest that the bound metals could be removed by lowering the pH of the solution. It is presumed that protons would then displace the adsorbed metal ions. Table 5 shows the percentage of metal that was recovered from the capacity experiments by reacting the biomass pellets with 0.1 M HCl. By using a low-strength acid, the biomass might not be destroyed and could be reused again. As can be seen from Table 5, greater than 80 percent of the bound metal was recovered for all metals with the exception of Cr(III) and Cr(VI). Again the leaves were surprisingly different from the stems and roots. The leaves had a recovery of approximately 90 percent or better in most cases. We are currently investigating other complexing agents to remove Cr(III). Variations in metal recoveries may also depend on the type of metal binding ligands involved, as well as their available concentration. This is under investigation.

CONCLUSIONS

The binding of Ni(II), Cd(II), Pb(II), Zn(II), Cr(III), and Cr(VI) to *Larrea tridentata* roots, stems, and leaves has been shown to be dependent upon pH, with best binding occurring between pH 5 and 6. This effect in pH suggests that the binding mechanism may be an ion-exchange type process. Also, the binding mechanism for these metals is a stable, rapid process which implies that the binding is taking place on the cell wall surface of the creosote bush. Capacity and recovery experiments have demonstrated that *Larrea tridentata* possessed the ability to bind appreciable amounts of Ni(II), Cd(II), Pb(II), Zn(II), and Cr(III) as compared to other biosorbents. This ability to remove and recover heavy metals from solution indicates the tremendous potential that the creosote bush could have for cleansing the environment and industrial waste effluents from toxic metal ions.

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Table 1. Time dependency batch experiments for the binding of metal ions by creosote bush stems.

Time Interval in Minutes	**% Metal Bound**											
	Cd^{2+}		Cr^{3+}		Cr^{6+}		Pb^{2+}		Ni^{2+}		Zn^{2+}	
5	71.7	±4.5	36.2	±6.6	9.0	±18.0	75.5	±5.5	71.3	±11.9	76.1	±2.9
10	71.8	±2.1	37.2	±1.2	10.6	±1.6	77.0	±8.8	72.1	±3.20	77.0	±4.2
15	71.4	±4.3	39.2	±6.6	11.5	±16.2	76.7	±12.6	73.1	±1.53	73.9	±9.2
30	73.2	±1.7	40.6	±1.3	12.4	±24.3	79.8	±3.2	71.3	±1.88	76.5	±2.0
60	72.8	±3.9	45.4	±4.1	14.2	±13.4	80.8	±2.1	70.7	±1.06	72.6	±19.7
90	74.8	±1.2	47.5	±4.8	10.6	±9.86	79.7	±1.4	70.7	±3.55	71.2	±16.4
120	73.7	±4.5	49.9	±9.6	15.2	±19.6	76.0	±10.3	70.1	±1.05	74.1	±4.3

NOTE: 95% confidence interval was used to determine error. Biomass was shaken for appropriate time with 0.3 mM solution of each metal ion, independently.

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Table 2. Time dependency batch experiments for the binding of metal ions by creosote bush roots.

Time Interval in Minutes	**% Metal Bound**											
	Cd^{2+}		Cr^{3+}		Cr^{6+}		Pb^{2+}		Ni^{2+}		Zn^{2+}	
5	68.6	±2.6	39.2	±6.2	0.0	±0.0	87.2	±0.8	76.4	±5.9	76.6	±5.2
10	74.0	±1.7	43.0	±5.5	2.7	±7.9	88.3	±6.8	77.7	±1.2	79.8	±5.5
15	78.7	±9.3	46.3	±3.5	3.2	±13.6	90.0	±1.1	80.5	±2.8	74.1	±16.7
30	76.4	±1.0	49.1	±10.6	3.3	±9.4	90.6	±2.4	81.1	±2.0	82.0	±5.4
60	78.1	±3.3	54.4	±3.2	4.8	±7.2	91.2	±1.1	80.3	±3.2	80.5	±5.8
90	80.2	±1.8	56.8	±4.7	7.0	±5.2	91.2	±1.1	77.7	±3.5	83.1	±6.8
120	77.8	±6.6	57.2	±0.7	10.4	±3.8	91.1	±4.1	76.8	±4.0	81.1	±3.3

NOTE: 95% confidence interval was used to determine error. Biomass was shaken for appropriate time with 0.3 mM solution of each metal ion, independently.

Table 3. Time dependency batch experiments for the binding of metal ions by creosote bush leaves.

Time Interval in Minutes	**% Metal Bound**											
	Cd^{2+}		Cr^{3+}		Cr^{6+}		Pb^{2+}		Ni^{2+}		Zn^{2+}	
5	65.8	±5.3	33.9	±20.4	3.9	±9.2	58.8	±13.5	68.6	±7.5	76.6	±5.2
10	68.3	±2.2	34.7	±9.2	2.7	±6.9	52.4	±22.0	63.7	±2.4	79.8	±5.5
15	68.0	±3.9	36.2	±4.9	5.0	±3.8	56.5	±12.0	64.5	±3.5	74.1	±16.7
30	67.8	±0.3	39.2	±7.1	4.3	±5.3	56.6	±13.1	64.5	±4.9	82.0	±5.4
60	67.7	±2.4	43.6	±7.0	10.0	±9.5	61.2	±9.0	62.5	±2.4	80.5	±5.8
90	67.3	±0.7	47.5	±5.2	11.0	±10.2	36.1	±21.3	61.3	±3.7	83.1	±6.9
120	66.9	±4.1	48.3	±4.6	16.3	±6.4	61.4	±5. 8	59.0	±8.2	81.1	±3.3

NOTE: 95% confidence interval was used to determine error. Biomass was shaken for appropriate time with 0.3 mM solution of each metal ion, independently.

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Table 4. Adsorption capacity for metal uptake by creosote

METAL ION		mg metal / g biomass	
CADMIUM (II)	Roots	10.10	±1.23
	Stems	8.39	±0.48
	Leaves	9.61	±1.76
CHROMIUM (III)	Roots	30.57	±7.24
	Stems	52.07	±6.74
	Leaves	39.60	±6.34
CHROMIUM (VI)	Roots	0.82	±0.55
	Stems	0.42	±0.70
	Leaves	0.71	±0.51
NICKEL (II)	Roots	5.60	±2.05
	Stems	4.60	±1.16
	Leaves	5.90	±2.32
LEAD (II)	Roots	27.00	±10.42
	Stems	24.06	±12.5
	Leaves	36.40	±13.45
ZINC (II)	Roots	6.24	±2.78
	Stems	5.05	±1.46
	Leaves	6.20	±1.0

NOTE: 95% confidence interval was used to determine error.

Table 5. Desorption of bound metals with 0.1M

METAL ION		% Metal Recovered
CADMIUM (II)	Roots	95.2
	Stems	94.5
	Leaves	118.9
CHROMIUM (III)	Roots	32.5
	Stems	16.3
	Leaves	18.2
CHROMIUM (VI)	Roots	26.5
	Stems	127.8
	Leaves	56.7
NICKEL (II)	Roots	99.3
	Stems	92.8
	Leaves	88.9
LEAD (II)	Roots	97.0
	Stems	81.3
	Leaves	104.8
ZINC (II)	Roots	125.3
	Stems	135.5
	Leaves	126.0

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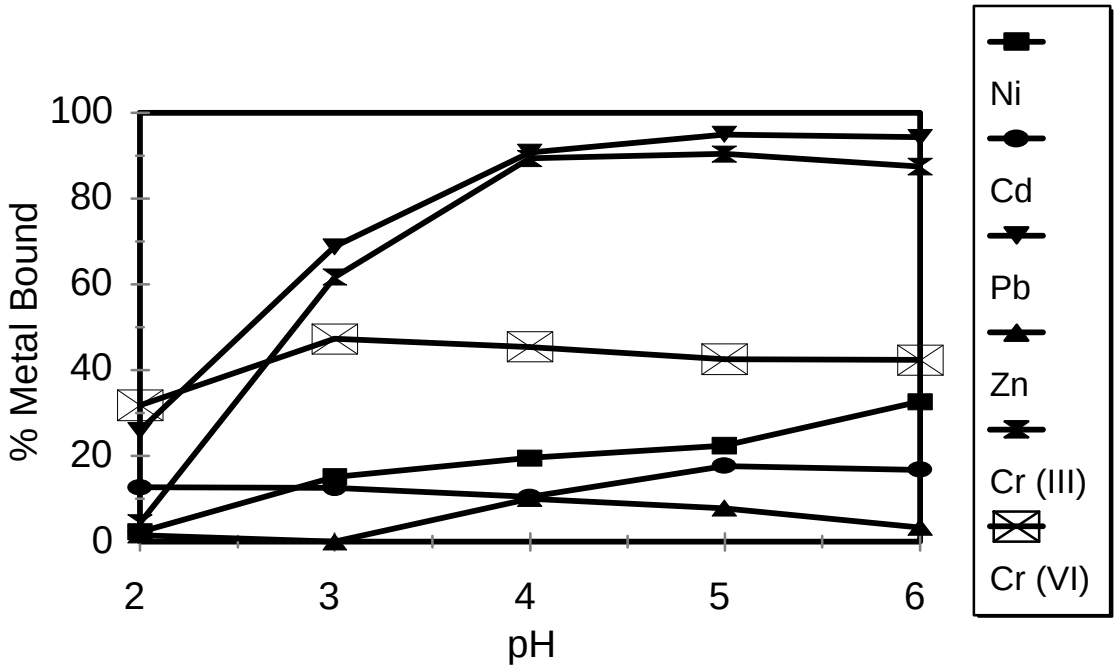

Figure 1: Effect of pH on the binding of metal ions by creosote bush roots. Biomass (5 mg/mL) was shaken for 1 hour at the appropriate pH with 0.1 mM of each metal ion, independently.

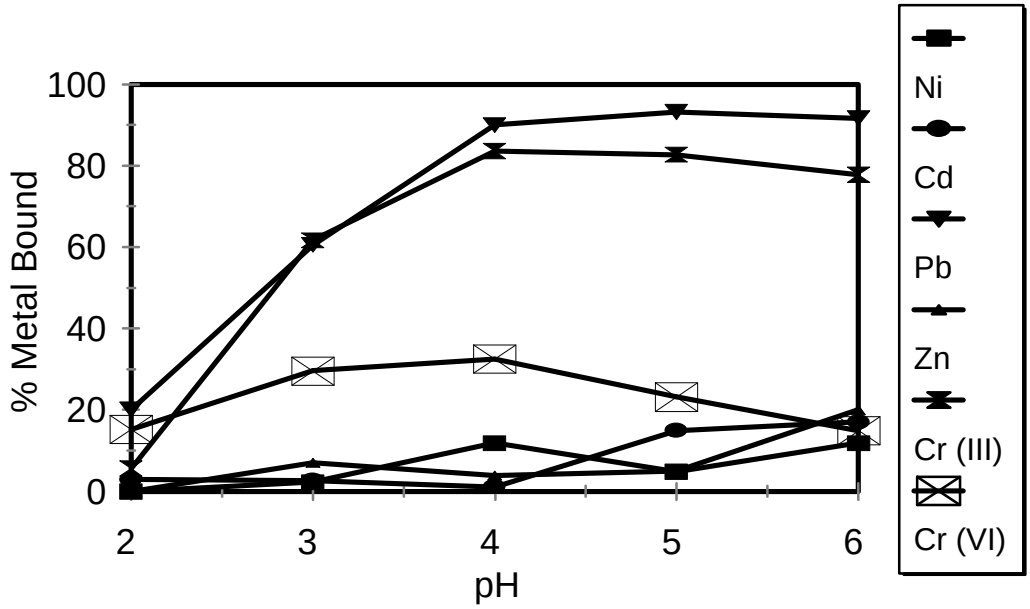

Figure 2: Effect of pH on the binding of metal ions by creosote bush stems. Biomass (5 mg/mL) was shaken for 1 hour at the appropriate pH with 0.1 mM of each metal ion, independently.

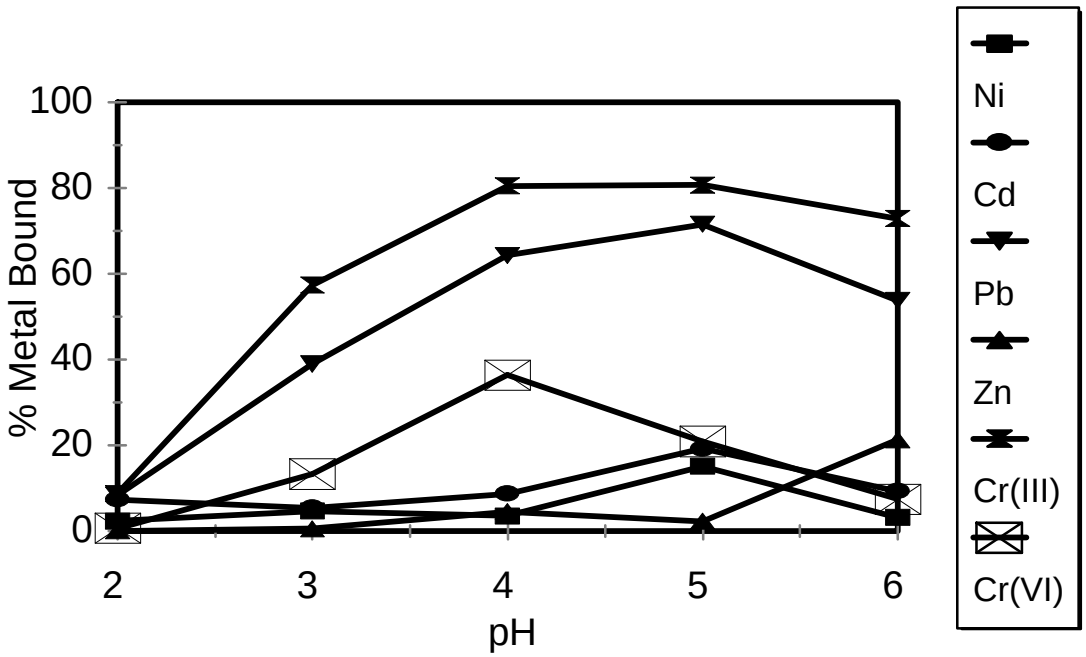

Figure 3: Effect of pH on the binding of metal ions by creosote bush leaves. Biomass (5 mg/mL) was shaken for 1 hour at the appropriate pH with 0.1 mM of each metal ion, independently.

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