BIOSORPTION OF CADMIUM, CHROMIUM, LEAD, AND ZINC BY BIOMASS OF *MEDICAGO SATIVA* (ALFALFA)

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ABSTRACT Previous laboratory batch experiments of *Medicago sativa* (alfalfa) indicated that the African shoots population had an excellent ability to bind copper(II) and nickel(II) ions from aqueous solution. Batch laboratory pH profile, time dependency, and capacity experiments were performed to determine the binding ability of the African shoots to cadmium(II), chromium(III), chromium(VI), lead(II), and zinc(II). Batch pH profile experiments for the mentioned ions indicated that the optimum pH for metal binding is approximately 5.0. Time dependency experiments for the metal ions showed that for all the metals studied, binding to the African alfalfa shoots occurred within five minutes. Binding capacity experiments revealed the following amounts of metal ions bound per gram of biomass: 7.1 mg Cd, 7.7 mg Cr(III), 43 mg Pb(II), and 4.9 mg Zn(II). However, no binding occurred for chromium(VI). Nearly all of the metals studied were recovered by treatment with 0.1M HCl, with the exception of chromium(III). Column experiments were performed to study the binding of Cd(II), Cr(III), Cr(VI), Pb(II), and Zn(II) to silica-immobilized African alfalfa shoots under flow conditions. These experiments showed that the silica-immobilized African alfalfa shoots were effective for removing metal ions from solution, and over 90% of the bound Pb(II), Cu(II), Ni(II), and Zn(II), and over 70% Cd(II), were recovered after treatment with four bed volumes of 0.1M HCl. The results from these studies will be useful for a novel phytofiltration technology to remove and recover heavy metal ions from aqueous solution.

KEYWORDS: phytofiltration, alfalfa, *Medicago sativa*, heavy metal binding

INTRODUCTION

The accumulation of heavy metal contaminants in the environment has become a concern due to growing health risks to the public. These contaminants, such as cadmium, chromium, lead, and zinc, enter the environment through industrial waste, mill tailings, and landfill run off. Exposure to heavy metal contamination has been found to cause kidney damage, liver damage, and anemia in low doses, and in high concentrations, heavy metals can be carcinogenic and teratogenic if not fatal [1]. The Environmental Protection Agency has regulated industrial waste disposal; however, when industrial waste discharges exceed the regulated disposal amounts, many industries respond by diluting the hazardous materials. Once the diluted hazardous substances are released into the environment, they naturally concentrate in wetlands and soils. The natural process of transportation of metal ions between the soil and water consolidate heavy metal contamination in high concentrations that affect the natural ecosystems [2]. Because of the increasing environmental concern regarding heavy metal contamination, there has been an abundance of interest in the removal of heavy metal ions from contaminated soils and waste streams [3-8]. Although cleanup is necessary to prevent any further discharge of contaminated wastes into the environment, a technology needs to be developed that is cost effective for industry to use. Methods traditionally employed for water remediation consist of removal of

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heavy metals by filtration, flocculation, activated charcoal, and ion exchange resins. However, because of the high cost of these methods, development of a more cost-effective remediation system is necessary.

There has been a tremendous amount of attention given to the use of biological systems for removal of heavy metal ions from contaminated areas [9-14]. More recently, phytoremediation has emerged as one of the alternative technologies for removing pollutants from the environment. Interest in using plants for environmental remediation is increasing due to their natural capacity to accumulate heavy metals and degrade organic compounds [15-17]. Since chemical functional groups are most likely responsible for metal binding, it is likely that higher plant cells might also be capable of metal binding. Various research groups have conducted studies on the metal binding properties of plant tissues [18-22]. Investigations performed by the genetic research group at Los Alamos National Laboratories have shown binding of cadmium(II) by cells of *Datura innoxia* [23]. Plant species that have been found growing on heavy metal-contaminated soils have become tolerant to the toxic effects of heavy metals [24, 25]. Heavy metal tolerance of the plants may be due to the evolution of chemical functional groups that reduce the toxic effects of the heavy metals. Therefore plants may be a good source for naturally occurring biological compounds that may have the potential for heavy metal contamination removal from waste water.

Alfalfa (*Medicago sativa*) has been found growing in soils being irrigated by heavy metal-contaminated waters [24, 25]. It has been reported that alfalfa has the ability to accumulate concentrations of heavy metals well above the tolerance levels of other plants [26-30]. This ability may be due to specialized chemical functional groups that could be responsible for metal tolerance and accumulation [31]. Because alfalfa possesses these chemical characteristics and is inexpensive and easily obtained, it may be a potential biosource for the extraction and recovery of heavy metals from contaminated waters. The metal ions that were used for this study were chosen because of their threat to the public health and environment. Each of the metal ions studied are found in high concentrations at EPA Superfund sites in Texas. Since it is the goal of the project to remove metal contaminants from water, it was essential to choose metals that are actually found at contaminated sites. For these reasons we chose to study cadmium(II), chromium(III), chromium(VI), lead(II), and zinc(II), due to their toxic characteristics and actual concentrations in the environment.

The objective of this study was to investigate the binding of cadmium(II), chromium(III), chromium(VI), lead(II), and zinc(II) to African alfalfa shoots. Batch laboratory pH profile, time dependency, and capacity experiments were performed to determine the binding ability of the African shoots to the above-mentioned metals. In addition, column experiments were performed with silica-immobilized African alfalfa shoots to determine the extraction and recovery ability of cadmium(II), chromium(III), chromium(VI), lead(II), and zinc(II) under flow conditions.

METHODOLOGY

Alfalfa collection

Alfalfa plants were collected from field studies conducted by Dr. John Henning at New Mexico State University near Las Cruces, New Mexico. Plants were removed from the soil, washed, and the roots were separated from the shoot material (stems and

leaves). All samples were oven dried at 90ºC for one week. Dried samples were ground to pass through a 100-mesh screen using a Wiley mill.

pH profile studies for metal binding

This experiment was carried out using the pH profile method previously reported by Gardea-Torresdey, *et al.* [32]. A 250 mg sample of biomass was washed twice with 0.01 M hydrochloric acid (HCl) to remove any debris or soluble biomolecules that might interact with metal ions. Each biomass sample was resuspended in 50 ml of 0.01 M HCl with tissue concentration of approximately 5 mg per ml solution. A metal solution of 0.1 mM was prepared for the following metal ions: cadmium(II), chromium(III), chromium(VI), lead(II), and zinc(II). The pH was adjusted to 2.0, 3.0, 4.0, 5.0, and 6.0. At each pH, 2 ml of the metal solution were added to the respective pH biomass pellet and to the separated supernatant solutions. In addition, at each respective pH, 2 ml of the 0.1 mM metal solution were transferred to three tubes for controls. All the tubes were equilibrated on a rocker for one hour. The samples were then centrifuged at 3,000 rpm for five minutes and the supernatants for the pellets were transferred to clean tubes. Final pHs for all tubes were recorded, and analyses for metal ions were performed by flame atomic absorption spectroscopy.

Time dependence studies for metal binding

The time dependence batch experiments were performed using a procedure reported previously [32]. The procedure was followed to yield a concentration of biomass of approximately 5 mg per ml of solution. The solution was then adjusted to pH 5.0 and allowed to equilibrate. The time intervals chosen for the time dependence studies were 5, 10, 15, 20, 25, 30, 45, and 60 minutes. After centrifugation and decantation, 2 ml of 0.3 mM metal solution were added to each of the tubes and controls. This procedure was repeated for each different metal ion 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), and Zn(II) to the African shoots were performed as reported previously for the binding of copper and nickel to different species of *Medicago sativa* [32]. For these experiments, 100 mg of biomass were washed twice with 0.01 M HCl, and washings were collected and weighed to determine biomass loss. Washed biomass was resuspended in 20 ml of deionized water and the pH adjusted to 5.0. This was repeated for each one of the metal ions being studied, using 0.3 mM concentration of each metal ion at pH 5.0. Final pHs for all tubes were recorded. Samples were diluted as required to remain within the calibration linear range, and metal concentrations were determined by flame atomic absorption.

Desorption of the adsorbed metal ions

In order to remove the bound metal ions from the alfalfa biomass, pellets from binding capacity studies with the adsorbed metal were exposed to 2 ml of 0.1 M HCl, equilibrated by rocking for five minutes, and then centrifuged as indicated by Gardea-Torresdey, *et al.* [32]. Supernatants were collected for analysis and diluted as required to stay within the calibration range. Pellets were then exposed to 2 ml of 1 M HCl to remove remaining metal and equilibrated by

rocking for five minutes. After centrifugation, the supernatants were analyzed. All metal analyses were performed by flame atomic absorption spectroscopy.

Immobilized alfalfa biomass and column experiments

The immobilization of the African alfalfa biomass was performed as indicated previously by Gardea-Torresdey, *et al.* [34-35]. Samples of 5 g were washed twice with water, and the cell debris was removed by centrifugation. The following part of this experiment is similar to that reported before for the binding of copper and nickel to different species of *Medicago sativa* [34, 35]. The experiments also were performed for cadmium(II), chromium(III), chromium(VI), lead(II), and zinc(II). One bed volume of solution that is passed through the column is equivalent to the volume of immobilized biomass within the column. In this case the volume of immobilized biomass used was 6 ml; therefore one bed volume is equal to 6 ml. The metal solutions were passed at a flow rate of 2 ml per minute.

Recovery of metal ions from columns

To remove the bound metal from the immobilized African alfalfa shoots, 10 bed volumes of 0.1M HCl was passed through the column at a flow rate of 2 ml per minute. Each effluent bed volume was collected and analyzed by flame atomic absorption spectroscopy. The amount of metal found in each bed volume of effluent was added up, and the total was taken to be the total amount of metal recovered from the column.

Metal analyses

The metal content in all the experiments was performed 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 value and the relative standard deviation were computed. The following wavelengths were used for the metals studied: cadmium—228.8 nm; chromium—358.2 nm; lead—283.3 nm; and zinc—213.9 nm. Impact bead was utilized to improve the sensitivity, but in the case of zinc a flow spoiler was used. Confidence intervals of 95% were calculated for each set of samples to determine the error margin. 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 binding of cadmium(II), lead(II), zinc(II), chromium(III), and chromium (VI) to African alfalfa shoots as a function of pH. Binding of every metal by the alfalfa biomass was unique. It can be observed that as the pH was increased, the amount of metal bound also increased, with most of the binding of zinc(II), lead(II), chromium(III), and cadmium(II) occurring between pH 5 and 6. It can also be observed from Figure 1 that lead binding even occurred at pH 2. Chromium(III) and chromium(VI) were studied because of their oxidation states, existence in contaminated waters, and differences in chemical properties. Chromium(VI) had no binding to the biomass because chromium(VI) exists in aqueous solution as oxo-anion (CrO_4^{-2}) with an overall charge of -2. If there is an electrostatic interaction between the metal ion and the alfalfa biomass, a negatively-charged ion will not bind to a negatively-charged ligand. This could be the case if the

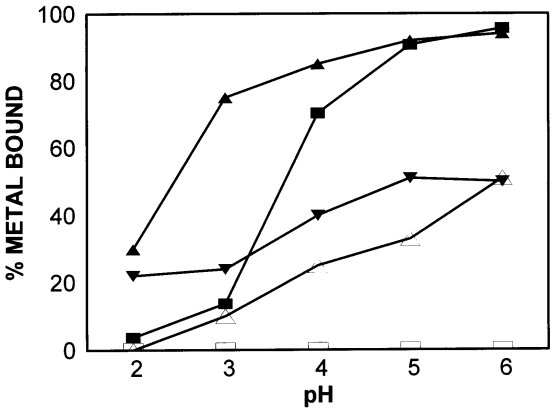

FIGURE 1. EFFECT OF pH ON THE BINDING OF CADMIUM(II) ■, CHROMIUM (III) ▼, CHROMIUM (VI) □, LEAD (II) ▲, AND ZINC (II) △ BY AFRICAN ALFALFA SHOOTS. BIOMASS (5 mg/ml) WAS SHAKEN FOR ONE HOUR AT THE APPROPRIATE pH WITH 0.1 mM OF EACH OF METAL ION, INDEPENDENTLY.

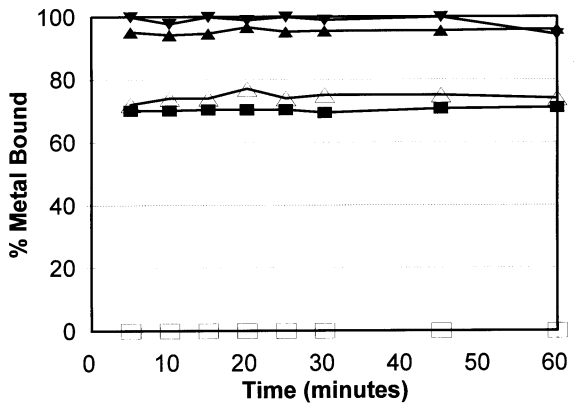

FIGURE 2. TIME DEPENDENCY BATCH EXPERIMENTS FOR THE BINDING OF CADMIUM(II) ■, CHROMIUM (III) ▼, CHROMIUM (VI) □, LEAD (II) ▲, AND ZINC (II) △ BY AFRICAN ALFALFA SHOOTS. BIOMASS WAS SHAKEN FOR APPROPRIATE TIME WITH 0.3 mM OF EACH OF METAL ION, INDEPENDENTLY.

carboxylate groups are the binding sites on the alfalfa biomass, as has been shown with other biomasses [36]. Therefore, the binding sites on the African shoot biomass responsible for this heavy metal uptake may only bind positively-charged ions. This trend in pH suggests that these metal ions bind by a similar ion exchange mechanism as observed with copper and nickel [32-35]. The pH-dependent trend also may suggest that those metal ions that can be bound by African shoots may also be recovered by reducing the pH.

Figure 2 demonstrates that the heavy metal uptake by the African shoots is very rapid for all the metals studied, with the exception of Cr(VI), for which no binding occurred. Binding occurred within five minutes and was relatively stable thereafter. This trend was observed for all the metals under investigation. Due to the extensive prior washings, soluble compounds are eliminated as a possible means for heavy metal adsorption. The rapid uptake of Cd(II), Cr(III), Pb(II), and Zn(II) from solution suggests that the binding sites are cell wall components and that the metal ions are not diffusing through the cell wall. Since the tissues of the alfalfa plants studied were inactivated, we expected rapid binding of Cd(II), Cr(III), Pb(II), and Zn(II) by the plant cell wall functional groups. Further studies using adsorption isotherms will be performed to give us more information on binding that is occurring, and to determine the binding constants as well as the binding mechanism of the metal ions.

Table 1 exhibits the amount of Cd(II), Cr(III), Cr(VI), Pb(II), and Zn(II) that was adsorbed from solution as the saturation point was reached. These studies were performed at pH 5.0. The binding capacities of the different populations are given in mg of metal adsorbed per gram of biomass. The range of adsorption for the metals studied was from 4.9 mg/g for zinc(II) up to 43 mg/g for lead(II). The binding capacities obtained in these studies are comparable to those seen previously in other biomasses [20, 32-35].

pH profile experiments for heavy metal binding by the African shoots demonstrated that heavy metal uptake was low at low pHs. This suggested that we could remove the adsorbed copper by lowering the pH. Presumably protons would then displace the adsorbed heavy metal ions. Table 2 shows the various percentages of metal recovered after reacting the alfalfa with 0.1 M HCl. As can be seen in the table for Cd(II) and Pb(II), more than 99% was recovered. In addition, almost 70% of the zinc was recovered from the biomass. However, the recovery for Cr(III) was only 13.9%. The reason for this low recovery is still not understood. By using acid at this low strength the biomaterial is not destroyed and it can be reused. The reversibility of the heavy metal binding could have very important implications for the removal of metal ions from waste waters, since the metal ions can be adsorbed and also reclaimed. This may represent an innovative method for the removal and recovery of heavy metal ions from water by alfalfa biomass.

Our previous batch capacity experiments showed that African alfalfa shoots bind some heavy metal ions well. However, it was necessary to conduct experiments with immobilized African shoots under flow conditions to determine if the immobilized alfalfa biomass could remove other metal ions from solution in a more practical way. These experiments were conducted in triplicate to maintain quality control. As seen in Figure 3, the immobilized African shoots showed to be efficient in removing these metal ions from solution. The same molarity of metal solution was used for each of the metal ions studied (0.3 mM). Cadmium(II), lead(II), and zinc(II) showed to have binding capacities over 500 ppm. After 120 bed volumes of metal solutions had been passed through the column containing the immobilized alfalfa biomass, lead(II) showed to have the highest capacity of all the metal ions under flow conditions. The second closest was cadmium, followed by zinc(II) and chromium(III). After 120 bed volumes of metal ion solution had been passed, only chromium(III) was near saturation; the rest of the columns still had the ability to bind more metal ions. Chromium(VI) was not bound by the immobilized African shoots. We also used a control column, which contained only the polysilicate that was used to entrap the biomass. The silica column did not bind any of the metal ions (data not shown).

TABLE 1. METAL ION BINDING CAPACITIES OF AFRICAN ALFALFA SHOOTS (BATCH EXPERIMENTS).

Metal Ion	Capacity (mg/g)
Cadmium(II)	7.1
Chromium(III)	7.7
Chromium(VI)	0.0
Lead(II)	43.0
Zinc(II)	4.9

TABLE 2. PERCENT RECOVERY OF METAL IONS BOUND TO AFRICAN ALFALFA SHOOTS USING 0.1 M HCl (BATCH EXPERIMENTS).

Metal Ion	% Recovery (mg/g)
Cadmium(II)	100.0
Chromium(III)	13.9
Chromium(VI)	0.0
Lead(II)	99.6
Zinc(II)	69.3

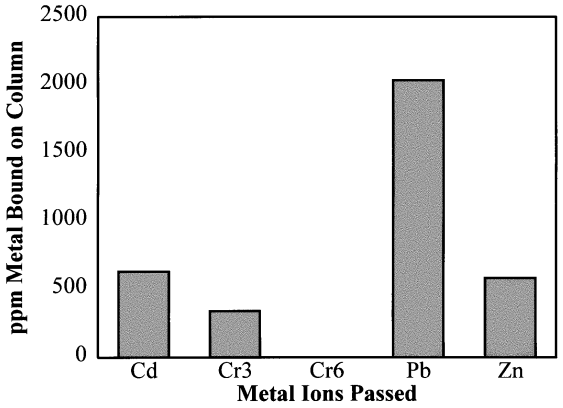

FIGURE 3. METAL IONS BOUND BY IMMOBILIZED AFRICAN ALFALFA BIOMASS IN A COLUMN UNDER FLOW CONDITIONS. AFTER 120 BED VOLUMES OF 0.1 mM METAL SOLUTION WERE PASSED, THE EFFLUENTS WERE ANALYZED FOR METAL CONCENTRATIONS BY FLAME ATOMIC ABSORPTION. THE X-AXIS REPRESENTS THE PARTICULAR METAL ION, AND THE Y-AXIS REPRESENTS THE ppm OF METAL ION BOUND AFTER 120 BED VOLUMES WERE PASSED.

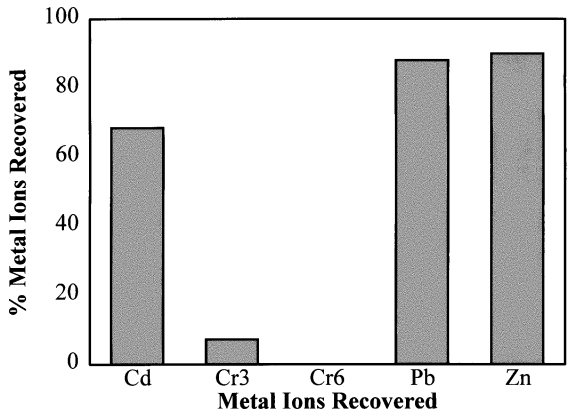

FIGURE 4. PERCENT METAL ION RECOVERED FROM THE COLUMN CONTAINING ALFALFA BIOMASS WITH METAL BOUND. TEN BED VOLUMES OF 0.1 M HCl WERE PASSED THROUGH THE COLUMN CONTAINING THE PARTICULAR BOUND METAL. EFFLUENTS WERE ANALYZED FOR METAL CONTENT BY FLAME ATOMIC ABSORPTION. THE X-AXIS REPRESENTS THE PARTICULAR METAL ION, AND THE Y-AXIS REPRESENTS THE PERCENT OF METAL ION DESORBED.

As indicated by the pH profiles, by using low strength acid, the bound metal ions can be recovered from the immobilized African shoots. Figure 4 shows the percentage of bound metal ions that were recovered after passing 10 bed volumes of 0.1 M HCl through the column. It can be seen that almost 90% of the lead(II) and zinc(II) was removed from the columns. For cadmium(II), recovery was almost 70%, and hardly any of the chromium(III) was recovered. Upon repeating these experiments three times, little change was observed for the recovery percentages. The +2 charge ions showed to have good recoveries which may be due to the metal displacement by protons. Chromium(III) may have been more tightly bound to its binding site, and it may be necessary to use a stronger acid concentration or other stripping agents to remove the metal ion. No chromium(VI) bound to the column; therefore, none was recovered. Since most of the metals that bound to the columns were recoverable, this could be an effective way to reclaim these metal ions from waste waters; however, other stripping agents need to be studied.

CONCLUSIONS

It was determined that the binding of Cd(II), Cr(III), Pb(II), and Zn(II) to African alfalfa shoots is pH dependent, with most of the binding occurring around pH 5.0. Furthermore, experiments showed that most binding occurred within less than five minutes. In addition, metal ion binding remained constant throughout longer periods of time, without any desorption occurring. The fast binding of the heavy metals studied suggests that most binding occurs on the cell

walls of the African shoots. The lack of Cr(VI) binding leads us to believe that the adsorption occurs through an ion-exchange mechanism. Capacity and recovery batch experiments showed that African alfalfa shoots have a remarkable ability to take up heavy metal ions. The recovery of the heavy metal ions is very feasible since over 99% of lead(II) and cadmium (II) was removed from the alfalfa biomass in the batch experiments. It was also shown that the adsorption and recovery of heavy metal ions from immobilized alfalfa shoots under flow conditions was as successful as in the batch experiments. The fact that the alfalfa shoots have the same efficiency under flow conditions suggests that they can be used in a more practical and economical method. Furthermore, the column experiments showed that the columns are reusable. After they have been stripped of the heavy metal adsorbed, the columns containing the immobilized alfalfa biomass can be recycled.

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