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Phytofiltration of hazardous metal ions by alfalfa: a study of calcium and magnesium interferences

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Abstract

Previous batch laboratory experiments performed to determine the potential ability of seven different varieties of Medicago sativa (Alfalfa, African variety) revealed that the shoots tissue-derived population was able to efficiently bind copper(II) and nickel(II) from aqueous solutions. Batch laboratory interference studies were performed with various calcium and magnesium concentrations (0.1 mM–1 M) in order to ascertain the effects of these ions on the heavy metal binding ability of African alfalfa shoots. Results from these studies have shown that calcium and magnesium did not seriously reduce the binding of copper(II), lead(II), to African alfalfa shoot tissues. However, high concentrations of calcium and magnesium, to some extent, reduced chromium(III), cadmium(II), nickel(II), and zinc(II) binding to African shoot tissues. In addition, all these experiments were repeated maintaining constant ionic strength, and similar results were obtained. Interference studies were also conducted in order to determine the effects of hard cations under flow conditions with silica immobilized African alfalfa shoots. Column experiments under flow conditions produced similar results than batch laboratory experiments. The information obtained from these studies will be useful for an innovative method to remove and recover heavy metal ions from contaminated waters. © 1997 Elsevier Science B.V.

Keywords: Phytofiltration; Alfalfa; Medicago sativa; Interference; Heavy metal binding; Recovery

1. Introduction

Due to the increase in industrial activity, an alarming amount of toxic heavy metals have been released into the environment, endangering natural ecosystems and public

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human health [1–3]. Industries such as smelters, metal refineries, and mining operations have been indicated as major sources of metal release into the environment [4–8]. The United States has invested billions of dollars attempting to clean heavy metal polluted ground waters and soils. However, recent studies have shown that many of the treated polluted ground water sites have not been restored back to drinking water standards [9]. Current technologies that are employed are not only costly and inefficient, but they also increase the contaminant exposure to cleanup crews. The limitations of conventional ground water remediation have spurred many investigations into alternative methods which are more cost effective. One of these technologies includes bioremediation; the use of biological systems such as those employed to remediate petroleum that can change the offending organic contaminant into substrates such as CO_2 [10]. The problem existing with bioremediation and bioaccumulation of toxic metals is that although the metals' valence or oxidation state may be biologically converted, the metal is still present and poses an environmental threat. Many bioremediation techniques have been studied as a safer, more conventional technology, yet these processes are not universally understood nor accepted. This is in part due to the fact that these processes rely on microorganisms which precipitate or solubilize the metal ions [11–13].

An innovative technology that is gaining momentum in the environmental field is phytoremediation. Phytoremediation is the use of plants to remove toxic contaminants from the environment. J.L. Schnoor et al., have proven in several applications that hybrid poplar trees have the ability to phytoremediate contaminated soils at a low cost [14]. W.F. Mueller and coworkers also have shown the potential use of *Datura innoxia* and *Lycopersicon peruvianum* to biotransform TNT wastes [15]. Many studies such as those conducted by Ernst et al., have demonstrated the heavy metal resistance in plants [16]. Only recently has the value of heavy metal accumulating plants been realized in the process termed phytoextraction to remove the metals from the soils. Raskin et al., have proven that crops such as *Brassica junica* (L.) possess great potential for phytoextraction of various metals from soils [17,18]. In addition, many other investigators have shown metal uptake by cultivars such as lettuce, corn, and mushrooms [19–22]. Alfalfa (*Medicago sativa L.*) has also shown to be extremely resistant to high levels of contaminants as well as a bioaccumulator [23–25]. Only within the last few years has this technology been implemented for cleaning up heavy metal contaminated waters. Other investigators have proven that rhizofiltration is a practical way to remove toxic heavy metals from ground waters [26–29]. Although these are cost-effective alternatives to the more traditional methods, rhizofiltration takes time and poses a threat to the cleanup crews who must transport the metal accumulated plants. In addition, the plants have to be disposed of either by incineration or in landfills.

Since plants have shown to be accumulators of heavy metals, a more practical application would be 'phytofiltration': the use of plant material to accumulate toxic compounds in a filtration apparatus. Previous studies such as those conducted by Ramelow, Rayson, and Volesky as well as other investigators indicate that concentrating heavy metals with an immobilized biomass in a column form would be practical and cost effective [30–33]. Gardea-Torresdey et al., have shown alfalfa as a potential source for 'phytofiltration' [34–37]. Several batch laboratory experiments determined that alfalfa possesses the ability to bind copper and nickel ions from aqueous solutions

[35,36]. In addition, considerable amounts of the bound metal ions were recovered from the reusable immobilized biomaterial. This biorecovery process for toxic metals may be employed for a cheaper and more efficient strategy to recycle waste metals. At least one problem that arises with most metal filtration systems is the presence of hard cations such as magnesium and calcium in contaminated waters. These cations have been reported as high as 4,000 ppm in some of the EPA superfund contaminated sites in Texas. In addition, these hard cations usually saturate conventional ion-exchange type filtration systems.

The objective of our study is to determine the effects of various concentrations of calcium and magnesium on the ability of immobilized alfalfa to remove different metal ions from aqueous solutions. Several batch laboratory experiments were carried out with various concentrations of calcium and magnesium while keeping the studied metal ion concentration constant. Also, column experiments were performed under similar conditions to determine the effects of various concentrations of calcium and magnesium on metal ion binding under flow conditions.

2. Methodology

2.1. Alfalfa collection

Previous studies were performed with seven alfalfa populations which were selected as representatives from the many different varieties of alfalfa due to their individual characteristics. The populations studied include the alfalfa basic germplasms (African, Peruvian, Flemish, Ladak) and two cultivars (Malone, Moapa 69) which were obtained from plots that had received irrigation every two weeks during the growing season. In addition, one cultivar (Cal West 630) was examined from a dryland test, which received no irrigation. These studies have shown that some populations bind metal ions better than others, and the best selection was used for further studies [34]. The African germplasm had shown to bind both copper and nickel very well and was chosen as the biomass source for this study.

The alfalfa tissues were collected from field studies conducted by Dr. John Henning and Shawn Townsend at New Mexico State University near Las Cruces, New Mexico. The 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 90°C, then ground to pass through a 100-mesh screen using a Wiley mill.

2.2. Batch laboratory interference experiments for metal binding

These studies were conducted to determine the interference of calcium and magnesium ions with the binding of metal ions to African shoots. For each experiment, 300 mg of African shoot tissue materials were washed twice with 0.01 M HCl by vortexing to remove any debris or soluble biomolecules that might interact with the metal ions. This was then followed by centrifugation to obtain a pellet after each wash. The washings were collected, dried, and weighed to account for any biomass weight loss. The washed

biomass was resuspended into 60 mL of deionized water with the final tissue concentration approximately 5 mg per ml solution and the pH of the solution was adjusted to 5.0. Two ml of the African shoot suspension (10 mg in 2 ml) was transferred into each of three test tubes. Solutions were prepared to include 0.1 mM metal ion solution at pH 5.0 for the following calcium and magnesium concentrations (as nitrate salts): 0.0 mM, 0.1 mM, 0.2 mM, 1 mM, 2 mM, 10 mM, 20 mM, 0.1 M, 0.2 M, and 1 M. The metal solutions were prepared from $Cd(NO_3)_2$, $Pb(NO_3)_2$, $Ni(NO_3)_2$, $Cr(NO_3)_3$, $ZnCl_2$, $CuSO_4$ and $K_2Cr_2O_7$. Because these hard cations coexist in the environment, these studies were also carried out with both the calcium and magnesium concentrations combined as well as separate to determine their effects. From each solution, 2 ml of the various concentrations of hard cations and 0.1 mM metal solutions were added to the three respective tubes and reacted with the biomass. In addition, three controls containing the 0.1 mM metal of interest were retained at each of the various concentrations of calcium and magnesium. All the tubes were equilibrated on a rocker for 1 hour. The samples were then centrifuged at 3,000 rpm for five minutes and the supernatant solutions for the pellets were transferred to clean respective tubes. Final pHs for all tubes were recorded and all metal analyses were performed by flame atomic absorption.

2.3. Immobilization of alfalfa biomass

In order to prevent the clumping of the alfalfa cell material, it was necessary to use a support material composed of a polysilicate matrix. The ground African alfalfa shoots were immobilized following the method described by Gardea–Torresdey et al. [35]. The polymerized gel which supported the plant material was oven dried overnight at 60°C and then ground and sieved to pass between the 20–40 mesh size.

2.4. Column experiments

One bed volume which consisted of 6 ml of immobilized shoot tissues were packed into columns. The columns were conditioned by passing 10 bed volumes of 0.01 M sodium acetate buffer at pH 5.0. For each experiment, 120 bed volumes of 0.1 mM metal solution in 0.01 M sodium acetate at pH 5.0 was passed at a flow rate of 2 ml per minute for each of the various concentrations of calcium and magnesium. This experiment was repeated for 0.0 mM, 0.1 mM, 10 mM, and 0.1 M solution concentrations of calcium and magnesium. Each bed volume was collected and analyzed by flame atomic absorption.

2.5. Recovery of adsorbed metal ions from immobilized alfalfa

To remove the bound metal ions being studied, 10 bed volumes of 0.1 M HCl were passed through the columns of immobilized African alfalfa shoots at a flow rate of 2 ml per minute. Each bed volume passed was collected and analyzed for metal content by flame atomic absorption. Calculations were performed to determine the percent of metal recovery from the amount of metal bound to the columns for each experiment.

2.6. Analytical procedure

Analyses for the metal ions studied were performed using a Perkin Elmer model 3110 Atomic Absorption Spectrometer with deuterium background subtraction. The methods and conditions followed for each metal analysis were obtained from the Perkin Elmer model 3110 Atomic Absorption Spectrometer manual. Analytical wavelengths used for the various metals were as follows: 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 calibration of the instrument was performed within the range of analysis and a correlation coefficient for the calibration curve of 0.98 or greater was obtained. Periodically, the instrument response was checked throughout the analysis with known standards. Samples were read three times, and a mean value and relative standard deviation was computed. The difference between the initial control metal concentration and that observed in the effluent was assumed to be bound by the alfalfa biomass.

2.7. Electron microscopy

An Environmental Scanning Electron Microscope (ESEM) was used to gain insight into the biomass interaction with the polysilicate support matrix that was used. An Electroscan ESEM model 2020 was used to take micrographs of uncoated oven-dried silica-immobilized African alfalfa shoots in a water vapor atmosphere at three torr.

3. Results and discussion

Previous screening experiments performed to determined the copper and nickel binding characteristics of seven populations of Medicago sativa (alfalfa) indicated that the African germplasm was among the best candidates for further metal binding studies [34]. African alfalfa shoots have shown to bind cadmium(II), chromium(III), copper(II), nickel(II), lead(II), and zinc(II) in considerable levels. However, due to the presence of hard cations such as calcium and magnesium found in contaminated ground waters, batch laboratory interference studies were performed with African alfalfa shoot tissue materials to determine the effects of the hard cations on the binding of the above-mentioned metal ions.

Fig. 1 summarizes the data collected from the calcium interference study. As indicated on Fig. 1, the amounts of lead(II) and copper(II) bound to the alfalfa biomass remain moderately constant, until concentrations of calcium exceed 10 mM. A small reduction in the binding was observed thereafter, but appreciable levels of lead(II) and copper(II) were still removed from solution with calcium concentrations as high as 1 M. These concentrations of calcium which are nearly 40,000 ppm, are ten times the levels reported at some of the contaminated superfund sites. The binding of cadmium(II), nickel(II), and zinc(II) were affected by increased concentrations of calcium more drastically than lead(II) and copper(II). Overall, there was nearly a 30% reduction in the binding ability of the African alfalfa shoots when exposed to the highest concentration of calcium. The ability to bind heavy metal ions at concentrations of 0.1 mM while being immersed in 10,000 times greater concentration of calcium indicates that the African alfalfa shoots are selectively binding the heavy metals in solution. Therefore,

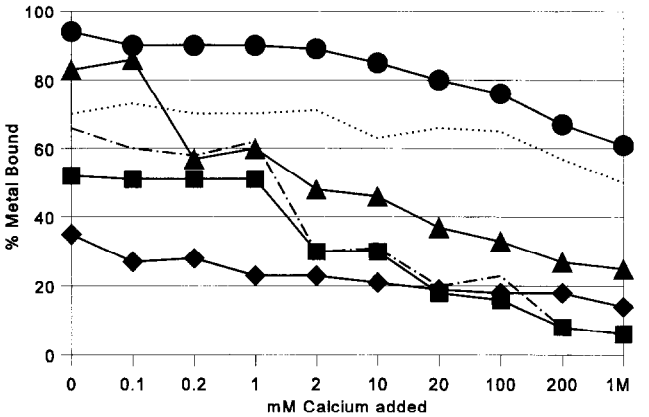

Fig. 1. Effects of Calcium on metal binding by African alfalfa Shoot Tissues. (Cadmium(II) ■, Chromium(III) ▲, Copper(II) · · ·, Nickel(II) – · –, Lead(II) ●, Zinc(II) ♦).

specific binding sites with chemical functional groups that have higher affinities for heavy metals may be responsible.

Fig. 2 represents the data observed from the magnesium interference studies. From Fig. 2, it can be seen that there is nearly no effect of magnesium interference on the African alfalfa shoots' ability to bind copper(II) and lead(II). However for cadmium(II), chromium(III), nickel(II), and zinc(II), a significant decline was observed. Overall, again there was nearly a 30% reduction in the binding ability of the African alfalfa shoots when exposed to the highest concentrations of magnesium.

Fig. 3 illustrates the combined effects of calcium and magnesium upon African alfalfa shoots binding ability. Only a slight decline was encountered for copper(II) binding, and a reduction in lead(II) binding was observed again after 10 mM of calcium

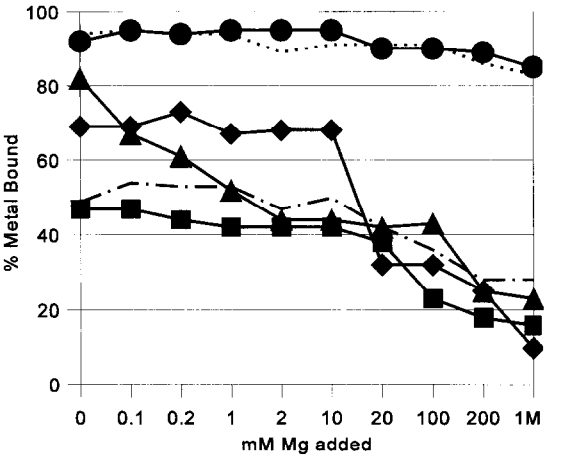

Fig. 2. Effects of Magnesium on metal binding by African alfalfa Shoot Tissues. (Cadmium(II) ■, Chromium(III) ▲, Copper(II) · · ·, Nickel(II) – · –, Lead(II) ●, Zinc(II) ♦).

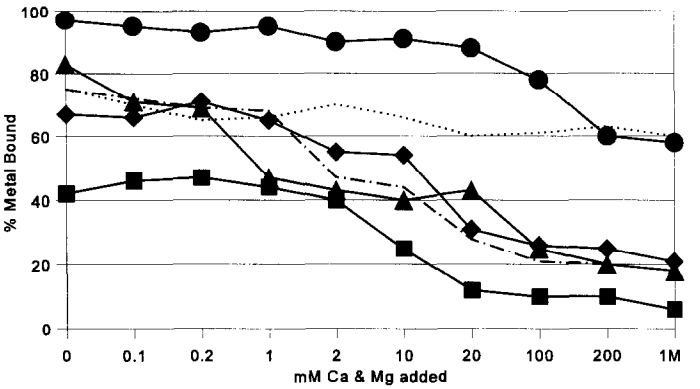

Fig. 3. Effects of Calcium and Magnesium on metal binding by African alfalfa Shoot Tissues. (Cadmium(II) ■, Chromium(III) ▲, Copper(II) · · ·, Nickel(II) – · –, Lead(II) ●, Zinc(II) ◆).

and magnesium were added. This reduction of lead(II) and copper(II) binding follows the same trend as that seen in Fig. 1. Since magnesium concentrations had little effect on copper(II) and lead(II) binding to the alfalfa biomass, this decrease should be due to the calcium concentrations alone.

As seen in Figs. 1 and 2, cadmium(III), chromium(II), nickel(II), and zinc(II) binding was reduced by the increasing concentrations of calcium and magnesium. An overall reduction of 40% of the heavy metal binding to the alfalfa biomass was observed. The combined effects of the calcium and magnesium at the highest concentrations only increased the overall interference by 10% rather than the 30% as one would expect. This overall difference may indicate that it is the quantity of hard cations in solution rather than the identity of the hard ions that plays a role in the reduction of heavy metal binding to alfalfa biomass.

Previous batch laboratory experiments indicated that metal ion binding to biomass functional groups may occur via an ion-exchange type mechanism [34]. Because the alfalfa biomass demonstrates selective binding, the interference observed may be due to the increased concentration of ions in solution, instead of competition for binding locations. These batch interference experiments were carried out again keeping the ionic strength constant (data not shown) and similar results were seen as those found in Figs. 1–3.

Selective binding of specific metal ions in an ion rich solution might be explained by the binding constants of various ligands that could be responsible for the heavy metal binding. The binding constants for functional groups such as carboxylates, sulfuhydryl groups, and amino groups have an overall higher binding affinity for the various metal ions studied than for calcium and magnesium [38]. Because the binding complexes of the heavy metals with the various functional groups have higher stability constants, it stands to reason that the metals would bind before the hard cations. This would explain the specificity of the alfalfa binding for metal ions such as copper(II) and lead(II).

In addition to performing batch laboratory interference studies, column experiments were conducted to determine whether various concentrations of calcium and magnesium

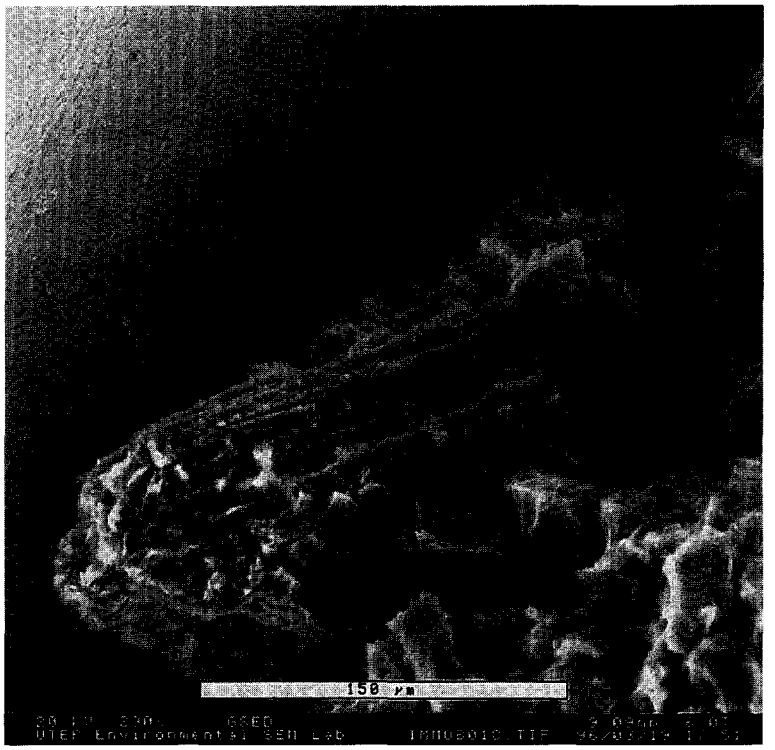

Fig. 4. Environmental Scanning Electron Micrograph of African alfalfa shoot biomass embedded inside a polysilicate matrix material used to support the biomass in columns.

would affect the heavy metal binding ability of African alfalfa shoots under flow conditions. In order to maintain optimal flow rates, a polysilicate support material was used to immobilize the alfalfa biomass. Fig. 4 shows an environmental scanning electron

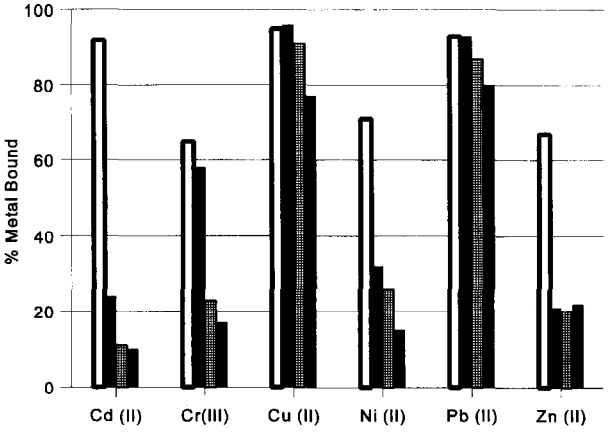

Fig 5.

Table 1
Desorption of bound metal with 0.1 M HCl

	% Metal recovered for the mM Ca & Mg concentrations added			
	0.0 mM	0.1 mM	10 mM	0.1 M
Cd(II)	84	85	82	70
Cr(III)	10	18	22	42
Cu(II)	90	95	100	92
Ni(II)	80	65	47	50
Pb(II)	90	100	96	93
Zn(II)	72	92	87	89

micrograph obtained of the silica immobilized African alfalfa shoots. Fig. 4 shows a piece of the alfalfa biomass surrounded by the silica polymer. The process of biomass immobilization is believed to be entrapment, and the exposed biomass as seen in Fig. 4, could be responsible for the metal binding activity. Control experiments were conducted with the silica matrix material alone and resulted in no metal binding.

Fig. 5 indicates the amount of metal ion that was bound to the column of immobilized alfalfa biomass after passing 120 bed volumes of 0.1 mM metal solution containing various levels of calcium and magnesium through the column. One column was used for each heavy metal ion studied. After passing 10 bed volumes of 0.1 M HCl to recover any of the bound metal ion, the same columns were conditioned to pH 5.0 and again run with the increased concentration of hard cations. As indicated in the batch experiments, small decreases in binding was observed for copper(II) and lead(II). More significant reductions in binding were observed for cadmium(II), chromium(III), nickel(II), and zinc(II). The same trend was observed for the binding of all the metals, as the hard cation concentration increased, the binding of heavy metals from solution slightly decreased. Nickel(II), cadmium(II), and zinc(II) showed a dramatic drop in binding upon hard cation exposure, but zinc(II) seemed to stabilize afterward. This may be explained by the biorecovery rates for all the metals studied as seen in Table 1. The first percent recovery rate for zinc was lower than the rest, suggesting that some of the bound zinc was not removed from the column by exposure to 0.1 M HCl, therefore hindering some of the binding. The concentration of calcium and magnesium may have also reduced the recovery of nickel(II) from the biomass. In addition, calcium and magnesium concentrations seemed to enhance the recovery of chromium(III) ions from the immobilized African alfalfa shoots. This occurrence may help in determining a better method for recovery of the strongly bound chromium(III) from the alfalfa biomass. Excellent recoveries were obtained for copper(II) and lead(II).

4. Conclusions

These studies have shown that alfalfa has the potential to work like a 'biological' mixed-bed-ion-exchange resin to bind heavy metals from aqueous solutions and recover some of these ions in a reusable form. Like ion-exchange resins, the silica immobilized

alfalfa can be recycled, but unlike ion-exchange resins, they can be made through inexpensive methods that will not contribute to environmental problems. In addition, the use of the silica immobilized alfalfa as a 'Phyto-filter' will allow for selective removal and recovery of various heavy metal ions, even under hard water conditions which would foul typical ion-exchange type systems. We have shown that high concentrations of hard cations do not seem to greatly reduce the binding of lead(II) and copper(II). Even with calcium and magnesium concentrations at 10,000 times that of the contaminant metals studied, the overall binding ability of silica immobilized African alfalfa shoots was only reduced by about 30%. This innovative technology has the potential to be used for the cleanup of contaminated waters through environmentally friendly methods.

Further studies are being performed in our laboratories to determine the chemical functional groups responsible for various metal binding by the alfalfa biomass. We would also like to conduct in-situ experiments with actual heavy metal contaminated waters.

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