INTERFERENCE STUDIES FOR MULTI-METAL BINDING BY *MEDICAGO SATIVA* (ALFALFA)

K.J. Tiemann, J.L. Gardea-Torresdey, G. Gamez, and K. Dokken

Department of Chemistry and Environmental Sciences and Engineering, University of Texas at El Paso, El Paso, Texas 79968; Phone: (915) 747-5359, Fax: (915) 747-5748

ABSTRACT

Previous studies have shown that alfalfa shoot biomass has the ability to bind a significant amount of various individual metals from aqueous solutions. Since most heavy metal-contaminated waters contain more than one heavy metal, it was necessary to determine the binding abilities of the alfalfa biomass with multi-metal solutions. Multi-metal binding capacity experiments resulted in the following order of affinity for the multi-metal binding by the alfalfa biomass: 42.1mg/g for lead(II), 11.4 mg/g for chromium(III), 6.5 mg/g for copper(II), 4.4 mg/g for cadmium(II), 4.3 mg/g for zinc(II), and 0.5 mg/g for nickel(II). In order to better understand how this system may behave with hard cations such as magnesium and calcium which are found in contaminated waters, batch laboratory interference studies were performed with various calcium and magnesium concentrations (0.1mM - 1M). In general, the alfalfa biomass seems to have a high selectivity to bind copper, lead, and chromium in the presence of high concentrations of calcium and magnesium from a multi-metal-containing solution. The information obtained will be useful for the future development of an innovative technology to remove heavy metal contaminants from polluted groundwaters

Key words: *interference, heavy metal binding, phytofiltration, alfalfa*

INTRODUCTION

The presence of toxic heavy metals in surface and groundwaters has become a serious concern due to the possible health threat these contaminants pose to the public. These pollutants enter the environment through a variety of sources, such as mining, refining, and electroplating activities. The effluents from these industries contain an array of heavy metals, like cadmium, copper, chromium, nickel, lead, and zinc which contribute to the release of toxic metals into the aquatic environment. These metals can be carcinogenic and teratogenic, if not fatal, in high concentrations (Carson et al., 1986). Once in the environment, heavy metal ions naturally concentrate into wetlands and soils, which may leach into groundwaters and eventually affect human health (Runnells et al., 1992). Because of this concern regarding heavy metal contamination, there has been an abundance of interest in remediation of heavy metal ions from the environment (Godtfredsen et al., 1994; Mays et al., 1991; El-Aziz et al., 1991; Mench, 1994; Basta et al., 1992; Jewell, 1994). Traditional methods utilized for the removal of heavy metal ions from industrial waste solutions may prove to be cost prohibitive. Therefore, there is a need for the development of new, cost-effective methods for the removal of heavy metal contaminants from aqueous solutions. The use of biological materials for biosorption of toxic metal ions may be a cost-effective alternative technique for the treatment of industrial effluents [Atlas; 1995]. In fact, many studies have been performed with bacteria, algae, and fungi to determine the abilities of these biomasses to absorb metal ions (Cervantes et al.,1994; Zhang et al., 1994; Zhang et al., 1993; Gardea-Torresdey et al., 1990). More recently, plants have been of interest for their unique ability to bind heavy metals. Both tomato and tobacco plants have

been studied by Lue-Kim et al. and Scott for their metal uptake abilities (Lue-Kim et al., 1986; Scott, 1992). Romheld et al. studied the metal binding by peanuts, and Delhaize et al. studied cadmium binding in *Datura innoxia* (Romheld et al., 1983; Delhaize et al., 1989). Lujan et al. found that metal binding by higher plant tissues was pH dependent (Lujan et al.,1994). Therefore, plant tissues may be a good source of biological materials for the biosorption of toxic metal ions from aqueous industrial waste effluents.

Medicago sativa (alfalfa) may be a good source of plant tissues, because it has been found to tolerate heavy metals and grow well in contaminated soils (El-Kherbawy et al., 1989, Cajuste et al., 1991; Baligar et al., 1993; Recheigl et al., 1988). Gardea-Torresdey et al., have shown that alfalfa is a potential source of biomaterials for the removal and recovery of heavy metal ions (Gardea-Torresdey et al., 1996a; 1996b; 1996c; 1996d). Batch laboratory experiments have determined that alfalfa possesses the ability to bind various heavy metal ions. Alfalfa shoot biomass has demonstrated the ability to bind an appreciable amount of copper(II), nickel(II), cadmium(II), chromium(III), lead(II), and zinc(II) from aqueous solutions. In addition, alfalfa has shown to bind heavy metals well even in hard waters containing high concentrations of calcium and magnesium which typically foul conventional filtration systems. However, single element binding is good for some circumstances but these conditions are rarely seen with industrial waste effluent and contaminated waters in the environment. It is more common to find toxic metal ions in a mixed solution containing more than one heavy metal. Therefore, it was necessary to determine the metal binding abilities of the alfalfa biomass with multi-metal-containing solutions in a variety of hard cation conditions (such as calcium(II) and magnesium(II)).

The objective of this study was to investigate the binding affinities of each metal ion in a mixed-metal solution containing both calcium and magnesium to Malone alfalfa shoots. Batch laboratory experiments were performed with a solution containing each of the following metal ions: copper(II), nickel(II), cadmium(II), chromium(III), lead(II), and zinc(II). In addition, column experiments were performed with silica-immobilized Malone alfalfa shoots to determine the extraction and recovery ability of copper(II), nickel(II), cadmium(II), chromium(III), lead(II), and zinc(II) in a mixed-metal solution under flow conditions. Also, the effects of increasing concentrations of calcium and magnesium on multi-metal binding by alfalfa biomass were studied. These experiments will determine the ability of *Medicago sativa* alfalfa to bind several metal ions under multi-contaminant conditions. The information obtained will be useful for the future development of an innovative technology to remove heavy metal contaminants from polluted groundwaters.

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. 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 for one week. Dried samples were then 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., (Gardea-Torresdey et al., 1996b). In summary, a multi-metal solution was prepared containing 0.1 mM of the each of the following metal ions: cadmium(II), copper(II), chromium(III), lead(II), nickel(II), and zinc(II) and the pH was adjusted to 2.0, 3.0, 4.0, 5.0, and 6.0. The metal solution was prepared from the corresponding salts: Cd(NO3)2,CuSO4, Cr(NO3)3, Pb(NO3)2, Ni(NO3)2, and ZnCl2. At each pH, the multi-metal solution was added to the respective pH biomass pellet. The biomass concentration was maintained at 5 mg biomass per mL. This experiment was carried out three times for quality control. 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 from the pellets were transferred to clean tubes for analysis. Final pHs for all samples 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 (Gardea-Torresdey et al., 1996b). The multi-metal solution was prepared containing 0.3 mM of the each of the following metal ions: Cd(II),Cu(II), Cr(III), Pb(II), Ni(II), and Zn(II). The solution was adjusted to pH 5.0 and allowed to equilibrate with the biomass for different time intervals. The time intervals chosen for the time dependence studies were: 5, 10, 15, 20, 25, 30, 45, 60, and 90 minutes. A biomass concentration of approximately 5 mg per mL of solution was maintained. This experiment was carried out three times for quality control. Final pHs for all samples were recorded and metal concentrations were determined by flame atomic absorption spectroscopy.

Metal Binding Capacity Studies

Batch laboratory methods were used to determine the binding affinities and capacities for the individual metal ions by the alfalfa biomass (Gardea-Torresdey et al., 1996b). The alfalfa biomass was reacted with a solution containing 0.3 mM of each of the previously described metal ions at pH 5.0. The biomass concentration was maintained at 5 mg biomass per mL. After equilibration for

10 minutes, the samples and controls were centrifuged, and the decanted supernatants were stored for metal analysis and fresh multi-metal solution was again reacted with the biomass. This was repeated for 10 cycles or until the biomass became saturated. 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 spectroscopy.

Batch Interference Studies

Batch laboratory metal binding capacities were repeated (as indicated above) with solutions containing the following calcium and magnesium concentrations (as nitrate salts): 0.001M, 0.01M, 0.1M, 1M. Final pH's were recorded and all metal analyses were performed by flame atomic absorption.

Desorption of the Adsorbed Metal Ions

In order to remove the bound metal ions from the alfalfa biomass, the pellets from binding capacity studies, with the adsorbed metals, were equilibrated with 0.1 M HCl. The samples were then centrifuged and the supernatants were removed as indicated by Gardea-Torresdey et al., (Gardea-Torresdey et al., 1996b). The resulting supernatant solutions were collected for analyses and diluted as required to stay within the calibration range of the measuring instrument. The pellets were then exposed to 0.2 M HCl and 0.2M thiourea to remove any remaining metals and equilibrated by rocking for five minutes. After centrifugation, the supernatant solutions were analyzed. All metal analyses were performed by flame atomic absorption spectroscopy.

Immobilized Alfalfa Biomass and Column Experiments

The immobilization of the alfalfa biomass was performed as indicated previously by Gardea-Torresdey et al., (Gardea-Torresdey et al., 1996a; 1996c). Samples of 5 g were washed twice with water and the cell debris were 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 (Gardea-Torresdey et al., 1996a; 1996c). Seventy-five mL of 5% sulfuric acid (H2SO4) were mixed with enough 6% sodium silicate (Na2SiO3) solution to raise the pH to 2.0. Once the solution was at pH 2.0, 5 grams of washed biomass were added to the silica solution and allowed to stir for 15 minutes. The pH was then raised slowly by addition of 6% Na2SiO3 to reach a final pH of 7.0. The polymer gel with the immobilized biomass was dried overnight at 60°C and then ground by using a mortar and pestle and sieved to pass the 20-40 mesh size. The multi-metal solutions were passed through the column and the effluents were analyzed for metal content. 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. Column interference studies were performed with solutions containing the following

calcium and magnesium concentrations combined: 0.001M, 0.01M, 0.1M, and 1M. Each effluent bed volume was collected and analyzed by flame atomic absorption spectroscopy.

Recovery of Metal Ions from Columns

To remove the bound metals from the immobilized alfalfa shoots, 10 bed volumes of 0.2M HCl and 0.2M thiourea were 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 metals recovered in each bed volume of effluent was summed 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, as well as the relative standard deviation, were computed. Samples were diluted as required to remain within the calibration linear range. The following wavelengths were used for the metal ions studied: cadmium 228.8 nm; copper 327.2 nm; chromium 358.2 nm; nickel 352.5: lead 283.3 nm; and zinc 213.9 nm. 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 ion concentration and the remaining metal ion concentration was assumed to be bound to the biomass.

RESULTS AND DISCUSSION

Figure 1 shows the percentage of each metal that was bound onto the biomass from the multi-metal solution, as the pH was raised from 2.0 to 6.0. On the other hand, Figure 2 represents the percent of metal bound by the alfalfa biomass at different time exposures to the multi-metal solution. As can be seen from Figure 1, the metal binding by the alfalfa biomass is pH dependent. At lower pHs, the binding is relatively low; however, as the pH is raised, the metal binding increases for all the metal ions studied. This phenomenon may be due to metal binding by carboxylate ligands. At low pH values, the carboxyl ligands are protonated and are unavailable for metal binding, while at higher pH values they are deprotonated and possess a negative charge . Therefore, the metal binding by the alfalfa biomass may be through an electrostatic interaction with carboxyl ligands at higher pH values. This trend in pH-dependent metal binding corresponds to the data observed by Lujan et al (Lujan et al., 1994). This behavior has also been observed when the experiment was conducted with single metal solutions (Gardea-Torresdey et al., 1996a; 1996b; 1996c; 1996d). As shown in Figure 2, the metal binding is relatively stable over the 90 minute period of reaction time with the exception of chromium which increases slightly after 30 minutes. Also, Figure 2 shows that the metal binding is rapid, since metal binding occurred within the first five minutes for most of the

metals. Therefore, the majority of the metal binding is taking place within the time it takes to shake the biomass, centrifuge the tubes, and separate the supernatant. The rapid binding of the metal ions by the alfalfa biomass could indicate that the metals are being adsorbed onto the surface of the plant tissues, instead of absorption within the plant cells. Because the plant tissues were inactivated, metal adsorption does not occur through an active process.

Table 1 shows the metal-binding capacities by the alfalfa biomass for each metal ion in the multi-metal solution. It is important to point out that these capacities were obtained at pH 5.0. The range in metal binding was from 8.0 µmol/g for nickel(II) to as high as 368.5 µmol/g for chromium(III). As seen in Table 1, the µmol units show that there is an affinity for the metal ions in the order of Cr(III) ñ Pb(II) ñ Cu(II) ñ Zn(II) ñ Cd(II)ñ Ni(II). The difference in the metal affinity may be due to the metal binding affinities for the same ligand and for different ligands. Since the metals are all in solution, they are in competition with each other for the available binding ligands. Therefore, a metal that has a higher affinity for carboxylate ligands should bind in greater quantities in an environment that contains many available carboxylate groups. However, a metal that has a higher affinity for other metal binding ligands will not bind as well in an environment containing more carboxylate groups. This might help to explain the preferential binding observed for chromium,lead, and copper over the other metals studied. Similar findings for metal binding to carboxylic acids was observed by Preston et al. (1985). This further indicates the importance of the carboxyl ligand for metal binding by the alfalfa biomass.

As can be seen in Figure 1, the binding of the metal ions from solution was pH dependent. The binding of metal ions was low when the pH of the solution was low. This trend in pH-dependent binding could be very useful for the recovery of the bound-metal ions. Table 2 shows the percentage of metal ions that was recovered from the metal-bound biomass by treatment with 0.1M HCl. Table 2 demonstrates that the recovery of the metal ions was very good by treatment with dilute acid (nearly 100%), with the exception of cadmium and chromium which was 60% and 73%, respectively.

Because calcium and magnesium are common metal cations found in natural waters, it was necessary to determine the effects of various concentrations of these cations on the metal-binding ability of alfalfa biomass with multi-metal-containing solutions. Batch laboratory-binding capacities were carried out with various concentrations of calcium and magnesium while maintaining a constant concentration of each of the metals in the multi-metal solutions. Figure 3 illustrates the combined effects of both calcium and magnesium on the binding of metal ions by alfalfa shoots. A significant decrease was observed in metal binding as the concentration of hard cations was raised from 0.0 M to 0.001 M. However, as the concentrations of calcium and magnesium were raised to 20,000 times the concentration of the individual metal ions , smaller reductions in metal binding were observed. Although appreciable levels of lead(II) and copper(II) were still removed from the solution

containing 1M of calcium and 1M magnesium combined, the binding of cadmium(II), nickel(II), chromium(III), and zinc(II) were affected by the increase in hard cation concentrations. This decrease may be due to changes in ionic strength rather than competition between the heavy metals and hard cations for available binding sites. Even though a decrease in overall metal binding was observed at high concentrations of hard cations, the preferential order of metal binding was maintained with the exception of Cr(III). This trend might be explained by differences in binding constants and the order of stability for metal-ligand complexes can be predicted using the Irvin Williams Series (Basolo et al.,1973).

While batch laboratory experiments provide useful information, column experiments under flow conditions provide more practical data concerning the binding of metal ions to an adsorbent compound. In addition, when performing column experiments, the biomass needs to be immobilized in order to avoid reduction in flows due to biomass clumping. Table 3 shows the amount of individual metal ions bound by the silica-immobilized alfalfa shoot biomass, while Table 4 shows the percent of each metal ion recovered from the laden silica-immobilized biomass by using 0.1M HCl. As observed in Table 3, the silica-immobilized alfalfa biomass adsorbs metal ions in the following order: Pb(II) ñ Cu(II) ñ Cr(III) ñ Zn(II) ñ Cd(II) ñ Ni(II). This is similar with the batch-adsorption capacity experiments described above where Cu(II), Cr(III), and Pb(II) have the highest affinities for binding by the alfalfa biomass (although the order changed slightly). Good recoveries were observed for most of the metal ions with the exception of Cr(III) (Table 4). The data shown herein represents only one adsorption / acid recovery cycle. Therefore, more cycles are required to determine the recyclability of the column in the presence of a solution containing six different heavy metal ions. In addition, it can be clearly seen that 0.1M solution of hydrochloric acid is not enough concentration to completely remove Cr(III), as well as some of the other metal ions. More research is needed to ascertain the optimum conditions for the removal of the bound-metal ions.

Figure 4 indicates the average amount of metal ions bound by three columns of silica-immobilized alfalfa biomass after 120 bed volumes of a 0.1 mM multi-metal solution containing various levels of calcium and magnesium were passed. Although overall decreases in metal binding was observed as the concentrations of hard cations were increased, a similar trend of preferential metal binding was seen. Therefore, the alfalfa biomass exhibits selective binding for metal ions even under hard-cation exposure. Consequently, the reduced binding may not be due to heavy metal and hard-cation competition for the binding ligands, but instead due to differences in ionic strength.

In summary, these experiments showed the ability of *Medicago sativa* alfalfa to bind several metal ions under multi-contaminant conditions. However, the alfalfa biomass was shown to have a high selectivity to bind copper, lead, and chromium ions from a multi-metal solution even when high concentrations of calcium and magnesium were present. The information obtained will be useful for future development of an innovative technology to remove heavy metal contaminants from industrial effluents and polluted waters.

ACKNOWLEDGMENTS

The authors acknowledge financial support from the National Institutes of Health (NIH), (grant # GM 08012-25) and financial support from the University of Texas at El Paso's Center for Environmental Resource Management (CERM) through funding from the HBCU/MI Environmental Technology Consortium, which is funded by the Department of Energy and the Office of Exploratory Research of the U.S. Environmental Protection Agency (cooperative agreement CR- 819849 - 01 - 4).

REFERENCES

Atlas, R. M.,1995. Bioremediation, *Chem. Eng. News*, 3, pp. 32-42.

Baligar, V. C., T.A. Campbell, and R.J. Wright, Jr., 1993. Differential Responses of Alfalfa Clones to Aluminum-Toxic Acid Soil, *Plant Nutrition*, 16, pp. 219-223.

Basta, N.T., and M.A. Tabatabai,1992. Effects of Cropping Systems on Adsorption of Metals by Soils, *Soil Science.*, 153, pp. 108-117.

Cajuste, L.J., R. Carrillo G., E. Cota, and R.J. Laird, 1991. The Distribution of Metals from Wastewater in the Mexican Valley of Mezquital, *Water, Air and Soil Pollution*, 57, pp. 763-781.

Carson, B.L., H.V. Ellis, and J.L. McCann, 1986. *Toxicology and Biological Monitoring of Metals in Humans*, Lewis Publishers, Chelsea, Michigan, pp. 65, 71, 97, 133,165, 297.

Cervantes C., and F. Gutierrez-Corona, 1994. Copper Resistance Mechanisms in Bacteria and Fungi, *FEMS Microbiol. Rev.*, 14, pp. 121-138.

Delhaize E., P.J. Jackson, L.D. Lujan, and N.J. Robinson, 1989. Poly (g- glutamylcysteinyl) glycine Synthesis in Datura innoxia and Binding with Cadmium, *Plant Physiol*., 89, pp. 700-706.

El-Aziz, R., J. S. Angle, and R. L. Chaney, 1991. Metal Tolerance of *Rhizobium meliloti* Isolated from Heavy Metal-Contaminated Soils, *Soil Biol. Biochem*., 23, pp. 795-807.

El-Kherbawy, M., J.S. Angle, A. Heggo, and R.L. Chaney, 1989. Siol pH Rhizobia, and Vesicular-arbuscular Mycorrhizae Innoculation Effects on Growth and Heavy Metal Uptake of Alfalfa (Medicago sativa), *Biol. Fertil. Soils*, 8, pp. 61-68.

Gardea-Torresdey, J.L., M.K.Becker-Hapak, J.M.Hosea, and D.W. Darnall, 1990. Effect of Chemical Modification of Algal Carboxyl Groups on Metal Ion Binding, *Environ. Sci.Technol*., 24, pp. 1372-1378.

Gardea-Torresdey, J.L., K. J. Tiemann, J. H. Gonzalez, J. A. Henning, and M. S.Towsend, 1996a. Ability of Silica-Immobilized *Medicago sativa* (Alfalfa) to Remove Copper Ions from Solution, *J. of Hazardous Materials*, 48, pp. 181-190.

Gardea-Torresdey, J.L., K.J. Tiemann, J. H. Gonzalez, J. A. Henning, and M.S. Townsend,1996b. Uptake of Copper Ions from Solution by Different Populations of *Medicago Sativa* (Alfalfa), *Solvent Extraction and Ion-Exchange*, 14, pp. 119-140.

Gardea-Torresdey, J.L., K.J. Tiemann, J. H. Gonzalez, J. A. Henning, M.S. Townsend, and I. Canno-Aguilera,1996c. Removal of Nickel Ions from Aqueous Solution by Biomass and Silica -Immobilized Biomass of *Medicago sativa* (Alfalfa). *J. of Hazardous Materials*, 49, pp. 205-223.

Gardea-Torresdey, J.L., K.J. Tiemann, J.H. Gonzalez, and O. Rodriguez,1996d. Biosorption of Cadmium, Chromium, Lead, and Zinc, by Biomass of *Medicago sativa* (Alfalfa), *Proceedings of the 11th Annual Conference on Hazardous Waste Research. Edited by L.E. Erickson, D.L. Tillison, S.C. Grant, and J.P. McDonald, Kansas State Univ., Manhattan, KS*, pp. 209-214.

Godtfredsen, K.L., and A. T. Stone, 1994. Solubilization of Manganese Dioxide Bound by Naturally Occurring Organic Compounds, *Environ. Sci. Technol.*, 28, pp.1450-1458.

Jewell, W. J., 1994. Resource-Recovery Wastewater Treatment, *American Scientist*, 82, pp. 366-375.

Lue-Kim, H., and W.E. Rauser, 1986. Partial Characterization of Cadmium-Binding Protein from Roots of Tomato, *Plant Physiol*., 81, pp. 896-900.

J.R. Lujan, D.W. Darnall, P.C. Stark, G.D. Rayson, and J.L. Gardea-Torresdey, 1994. Metal Ion-Binding by Algae and Higher Plant Tissues: A Phenomenological Study of Solution pH Dependence, *Solvent Extr. Ion Exch*., 12, pp. 803-816.

Mays, D.A., P.M. Giordano, and A.D. Behel Jr., 1991. Impact of Fluidized-Bed Combustion Waste on Metal Content of Crops and Soil, *Water, Air, and Soil Pollution*, 57, pp. 307-317.

Mench, M.J., 1994. A Mimicked *In situ* Remediation Study of Metal-Contaminated Soils with Emphasis on Cadmium and Lead, *J. Environ.Qual*., 23, pp. 58-65.

Peterson, J.S., 1985. Solvent Extraction of Metals by Carboxilic Acids, *Hydrometallurgy*, 14, pp. 171-188.

Rechcigl, J.E., R.B. Reneau, Jr., and L.W. Zelazney, 1988. Soil Solution Al as a Measure of Al Toxicity to Alfalfa in Acid Soils, *In Soil Sci. Plant Anal*. , 19, pp.989-993.

Romheld, V., and H. Marschner, 1983. Mechanism of Iron Uptake by Peanut Plants, *Plant Physiol*., 71, pp. 949-954.

Runnells, D.D., and T.A. Shepherd, 1992. Metals in Water: Determining Natural Background Concentrations in Mineralized Areas, *Environ. Sci. Technol.*, 26, pp.2316-2323.

Scott, C.D., 1992. Removal of Dissolved Metals by Plant Tissue, *Biotechnol. and Bioeng*., 39, pp. 1064-1068.

Zhang, W., and V. Majidi, 1993. Study of Influences on the Binding of Metals to *Stichococcus bacillaris* with ^{113}Cd NMR, *Applied Spectroscopy*, 47, pp. 2151-2156.

Zhang, W., and V. Majidi, 1994. Monitoring the Cellular Response of *Stichococcus bacillaris* to exposure of Several Different Metals Using in Vivo ^{31} P NMR and Other Spectroscopic Techniques, *Environ. Sci. Technol.*, 28, pp. 1577-1581.

Table 1. Adsorption capacities for metal binding by alfalfa shoot biomass (µmol metal / g biomass.

Cr(III)	219.4
Pb(II)	203.2
Cu(II)	103.1
Zn(II)	65.6
Cd(II)	38.7
Ni(II)	8.0

Table 2. Percent metal desorption from saturated alfalfa shoot biomass with 0.1 M HCl.

Cr(III)	73%
Pb(II)	100%
Cu(II)	100%
Zn(II)	99%
Cd(II)	60%
Ni(II)	100%

Table 3. Column experiments.

	ppm bound	mM
Cr(III)	158.3	3.0
Pb(II)	1859.0	9.0
Cu(II)	355.4	5.6
Zn(II)	89.9	1.4
Cd(II)	150.1	1.3
Ni(II)	47.2	0.4

Table 4. Percent metal ions recovered from the silica-immobilized alfalfa shoot biomass by treatmentt with 0.1 M HCl.

	% metal recovery
Cr(III)	100%
Pb(II)	18.5%
Cu(II)	71.2%
Zn(II)	79.7%
Cd(II)	77.2%
Ni(II)	100%

Figure 1. Percent of each metal bound by the alfalfa biomass after one hour equilibration with a multi-metal solution at different pH values. The solution contained 0.1mM of the following metals: Cd (■) , Cr (—), Cu (····), Ni (♦), Pb (●), Zn (-···-).

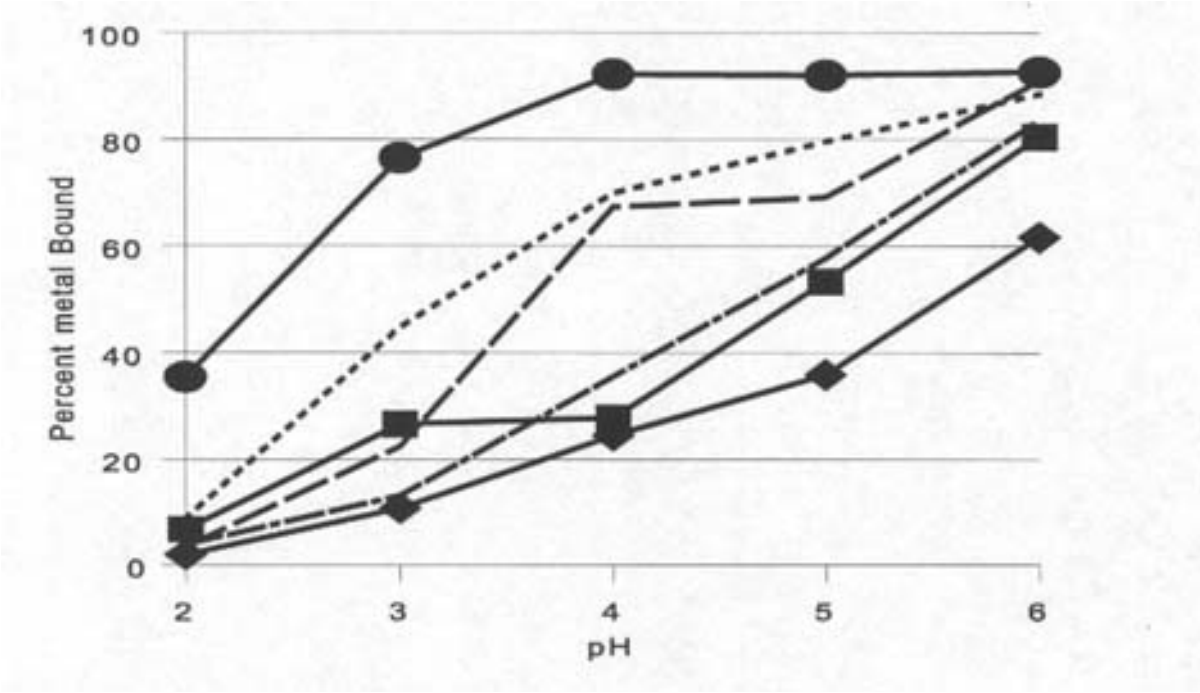

Figure 2. Percent of each metal bound by the alfalfa biomass after equilibration for different times with a multi-metal solution at pH 5.0. The solution contained 0.1mM of following metals: Cd (■), Cr (—), Cu(····), Ni (♦), Pb (●), Zn (-···-).

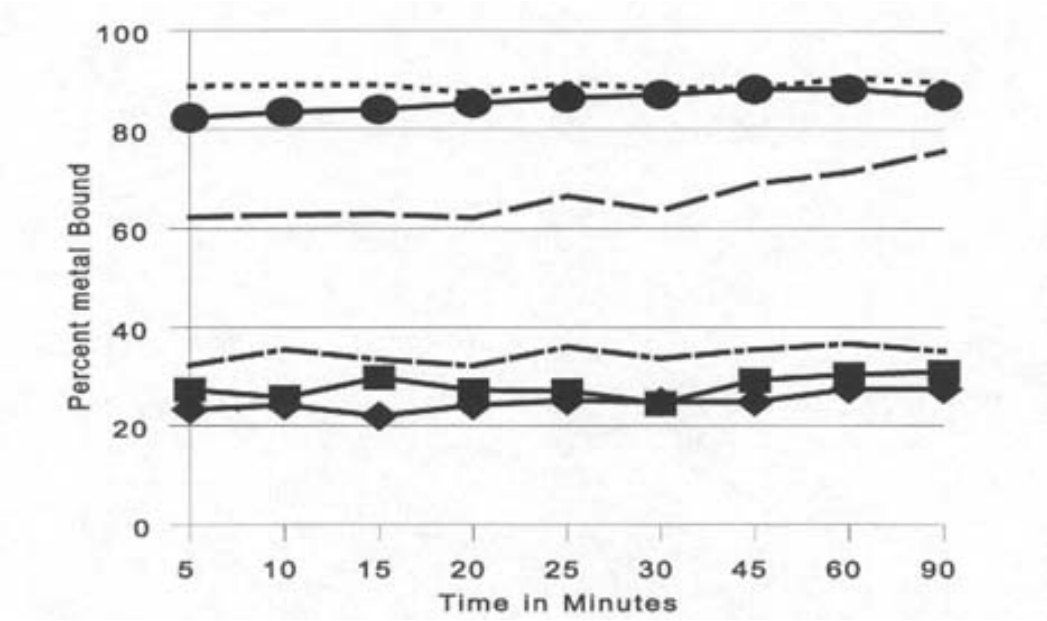

Figure 3. Effects of various calcium and magnesium on batch adsorption metal binding by alfalfa shoot tissues from a solution containing 0.1mM of the following metals: Cd(II), Cr(III), Cu(II),

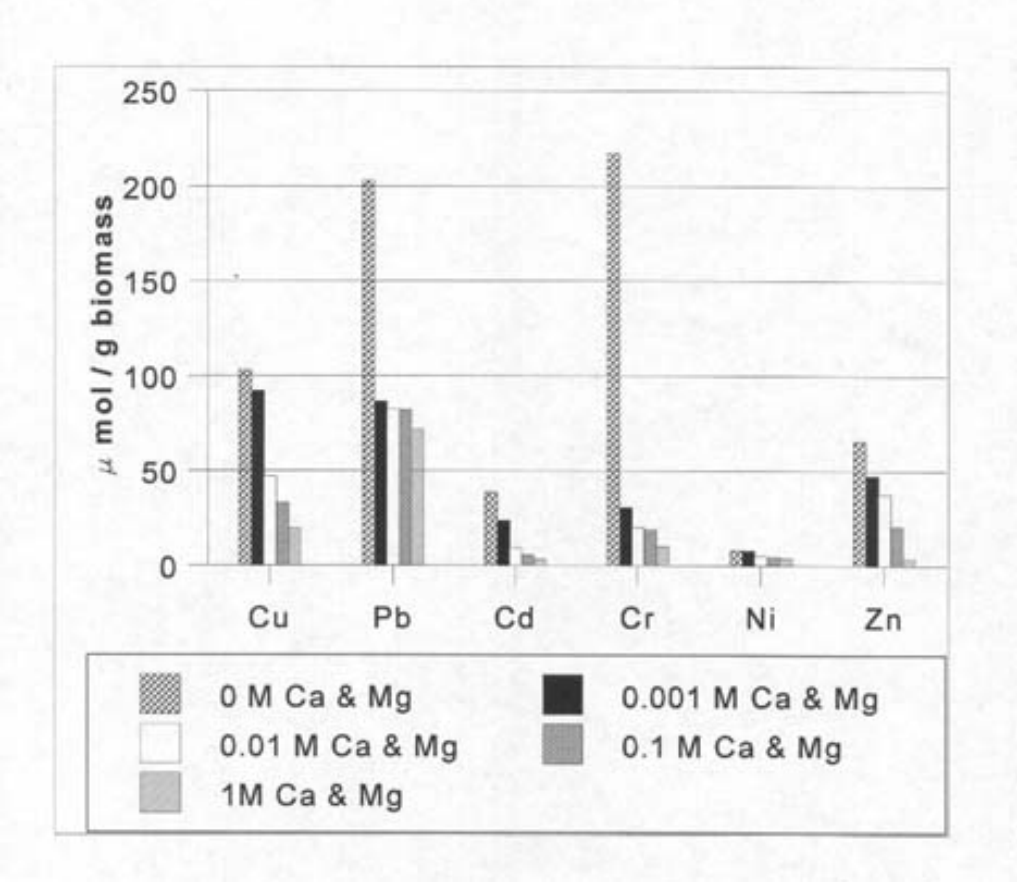

Figure 4. Effects of various calcium and magnesium on column experiments with silica-immobilized alfalfa shoot tissues after passing a solution containing 0.1mM of the following metals: Cd(II),