

REMOVAL OF COPPER IONS FROM SOLUTION BY SILICA-IMMOBILIZED MEDICAGO SATIVA (ALFALFA)

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ABSTRACT

Previous screening laboratory batch experiments to determine the binding ability of seven different populations of *Medicago sativa* (alfalfa) showed good copper binding characteristics of the biomasses studied. All seven populations examined had similar trends for binding copper as a function of pH. The copper binding by the different alfalfa populations occurred in less than five minutes. All the alfalfa biomasses showed high copper binding, but the capacities varied according to the alfalfa population studied. The pH dependence of the copper ion binding to the alfalfa biomasses suggested that it might be possible to recycle the system much like an ion-exchange resin. However, the alfalfa cells can not be packed into a column since the cells clump together and restrict the flow. We have immobilized the cells of alfalfa Malone shoots in a silica matrix. Column experiments for copper binding by the silica-immobilized Malone demonstrated that the alfalfa tissues were capable of removing considerable amounts of copper ions under flow conditions. After every copper binding cycle most of the copper was desorbed with a few bed volumes of 0.1 M HCl. Our work indicates that the Malone-silica preparations are highly durable. We have subjected the biomaterial to as many as 10 cycles of binding and elution without observing any significant decrease in copper binding capacity.

KEY WORDS

biofiltration, phytoremediation, *Medicago sativa*, alfalfa, copper, removal, recovery

INTRODUCTION

Due to the accumulation of many toxic chemicals in the environment which threaten the public health, there has been an increase in research and development aimed at environmental remediation [1]. Traditionally, contaminated areas required soil removal and transport to hazardous landfills or wastewater treatment by activated charcoal and ion exchange resin filters. Due to the high cost of these methods there is a crucial need for the development of a method that is not only cost effective, but can be easily performed. Biological

systems are the target of recent research for environmental remediation. This is due in part to an increase in environmental awareness and governmental regulations and policies that favor green technologies. Biological systems have great potential for remediation because they are easily obtained, low in cost, and prevent further pollution to the environment [2].

Bioremediation has emerged as a technology for accumulation of heavy metal contaminants by the use of living organisms. Many researchers have done studies with live microbial and fungal systems to re-

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move heavy metals from contaminated waters [3-8]. Bioremediation works well in low concentrations, but live systems are limited by the toxicological effects of high levels of contamination. The advantages of using dead systems are the nondestructive effects from high levels of contamination and low maintenance as well as low cost. Dead or inactivated systems using peat moss have proven to be very effective for decontamination of water [9]. Recent research done with dead algal biomass has shown a great potential for removal and recovery of heavy metal ions [10]. More recently, phytoremediation has emerged as one of the alternative technologies for removing pollutants from the environment. Significant new research projects use plants for environmental remediation due to their enormous natural capacity to accumulate heavy metals and degrade organic compounds [11-17]. Studies done with *Datura innoxia* (gympsyn weed), the roots of garlic, and dried roots of the tomato plant have shown metal binding properties [18-19]. Gardea-Torresdey and coworkers demonstrated that carboxyl groups found on the cell wall of algae are responsible for copper binding [20]. Therefore, higher plant cells which contain these functional groups might also be capable of metal binding.

Alfalfa may be a potential source of biomaterial for the removal of heavy metal ions from water. Alfalfa has been found growing in fields irrigated with water high in heavy metal contamination [21, 22]. Studies have shown that alfalfa has tolerance levels to heavy metals above other plants [23-26]. This tolerance may be due to its high protein content or the evolution of chemical functional groups in the plant cells that inhibit the toxic effects of the heavy metals [27]. Because alfalfa shows itself to have a high affinity for metal ions and can be obtained inexpensively and easily, it has attractive applications for the removal of metal ions from contaminated waters. We chose to perform experiments with dead alfalfa plant tissues, hoping to find a way to

remove metal ions from solution through green chemistry.

The objective of our study was to investigate the binding of copper ions to silica-immobilized alfalfa under flow conditions. Column experiments were performed with immobilized alfalfa biomass to examine copper removal and recovery, as well as the ability to recycle the column and determine its efficiency.

METHODOLOGY

Alfalfa collection

The seven alfalfa populations were selected as representatives from the many different varieties of alfalfa by their individual characteristics. The different characteristics of each population may be due to differences in plant composition and may provide different chemical functional groups that could affect copper binding to the biomasses.

Alfalfa tissues were collected from field studies conducted by Dr. John Henning at New Mexico State University near Las Cruces, New Mexico. Four alfalfa basic germplasms (African, Peruvian, Flemish, Ladak) and two cultivars (Malone, Moapa 69) were obtained from plots that had received irrigation every 2 weeks during the growing season. One cultivar (Cal West 630) was taken from a dryland test, which received no irrigation. 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 while Malone and CW630 were also lyophilized. Dried samples were ground to pass through a 100-mesh screen using a Wiley mill.

pH profile studies for copper binding

Batch laboratory techniques were used for the pH studies. A 250 mg sample of biomass was washed twice with 0.01 M hy-

drochloric acid (HCl) to remove any debris or soluble biomolecules that might interact with metal ions. Washings were collected, dried and weighed to account for any biomass weight loss. Each biomass sample was resuspended in 50 ml of 0.01 M HCl with tissue concentration approximately 5 mg per ml solution. The pH was adjusted to 2.0, allowed to equilibrate, and 2 ml aliquots of the suspension were transferred into three 5 ml plastic tubes. The pH was then adjusted and allowed to equilibrate at pH 3.0, 4.0, 5.0, 6.0, and 2 ml aliquots of the suspension at each pH were transferred into 3 tubes for each pH. The suspensions were centrifuged at 2,500 rpm for 5 minutes, and the supernatants were kept for testing to determine if soluble materials were binding the metal. A solution of 0.1 mM copper sulfate (CuSO_4) was prepared and pH adjusted to 2.0, 3.0, 4.0, 5.0 and 6.0. At each pH, 2 ml of the copper solution was added to the respective pH biomass pellet and separated supernatant. In addition, at each respective pH, 2 ml of the 0.1 mM Cu^{2+} solution was transferred to 3 tubes for controls. All the tubes were equilibrated on a rocker for 1 hour. The samples were then centrifuged at 3,000 rpm for 5 minutes, and the supernatants for the pellets were transferred to clean respective tubes. Final pHs for all tubes were recorded, and analysis for copper was performed by flame atomic absorption.

Time dependence studies for copper binding

A 500 mg sample of biomass was washed twice with 0.01 M HCl to remove any debris or soluble biomolecules that might interact with metal ions. The washings were collected, dried and weighed to account for any biomass weight loss. Each biomass sample was resuspended in 100 ml of deionized water with tissue concentration of approximately 5 mg per ml solution. The solution was then adjusted to pH 5.0 and allowed to equilibrate. Two ml of the suspension was transferred to 24 tubes; 3

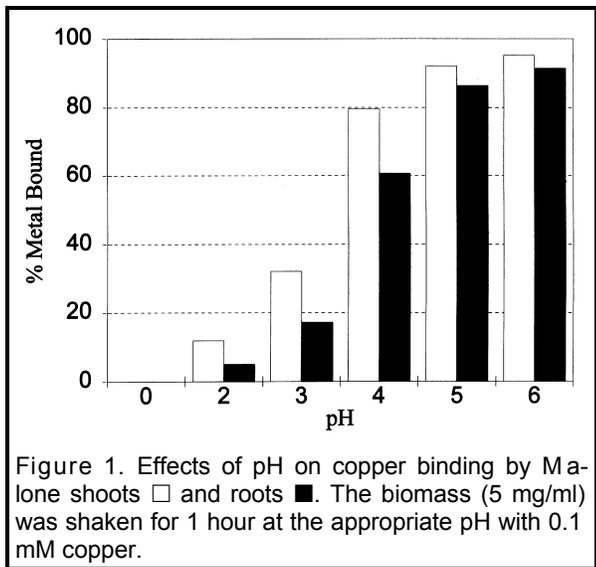
tubes for each time interval of 5, 10, 15, 20, 25, 30, 45 and 60 minutes. After centrifugation, 2 ml of 0.1 mM copper solution was added to each of the tubes and controls. All the tubes were equilibrated by rocking and were removed at the appropriate time intervals. The samples were then centrifuged at 3,000 rpm for 5 minutes, and the supernatants from the pellets were transferred to clean respective tubes. Final pHs for all tubes were recorded and analysis for copper was performed by flame atomic absorption.

Copper binding capacity studies

Samples of 100 mg of biomass were washed twice with 0.01 M HCl, and washings were collected and weighed to determine biomass loss. The washed biomass was resuspended in 20 ml of deionized water and the pH adjusted to 5.0. Two ml of the suspension was transferred to 3 tubes and then centrifuged. The supernatants were saved for testing. Two ml of 0.3 mM Cu^{2+} solution was added to each of the tubes and controls and were equilibrated for 10 minutes. After centrifugation, the supernatants were saved for analysis and again 2 ml of 0.3 mM copper solution was added. This was repeated 12 times or until the saturation point was achieved, and a final pH for all tubes was recorded. Samples were diluted as required to stay within the calibration linear range, and analysis for copper was performed by flame atomic absorption.

Desorption of the adsorbed copper

Pellets from capacity studies with adsorbed copper were exposed to 2 ml of 0.1 M HCl, equilibrated by rocking for 5 minutes, and then centrifuged. 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 strip any remaining metal and equilibrated by rocking for 5 minutes. After centrifugation, the supernatants were analyzed. All



analysis for copper was performed by flame atomic absorption.

Immobilization of alfalfa biomass

The method for immobilization of cell wall material within a polysilicate matrix was similar to that reported by Huei-Yang and Rayson [28]. A 5 g sample of biomass was washed twice by vortexing the sample with water and was centrifuged for five minutes at 3,000 rpm. This step will remove solubles and debris. Next, 75 ml of 5% sulfuric acid (H_2SO_4) was mixed with enough 6% sodium silicate (Na_2SiO_3) solution to raise the pH to 2.0. At pH 2.0, the 5 grams of washed biomass was added to the silica solution and allowed to stir for 15 minutes. The pH was then raised slowly by the addition of 6% Na_2SiO_3 to reach a final pH of 7.0. The polymer gel was washed with water enough times so that by the addition of two drops of barium chloride ($BaCl_2$), there was no white precipitate forming. $BaCl_2$ was used to indicate whether the sulfates had been removed. The polymer gel with the immobilized biomass was dried overnight at 60°C and then ground by mortar and pestle and sieved to pass 20-40 mesh size.

Column experiments

One bed volume equals the volume of immobilized biomass inside the column. Six ml of the immobilized alfalfa was used in the column. The column was washed with 10 bed volumes of 0.01 M sodium acetate buffer at pH 5.0 and the effluent pH was checked to ensure that the column was at the optimal binding pH. A flow rate of 2 ml per minute was used to pass 120 bed volumes of 5.0 ppm Cu solution in 0.01 M sodium acetate at pH 5.0. Each bed volume was collected and analyzed by flame atomic absorption.

Recovery of copper from column

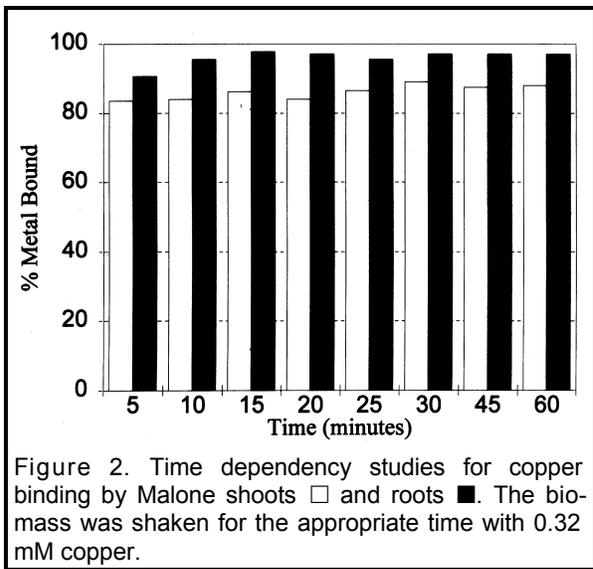
To remove the bound copper, 0.01 M HCl was passed at a flow rate of 2 ml per minute. Each bed volume was collected and analyzed by flame atomic absorption.

Analytical procedure

Analysis for copper was performed using a Perkin Elmer model 3110 Atomic Absorption Spectrometer with deuterium background subtraction. Impact bead was utilized to improve the sensitivity at a wavelength of 327.4 nm. Samples were read three times and a mean value and relative standard deviation was computed. Calibrations were performed in the range of analysis and a correlation coefficient for the calibration curve of 0.98 or greater was obtained. The instrument response was periodically checked with known copper standards. The difference between the initial metal concentration and the remaining metal concentration in effluents were assumed to be taken up by the biomass.

Data analysis

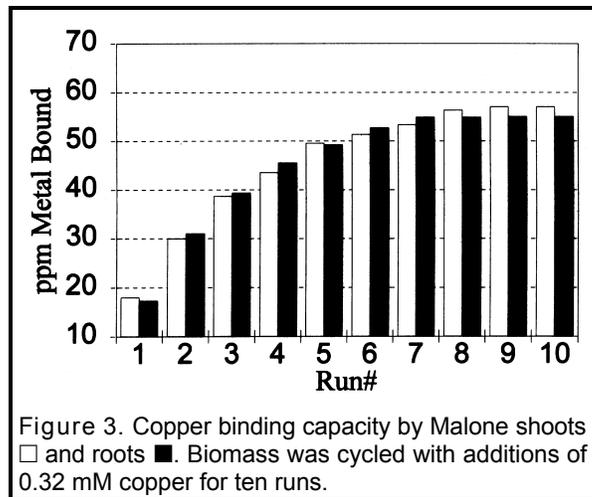
The experiments were performed in triplicate and the samples were analyzed in triplicate. For each set of given data, standard statistical methods were used to determine the mean values and standard deviations. Confidence intervals of 95% were



calculated for each set of samples to determine the error margin.

RESULTS AND DISCUSSION

Previous screening experiments performed to determine the copper binding characteristics of seven populations of *Medicago sativa* alfalfa showed that the Malone variety had good binding capabilities [29]. Figure 1 is the pH profile for copper binding by Malone roots and shoots. The shoots show only slightly higher binding than the roots. All seven of the populations examined had similar trends in pH for copper binding. As can be seen in Figure 1, the binding of copper ion by the Malone biomass is pH dependent, with a maximum binding observed between pH 5.0 and 6.0. At pHs higher than 6.0, the copper ions start to precipitate out of solution. This trend in pH-dependent binding suggests that carboxyl groups may play a role in the copper binding by the biomass. The ionization constants (pK 's) for various carboxyl groups have been reported to be around 3-4 [30-31]. Free carboxyl groups are protonated at pHs lower than 3 and reduce any metal binding. At pHs greater than 4, the carboxyl groups are deprotonated and attract positively charged copper ions. Metal ions bind to the carboxyl groups through an ion ex-



change-type mechanism. Therefore if carboxyl groups do play a role in the binding of metal ions, lowering the pH would cause the metal ions to be released back into solution.

Studies were conducted to determine how long it would take the copper ions to bind to the alfalfa biomass. Figure 2 demonstrates the percent of copper ions removed from solution by Malone germplasm roots and shoots when exposed to 0.1 mM copper solution at pH 5 over a 60 minute period [29]. It can be seen that the mechanism for copper ion binding occurs in less than 5 minutes. Even after one hour of reaction, relatively the same amount of copper was bound. This shows that the binding of copper is relatively stable. Since all of the soluble components were eliminated in prior washing, the binding must be due to the alfalfa biomass. Because the alfalfa plant tissues were inactivated during drying, the rapid binding of the copper ions may be due to functional groups located on the cell wall and not due to any cellular processes.

Capacity experiments were previously performed and showed that various populations of alfalfa were capable of binding copper ions from solution. Previous experiments indicated that the shoots bind more copper than the roots for most of the

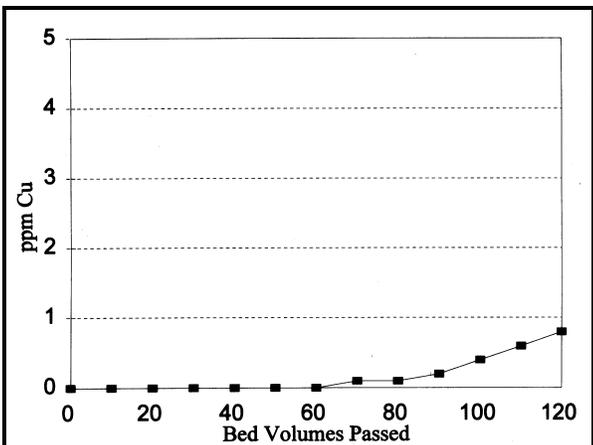


Figure 4. Concentration of copper remaining in the effluent after being passed through a column of immobilized Malone shoots. Five ppm copper solution was passed at a flow rate of 2 ml/minute. Column effluents were analyzed for copper. One bed volume equals the volume of silica immobilized alfalfa in the column (6 ml).

populations studied. This phenomena may be attributed to the different chemical compositions in the plant's roots and shoots. Figure 3 shows the amount of copper bound by the Malone population [29]. Saturation of the Malone biomass was achieved by cycle 7. Malone shoots had proven to bind copper well and also showed good recovery rates by treatment with 0.1 M HCl; therefore, Malone shoots were chosen as one of the biomasses to be further investigated. The reversibility of the copper binding could have very important implications for the reclamation of metal ions from contaminated waters.

Instead of removal of copper ions from solution by batch experiments, it would be most useful if the alfalfa plant tissues could be packed into a column so that contaminated waters could simply be passed through the column. Unfortunately when the alfalfa cells are packed into columns, the alfalfa clumps together and the flow rates are reduced significantly. This problem can be solved by immobilizing the alfalfa cells in a polymer matrix. The immobilized biomass can then be packed into columns through which high flow rates can be

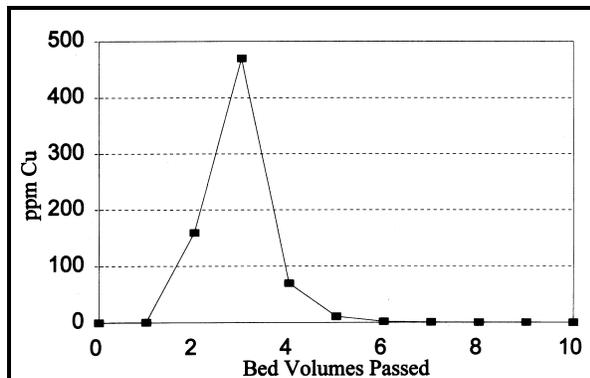
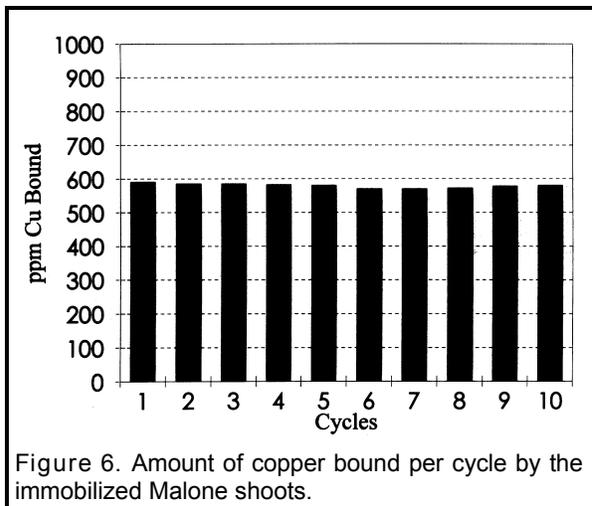


Figure 5. Recovery of copper ions from the column of immobilized Malone shoots by the addition of 0.1 M HCl. Flow rate of 2 ml/minute was used.

achieved. In order to maintain optimal flow through the columns, a polysilicate matrix support material was used to immobilize the alfalfa biomass. This would give the physical properties of a polymer resin and the binding properties of the alfalfa. Column experiments were conducted to study the effects of copper binding by the alfalfa biomass under flow conditions. Figure 4 shows the amount of copper remaining after a solution of 5 ppm copper at pH 5.0 was passed through the column of immobilized Malone shoots. It was not until after 60 bed volumes had been passed that trace amounts of copper emerged in the effluent. Even after 120 bed volumes had been passed, the concentration of copper in the effluent was still below 1 ppm, and 97% of the copper passed was bound in the column. Some of the experiments were carried out to approximately 230 bed volumes, and the saturation of the column was still not achieved.

The pH profile experiments (Figure 1) suggested that the copper ion could be removed by lowering the pH. By using low strength acid, the copper can be stripped from the column without damaging the alfalfa biomass or the polysilicate matrix. Figure 5 shows the effects of passing 0.1 M HCl through the column of immobilized shoots which contained bound copper ions. The initial lag was due to the movement of



the solution through the tubing. The immediate effects of the acid are seen in bed volumes 1-5. Most of the bound copper was recovered in approximately 3 bed volumes of low concentration acid.

In order to verify that the alfalfa biomass was not affected during the recovery cycle, the column was cycled again with 120 bed volumes of 5 ppm copper solution at pH 5.0. As expected, the amount of copper bound in the second cycle was very similar to that seen in the first cycle. This demonstrates that the low concentration of acid used to remove the bound copper ions had little effect on the binding by the immobilized alfalfa biomass. Figure 6 illustrates 10 cycles of binding after 120 bed volumes of 5 ppm copper solution had been passed and recovered by immobilized Malone shoots. It can be seen that the efficiency of the column remained relatively steady even after ten cycles of low concentration of acid were used for the recovery. This innovative technology has potential for the removal and recovery of copper ions from contaminated waters.

CONCLUSIONS

Alfalfa has shown to be successful in binding copper ions from aqueous solutions. These studies provide preliminary data that shows the potential for the silica immobi-

lized alfalfa biomass to be used as a biofilter for the removal and recovery of metal ions from contaminated waters. The alfalfa silica polymer matrix functions like a "biological" mixed-bed ion-exchange resin. Like ion-exchange resins, the alfalfa silica biomaterial can be recycled. We sorbed and desorbed copper ions over as many as 10 cycles with no significant loss in binding efficiency. Alfalfa is inexpensive and easily obtained. This innovative technology provides a reusable material that is not only biodegradable, but also environmentally friendly.

Further experiments are being performed in our laboratory to determine the binding of several different metal ions by the different populations of alfalfa. We will also be conducting interference studies to find what effects other cations will have on metal binding under flow conditions.

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REFERENCES

1. D.D. Runnells, T.A. Shepherd and E.E. Angino, Metals in Water Determining Natural Background Concentrations in Mineralized Areas, Environ. Sci. Technol., 26 (1992) 2316.
2. R.M. Atlas, Bioremediation, Chem. Eng. News, 3 (1995) 32-42.
3. C. Cervantes and F. Gutierrez-Corona, Copper Resistance Mechanisms in

- Bacteria and Fungi, FEMS Microbiol. Rev., 14 (1994) 121-138.
4. W. Zhang and V. Majidi, Monitoring the Cellular Response of *Stichococcus bacillaris* to Exposure of Several Different Metals Using in Vivo ³¹P NMR and Other Spectroscopic Techniques, Environ. Sci. Technol., 28 (1994) 1577-1581.
 5. J. Bender, S. Rodriguez-Eaton, U.M. Ekanemesang and P. Phillips, Characterization of Metal-Binding Biofloculants Produced by Cyanobacterial Component of Mixed Microbial Mats, App. Environ. Microbiol., 60 (1994) 2311-2315.
 6. C.R. Nagendra Rao, L. Iyengar and C. Venkobachar, Sorption of Copper(II) from Aqueous Phase by Waste Biomass, J. Environ. Eng., 119 (1993) 369-377.
 7. L. de Rome and G.M. Gadd, Use of Pellet and Immobilized Yeast and Fungal Biomass for Heavy Metal and Radio Nuclide Recovery, J. Industrial Microbiol., 7 (1991) 97-104.
 8. G.J. Ramelow, L. Liu, C. Himel, D. Fralick, Y. Zhao and C. Tong, The Analysis of Dissolved Metals in Natural Water after Preconcentration on Biosorbents of Immobilized Lichen and Seaweed Biomass in Silica, Intern. J. Anal. Chem., 53 (1993) 219-232.
 9. T. Viraraghavan, R. Saskatchewan and M.M. Dronamraju, Removal of Copper, Nickel and Zinc from Wastewater by Adsorption Using Peat, J. Environ. Sci. Health, A28 (1993) 1261-1276.
 10. J.R. Lujan, D.W. Darnall, P.C. Stark, G.D. Rayson and J.L. Gardea-Torresdey, Metal Ion Binding by Algae and Higher Plant Tissues: A Phenomenological Study of Solution pH Dependence, Solvent Extr. Ion Exch., 12 (1994) 803-816.
 11. S.K. Jain, P. Vasudevan and N.K. Jha, Azolla Pinnata R. Br. and Lemna Minor L. For Removal of Lead and Zinc from Polluted Water, Wat. Res., 24 (1990) 177-183.
 12. P.J. Jackson, C.J. Unkefer, J.A. Doolen, K. Watt and N.J. Robinson, Poly (γ -glutamylcysteinyl) Glicine: Its Role in Cadmium Resistance in Plant Cells, Proc. Natl. Aca. Sci., 84 (1987) 6619-6623.
 13. C.D. Scott, Removal of Dissolved Metals by Plant Tissue, Biotechnol. and Bioeng., 39 (1992) 1064-1068.
 14. R.E. Joost, C.S. Hoveland, E.D. Donnelly and S.L. Fales, Screening *Sericea Lespedeza* for Aluminum Tolerance, Crop Science, 26 (1986) 1250-1251.
 15. V. Romheld and H. Marschner, Mechanism of Iron Uptake by Peanut Plants, Plant Physiol., 71 (1983) 949-954.
 16. G. Micera and A. Dessi, Chromium Adsorption by Plant Roots and Formation of Long-Lived Cr(V) Species: An Ecological Hazard?, J. Inorg. Biochem., 34 (1988) 157-166.
 17. H. Tan, J.T. Champion, J.F. Artiola, M.L. Brusseau and R.M. Miller, Complexation of Cadmium by a Rhamnolipid, Environ. Sci. Technol., 26 (1994) 2402-2406
 18. E. Delhaize, P.J. Jackson, L.D. Lujan and N.J. Robinson, Poly (γ -glutamylcysteinyl) Glicine Synthesis in *Datura innoxia* and Binding with Cadmium, Plant Physiol., 89 (1989) 700-706.

19. H. Lue-Kim and W.E. Rauser, Partial Characterization of Cadmium-Binding Protein from Roots of Tomato, *Plant Physiol.*, 81 (1986) 896-900.
20. J.L. Gardea-Torresdey, M.K. Becker-Hapak, J.M. Hosea and D.W. Darnell, Effect of Chemical Modification of Algal Carboxyl Groups on Metal Ion Binding, *Environ. Sci. Technol.*, 24 (1990) 1372-1378.
21. L.J. Cajuste, R. Carrillo G., E. Cota and R.J. Laird, The Distribution of Metals from Wastewater in the Mexican Valley of Mezquital, *Water, Air and Soil Pollution*, 57-58 (1991) 763-771.
22. R. Carrillo G. and L.J. Cajuste, Heavy Metals in Soils and Alfalfa (*Medicago sativa* L.) Irrigated with Three Sources of Wastewater, *J. Environ. Sci. Health*, A27 (1992) 1771-1783.
23. V.C. Baligar, T.A. Campbell and R.J. Wright, Differential Responses of Alfalfa Clones to Aluminum-Toxic Acid Soil, *J. Plant Nutrition*, 16 (1993) 219-233.
24. J.S. Angle and R.L. Chaney, Heavy Metal Effect on Soil Populations and Heavy Metal Tolerance of *Rhizobium Meliloti*, Nodulation, and Growth of Alfalfa, *Water, Air, and Soil Pollution*, 57-58 (1991) 597-604.
25. J.E. Rechcigl, R.B. Reneau Jr. and L.W. Zelazney, Soil Solution Al as a Measure of Al Toxicity to Alfalfa in Acid Soils, *Commun. in Soil Sci. Plant Anal.*, 19 (1988) 989-1001.
26. P.W.G. Sale, D.I. Couper, P.L. Cachia and P.J. Larkin, Tolerance to Manganese Toxicity Among Cultivars of Lucerne (*Medicago sativa* L.), *Genetic Aspects of Plant Mineral Nutrition* (1993) 45-52.
27. Alfalfa Germplasm in the United States, Genetic Vulnerability, Use, Improvement, and Maintenance, Technical Bulletin Number 1571, USDA, November 1977.
28. D.K. Huel-Yang and G.D. Rayson, Luminescence Study of UO_2^{2+} Binding to Immobilized *Datura innoxia* Biomaterial, *App. Spectroscopy*, 47 (1993) 44-51.
29. J.L. Gardea-Torresdey, K.J. Tiemann, J.H. Gonzalez, J.A. Henning and J.H. Gonzalez, Uptake of Copper Ions from Solution by Different Populations of *Medicago Sativa*, Solvent Extraction and Ion Exchange, (1995) submitted.
30. S. Hunt, In: H. Eccles and S. Hunt (Eds.), *Immobilization of Ions by Biosorption*, Ellis Horwood Limited, Chichester, England, 1986.
31. I.H. Segel, *Biochemical Calculations*, John Wiley and Sons Inc., New York, 2nd ed., 1976.