ELSEVIER

Journal of Hazardous Materials B69 (1999) 41–51

Journal of Hazardous Materials

www.elsevier.nl/locate/jhazmat

Effects of chemical competition for multi-metal binding by *Medicago sativa* (alfalfa)

J.L. Gardea-Torresdey [a,b,*], K.J. Tiemann [a,b], G. Gamez [a], K. Dokken [a]

[a] *Department of Chemistry, University of Texas at El Paso, El Paso, TX 79968, USA*
[b] *Environmental Sciences and Engineering Program, University of Texas at El Paso, El Paso, TX 79968, USA*

Received 24 December 1998; received in revised form 8 April 1999; accepted 19 April 1999

Abstract

Alfalfa shoot biomass has demonstrated the ability to bind an appreciable amount of cadmium(II), chromium(III), copper(II), lead(II), nickel(II), and zinc(II) separately from aqueous solutions. Since most heavy metal contaminated waters contain more than one heavy metal ion, it was necessary to determine the binding abilities of the alfalfa biomass with multi-metal solutions. Batch laboratory experiments were performed with a solution containing 0.1 mM of each of the following metal ions: cadmium(II), chromium(III), copper(II), lead(II), nickel(II), and zinc(II). We determined the pH profile, time dependency, and binding capacity by the alfalfa biomass of each metal ion under multi-elemental conditions. For all the metal ions studied, the alfalfa biomass showed to have a high affinity for metal binding around pH 5.0 within a time period of approximately 5 min. The binding capacity experiments showed that there was a preferential binding of the metal ions from the multi-elemental solution with the following amounts of metal ion bound per gram of biomass: 368.5 μmol/g for copper(II), 215.4 μmol/g for chromium(III), 168.0 μmol/g for lead(II), 56.9 μmol/g for zinc(II), 49.2 μmol/g for nickel(II), and 40.3 μmol/g for cadmium(II). Reacting the biomass from the capacity experiments with 0.1 M HCl resulted in 90% or greater recovery of bound cadmium, copper, lead, nickel, and zinc. However, only 44% of the bound chromium was recovered. These experiments show the ability of *Medicago sativa* (alfalfa) to bind several metal ions under multi-contaminant conditions. Similar results were obtained when the experiments were performed under flow conditions using silica-immobilized alfalfa biomass. Chromium bound on the silica-immobilized biomass was also difficult to be desorbed with 0.1 M HCl. The information obtained will be useful for the future

* Corresponding author. Tel.: +1-915-747-5359; fax: +1-915-747-5748; e-mail: jgardea@utep.edu

PII: S0304-3894(99)00057-6

development of an innovative technology to remove heavy metal contaminants from polluted ground waters.

Keywords: Mix metal solutions; Heavy metal binding; Phytofiltration; Alfalfa; Multi-element

1. Introduction

The presence of toxic heavy metal ions in surface and ground waters 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. The effluents from these industries contain an array of heavy metal ions 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 [1]. Once in the environment, heavy metal ions naturally concentrate into wetlands and soils, which may leach into ground waters and eventually affect human health [2]. Because of this concern regarding heavy metal contamination, there has been an abundance of interest in remediation of heavy metal ions from the environment [3–8]. 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 [9]. In fact, many studies have been performed with bacteria, algae, and fungi to determine the abilities of these biomasses to adsorb metal ions [10–15]. More recently, plants have been of interest for their unique ability to bind heavy metals and phytoremediate contaminated areas [16]. Both tomato and tobacco plants have been studied by Lue-Kim and Rauser [17] and Scott [18] for their metal uptake abilities. Romheld and Marschner [19] studied the metal binding by peanuts, and Delhaize et al. [20] studied cadmium binding in *Datura innoxia*. Lujan et al. [21] found that metal binding by higher plant tissues was pH dependent. 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 [22–25]. Gardea-Torresdey et al. [26–29,31], Peterson [30] and Tiemann et al. [32] have shown that alfalfa is a potential source of biomaterials for the removal and recovery of heavy metal ions. 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. Single element binding is good for some circumstances, however, 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 ion. Therefore, it was necessary to determine the metal binding abilities of the alfalfa biomass with multi-metal containing solutions.

The objective of this study was to investigate the binding affinities of each metal ion in a mixed metal solution 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. These experiments will determine the ability of *M. 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 ground waters.

2. Methodology

2.1. Alfalfa collection

Alfalfa plants were collected from field studies conducted at New Mexico State University near Las Cruces, NM. 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 1 week. Dried samples were then ground to pass through a 100-mesh screen using a Wiley mill.

2.2. pH profile studies for metal binding

This experiment was carried out using the pH profile method previously reported by Gardea-Torresdey et al. [27]. 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 multi-metal solution was prepared from the corresponding salts: $Cd(NO_3)_2$, $CuSO_4$, $Cr(NO_3)_3$, $Pb(NO_3)_2$, $Ni(NO_3)_2$, and $ZnCl_2$. At each pH, the multi-metal solution was added to the respective pH biomass pellet. The biomass concentration was maintained at 5 mg biomass/ml of multi-metal solution. This experiment was carried out three times to maintain quality control. All the tubes were equilibrated on a rocker for 1 h. The samples were then centrifuged at 3000 rpm for 5 min 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.

2.3. Time dependence studies for metal binding

The time dependence batch experiments were performed using a procedure reported previously by Gardea-Torresdey et al. [27]. The multi-metal solution was prepared

containing 0.3 mM of each of the following metal ions: cadmium(II), copper(II), chromium(III), lead(II), nickel(II), and zinc(II). The solution was adjusted to pH 5.0 in a 0.01 M sodium acetate buffer 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 min. A biomass concentration of approximately 5 mg/ml of multi-metal solution was maintained. This experiment was carried out three times to maintain quality control. Final pHs for all samples were recorded and metal concentrations were determined by flame atomic absorption spectroscopy.

2.4. 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 [27]. 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/ml of multi-metal solution. After equilibration for 10 min, the samples and controls were centrifuged and the decanted supernatants were stored for metal analysis, while 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.

2.5. 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. [27]. The resulting supernatant solutions were collected for analyses and diluted as required to stay within the calibration range. The pellets were then exposed to 1 M HCl to remove any remaining metals and equilibrated by rocking for 5 min. After centrifugation, the supernatant solutions were analyzed. All metal analyses were performed by flame atomic absorption spectroscopy.

2.6. Immobilized alfalfa biomass and column experiments

The immobilization of the Malone alfalfa biomass was performed as indicated previously by Gardea-Torresdey et al. [26,28]. 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 *M. sativa* [26,28]. Seventy-five milliliters 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. Once the solution was at pH 2.0, 5 g of washed biomass were added to the silica solution and allowed to stir for 15 min. The pH was then raised slowly by addition of 6% Na_2SiO_3 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 mortar and pestle and finally sieved. A

particle size of 20–40 mesh was used to pack the columns. 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/min.

2.7. Recovery of metal ions from columns

To remove the bound metals from the immobilized Malone alfalfa shoots, 10 bed volumes of 0.1 M HCl were passed through the column at a flow rate of 2 ml/min. 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.

2.8. 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 nm; 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. Confidence intervals of 95% were calculated for each set of samples to determine the margin of error. The difference between the initial metal ion concentration and the remaining metal ion concentration was assumed to be bound to the biomass.

3. Results and discussion

Since contaminated waters with metal ions have been observed at various pHs, the effects of pH on metal binding by alfalfa biomass was studied. Fig. 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. As can be seen from Fig. 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

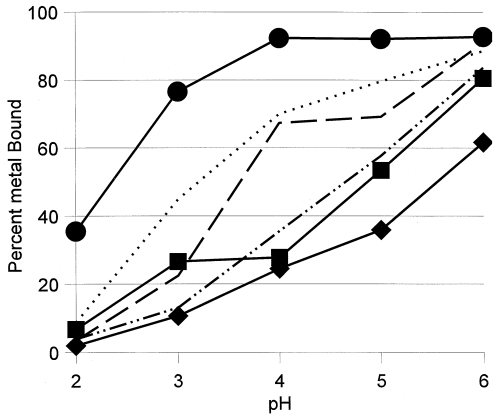

Fig. 1. Percent of each metal bound by the alfalfa biomass after 1 h equilibration with a multi-metal solution at different pH values. The solution contained 0.1 mM of the following metals: Cd (■), Cr (---), Cu (· · ·), Ni (♦), Pb (●), Zn (-· ·-).

carboxyl ligands at higher pH values [27]. Also, as seen in Fig. 1, the metal ions showed different affinities for binding to the biomass at different pH values. Furthermore, simply changing the pH of the solution could be employed to selectively bind certain metals out of the multi-ion solution. For example, lead(II) ions can be separated from the other metal ions at pH 2.0, since nearly 40% of the lead(II) is bound at this pH. On the other hand, very low percentages of the other metal ions are bound at pH 2.0. This could allow for separation of metal ions from multi-metal containing solutions. This trend in pH dependent metal binding corresponds to the data observed by Lujan et al. [21]. This behavior has also been observed by the alfalfa biomass when the experiment was conducted with single metal solutions [26–29].

In addition to knowing the optimal pH of metal ion binding by the alfalfa biomass, it was also necessary to determine reaction times required for metal ion binding. Fig. 2 represents the percentage of metal bound by the alfalfa biomass at different time exposures to the multi-metal solution. As shown in Fig. 2, the metal binding is relatively stable over the 90-min period of reaction time, with the exception of chromium(III). Also, Fig. 2 shows that the metal binding is rapid, since metal binding occurred within the first 5 min. 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 should not be through an active process.

In order to better understand how these metal ions compete for binding sites on the alfalfa biomass, batch binding capacities were performed. 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 40.3 μmol/g for cadmium(II) to as high as 368.5 μmol/g for copper(II). As seen in Table 1, the μmol units show that there is an affinity for the metal

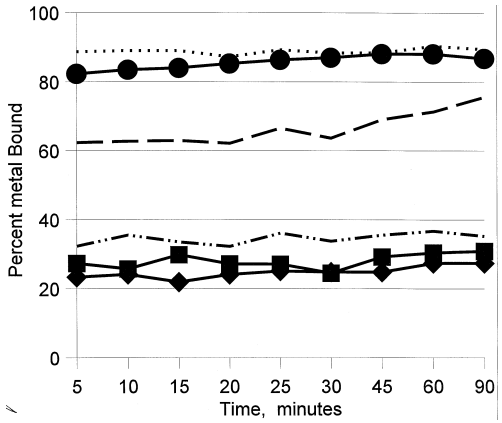

Fig. 2. Percent of each metal bound by the alfalfa biomass after equilibration for different times with a multi-metal solution at pH 5.0. The solution contained 0.3 mM of the following metals: Cd (■), Cr (- - -), Cu (· · ·), Ni (♦), Pb (●), Zn (-· · -).

ions in the order of Cu(II) > Cr(III) ≈ Pb(II) > Zn(II) > Ni(II) ≈ Cd(II). The difference in the metal affinity may be due to the metal binding affinities for the same ligand and/or for different ligands. Since the metals are all in solution, they are in competition with each other for the available binding ligands. However, the binding affinities observed resemble, in some aspect, the Irvin–Williams sequence where copper has a higher coordination affinity with oxygen containing ligands [33]. 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. Nevertheless, 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 copper(II), chromium(III), and lead(II) over the other metals studied. Similar findings for metal binding to carboxylic acids were observed by Peterson [30]. This further indicates the importance of the carboxyl ligand for metal binding by the alfalfa biomass.

As can be seen in Fig. 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.

Table 1
Batch laboratory adsorption saturation capacities for metal binding by alfalfa shoot biomass

Adsorption capacities		
	μmol metal/g biomass	95% C.I.
Cu(II)	368.5	±17.9
Cr(III)	215.4	±21.5
Pb(II)	168.0	±12.5
Zn(II)	56.9	±8.9
Ni(II)	49.2	±7.0
Cd(II)	40.3	±9.7

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

Metal desorption		
	% Metal recovery	95% C.I.
Cu(II)	98.7	±0.6
Cr(III)	43.8	±5.9
Pb(II)	96.1	±7.3
Zn(II)	99.8	±2.4
Ni(II)	90.4	±2.4
Cd(II)	94.9	±2.5

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 0.1 M HCl. Table 2 demonstrates that the recovery of the metal ions was very good by treatment with dilute acid, with the exception of chromium. Since over 90% recoveries of the bound metals were observed for cadmium, copper, lead, nickel, and zinc from the multi-metal solutions, alfalfa biomass has shown to be very effective at adsorption and recovery of these metal ions. However, only 44% of the bound chromium was recovered by treatment with dilute acid. This may be due to the difference in oxidation state which could allow for stronger coordination with the metal binding site.

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 0.1 M HCl. As observed in Table 3, the silica-immobilized alfalfa biomass adsorbs metal ions in the following order: Cu(II) > Pb(II) ≈ Cr(III) > Zn(II) > Cd(II) ≈ Ni(II). This is similar with the batch adsorption capacity experiments described above where Cu(II), Cr(III), and Pb(II) have

Table 3
Results of column experiments performed with silica-immobilized alfalfa when a mixed-metal solution was passed. The data represents the amount of metal bound from three cycles using the same column

Metals	Cycles		
	1 (mmol bound)	2 (mmol bound)	3 (mmol bound)
Cu(II)	8.0	6.5	5.0
Pb(II)	7.7	5.8	5.3
Cr(III)	7.6	4.9	4.6
Zn(II)	4.0	2.4	2.4
Cd(II)	2.6	2.1	1.7
Ni(II)	2.4	1.8	1.8

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

Metals	Cycles		
	1 (% removed)	2 (% removed)	3 (% removed)
Cu(II)	92.9	100.0	100.0
Pb(II)	94.9	100.0	94.0
Cr(III)	46.7	58.6	44.5
Zn(II)	91.1	100.0	97.5
Cd(II)	93.3	90.8	99.5
Ni(II)	96.8	100.0	94.4

the highest affinities for binding by the alfalfa biomass (although the order slightly changed). Good recoveries were observed for all of the metal ions with the exception of Cr(III) (Table 4). In addition, it can be clearly seen that 0.1 M solution of hydrochloric acid is not enough to completely remove Cr(III). Additionally, three binding–desorption cycles were performed to determine the recyclability of the column in the presence of a solution containing six different heavy metal ions. Overall, there appears to be a decrease in the metal binding of every ion after the first cycle. However, following the second cycle, the binding seems to be more constant. This decrease in binding from the first cycle to the second is most likely due to the low recovery of chromium(III) by 0.1 M HCl. More than 50% of the bound chromium(III) remained bound to the biomass and occupied the potential metal binding sites. This further reduced the available metal binding sites where other metal ions could coordinate, hence reducing the binding capacity of the silica-immobilized alfalfa biomass. Excellent recoveries were observed for cadmium, copper, nickel, lead, and zinc. Only chromium seems to difficult to be recovered. Nonetheless, we have previously found that carboxylate ligands may be responsible for nickel(II) and chromium(III) binding in alfalfa biomass [32]. In addition, carboxylic groups have shown to play an important role in the binding of other metal ions to other biomaterials [21,30]. Thus, the only explanation for the difficulty in recovery of chromium(III) should be due to difference in oxidation state. Furthermore, it can be clearly seen that 0.1 M solution of hydrochloric acid did not completely remove the bound chromium from the alfalfa biomass. Higher HCl concentrations were tested but similar results were obtained (data not shown). Therefore, the other metal ions must compete with the bound chromium for available carboxylic ligands. If chromium is not recovered from the biomass, fewer ligands will be available and consequently reduce the overall metal binding capacity. More research is needed to ascertain the optimum conditions (and possibly other stripping agents) for the removal of the bound chromium ions.

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

Acknowledgements

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

References

[1] B.L. Carson, H.V. Ellis, J.L. McCann, Toxicology and Biological Monitoring of Metals in Humans, Lewis Publishers, Chelsea, MI, 1986, p. 65.
[2] D.D. Runnells, T.A. Shepherd, Environ. Sci. Technol. 26 (1992) 2316.
[3] K.L. Godfredsen, A.T. Stone, Environ. Sci. Technol. 28 (1994) 1450.
[4] D.A. Mays, P.M. Giordano, A.D. Behel Jr., Water, Air, Soil Pollut. 57–58 (1991) 307.
[5] R. El-Aziz, J.S. Angle, R.L. Chaney, Soil Biol. Biochem. 23 (1991) 795.
[6] M.J. Mench, J. Environ. Qual. 23 (1994) 58.
[7] N.T. Basta, M.A. Tabatabai, Soil Sci. 153 (1992) 108.
[8] W.J. Jewell, Am. Sci. 82 (1994) 366.
[9] R.M. Atlas, Chem. Eng. News 3 (1995) 32.
[10] C. Cervantes, F. Gutierrez-Corona, FEMS Microbiol. Rev. 14 (1994) 121.
[11] W. Zhang, V. Majidi, Environ. Sci. Technol. 28 (1994) 1577.
[12] W. Zhang, V. Majidi, Appl. Spectrosc. 47 (1993) 2151.
[13] J.L. Gardea-Torresdey, M.K. Becker-Hapak, J.M. Hosea, D.W. Darnall, Environ. Sci. Technol. 24 (1990) 1372.
[14] D. Chen, Z. Lewandowski, F. Roe, P. Surapaneni, Biotechnol. Bioeng. 41 (1993) 755.
[15] K. Fukushi, D. Chang, S. Ghosh, Water Sci. Technol. 34 (1996) 267.
[16] D.E. Salt, R.D. Smith, I. Raskin, Annu. Rev. Plant Physiol. Plant Mol. Biol. 49 (1998) 643.
[17] H. Lue-Kim, W.E. Rauser, Plant Physiol. 81 (1986) 896.
[18] C.D. Scott, Biotechnol. Bioeng. 39 (1992) 1064.
[19] V. Romheld, H. Marschner, Plant Physiol. 71 (1983) 949.
[20] E. Delhaize, P.J. Jackson, L.D. Lujan, N.J. Robinson, Plant Physiol. 89 (1989) 700.
[21] J.R. Lujan, D.W. Darnall, P.C. Stark, G.D. Rayson, J.L. Gardea-Torresdey, Solvent Extr. Ion Exch. 12 (1994) 803.
[22] M. El-Kherbawy, J.S. Angle, A. Heggo, R.L. Chaney, Biol. Fertil. Soils 8 (1989) 61.
[23] L.J. Cajuste, R. Carrillo, G.E. Cota, R.J. Laird, Water, Air, Soil Pollut. 57 (1991) 763.
[24] V.C. Baligar, T.A. Campbell, R.J. Wright, Plant Nutr. 16 (1993) 219.
[25] J.E. Rechcigl, R.B. Reneau Jr., L.W. Zelazney, Soil Sci. Plant Anal. 19 (1988) 989.
[26] J.L. Gardea-Torresdey, K.J. Tiemann, J.H. Gonzalez, J.A. Henning, M.S. Towsend, J. Hazard. Mater. 48 (1996) 181.
[27] J.L. Gardea-Torresdey, K.J. Tiemann, J.H. Gonzalez, J.A. Henning, M.S. Townsend, Solvent Extr. Ion Exch. 14 (1996) 119.
[28] J.L. Gardea-Torresdey, K.J. Tiemann, J.H. Gonzalez, J.A. Henning, M.S. Townsend, I. Cano-Aguilera, J. Hazard. Mater. 49 (1996) 205.
[29] J.L. Gardea-Torresdey, K.J. Tiemann, J.H. Gonzalez, O. Rodriguez, G. Gamez, J. Hazard. Mater. 57 (1998) 29.
[30] J.S. Peterson, Hydrometallurgy 14 (1985) 171.

[31] J.L. Gardea-Torresdey, K.J. Tiemann, J.H. Gonzalez, O. Rodriguez, J. Hazard. Mater. 56 (1997) 169.
[32] K.J. Tiemann, J.L. Gardea-Torresdey, G. Gamez, K. Dokken, S.M. Renner, L. Furenlid, Environ. Sci. Technol. 33 (1999) 150–154.
[33] H. Sigel, D.B. Mc Cormick, Collected accounts of transition metal chemistry, ACS Reprints 1 (1970) 224.