ADSORPTION OF COPPER IONS FROM SOLUTION BY HEAVY METAL STRESSED *LARREA TRIDENTATA* (CREOSOTE BUSH) BIOMASS

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ABSTRACT *Larrea tridentata* (creosote bush) was found naturally growing in heavy metal contaminated soils. Samples of *Larrea tridentata* were collected from seven different locations to study their ability to bind copper ions from solution. Samples from the same locations were either oven dried at 90ºC or lyophilized in order to determine differences in drying conditions. Batch laboratory experiments were conducted with the leaves, stems, and roots of *Larrea tridentata* in order to determine pH profiles, time dependencies, and total copper binding capacities. It was determined by the pH profile experiments that the optimum copper binding pH was between 5 and 6. A maximum adsorption of copper ions was observed within five minutes of reaction time for most of the biomass collected from the various sites. The copper binding capacity experiments showed that one gram of biomass can bind as high as 23.7 mg and as low as 7.6 mg of copper. The capacity to bind copper ions by the biomasses varied according to the location of the sample site. The leaves generally bound more copper than the roots and stems. The stems bound the least amount. These differences in capacities correlate with the distances from the collection site to the possible contamination source. The closer the sample to the possible source, the greater the copper binding capacity. The desorption studies showed that once bound to the creosote biomass, it was possible to remove as much as 99.9% of the bound copper.

KEYWORDS: *Larrea tridentata,* phytoremediation, copper, metal binding

INTRODUCTION

Toxic metals released into the environment affect human and plant life, as well as our water supply [1-3]. These heavy metals are released into the environment in a number of different ways. Coal combustion, sewage waste waters, automobile emissions, mining activities, and the utilization of fossil fuels are just a few examples [4-7]. These metals are taken up by plants and can cause a variety of effects on them which include: decreased uptake of nutrients, changed architecture of root systems, and decreased water uptake. An excess of copper can affect root extension, root cell elongation, and root tip mitosis [8]. In humans some trace elements have been associated with cancer and cardiovascular disease [1]. Copper in particular has been associated with stomach and intestinal distress, as well as anemia in humans. Due to these problems in the environment, there has been an increase in research geared at environmental remediation [9]. Current techniques, excavation and reburial, for cleaning up metal-contaminated soils are expensive and only used for small areas [10]. Methods for cleaning contaminated water, which include ion-exchange resins and filtration, are cost inefficient as well. An alternative to these expensive methods is the use of biological systems. Bioremediation, the usage of live organisms for the accumulation of heavy

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metals, and phytoremediation, the usage of plant materials, are emerging as inexpensive techniques for environmental clean up.

Bioremediation studies have been conducted by a number of researchers using live microbial and fungal systems in the removal of heavy metals from contaminated waters [11-16]. Although live systems work well for low amounts of metals, high levels may be so toxic to the system that the system does not survive. This was shown in an experiment conducted by Narwal and associates when working with corn and large amounts of nickel [5]. Phytoremediation experiments conducted by Gardea-Torresdey and colleagues showed that dead (inactivated) alfalfa biomass was able to bind copper effectively [17]. Other studies conducted by Gardea-Torresdey and co-workers showed that carboxyl groups on the cell wall of algae were responsible for binding copper [18]. As far back as 60 years ago it was thought that plants demonstrated copper resistance as a result of natural selection [19]. These heritable resistances occur in a small number of plants and have been shown for Zn, Pb, Cu, and some other metals [20-23]. More recently, it has been thought that tolerance may be due to a high protein content or by the evolution of chemical functional groups that inhibit the effects of the heavy metals [24]. It has been shown that living organisms induce the production of metallothioneins, which are proteins that contain large amounts of cysteine and bind heavy metal ions, in order to respond to the effects of heavy metals [25]. Some plants and yeasts employ molecules called phytochelatins. These small peptides bind metals in forms that are less toxic to the plant [10].

Due to the levels of metal tolerance in alfalfa [26-27], similar studies using *Larrea tridentata* (creosote bush), which grows in heavy metal-contaminated soils, were conducted in order to determine if this plant was also capable of removing copper ions from solution. Creosote bush was also chosen for its abundance in the Chihuahuan desert, as well as for its growth conditions. Two important soil factors in determining the growth of creosote are the presence of $CaCO_3$ and gravel. Creosote does not grow well in soils lacking these two factors [28]. Creosote does not require large amounts of water for growth and is therefore easily grown in some desert environments such as west Texas.

The purpose of this study was to investigate a plant that has never been associated with heavy metal binding abilities and that grows in a heavy metal-contaminated area. We investigated the adsorption specifically of copper ions from solution by biomass of creosote grown naturally in seven different sites, which contain varying amounts of heavy metals. Experiments were conducted to determine the pH profiles, time dependencies, and capacities of copper uptake. The optimal creosote drying conditions (oven-dried and lyophilized) were also examined. The differences in the roots, stems, and leaves were observed in order to determine the metal binding properties of *Larrea tridentata*.

METHODOLOGY

Creosote bush collection

Creosote bush samples were collected during the summer of 1995 from seven different sites in the El Paso, Texas, area. Six of these sites (Figure 1) were in close proximity of each other and were collected on The University of Texas at El Paso (UTEP) property off of Interstate-10 close to a copper smelting facility. The seventh site (Figure 1), which was approximately 25 miles east of the other sites, was used as a

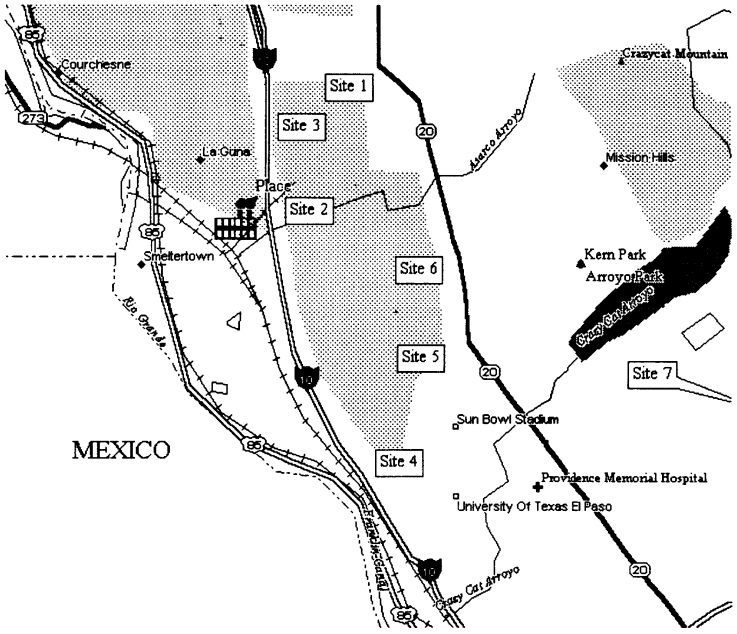

FIGURE 1. MAP OF AREA WHERE DIFFERENT CREOSOTE SAMPLES WERE TAKEN.

control due to its distance from the smelter. The plants collected were all found naturally growing.

Three plants were removed from each site and they all shared similar characteristics such as height (approximately 3 feet) and maturity. Once in the laboratory the plants were washed with deionized water and separated into roots, stems, and leaves. The samples were oven dried at 90ºC for four days, while site 1 and site 7 samples were also lyophilized. Dried samples were ground using a mill and passed through a 100-mesh screen.

pH profile studies for copper binding

Batch laboratory methods were employed for pH studies. A 250 mg sample of biomass was weighed and washed twice with 0.01 M HCl to remove any debris or soluble biomolecules that might interact with copper ions. Both washings were collected, dried, and weighed to account for any biomass weight loss. The biomass sample was then resuspended in 50 ml of 0.01 M HCl (tissue concentration 5 mg/ml). The pH was adjusted to 2.0, allowed to equilibrate, and the suspension was added to three 5 ml plastic tubes in 2 ml aliquots. The pH was then adjusted and allowed to equilibrate at pH 3.0, 4.0, 5.0, and 6.0. At each pH, 2 ml aliquots of the suspensions were transferred into three tubes for each pH. The suspensions were centrifuged at 2,500 rpm for 5 minutes and the supernatants were separated and kept for testing to determine if soluble materials were involved in the binding of copper. A solution of 0.1 mM copper (6.3 ppm) 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 appropriate pH biomass pellet, separated supernatant, and into three clean tubes for controls. All the tubes were allowed to equilibrate for 1 hour on a rocker. The samples were then centrifuged at 2,500 rpm for 5 minutes, and the supernatants for the pellets were transferred to clean

respective tubes. The final pHs for all tubes were recorded, and analysis for copper was conducted by flame atomic absorption.

Time dependence studies for copper binding

A 250 mg sample of biomass was weighed and washed twice with 0.01 M HCl in order to remove any debris or soluble biomolecules that might interact with copper ions. Both washings were collected, dried, and weighed to account for any biomass weight loss. The biomass sample was then resuspended in 50 ml of deionized water (tissue concentration 5 mg/ml). The pH was then adjusted to 5.0 and allowed to equilibrate. Two ml of the suspension were transferred to 21 tubes; three tubes for each time interval of 5, 10, 15, 30, 60, 90, and 120 minutes. After centrifugation and decantation, 2 ml of 0.3 mM (18.9 ppm) copper solution buffered with 0.01 M sodium acetate was added to each of the tubes and controls. This was also done for one site using an unbuffered solution of 0.3 mM copper at pH 5.0. All the tubes were equilibrated by rocking and were removed at each appropriate time interval. The samples were then centrifuged at 2,500 rpm for 5 minutes, and the supernatants from the pellets were transferred to clean respective tubes. The final pHs for all tubes were recorded, and analysis for copper was conducted by flame atomic absorption.

Copper binding capacity studies

Samples of 50 mg of biomass were washed twice with 0.01 M HCl, and washings were collected and weighed to determine the amount of biomass loss. The washed biomass was resuspended in 10 ml of deionized water and the pH adjusted to 5.0. Two ml of the suspension were transferred to each of three tubes and then centrifuged. Each of the supernatants was saved for testing. Two ml of 0.3 mM copper solution buffered with 0.01 M sodium acetate was added to each of the tubes and controls. This was also done for two of the sites using an unbuffered solution of 0.3 mM copper at pH 5.0. After equilibration for 15 minutes, the tubes were centrifuged and the decanted supernatants were stored for copper analysis and again 2 ml of 0.3 mM copper solution was added to each tube. This was repeated for a total of seven times or until the saturation point was achieved and a final pH for all tubes was recorded. Samples were diluted as required in order to stay within the calibration linear range, and copper analysis was performed by flame atomic absorption.

Desorption of the adsorbed copper

Pellets from the capacity studies with adsorbed copper were exposed to 2 ml of 0.1 M HCl, equilibrated by rocking for 15 minutes, and then centrifuged. Supernatants were then collected for analysis and diluted if needed to stay within the calibration range. Pellets were then again exposed to 2 ml of 0.1 M HCl to strip any remaining copper and equilibrated by rocking for 15 minutes. After centrifugation, the supernatants were analyzed and diluted if necessary. All analyses for copper were performed by flame atomic absorption.

Analytical procedure

Analysis for copper was performed using a Perkin Elmer model 3110 Atomic Absorption Spectrometer with deuterium background subtraction. The sensitivity was improved by utilizing the impact bead. The wavelength used for copper for maximum absorbance was 327.8 nm. Each sample was 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

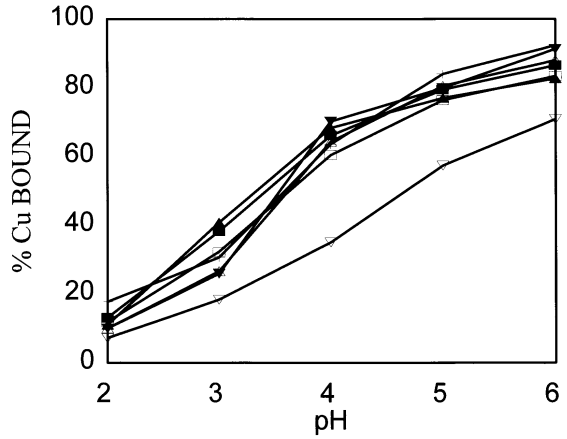

FIGURE 2. EFFECT OF pH ON COPPER BINDING BY CREOSOTE ROOTS GROWN AT VARIOUS SITES (SITE 1■, SITE 2❒, SITE 3▲, SITE 4Δ, SITE 5▼, SITE 6▽, SITE 7✛). THE BIOMASS (5 mg/ml) WAS EQUILIBRATED FOR 1 HOUR WITH 0.1 mM COPPER (6.3 ppm) AT EACH pH.

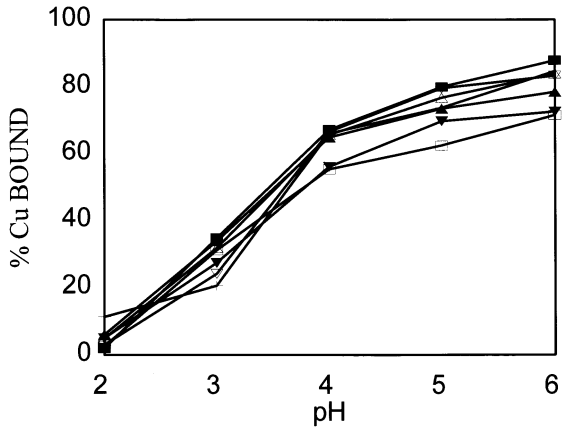

FIGURE 3. EFFECT OF pH ON COPPER BINDING BY CREOSOTE STEMS GROWN AT VARIOUS SITES (SITE 1■, SITE 2❒, SITE 3▲, SITE 4Δ, SITE 5▼, SITE 6▽, SITE 7✛). THE BIOMASS (5 mg/ml) WAS EQUILIBRATED FOR 1 HOUR WITH 0.1 mM COPPER (6.3 ppm) AT EACH pH.

obtained. The instrument response was periodically checked with known copper standards.

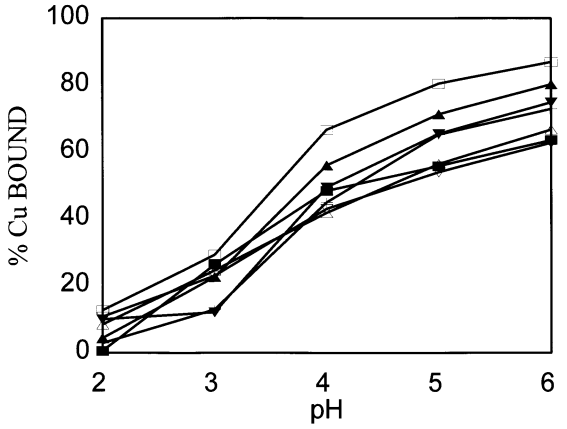

FIGURE 4. EFFECT OF pH ON COPPER BINDING BY CREOSOTE LEAVES GROWN AT VARIOUS SITES (SITE 1■, SITE 2❒, SITE 3▲, SITE 4Δ, SITE 5▼, SITE 6▽, SITE 7✛). THE BIOMASS (5 mg/ml) WAS EQUILIBRATED FOR 1 HOUR WITH 0.1 mM COPPER (6.3 ppm) AT EACH pH.

Data analysis

The experiments were performed in replicates of three and the samples were analyzed in replicates of three as well. For each set of data present, standard statistical methods were used to determine the mean values and standard deviations. Confidence intervals of 95% were calculated for each set of samples in order to determine the margin of error.

RESULTS AND DISCUSSION

Figures 2, 3, and 4 show the pH profile data for copper binding by the roots, stems, and leaves, respectively. These figures show the copper binding to be similar for all three components of the plant. The roots show the highest binding and leaves the lowest binding. Roots from site 7 (Figure 2) show the highest binding ability compared to the other sites. This was the control site, which was farthest from the smelter, and the plants grew in soil that contained the lowest

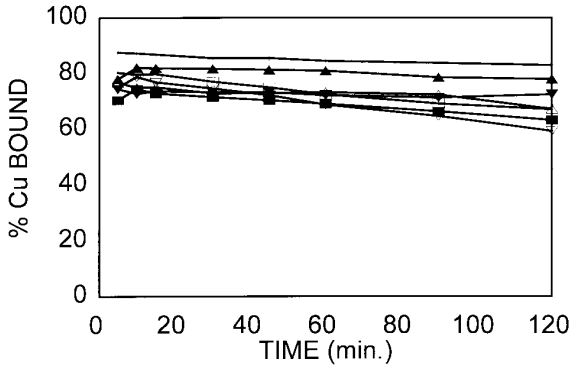

FIGURE 5. TIME DEPENDENCIES FOR COPPER BINDING BY CREOSOTE ROOTS GROWN AT VARIOUS SITES (SITE 1■, SITE 2❒, SITE 3▲, SITE 4Δ, SITE 5▼, SITE 6▽, SITE 7✛). THE BIOMASS WAS EQUILIBRATED FOR EACH TIME INTERVAL WITH 0.3 mM COPPER (18.9 ppm).

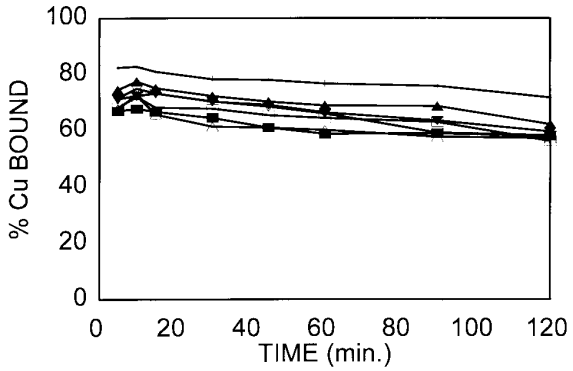

FIGURE 6. TIME DEPENDENCIES FOR COPPER BINDING BY CREOSOTE STEMS GROWN AT VARIOUS SITES (SITE 1■, SITE 2❒, SITE 3▲, SITE 4Δ, SITE 5▼, SITE 6▽, SITE 7✛). THE BIOMASS WAS EQUILIBRATED FOR EACH TIME INTERVAL WITH 0.3 mM COPPER (18.9 ppm).

amounts of copper. It was also shown by these experiments that the amount of copper bound is dependent on the solution pH. The maximum binding for most of the plant samples occurred between pH 5.0 and 6.0. pH 6.0 was the highest pH used because at a pH higher than 6.0 the copper ions may begin to precipitate out of solution. It has been proposed that the groups responsible for metal binding are carboxyl groups, which have pka's between 3 and 4 [29-30]. The large increase in binding (Figures 2, 3, and 4) that occurred at pH 4.0 as compared to pH 3.0 occurred because at pH 3.0 the carboxyl groups are still protonated and over pH 4.0 they are deprotonated and negatively charged. This negative charge attracts positively-charged copper ions, and binding occurs. This happens through an ion exchange mechanism. The final pH's measured were lower than the starting pH's. This occurred because the pH was higher than 4.0 and the binding groups began to lose their protons. Therefore, the solution became more acidic with the higher concentration of H^+ ions. The lyophilized samples for sites 1 and 7 (not shown) displayed similar binding properties as the oven-dried samples. The oven-dried samples were used for the rest of the experiments because they were more easily prepared than the lyophilized samples.

The time dependency studies showed the amount of time needed for maximum copper binding to occur. These experiments were conducted at pH 5.0, in 0.01 M sodium acetate buffer. The time was relatively consistent for each site and the greatest binding usually occurred within 5 minutes (Figures 5, 6, and 7), although sometimes it took as much as 15 minutes to reach the optimum binding. The roots and stems of site 7 displayed better binding of copper than any of the other sites. All sites displayed similar trends in their copper binding ability as well as in their ability to release the bound copper. After two hours of reaction time, copper binding decreased and copper was being removed from the biomass. The cause of this is unclear, but it may have occurred due to the buffer used. The acetate may have been complexing with the copper ions bound, therefore causing them to be released

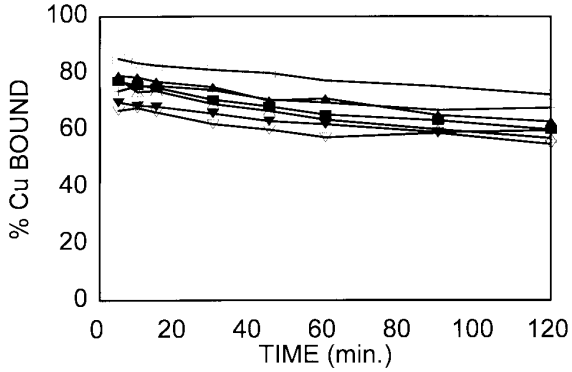

FIGURE 7. TIME DEPENDENCIES FOR COPPER BINDING BY CREOSOTE LEAVES GROWN AT VARIOUS SITES (SITE 1■, SITE 2❒, SITE 3▲, SITE 4Δ, SITE 5▼, SITE 6▽, SITE 7✛). THE BIOMASS WAS EQUILIBRATED FOR EACH TIME INTERVAL WITH 0.3 mM COPPER (18.9 ppm).

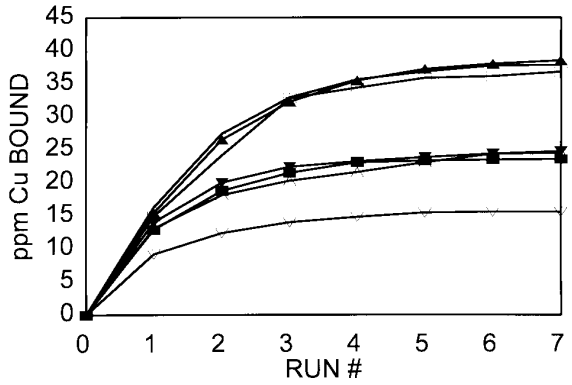

FIGURE 8. COPPER BINDING CAPACITIES FOR CREOSOTE ROOTS GROWN AT VARIOUS SITES (SITE 1■, SITE 2❒, SITE 3▲, SITE 4Δ, SITE 5▼, SITE 6▽, SITE 7✛). THE BIOMASS WAS EQUILIBRATED FOR 15 MINUTES SEVEN DIFFERENT TIMES WITH 0.3 mM COPPER (18.9 ppm).

from the plant biomass. The unbuffered site (not shown) examined showed considerably less binding than when it was buffered. For this reason all of the experiments conducted were buffered. Due to the fact that the creosote bush biomass was inactivated, it can be assumed that the cell wall functional groups were responsible for the binding.

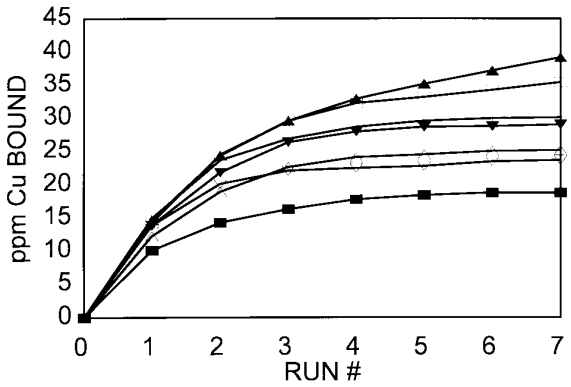

FIGURE 9. COPPER BINDING CAPACITIES FOR CREOSOTE STEMS GROWN AT VARIOUS SITES (SITE 1■, SITE 2❒, SITE 3▲, SITE 4Δ, SITE 5▼, SITE 6▽, SITE 7✛). THE BIOMASS WAS EQUILIBRATED FOR 15 MINUTES SEVEN DIFFERENT TIMES WITH 0.3 mM COPPER (18.9 ppm).

Similar studies have shown similar results [31].

The copper binding capacity experiments were performed at pH 5.0 for 15-minute time intervals to ensure maximum binding. Seven runs were performed in order to saturate the biomass. As far as binding capacity, site 3 was the best for all three categories (Figures 8, 9, and 10). The leaves bound as much 45 ppm of copper after seven runs. Both the stems and roots bound close to 40 ppm. The trends in the amount of copper bound varied greatly. Some sites became saturated at low concentrations of copper, whereas other sites were still binding copper after high concentrations were bound (Figures 8, 9, and 10). Table 1 shows the mg of copper bound per gram of biomass. Site 3 had the highest amounts of copper bound for the leaves and roots and was second for stems. Site 3 was one of the sites closest to the smelter and, due to its exposure to copper, it is possible that it may have developed more binding sites and therefore a higher capacity. Site 2, which was also in

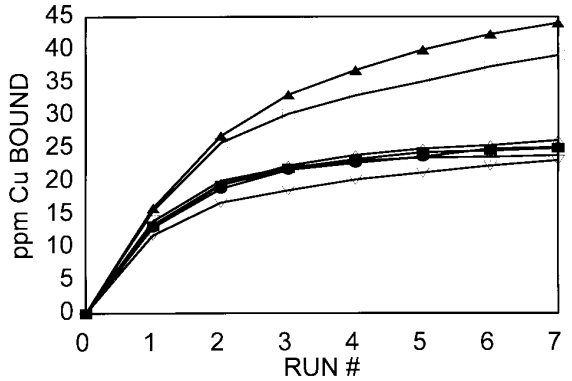

FIGURE 10. COPPER BINDING CAPACITIES FOR CREOSOTE LEAVES GROWN AT VARIOUS SITES (SITE 1■, SITE 2❒, SITE 3▲, SITE 4Δ, SITE 5▼, SITE 6▽, SITE 7✛). THE BIOMASS WAS EQUILIBRATED FOR 15 MINUTES SEVEN DIFFERENT TIMES WITH 0.3 mM COPPER (18.9 ppm).

close proximity to the smelter, had high capacities as well. Due to their high capacities to remove copper ions out of solution, these two sites may be useful in the removal of copper ions from contaminated water. The two sites which were also tested with the unbuffered copper solution were sites 1 and 2. They both bound half as much copper as their respective buffered experiments and their mg Cu/g biomass were also decreased by almost half. The reason this may have occurred is due to the fluctuation in pH. Their pHs went as high as 6.63 and as low as 4.33.

Once the creosote biomass was saturated with copper, we wanted to observe if the copper ions could be effectively removed by lowering the pH below the carboxyl group's pka (3-4). Below this pka the copper ions should be displaced by protons (H^+). We treated the copper-saturated creosote biomass with low concentrated acid solution in order to displace the copper ions. We chose 0.1 M HCl for both treatments because we found that 1.0 M HCl denatured the creosote biomass. The maximum recovery was as high as 99.9% for two of the sites, and the lowest amount of recovery was 75.7%. The average recovery for the copper ions was 91.2% (data not shown). These high recovery rates indicate that once the copper ions are bound, they can be effectively removed without damaging the plant tissues. This is important since the bound copper can be recycled for other uses.

In our original hypothesis we predicted that the closer the site to the source of contamination the greater the binding of the biomass would be. This held true for some of the sites but not for all of them. The biomass of site 4, which was found growing in soils which contained the highest amounts of copper, was an example of this. It did not bind copper as well as site 7 (control site),

TABLE 1. CAPACITY FOR COPPER UPTAKE BY CREOSOTE TISSUES GROWN AT VARIOUS SITES.

Sites		mg copper/g biomass
1	(ROOTS)	11.8
	(STEMS)	10.7
	(LEAVES)	14.7
2	(ROOTS)	18.9
	(STEMS)	18.2
	(LEAVES)	21.8
3	(ROOTS)	18.7
	(STEMS)	21.7
	(LEAVES)	23.7
4	(ROOTS)	11.4
	(STEMS)	14.0
	(LEAVES)	13.4
5	(ROOTS)	15.9
	(STEMS)	15.6
	(LEAVES)	15.8
6	(ROOTS)	7.6
	(STEMS)	12.2
	(LEAVES)	15.0
7	(ROOTS)	18.3
	(STEMS)	15.1
	(LEAVES)	16.4

which was the farthest site from the source of contamination. As mentioned earlier, site 7 was found to be very low in copper. We believe that the reason this occurred was because the copper bound to the cells in site 4 was bound very tightly and some of its binding sites were still occupied even after being washed with the 0.01 M HCl. Other studies have shown similar results in the removal of copper [1]. The binding sites in site 7 were unoccupied and they were therefore able to bind more copper than site 4. Future studies will concentrate on finding an agent that is capable of removing tightly bound copper from the plant tissue, while avoiding damage to the biomass. Other future studies will include column experiments for copper binding by the leaves, stems, and roots from the various locations, as well as binding abilities for other metals.

CONCLUSIONS

Larrea tridentata's cost and ability to remove copper ions from solution demonstrate its potential to be useful in the environment for the clean-up of contaminated waters. In general, most of the plant tissues of the various sites bound the copper ions within 5 minutes and were shown to do this well at pH 5.0. Once the binding sites were stripped from heavy metals, the biomass showed a high capacity for the copper ions before it became fully saturated. The removal of the copper bound from solution to the biomass was performed efficiently with low concentrated acid; therefore the copper could be recycled and reused for other purposes. The results of this study indicate the possibilities that exist in the clean-up of the environment with the use of natural resources.

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REFERENCES

1. R.H. Maier, S.M. Purser, J.T. Bray, and W.J. Pories, Trace metal characterization of some standard smokeless tobaccos, Trace Elements in Medicine, 10 (1993) 48-53.

2. T.C. Broyer, C.M. Johnson, and R.E. Paul, Some aspects of lead in plant nutrition, Plant Soil, 36 (1972) 301-313.

3. H. Loren, Binding forms of toxic heavy metals, mechanisms of entrance of heavy metals into the food chain and possible measures to reduce levels in foodstuff, Exotoxicol. Environ. Safety, 3 (1979) 47-58.

4. P.J. Peterson and C.A. Girling, Other trace metals, In: N.W. Lepp (Ed.), Effects of Heavy Metal Pollution on Plants, vol. 1, Applied Science Publishers, London, 1981, pp. 213-279.

5. R.P. Narwal, M. Singh, and J.P. Singh, Effect of nickel enriched sewage water on the accumulation of nickel and Other heavy metals in corn, J. Indian Soc. Soil Sci., 39 (1991) 123-128.

6. A. Fala-Ardakan, Contamination of environment with heavy metals emitted from automotives, Exotoxicol. Environ. Safety, 8 (1984) 152-161.

7. W. Thomas, A. Rohling, and H. Simon, Accumulation of airborne pollutants (PAH, chlorinated hydrocarbons, heavy metals) in various plant species and

humu, Environ. Pollut., Ser A. 36 (1984) 295-310.

8. H. Kahle, Response of roots of trees to heavy metals, Environmental and Experimental Botany, 33 (1993) 99-119.

9. 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.

10. A.S. Moffa, Plants proving their worth in toxic metal cleanup, Science, 269 (1995) 302-305.

11. C. Cervantes and F. Gutierrez-Corona, Copper resistance mechanisms in bacteria and fungi, FEMS Microbiol. Rev., 14 (1994) 121-138.

12. W. Zhang and V. Majidi, Monitoring the cellular response of *Stichococcus bacillaris* to exposure of several different metals using in vivo ^{32}P NMR and other spectroscopic techniques, Environ. Sci. Technol., 28 (1994) 1577-1581.

13. J. Bender, S. Rodriguez-Eaton, U.M. Ekanemesang, and P. Phillips, Characterization of metal-binding bioflocculants produced by the cyanobacterial component of mixed microbial ma, App. Environ. Microbiol., 60 (1994) 2311-2315.

14. C.R.N. Rao, L. Iyengar, and C. Venkobachar, Sorption of copper(II) from aqueous phase by waste biomass, J. Environ. Eng., 119 (1993) 369-377.

15. L. De Rome and G.M. Gadd, Use of pelleted and immobilized yeast and fungal biomass for heavy metals and radionuclide recovery, J. Ind. Microbiol., 7 (1991) 97-104.

16. G.J. Ramelow, L. Liu, C. Himel, D. Franlick, 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, Inter. J. Anal. Chem., 53 (1993) 219-232.

17. J.L. Gardea-Torresdey, K.J. Tiemann, J.H. Gonzalez, J.A. Henning, and M.S. Towsend, Ability of *Medicago sativa* (alfalfa) to remove copper ions from solution, J. of Hazardous Material, 48 (1996) 181-190.

18. 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.

19. W.H.O. Ernst, J.A.C. Verkleij, and H. Schat, Metal tolerance in plants, Acta Bot. Neerl., 41 (1992) 229-248.

20. W. Bröker, Genetisch-physiologische Untersuchungen in Silene inflata Sm., Inaugural-Dissertation, Universität Münster, 1962.

21. L. Wu and J. Antonovics, Experimental ecological genetics in *Plantago*. II. Lead tolerance in *Plantago lanceolata* and *Cynodon dactylon* from a roadside, Ecology, 57 (1976) 205-208.

22. M.R. Macnair, The genetic control of copper tolerance in the yellow monkey flower *Mimulus guttatus*, Heredity, 50 (1983) 283-293.

23. A.D. Watkins and M.R. Macnair, Genetics of arsenic tolerance in *Agrostis capillaris L.*, Heredity, 66 (1991) 47-54.

24. Alfalfa Germaplasm in The United States, Genetic Vulnerability, Use, Improvement and Maintenance, Tech. Bull. Number 157, USDA, November 1977.

25. K. Lerch and M. Beltramini, Spectroscopic studies on neurospora copper metallothionein, Biochemistry, 22 (1983) 2043-2048.

26. 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.

27. 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.

28. C.T. Hallmark and B.L. Allen, The distribution of creosote bush in west Texas and eastern New Mexico as affected by selected soil properties, Soil Sci. Soc. Amer. Proc., 39 (1975) 120-124.

29. S. Hunt, Diversity of biopolymer structure and its potential for ion-binding applications, In: H. Eccles and S. Hunt (Eds.), Immobilization of Ions by Biosorption, Ellis Horwood Limited, Chichester, England, 1986.

30. I.H. Segel, Biochemical Calculations, 2nd ed., John Wiley and Sons Inc., NY, 1976.

31. J.L. Gardea-Torresdey, K.J. Tiemann, J.H. Gonzalez, I. Cano-Aguilera, J.A. Henning, and M.S. Townsend, Ability of *Medicago sativa* (alfalfa) to remove nickel ions from aqueous solution, In: L.E. Erickson, D.L. Tillison, S.C. Grant, and J.P. McDonald (Eds.), Proceedings of the 10th Annual Conference on Hazardous Waste Research, Kansas State Univ., Manhattan, KS, 1995, pp. 239-248.