

COPPER(II) AND LEAD(II) BINDING BY ALFALFA BIOMASS: USE OF CHEMICAL MODIFICATION AND X-RAY ABSORPTION SPECTROSCOPY TO DETERMINE THE METAL BINDING MECHANISM

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ABSTRACT

Traditional methods currently employed to remediate heavy metal-contaminated waters prove to be cost prohibitive. Therefore, more cost-effective methods of remediating heavy metals from contaminated waters need to be developed. The use of plant materials as metal adsorbents may be a possible solution. Previously performed experiments have shown that alfalfa shoot biomass can bind an appreciable amount of heavy metal ions, even from multi-contaminant-containing solutions. Although alfalfa biomass has shown to be very effective at removing heavy metal ions from aqueous solution, more research is needed to understand the metal-binding mechanism. Carboxyl groups were chemically modified in order to determine their contribution to the metal-binding process. Batch experiments were performed with the modified biomass and suggest that carboxyl groups play a significant role in the binding of copper(II) and lead(II). In addition, X-ray absorption spectroscopic analysis (XANES and EXAFS) corroborates these results. These studies are important for determining the ligands that may be involved in the binding of copper and lead ions to alfalfa biomass, thus aiding in our understanding of the mechanisms involved in the removal and recovery of metal ions from contaminated waters through phytofiltration.

Key words: XANES, EXAFS, metal ligands, heavy metal binding, bioadsorption, alfalfa

INTRODUCTION

Throughout the last decade, there has been an increasing concern over heavy metal contamination of the aquatic environment and the potential health threat to public potable water sources (Runnels et al., 1992). Lead and copper accumulation in the aquatic environment could result in toxicity to both human and aquatic life (Honeyman and Santschi, 1988). Much of these contaminants can be traced back to industrial discharges and smelting operations. Copper is an essential nutrient in low concentrations, but in higher concentrations it has been found to cause stomach and intestinal distress, kidney damage, and anemia (Carson et al., 1986). In high enough concentrations, lead has been shown to interfere with red blood cell chemistry, cause damage to the nervous system, and cause physical and mental birth defects (Carson et al., 1986). For these reasons the EPA has regulated drinking water concentrations not to exceed 1.3 ppm for copper and 0.15 ppm for lead (Primary Drinking Water Rules, 1992).

The use of biological systems to remediate heavy metal-contaminated waters has been studied for some time (Baker et al., 1994; Chamberlain and Miller, 1982; Bewley, 1980; Zhang and Majidi, 1993; Cervantes and Gutierrez-Corona, 1994; Bender and Rodriguez-Eaton, 1994). Many researchers have found that nonliving biomaterials can be used to adsorb heavy metal ions from the environment (Nagendra et al., 1993; Viraraghavan et al., 1993; Rome and Gadd, 1991; Ramelow

et al., 1993; Lujan et al., 1994). The major chemical structures responsible for metal ion binding are found within these biomaterials. However, the actual mechanisms involved in this adsorption process are not fully understood. Identification of the actual chemical binding sites for heavy metal ion adsorption would be helpful in engineering a process to selectively remove contaminants from wastewaters.

Previously performed studies with *Medicago sativa* (alfalfa) have determined that it may be possible for alfalfa to be used as a natural product for adsorption of heavy metal ions (Gardea-Torresdey et al., 1996a, 1998). These studies have indicated that the alfalfa biomass is capable of binding considerable amounts of copper(II) and lead(II) from aqueous solution. However, up until now, the mechanism involved in adsorption of copper(II) and lead(II) has not been studied.

The objective of this study is to identify the possible binding mechanisms involved in copper(II) and lead(II) binding by the alfalfa biomass. In order to characterize the binding of copper(II) and lead(II) by alfalfa biomass, we performed chemical modification studies along with X-ray absorption spectroscopic analyses (XANES and EXAFS). These studies are important to understand the metal-binding mechanisms involved in copper(II) and lead(II) adsorption and possible modification of the biomass for selective binding of these metal ions.

METHODOLOGY

Sample Collection

The alfalfa plants were collected from controlled field studies at New Mexico State University. The plant tissues were prepared as previously described (Gardea-Torresdey et al., 1996a, 1996b, 1998). In short, the plants were washed and then dried in an oven at 90°C for one week. The biomass was then ground to pass a 100-mesh sieve to achieve a uniform particle size.

Chemical Modification

Carboxyl groups of the biomass were methanol-esterified following a similar method previously described (Gardea-Torresdey et al., 1990; Lin and Rayson, 1998; Tiemann et al., 1999). In summary, 9 g of biomass was suspended in 633 mL of 99.9% pure methanol and 5.4 mL of concentrated hydrochloric acid (HCl) was added to give a final acidic concentration of 0.1M HCl. While continuously stirring the solution, the samples were heated to 60°C for 48 h. Controls were maintained throughout the experiment by reacting alfalfa biomass with either pure methanol or 0.1M HCl to determine if either the methanol or acid alone affected the biomass. The biomass samples were then washed three times with de-ionized water (DI) to quench the reaction. Next, the biomass was lyophilized to be used in batch metal-binding experiments.

Base hydrolysis of the alfalfa biomass was performed following a similar procedure previously described (Gardea-Torresdey et al., 1990; Lin and Rayson, 1998; Tiemann et al., 1999). In summary, the 9 g of biomass were reacted with 100 mL of 0.1M sodium hydroxide (NaOH) for a

period of one hour at room temperature. The hydrolyzation reaction was quenched by washing the biomass with DI water three times and the biomass was then lyophilized for further batch metal-binding experiments.

Metal-Binding Experiments

Batch laboratory methods were implemented as previously described in order to determine the effect of chemical modification on the binding capacities for copper(II) and lead(II) by the alfalfa biomass (Gardea-Torresdey et al., 1996a, 1996b). In summary, the alfalfa biomass was reacted with a solution containing 0.3 mM of either copper (II) or lead(II) at pH 5.0. The concentration of biomass in the metal solution was maintained at 5 mg biomass per mL. Metal solution controls were also kept throughout the experiment to maintain quality control. The biomass-metal solutions were equilibrated for 10 minutes, centrifuged, and the decanted supernatants were stored for analysis while fresh metal solution was again reacted with the pelleted biomass. This process was repeated ten times, or until the biomass became saturated. The final pHs for all tubes were then recorded, and the supernatants were analyzed using flame-atomic absorption.

Metal Analysis

A Perkin Elmer model 3110 atomic absorption spectrometer with deuterium background subtraction was utilized to analyze the supernatants from the metal-binding experiments. The response of the instrument was checked periodically with known standards and the instrument was calibrated with a correlation coefficient of 0.98 or greater. All of the samples were read three times and the mean value, as well as the relative standard deviation, were computed. The wavelengths used for the metal analysis were 327.2 nm for copper and 283.3 nm for lead. An impact bead was utilized to improve the sensitivity. The difference between the initial metal ion concentration and the remaining metal ion concentration was assumed to be bound to the biomass.

X-ray Absorption Spectroscopic Analysis

Separate solutions of 1000ppm copper(II) and lead(II) were prepared from the salts of Cu(II)SO_4 and $\text{Pb(II)(NO}_3)_2$, respectively. Two 100 mg samples of alfalfa biomass were washed three times with 0.1 M HCl to remove any trace metal ions prior to analysis. This was followed by three washings with DI water to remove any remaining acid. The biomasses were reacted with either the 1000 ppm copper(II) or lead(II)solutions at pH 5.0 in order to saturate all available binding sites prior to X-ray absorption analysis. The X-ray absorption spectra were measured on beam lines X-18B and X-19A at the National Sychrotron Light Source. The data were collected with Si 111 monochromator crystals with slits adjusted to give approximately 1-2 eV resolution. All of the samples were measured in transmission mode using standard ion detectors for copper and lead. Model compounds consisting of Cu(II)SO_4 and $\text{Pb(II)(NO}_3)_2$, were measured as solid powders on tape. The data were analyzed using standard methods with the MacXAFS EXAFS

analysis package and the absorption edge step midpoint was used to determine the E_0 values. Quantitative comparisons between unknowns and standards were accomplished with nonlinear fits based on the general Extended X-ray Absorption Fine Structure (EXAFS) equation and verified with theoretical simulations carried out with FEFF 3.11, an *ab initio* curved-wave single-scattering EXAFS simulation code.

RESULTS AND DISCUSSION

Previous experiments have indicated that carboxyl groups play an important role in metal binding by alfalfa biomass (Gardea-Torresdey et al., 1996a, 1998; Tiemann et al., 1999). In order to determine if this was the case for copper(II) and lead(II), metal-binding experiments were carried out with esterified alfalfa biomass. The process used to chemically block the available carboxyl groups by transforming them into methyl esters is shown in the reaction below, where R represents all of the components in the biomass (Gardea-Torresdey et al., 1990; Lin and Rayson, 1998; Tiemann et al., 1999).

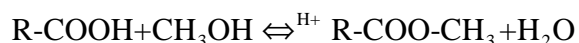


Table 1 displays the effects of esterification on the binding capacity (milligrams of metal per gram of biomass) of the alfalfa biomass for copper(II) and lead(II) binding. A dramatic decrease is observed in the binding capacity by the esterification of available carboxyl ligands. The binding capacity of the unmodified biomass was 19.7 mg/g (310 $\mu\text{mol/g}$) for copper and 43.0 mg/g (207 $\mu\text{mol/g}$) for lead(II), while the esterified biomass resulted in a binding capacity of 2.1 mg/g (33.1 $\mu\text{mol/g}$) for copper and 0.0 mg/g for lead(II). Therefore, a reduction of nearly 90% of the copper binding was observed and a 100% reduction of lead binding was observed. This indicates that carboxyl ligands must play a major role in the binding of copper(II) and lead(II) ions from solution. However, since only 90% of the copper binding was affected, other ligands may also be involved in copper(II) binding. In addition, control experiments conducted with either 0.1M HCl and methanol indicated little, if no effect, on the binding (data not shown).

Since it was determined that carboxyl ligands are important in the binding of copper(II) and lead(II) ions, increasing the number of these groups should increase the biomass binding ability. This was accomplished through hydrolysis (or saponification) of the biomass, where esters previously not active in the metal-binding process are transformed into carboxyl groups. This process of biomass hydrolyzation is shown in the reaction below, where R represents all of the components in the biomass (Gardea-Torresdey et al., 1990; Lin and Rayson, 1998; Tiemann et al., 1999).

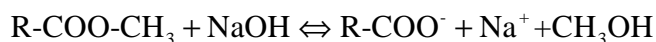


Table 1 also displays the effects of hydrolyzation on the binding capacity (milligrams of metal per gram of biomass) of the alfalfa biomass for copper(II) and lead(II) binding. As expected, a

significant increase in copper and lead binding was observed. After hydrolyzation of the alfalfa biomass, the binding capacity for copper(II) increased 28% to 25.3 mg/g (398 $\mu\text{mol/g}$) and 107% for lead (II) to 89.2 mg/g (430.5 $\mu\text{mol/g}$). This data indicates that the increase in metal binding was due to the formation of new carboxyl groups on the alfalfa biomass. Therefore, the reduction of metal binding through blocking the carboxyl ligands (esterification), and enhancement of metal binding through the formation of new carboxyl ligands (hydrolysis), implies that carboxyl groups are important in the binding of copper(II) and lead(II) ions out of solution. These results concur with the findings of other researchers (Gardea-Torresdey et al., 1990; Lin and Rayson, 1998; Tiemann et al., 1999).

X-ray absorption spectroscopy has been shown to be a sensitive method to determine the electronic and structural properties of metals. XANES spectra for a series of copper model compounds have shown that the edge energy and shape are sensitive to the metals-oxidation state and geometry. These data have shown that Cu(I) has a pre-edge peak (1s-4pz) at 8984 eV, whereas Cu(II) has a tail in this region from approximately the 8986 eV peak. For Cu(II), the first intense transition occurs at 8986 eV (1s-4p) and there is also a weak transition at 8979 eV (1s-3d). By normalizing and taking the difference, the XANES spectra for Cu(II) loaded biomass was obtained.

The XANES spectra for copper(II)-loaded biomass is shown in Figure 1. A weak 1s-3d peak is clearly seen for both copper(II)-loaded biomass and the model compound Cu(II)SO_4 . From Figure 1, it can be seen that the edge position for the copper(II)-loaded biomass (Cu-biomass) is within 5 eV of the model compound, thus indicating that the copper binds to the biomass as copper(II). The Fourier transform EXAFS (FT-EXAFS) and first shell peak indicate similar metal coordination environments between the model compound and the copper(II)-loaded biomass. The Cu-O distances were determined to be 1.99Å and 1.95Å for Cu(II)SO_4 and the copper(II)-loaded biomass, respectively. These data suggest that sulfur is not involved in copper(II) binding since the bond distance for Cu-cysteine is typically 2.3Å. Therefore, copper(II) coordination is occurring though an oxygen ligand, most likely a carboxyl group.

The difficulties and limitations of lead EXAFS data analysis have made this metal difficult to perform X-ray absorption analysis with. The problems associated with Pb EXAFS (multiple coordination numbers and distances, static and dynamic disorder, anharmonic disorder, and high thermal motion) result in broadening of the EXAFS oscillations and a poor signal to noise ratio. In order to reduce these effects, the X-ray absorption analysis were run at room temperature and 40K for the lead-loaded biomass (Pb-biomass) and at room temperature and 100K for the model compound $\text{Pb(NO}_3)_2$. The samples were cooled using a Displex closed-cycle refrigeration system. The XANES spectra for the lead-loaded biomass and $\text{Pb(NO}_3)_2$ are shown in Figure 2. As can be seen in Figure 2, the EXAFS oscillations and edges are broadened at room temperature. Similar E_0

values for the model compound $\text{Pb}(\text{NO}_3)_2$ and the lead-loaded biomass indicate that lead is binding to the biomass as lead(II). The FT-EXAFS data for the lead(II)-loaded biomass, lead foil, and model compound $\text{Pb}(\text{NO}_3)_2$ are shown in Figure 3. The best agreement between the standards and the lead-loaded biomass EXAFS data is for lead(II) binding to oxygen atoms, most likely carboxyl groups.

CONCLUSIONS

Chemical modification via esterification of available carboxyl groups on the alfalfa biomass resulted in a dramatic decrease in copper(II) and lead(II) binding. No binding was observed for lead(II) after esterification, while a small amount was noticed for copper(II). This indicates that other binding sites may still be involved in the binding of copper(II). In addition, hydrolysis of the biomass demonstrated an appreciable increase in both copper(II) and lead(II) binding. This further indicates that carboxyl groups are responsible for the binding of copper(II) and lead(II) from solution by alfalfa biomass. X-ray absorption experiments provided information about the oxidation state and coordination environment of copper(II) and lead(II) bound to the alfalfa biomass. In both cases, the metal ions bound without change in oxidation. From the EXAFS data, we have determined that both copper(II) and lead(II) binding occurred by coordination with an oxygen atom, most likely via carboxyl groups. Therefore, through the use of chemical modification and X-ray absorption spectroscopy, we have shown that carboxyl ligands play a major role in the binding of copper(II) and lead(II) by alfalfa biomass. This information will be useful in the understanding of the metal-binding mechanisms involved in copper(II) and lead(II) adsorption by alfalfa biomass and the possible modification of the biomass for selectively removing these metal ions from waste effluents and contaminated waters.

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Table 1. Alfalfa biomass capacity for copper(II) and lead(II) before and after esterification and hydrolysis.

Metal Ion	mg of metal/g of alfalfa biomass		
	before modification	after esterification	after hydrolysis
Cu(II)	19.7	2.1	25.3
Pb(II)	43.0	0.0	89.2

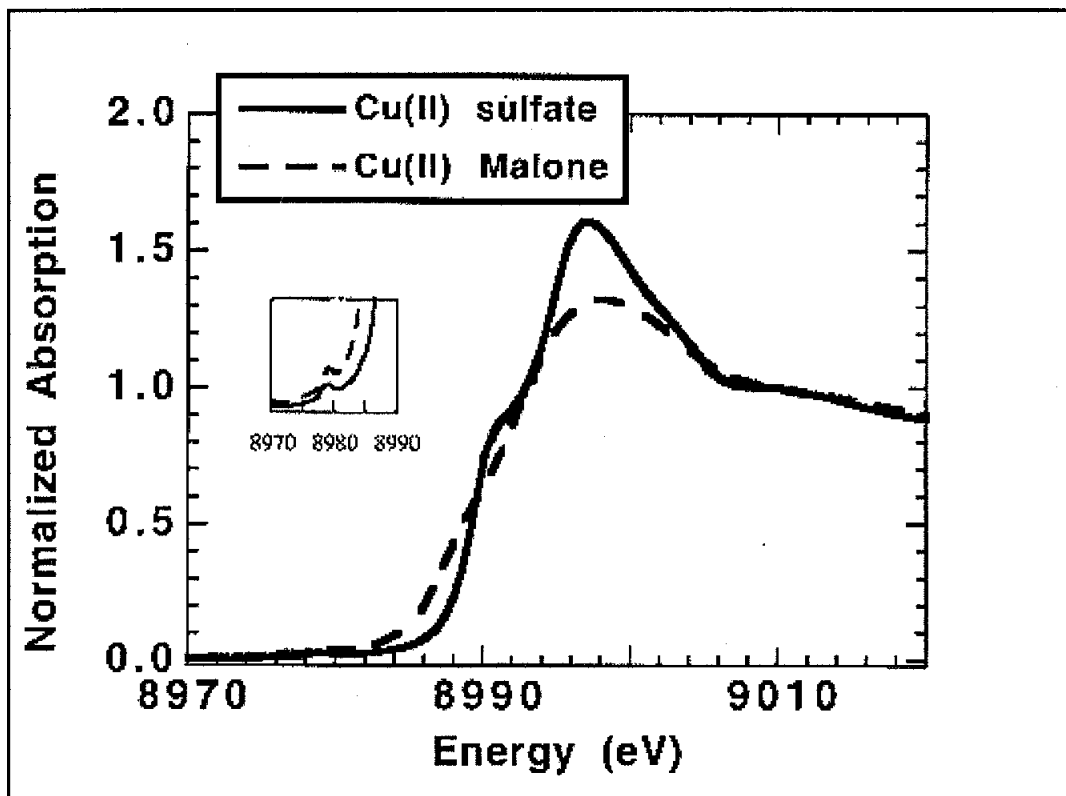


Figure 1. XANES spectra for Cu(II)SO₄- and Cu(II)- loaded biomass.

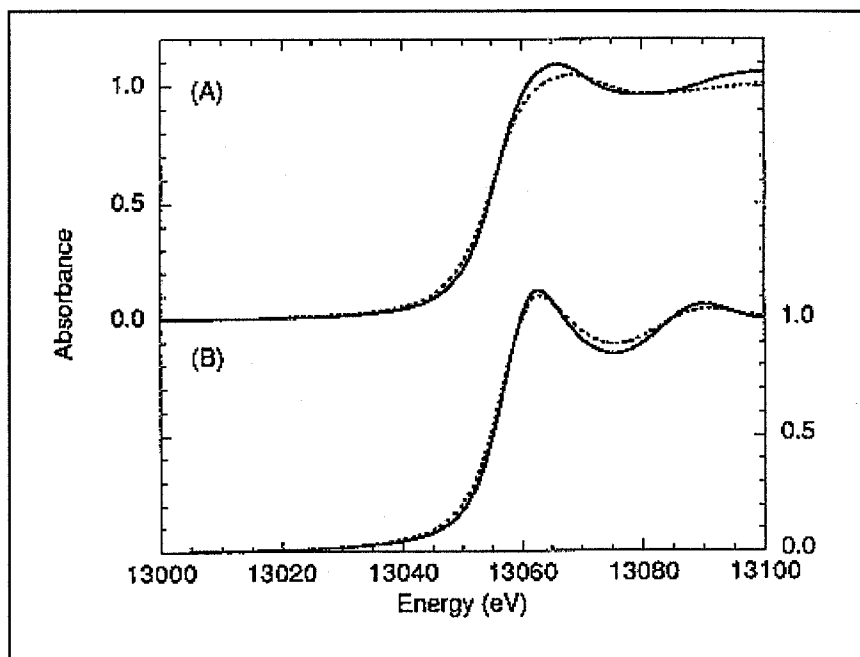


Figure 2. Normalized Pb LIII XANES spectra for (A) Pb biomass at room temperature (solid) and 40 K (dash), and (B) Pb(II)(NO₃)₂ at room temperature (solid) and 100 K (dash).

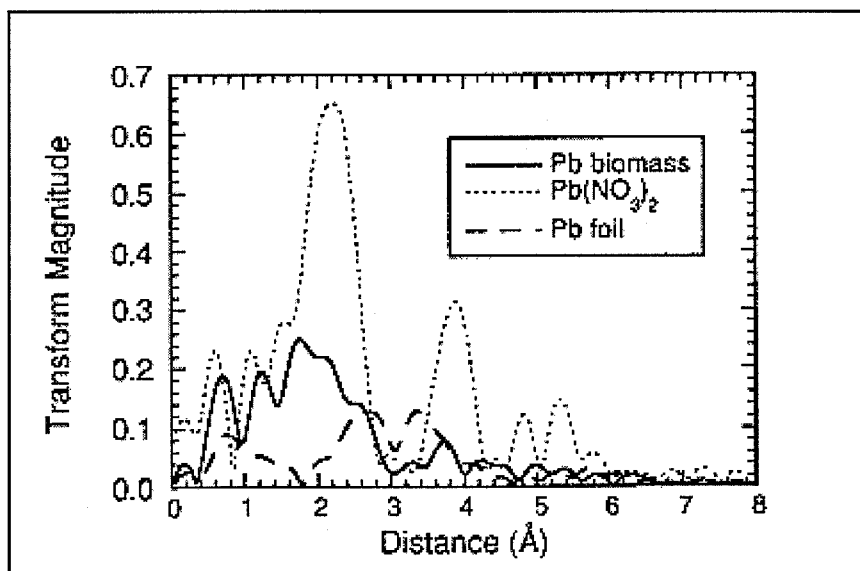


Figure 3. FT k²-weighted EXAFS oscillations for Pb biomass (solid), Pb(II)(NO₃)₂ (dash), and Pb foil (long dash).