STUDY OF THE BINDING MECHANISM OF HEAVY METALS BY INACTIVATED TISSUES OF SOLANUM ELAEAGNIFOLIUM

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ABSTRACT

Contamination caused by heavy metals has been long known as well as its toxic effects on the environment and mankind. It has also been observed that some native plants have survived within the areas polluted with heavy metals. This has been the case with the woody subshrub S*olanum elaeagnifolium*. We can take advantage of this singular feature to use its inactivated tissues as a biofiltration system. First, it is necessary to characterize the mechanism of the binding between the biomass tissues with heavy metals by using chemical modification techniques. These techniques include chemical esterification and hydrolyzation of carboxylic groups, and methyl esters, respectively. These studies have shown an overall decrease in metal binding for esterified biomass, and an overall increase for hydrolyzed biomass as compared to the unmodified biomass. These experiments were performed with Cu(II), Pb(II), Cr(III), Zn(II), and Ni(II). In addition, experiments conducted with modified biomass at different pH conditions were done in order to verify these results. Also, we used X-ray spectroscopy techniques (XANES and EXAFS) to elucidate the mechanism(s) of metal ion binding. Finding the mechanism of the metal binding by the *Solanum elaeagnifolium* biomass is the basis on which any bioremediation (biofiltration in this case) system should be built.

Key words: *bioremediation, binding mechanism, heavy metals, Solanum*

INTRODUCTION

The damaging effects that contamination with heavy metals impinges in our environment have become more obvious, especially in water (Spiro and Stigliani, 1996; Bolton and Gorby, 1995; Rich and Cherry, 1987). Several approaches have been taken for the cleaning up of such contamination (Jeffers et al., 1991), but none of them can efficiently counteract these toxic effects. Bioremediation is an environmentally friendly approach that offers many advantages over other methods of heavy metal decontamination (Kratochvil and Volesky, 1998.). However, to obtain an effective bioremediation system, it is imperative to understand the binding mechanism between the metal and the biomass. This understanding will ultimately allow us to design more functional bioremediation (more specifically, bioadsorption) technique(s). Several biomasses have been successfully used as metal binding materials (Tiemann et al., 2000; Rios et al.,1999; Korshin et al., 1998; and Gardea-Torresdey et al., 1996). The metal binding mechanism of some of these biomasses has been studied (Polette et al.,1997; Tiemann et al.,1999; Gamez et al., 1999; and Lytle et al., 1998), but this has not been the case for the biomass pertinent to the present study.

Solanum elaeagnifolium is a woody subshrub, commonly named Silverleaf Nightshade, which in many states and countries is considered as a plague (Parker, 1972; Reagents of the University of California, 1999). However, this aggressive weed is used for the production of a steroidal alkaloid called salasodine (Trione

and Cony, 1991). *Solanum e.* could be a novel and practical approach for bioremediation in arid regions, since it naturally grows well within these low precipitation regions. Furthermore, Gardea-Torresdey and coworkers (Gardea-Torresdey et al., 1998a) demonstrated *Solanum elaeagnifolium's* remarkable ability to bind certain heavy metal ions. Even though some researchers have shown that *Solanum e.* can bind metal ions (Baig et al., 1999), the metal binding mechanism is not well understood. The objective of this investigation is to gain further insight into the binding mechanism(s) and determine the nature of the chemical ligands that are involved in metal binding. To achieve this goal, *Solanum e.* inactivated tissues were chemical modified by the esterification of carboxyl groups and the hydrolysis of methyl ester groups. Then pH studies were performed by reacting the native and modified biomass with Cu(II), Pb(II), Cr(III), Zn(II), and Ni(II) solutions. Also, additional batch experiments were done to determine the metal adsorption capacities of the different metals to the *Solanum e.* unmodified and modified biomass. Finally, metal-saturated Silverleaf Nightshade biomasses were taken to Stanford Synchroton Radiation Laboratory (SSRL) for X-ray absorption spectroscopic (XAS) analysis. At SSRL, the biomass (native and modified) and model compounds were analyzed using X-ray absorption near-edge spectroscopy (XANES) and extented X-ray absorption fine structure (EXAFS) to determine oxidation states, coordination numbers, and nearest-neighbor distances for the metals. These X-ray studies permitted us to obtain a closer look at the actual binding sites of the biomass with the metals and clarified the role that carboxyl groups play in metal binding.

MATERIALS AND METHODS

Plant Collection and Preparation of the Biomass

The *Solanum elaeagnifolium* plants were collected from a control site located approximately four to five miles from a smelter located in El Paso, Texas. The control site is shielded from the smelter by a mountain, hence minimizing metal deposits in the soil. Subsequent to collection, the leaves were plucked and washed with deionized water; then they were dried in an oven at 60^{o}C for one week. The dried leaves were ground and sieved through a 100-mesh Tyler screen and the fine biomass obtained was used in the experiments described below.

Chemical Modification of the Biomass via Hydrolization

Hydrolysis was performed as follows. Four grams of the 100-mesh sample of *Solanum elaeagnifolium* were washed twice with 0.01M hydrochloric acid (HCl) and twice with deionized (DI) water to ensure that no debris would interfere in the experiment. Next the washed biomass was placed in a 150-ml beaker, and 50-ml of 0.1M sodium hydroxide (NaOH) were added. The solution was constantly stirred and left to react for an hour. After this, the hydrolized biomass was centrifuged for five minutes at 2,900 rpm and washed three times with deionized water. The pelleted biomass was then immersed in liquid nitrogen

for 45 minutes followed by lyophilization in a Labconco freeze-dryer.

Chemical Modification via Fisher Esterification

The modification was performed as previously described by Tiemann et al. (1999). Briefly, three grams of 100-mesh sample were washed twice with 0.01M HCl and twice with deionized water to remove debris and soluble matter. Then the washed biomass was placed in a round-bottom reaction flask with 211 ml of HPLC grade methanol and 1.8 ml of concentrated HCl. The flask was attached to a condenser and kept at 60°C for six hours. After this, the biomass was centrifuged for five minutes at 2,900 rpm, followed by two washings with 0.01M HCl and twice with deionized water. Then pelleted biomass was immersed in liquid nitrogen and lyophilized in a Labconco freeze-dryer.

pH Profile Studies for Metal Binding

These experiments were carried out according to a previously reported procedure (Gardea-Torresdey et al., 1998b). Individual metal solutions of 0.1 mM were prepared from the following salts: $CuSO_4$, $Cr(NO_3)_3$, $Pb(NO_3)_2$, $Ni(NO_3)_2$, and $ZnCl_2$. Metal analysis was performed by flame atomic absorption spectroscopy (Perkin Elmer FAAS Model 3110 Atomic Absorption Spectrophotometer with deuterium background subtraction). The calibration curves obtained with the metal standards yielded correlation coefficients greater than 0.98. The wavelengths used were 327.4 nm for Cu(II), 283.3nm for Pb(II), 359.4nm for Cr(III), 213.9 nm for Zn(II), and 352.5 nm for Ni(II). To improve nebulization efficiency, an impact bead was used with all the metals, except for zinc where a flow spoiler was utilized. Finally, the amount bound was determined by obtaining the difference between the final and initial supernatant metal concentrations.

Metal Binding Experiments

Batch laboratory methods were implemented in order to determine the effect of chemical modification on the separate binding capacities of copper(II), chromium(III), lead(II), nickel(II), and zinc(II) by the *Solanum elaegnofolium* biomass. For these experiments, 100 mg of biomass were washed twice with 0.01 M HCl and suspended in 20 mL of deionized water. The washings were collected and dried to account for any biomass lost during washing. Two mL aliquots of the suspension were transferred to three tubes and centrifuged. The supernatants were saved for further testing. Two ml of 0.3 mM metal solution at either pH 2.0 or pH 5.0 were added to each of the tubes and equilibrated by rocking for 15 minutes. After centrifugation, the supernatants were saved for analysis and again 2 mL of 0.3 mM metal solution were added. This was repeated 10 times or until the saturation point was achieved. Subsequently, the final pHs of all the supernatants were recorded. Samples were diluted as required to remain within the instrumental calibration linear range, and metal concentrations were determined by flame atomic absorption spectroscopy.

X-ray Absorption Spectroscopy

Separate solutions of 1,000 ppm

copper(II) and chromium(III) were prepared from the salts of Cu(II)SO_4 and Cr(III)$(NO_3)_3$, respectively. Two 100 mg samples of *Solanum elaegnofolium* biomass (native and modified) were washed three times with 0.01 M HCl to remove any trace metal ions. This was followed by three washings with DI water to remove any remaining acid. The biomasses (native and modified) were reacted for one hour with either the 1,000 ppm copper(II) or chromium(III) solutions at pH 5.0. This was carried out to saturate all available binding sites prior to X-ray absorption analysis. In addition, a weak cation exchange resin sample (Diaion®WT01S) containing carboxylic groups was analyzed by XAS for comparison. The resin was also reacted with a 1,000 ppm of either copper(II) or chromium(III) solution at pH 5.0.

X-ray absorption spectra were collected for the Cr K-edge (5989 eV) at beam line 7-3 at the Stanford Synchrotron Radiation Laboratory (SSRL). Standard operating conditions were 3 GeV and 50-100 mA beam current. An Si(111) double-crystal monochromator, with an entrance slit of 1 mm, was utilized for all the measurements. The monochromator was detuned to approximately 50% to reduce interference from higher order harmonics. All samples and model compounds were measured as solids and packed into 1 mm path-length aluminum holders with mylar tape windows. In addition, in order to reduce damping from the Debye-Waller factor, all samples were run at approximately 10 K by using a liquid helium cryostat. Fluorescence XAS data for the samples were collected with a Canberra 13-element germanium detector. On the other hand, transmission XAS data were collected for the $Cr(NO_3)_3 \times 9H_2O$ and $K_2Cr_2O_7$ model compounds using argon-filled ion chambers. The model compounds were ground and diluted with X-ray-transparent boron nitride prior to measurements. The calibration for all spectra obtained was performed against the edge position of Cr(0) foil. Several scans (between 2-4) were averaged for each of theXANES and EXAFS spectra to improve the signal-to-noise level.

The analysis of the experimental EXAFS data was performed with the EXAFSPAK software package obtained from SSRL using standard methods. In short, the background of the pre-edge region was removed by means of polynomial linear-fit subtraction. This was followed by a spline removal of three segments and normalization of the data by means of Victoreen polynomial. The EXAFS energy spectra were then converted to wavevector k space. The resulting scattering curve was weighted by k^3, to enhance damped scattering oscillations before Fourier transformation to yield the radial structure function.

RESULTS AND DISCUSSION

The binding of Cu(II), Cr(III), Ni(II), Pb(II), and Zn(II) by Silverleaf Nightshade showed to be pH dependent, with optimal binding occurring between pH 5 and 6 (Figure 1). This pH-dependent binding suggests that the binding of Cu(II), Cr(III), Ni(II), Pb(II), and Zn(II) by Silverleaf Nightshade is through carboxyl ligands. Via NaOH modification

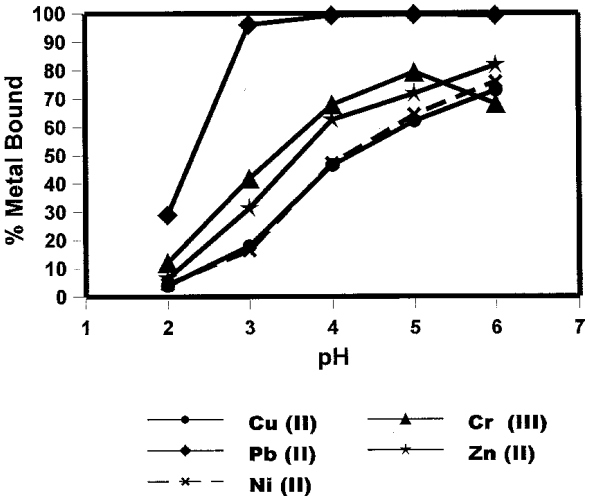

Figure 1. Effects of pH on the binding of copper(II), chromium(III), lead(II), nickel(II), and zinc(II) by the native Solanum elaeagnifolium biomass. The x-axis represents the pH values studied, and the y-axis represents the percentage of metal bound by the biomass.

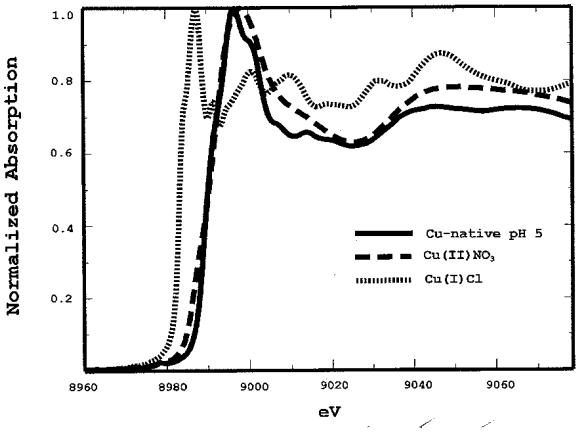

Figure 2. X-Ray absorption near edge structure of *Solanum elaeagnifolium* biomass loaded with Cu (II). Evidence of similarity between model compound of Cu (II) ($Cu(NO_3)_2$) and Cu-biomass as compared with Cu (I) model compound (CuCl).

(hydrolysis), we obtained greater adsorption for the metal ions studied, as compared to the native biomass (Table 2), which further suggested that the metal ion binding was occurring through carboxyl ligands. Moreover, "obstructing" or esterification of the carboxyl groups demonstrated a decrease in metal ion adsorption, which supports our hypothesis on the participation of Silverleaf Nightshade carboxyl moieties in metal binding. These two facts are displayed in Tables 1 and 2. X-ray absorption spectroscopic data showed that both copper(II) and chromium(III) are bound to the biomass of *Solanum elaeagnifolium* without change in oxidation state (Figures 2 and 3, respectively). From Figure 2, it can be seen that the absorption edge differs from the model compound Cu(I)Cl, but is very similar to the absorption edge of the model compound Cu(II)NO3. Hence, this XANES shows that the oxidation state of copper has not changed upon binding. On the other hand, Figure 3

Table 1. Metal- binding capacities for *Solanum elaeagnifolium* leaves at pH2.

Metal	Native (mg Metal/g Biomass)	Hydrolyzed (mg Metal/g Biomass)	Esterified (mg Metal/g Biomass)
Cu (II)	12.3	20.7	1.30
Cr (III)	0.00	2.40	0.00
Pb (II)	13.0	20.1	4.50
Ni (II)	0.00	0.00	0.00
Zn (II)	1.36	4.28	0.00

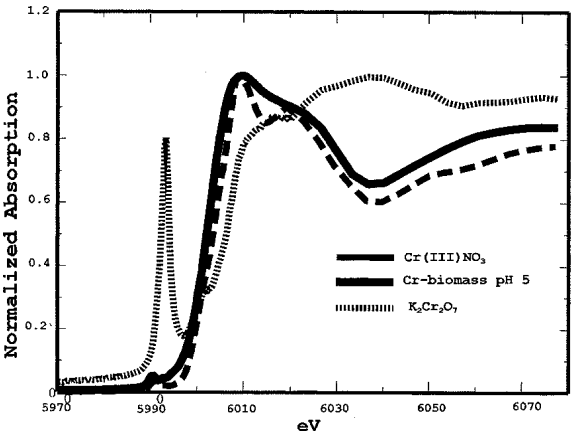

Figure 3. X-Ray absorption near- edge structure of *Solanum elaeagnifolium* loaded with Cr (III). A comparison with model compounds of Cr (III) as $Cr(NO_3)$, and Cr(VI) as $K_2Cr_2O_7$.

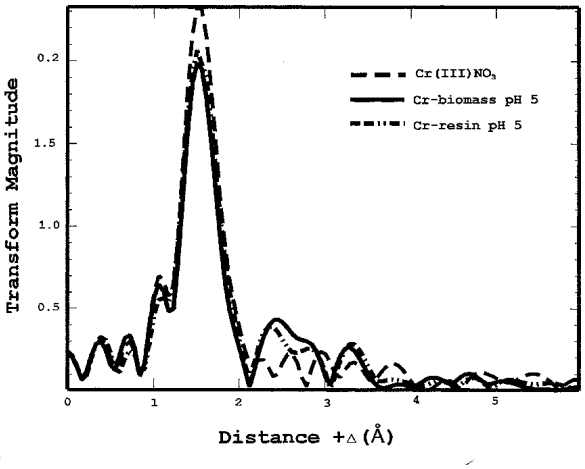

Figure 4. Extended X-Ray absorption fine structure (EXAFS) *Solanum elaeagnifolium* biomass. Showing model compound $Cr(NO_3)$ vs. Cr-biomass and Cr-resin at pH 5.

displays a pre-edge feature for the model compound belonging to Cr(VI) ($K_2Cr_2O_7$), which is absent in the Cr(III) model compound ($Cr(NO_3)_3$) and the XANES pertaining to Cr(III)-biomass. Thus, this indicates that the oxidation state of Cr(III) did not change upon binding to *Solanum* biomass. In addition, EXAFS studies showed that chromium(III) binds to the biomass via an oxygen ligand at pH 5, similar to that of a weak cation-exchange resin, further suggesting the involvement of carboxyl groups (Figure 4), with a very similar interatomic distance of 1.97 and 1.98 A, respectively. Figure 5 shows the EXAFS results for copper(II) binding at pH 5.0, indicating that copper(II) also binds through a ligand similar to that of a weak cation-exchange resin. This suggest that the ligands involved in copper(II) binding could also be carboxyl groups. Figure 6 displays the EXAFS for copper(II) to native, esterified, and hydrolyzed biomass. The EXAFS for the native and hydrolyzed biomasses is very similar but is different for the esterified biomass. This could be due to a different type of ligand involved in copper binding, which can only be observed when the

Table 2. Metal- binding capacities for *Solanum elaeagnifolium* leaves at pH5.

Metal	Native (mg Metal / g Biomass)	Hydrolyzed (mg Metal / g Biomass)	Esterified (mg Metal / g Biomass)
Cu (II)	45.9	70.5	10.3
Cr (III)	43.1	78.3	3.4
Pb (II)	31.9	80.9	12.6
Ni (II)	13.3	35.7	1.8
Zn (II)	11.5	32.8	2.35

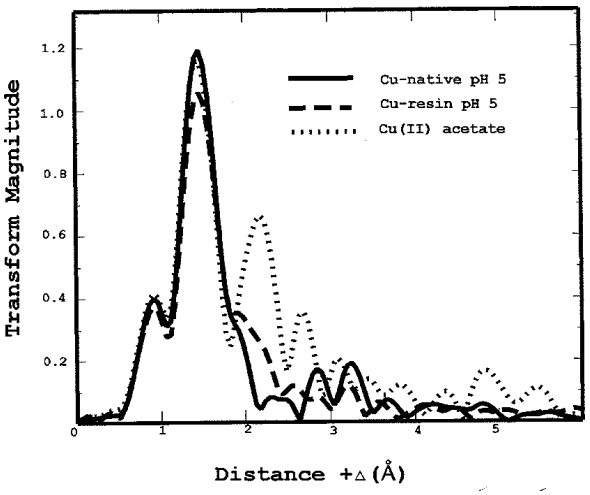

Figure 5. Extended X-Ray absorption fine structure (EXAFS) for *Solanum elaeagnifolium* loaded with Cu(II). Showing that Cu(II)-biomass binds through a ligand similar to that of a weak cation exchange (resin).

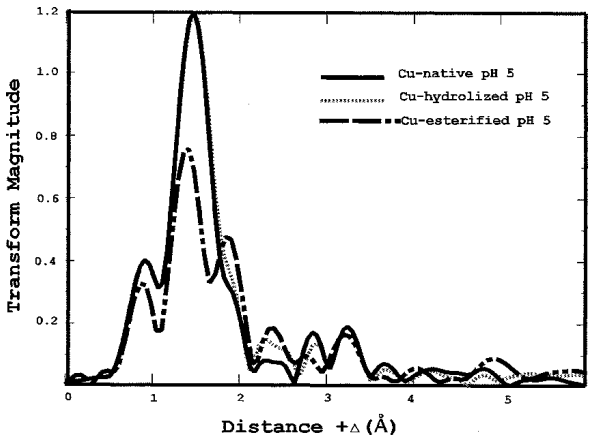

Figure 6. Extended X-Ray absorption fine structure (EXAFS) for modified *Solanum elaeagnifolium* biomass loaded with Cu(II). Featuring the equivalence between the binding of native and hydrolyzed biomass loaded with Cu(II), as well as the distinct EXAFS for the esterified Cu(II)-biomass.

carboxyl groups present in the biomass are blocked (esterified). Other model compounds such as Cu(II)S corroborate this observation, as shown in Figure 7. The EXAFS of the copper-esterified biomass indicates two possible ligands (oxygen and sulfur) as the responsible groups for copper binding to *Solanum elaeagnifolium* biomass. Finally, Figure 8 displays the very similar interatomic distances of Cr(III) binding to native, hydrolyzed, and esterified biomass (1.97, 1.98, and 1.98A, respectively). This indicates that Cr(III) could be bound to *Solanum e.* through carboxyl moieties in the biomass.

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 Cu(II) and Cr(III) by Silverleaf Nightshade biomass. This information will be useful in understanding the metal-binding mechanisms involved in metal adsorption by *Solanum elaeagnifolium* biomass and the possible modification of the biomass for selectively removing these metal ions from waste effluents and contaminated waters.

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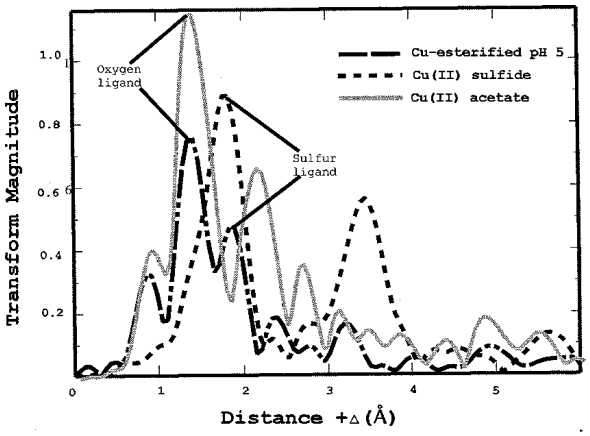

Figure 7. Extended X-ray absorption fine structure (EXAFS) of esterified *Solanum elaeagnifolium* biomass loaded with Cu(II). Evidence of oxygen and sulfur as possible ligands to biomass.

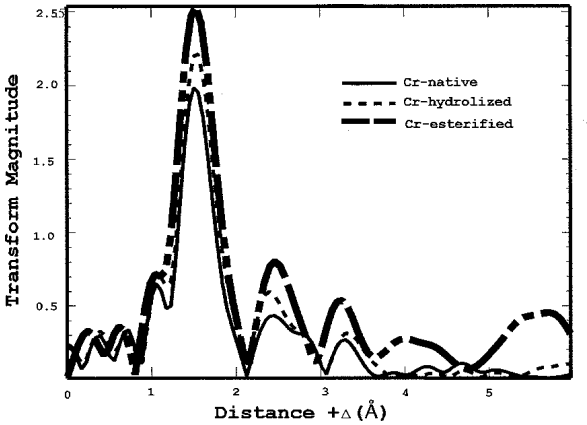

Figure 8. Extended X-ray absorption fine structure (EXAFS) of modified *Solanum elaeagnifolium* loaded with Cr(III). Comparison of native, hydrolyzed and esterified biomass.

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