ABSORPTION OF COPPER(II) BY CREOSOTE BUSH (LARREA TRIDENTATA): USE OF_ATOMIC AND X-RAY ABSORPTION SPECTROSCOPY

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Abstract—Larrea tridentata (creosote bush), a common North American native desert shrub, exhibits the ability to take up copper(II) ions rapidly from solution. Following hydroponic studies, U.S. Environmental Protection Agency method 200.3 was used to digest the plant samples, and flame atomic absorption spectroscopy (FAAS) was used to determine the amount of copper taken up in different parts of the plant. The amount of copper(II) found within the roots, stems, and leaves was 13.8, 1.1, and 0.6 mg/g, respectively, after the creosote bush was exposed to a 63.5-ppm copper(II) solution for 48 h. When the plant was exposed to a 635-ppm copper(II) solution, the roots, stems, and leaves contained 35.0, 10.5, and 3.8 mg/g, respectively. In addition to FAAS analysis, x-ray microfluorescence (XRMF) analysis of the plant samples provided further confirmation of copper absorption by the various plant parts. X-ray absorption spectroscopy (XAS) elucidated the oxidation state of the copper absorbed by the plants. The copper(II) absorbed from solution remained as copper(II) bound to oxygen-containing ligands within the plant samples. The results of this study indicate that creosote bush may provide a useful and novel method of removing copper(II) from contaminated soils in an environmentally friendly manner.

Keywords—X-ray absorption spectroscopy Copper(II) uptake Creosote bush

INTRODUCTION

In recent years a great deal of attention has been paid to the field of phytoremediation as many industrialized countries are faced with the challenge of cleaning sites polluted with toxic heavy metals. As the focus on phytoremediation has increased, many different species of plants have been studied for their potential application in removal of toxic waste from soils [1,2]. However, many of the species thought to be hyperaccumulators are slow growing, which inhibits their application in the field. Many different factors control the use of plants in the phytoremediation process, for example, the ability to grow fast, thereby producing large amounts of biomass, and the ability of the plant to accumulate large amounts of contaminant in its tissues [3]. The advantage that plants have over traditional methods of soil excavation or chemical extraction is the reduced cost of contaminant removal [4]. After growth and accumulation of contaminants, plants can be harvested in an inexpensive manner, which allows for a less intrusive and less expensive means of contaminant removal. Phytoremediation takes advantage of the plant’s natural ability to take up nutrients from the soil and the ability of the plant’s cellular components to store metal ions [5]. Therefore, the use of plants to remediate hazardous waste holds much promise as an inexpensive and effective way to recover toxic metal ions from soils.

Previous studies have documented the ability of nonliving tissues of alfalfa to bind copper(II), nickel(II), chromium(III), iron(II), and iron(III) from aqueous solution [6–8]. The ability of alfalfa tissues to bind metal ions from solution suggests that living alfalfa possesses the potential to extract metals ions from contaminated soils. Another example of a plant that has shown much promise in the treatment of polluted solutions is sunflower, which has the ability to take up uranium from contaminated water [9–11]. Additional studies with Thlaspi caerulescens have shown that this plant can take up large amounts of cadmium and zinc from soils and store the metals in its stem system [12].

While interest and research in the field continue to increase, several problems associated with phytoremediation remain [13]. For example, metal contamination may exist in areas of the soil that are unreachable by phytoremediation since they are beneath the root zone of the plants. In addition, many plants are unable to translocate significant quantities of the metal acquired by the roots into the stems and leaves. Furthermore, many of the plants that have been identified as metal accumulators for the purposes of phytoremediation grow in tropical and semitropical regions [14]. For future utility of phytoremediation, plants need to be identified that grow well in a variety of climates, especially in deserts and arid regions with strong mining activity.

Since Larrea tridentata, commonly known as the creosote bush, is native in the desert Southwest, it may posses potential for phytoremediation. This common desert shrub is found primarily in the arid southwestern regions of the United States and has adapted to this region by growing small, thick leaves and a very deep taproot (4–5 m) with massive lateral roots to scavenge scarce water resources [15,16]. In addition, creosote bush, which requires very little water or care, possesses antitherbivore compounds that make it unpalatable to animals, thus minimizing the risk to the ecological environment [15]. Furthermore, creosote bush has been found growing in soils highly contaminated with metals [17]. Previous studies have already shown that the creosote bush is capable of binding...
metal ions [18,19]. However, few studies have focused on the processes by which this binding occurs. The objective of this study was to gain a better understanding of the processes through which the creosote bush accumulates copper(II) ions and possibly ascertain the chemical functional groups responsible for the copper(II) binding. Hydroponic growth procedures were utilized to maintain better control over the growth conditions and reduce experimental times. Several spectroscopic techniques were employed for this investigation—flame atomic absorption spectroscopy, x-ray microfluorescence, and x-ray absorption spectroscopy—to aid in determining metal uptake and the possible mechanisms involved by the creosote bush.

**MATERIALS AND METHODS**

**Source of plant materials**

Seeds of *L. tridentata* (creosote bush) were obtained from a local vendor in Las Cruces (NM, USA). The seeds were planted in 250-cm³ polyethylene pots containing soil obtained from an unpopulated region East of El Paso, Texas, between the U.S. Interstate 10 and Americas Avenue intersection. The plants were maintained under controlled greenhouse conditions with a temperature of 29°C. The soil used to germinate the seedlings was tested for copper content utilizing U.S. Environmental Protection Agency method 3150 (acid digestion) and flame atomic absorption spectroscopy (no copper was found).

After the creosote plants were approximately 15 cm high (12 weeks old), each was transplanted into a 50-ml centrifuge tube, and the roots were covered with metal-free glass beads. A 3-mm hole was made at the bottom of the centrifuge tube so that a copper(II) sulfate solution (either 63.5 or 635 ppm) could be passed through the glass beads that supported the creosote plants. The tubes containing the plants were then set inside a plastic tray to receive the copper solution. Two copper(II) concentrations consisting of 63.5 and 635 ppm were tested, along with a deionized water control solution, with individual plants. The copper solutions were continually passed at a rate of 2 ml/h through the respective tubes containing the creosote plants, which were supported by the glass beads. The plants were exposed to the solutions for a 48-h period under normal fluorescent light at room temperature (25°C) and at an average relative humidity of 60%. The experiments were performed in triplicate for quality assurance. After 48 h, the plants were harvested from the glass beads and rinsed with tap water for 15 min, followed by three separate rinses with deionized water.

**Plant sample collection**

The collected samples were dried in an oven at constant temperature (59°C) for 2 d. The samples were then separated into three different parts: roots, stems, and leaves. The different plant parts were digested following U.S. Environmental Protection Agency method 200.3. A brief summary of the method of digestion is as follows: First, concentrated nitric acid (Trace Pure grade obtained from Fisher Scientific, Fair Lawn, NJ, USA) was added to a digestion flask containing the appropriate section of the plants, followed by 30% hydrogen peroxide (obtained from VWR Scientific, Chicago, IL, USA). Finally, concentrated hydrochloric acid (Trace Pure grade obtained from Fisher Scientific) was added. The control digestion was also performed with empty digestion flasks (no plant sections) to ensure accuracy, precision, and quality control. Certified copper standards (obtained from Fisher Scientific) were used in all the digestion procedures.

**Flame atomic absorption spectroscopy**

The solutions made from the digested plant material were analyzed for copper content using a Perkin-Elmer (Norwalk, CT, USA) model 3110 atomic absorption spectrometer with deuterium background correction (FAAS). To maximize the sensitivity of the instrument for copper analysis, an impact bead was used. The copper analysis was performed with a wavelength of 327.5 nm (recommended analytical line). Calibration of the instrument was performed in the linear working range for copper, and a correlation coefficient of 0.98 or greater was obtained for each calibration. The instrument response for copper was checked periodically with known copper standards. All the samples were analyzed in triplicate, and the mean value of the analysis was calculated.

**X-ray microfluorescence**

Dried plant tissues were placed on a glass slide and held in place with Kapton x-ray transparent tape. The instrumental parameters on the Kevek (Middleton, WI, USA) Omicron x-ray microfluorescence spectrometer were as follows: a 100-μm-diameter beam, a molybdenum x-ray source, and an energy dispersive solid-state detector. The operating parameters for the analysis were a 30-KeV energy source, a 5-min count time, an 8 to 10% dead time, and an operating current of 1.47 mA. The analysis was performed on the different plant sections for both copper(II) treatments (63.5 and 635 ppm) and the control creosote plants, which had no copper(II) exposure.

**Synchrotron XAS analysis**

All XAS measurements were performed at Stanford Synchrotron Research Laboratory (Stanford University, Stanford, CA, USA) on beam line 7-3 with the storage ring operating at 3 GeV and currents of 50 to 100 mA. A silicon (Si 220) monochromator crystal was used along with a 1.0-mm upstream aperture, and no focusing optics were used. The crystals of the monochromator were detuned by 50% at the absorption edge to reject any possible harmonics produced. The copper K-edge of the sample was measured by recording the Cu-K alpha fluorescence excitation spectrum with a Canberra 13-element germanium array detector (Canberra, Meriden, CT, USA), and the K-edge of the model compounds was measured using x-ray transmittance in nitrogen gas–filled ionization chambers. Samples of the creosote bush (roots, stems, and leaves) and the model compounds, copper(II) acetate and copper(II) sulfate, were kept within a temperature range between 2 and 4 K for the analysis using liquid helium. Instrumental calibration at the copper K-edge was achieved using a copper(0) foil, which has an inflection in the K-edge at 8908 eV. The spectra for both the model compounds and the samples were obtained by averaging four scans.

**XAS data analysis**

All the XAS data were processed using standard procedures for pre-edge subtraction, spline fit, spline removal, and Fourier filtering [20]. The software analysis of the data was performed using EXAFSPAK, a suite of computer programs commonly used for Extended X-ray Absorption Fine Structure (EXAFS) data analysis. The pre-edge subtraction on all the data was performed using a Gaussian function. To normalize the data,
dried biomass. In the stems of both sets of exposed plants, the 635 ppm of copper(II) was 35.1 mg of copper per gram of dried biomass, which could accumulate 13.8 mg of copper per gram of dried sample. During the growth and treatment periods, no signs of copper toxicity to the creosote bush were observed. The digestion for copper found in the roots, stems, and leaves of the creosote bush samples exposed to the two different copper(II) concentrations: 63.5 and 635 ppm

<table>
<thead>
<tr>
<th>Sample</th>
<th>63.5-ppm exposure</th>
<th>95% CI (±)</th>
<th>635-ppm exposure</th>
<th>95% CI (±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>0.6 mg/g</td>
<td>0.05</td>
<td>3.8 mg/g</td>
<td>0.9</td>
</tr>
<tr>
<td>Stems</td>
<td>1.1 mg/g</td>
<td>0.2</td>
<td>10.5 mg/g</td>
<td>0.6</td>
</tr>
<tr>
<td>Roots</td>
<td>13.8 mg/g</td>
<td>2.2</td>
<td>35.1 mg/g</td>
<td>11.0</td>
</tr>
</tbody>
</table>

* CI = confidence interval.

a Victoreen polynomial was used. The data was Fourier transformed with and without phase correction to obtain a frequency correlation between the absorbing and the neighboring atoms as a function of interatomic distance (R) in angstroms. Data fitting was performed by changing the interatomic distances, the number of backscatters, the threshold energy, and the Debye–Waller factor. The correlation coefficients guided the fittings.

**Model compounds**

The model copper compounds were obtained from Fisher Scientific (as pure reagents) to compare with copper(II) absorbed by the plants. The model copper compounds used were copper(II) acetate, copper(II) sulfate pentahydrate, and copper(I) chloride.

**RESULTS AND DISCUSSION**

The main objective of this study was to determine if creosote bush could be used as a practical alternative for the removal of contaminates from polluted areas in the desert Southwest via phytoremediation. The levels used for this study were 63.5 and 635 ppm, which fall within the range of concentrations found at hazardous waste sites. Texas Superfund sites have reported copper concentrations in contaminated groundwaters to be as high as 200 ppm and other sites with contamination as high as 2,900 ppm in surface waters, where the allowable U.S. Environmental Protection Agency maximum contaminant levels are only 1.3 ppm for copper [21,22]. When compared with other plant species, these levels seem somewhat similar. For instance, Tang et al. reported copper concentrations of *Elsholtzia haichowensis* leaf, stem, and root dry biomass to be within the ranges of 0.154, 0.079, and 0.987 mg/g, respectively, for plants growing in copper mining soils [23]. Additional studies have shown that grassland plants such as *Lolium perenne* have root and shoot dry biomass average copper concentrations of 0.0014 and 0.005 mg/g, respectively, [24]. Aquatic plants such as *Zostera marina* have shown to possess trace copper concentrations of 0.0033 mg/g in the roots and 0.0045 mg/g in the shoots [24]. As indicated by Nellessen and Fletcher, many other plants species have been studied for metal accumulation [25]. However, very few desert plants have been studied for phytoremediation purposes. The unique advantage that creosote bush has over the various plants studied is its applicability for phytoremediation in harsh desert environments. Nevertheless, the levels of metal accumulated within the plant’s roots (35 mg copper/g biomass) are high enough to be considered toxic if eaten by animals over an extended period. While it is the main goal of phytoremediation to remove toxic metals from the soils and concentrate them within the plant, it is important to keep this point in mind. However, one benefit that creosote bush has over other desert plant species is that it possesses a unique antiherbivore chemistry [15]. The specific resins found within the stems and leaves of the creosote plant make it unpalatable to nearly all wild and grazing animals. This unique feature makes creosote bush a very favorable candidate among desert plants for potential use in phytoremediation of arid desert Southwest environments, where much mining activity has occurred.

X-ray microfluorescence was also used to analyze the copper content of three different parts of the creosote plants. Figure 1A depicts the x-ray microfluorescence spectrum for the roots of the control plants. In Figure 1A the peak for copper is not present, showing that copper was below detection levels in the control plants. In contrast, Figure 1B shows the x-ray microfluorescence spectrum for the roots of the plants exposed to 63.5 ppm of copper(II), where a distinct copper peak (at about 8.01 keV) can be seen. Figure 1C shows the x-ray microfluorescence spectrum for the roots of the plants exposed to 635 ppm of copper(II) solution, where the copper peak has increased significantly as compared to the initial peak observed in Figure 1B. From the three spectra obtained by x-ray microfluorescence analyses, it can be seen that the uptake of
Cu(II) uptake by creosote bush using x-ray spectroscopy

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copper by the creosote plant from solution increases with exposure to increasing copper(II) concentrations.

X-ray absorption spectroscopy is a very powerful tool that can be used to determine the oxidation state of a bound metal in a sample using the X-ray Absorption Near Edge Structure (XANES) and the ligand involved in the metal binding by measuring the distance from the x-ray-absorbing atom to the next nearest using Extended X-ray Absorption Fine Structure (EXAFS). The XANES for the plant samples are depicted in Figure 2A, where a comparison between a copper(II) sulfate model compound and the plant sections is made. The inflection points for all the edges in the spectra are very similar, indicating that the copper is bound to the biomass as copper(II) in a six-member coordinated system. The coordination environment is determined by the inflection point on the spectra, which is a 1s to 4p transition for the three different plant
sections [26]. However, this contradicts current dogma that copper(II) is taken into the plant and is reduced to copper(I). Nevertheless, copper toxicity differs for different oxidation states. According to Furst et al., the lethal dose 50 (LD50) for copper(I) chloride and copper(II) acetate are 148 and 1,500, respectively, for oral toxicity testing in rats [27]. The U.S. Environmental Protection Agency primary drinking water rules indicate that the health effects of copper can be quite acute, ranging from stomach and intestinal disease to severe liver and kidney damage [22]. Therefore, the creosote bush exhibits an advantage by maintaining the oxidation state of copper(II), which has lower toxicity and shows an explicit potential for phytoremediation. Figure 2B compares the XANES spectra for the creosote plants exposed to copper(II) to the model compounds copper(II) acetate and copper(I) chloride. In this figure, the 1s to 4p transition is apparent in the model compound copper(II) acetate as well as in the plant samples (roots, stems, and leaves). The observed inflection point and edge position seen in the copper(II) acetate suggest that copper(II) is not reduced to copper(I) and that copper(II) may be coordinated to carboxyl groups. This agrees with the current opinion that metal ion absorption and adsorption by plants is mediated or controlled by carboxyl groups in the plant tissue [17–19,28]. Also, by looking at the actual energy of the edge for copper(II) in the plant samples and the edge position for the copper(II) acetate, one can suggest that the coordination environment is similar for the creosote plants and copper(II) acetate. In addition, the XANES analysis for the copper(II) sulfate and the creosote plant samples do not match, indicating different coordination environments.

While the potential for phytoremediation is apparent, the actual mechanism for copper uptake by most plants is not clear. It has been suggested by other researchers that uptake of copper(II) involves binding by organic acids [23,29,30]. In order to gain a better understanding of the mechanisms involved and the actual functional groups responsible for copper(II) binding by the creosote bush, creosote plant samples were compared to known model compounds using EXAFS. The Fourier-transformed magnitudes for the EXAFS are shown in Figure 3, which compares copper(II) acetate and the different sections of the plants. Several investigators have shown that the copper–oxygen binding distance in copper(II) acetate is approximately 1.969 Å [7,31]. The actual experimentally derived bond lengths are provided in Tables 2 and 3 for both the 63.5- and the 635-ppm copper(II) exposures, respectively. These data corroborate the data from Figure 3, which illustrates that the binding distance between the copper(II) and the nearest neighbor atom is similar for the creosote bush samples and copper(II) acetate. As seen in Tables 2 and 3, coordination of copper(II) in the creosote bush samples is via five or six oxygen atoms, resulting in a distance ranging between 1.93 and 1.96 Å for both concentration exposures. These data further demonstrate that the copper(II) absorbed by the creosote plant in all the samples remains in the plant as copper(II), which is coordinated to oxygen ligands, most likely via carboxyl groups.

CONCLUSIONS

The data presented in this study show that copper(II) absorbed by creosote bush plants is bound via oxygen-containing ligands, most likely carboxyl groups. The XANES comparisons of copper(II) acetate and the samples of the creosote bush show that the bound copper is in a similar oxidation state and a similar coordination environment for both the plant samples and the model compound copper(II) acetate. Although the nearest neighbor distance determined from the EXAFS data is
in agreement with other studies, the data suggest that copper(II) bound by the creosote bush remains as copper(II), as has been suggested as part of the plant metal binding mechanism. The significance of the lack of reduced copper oxidation state indicates that creosote bush, unlike several other plant species studied, has the ability to maintain the less toxic form of copper [27]. Consequently, reduced toxicological impact will result from creosote bush phytoremediation. In addition, we have found that the creosote bush is able to absorb and concentrate considerable amounts of metal ions through its root systems. Because of creosote’s limited transpiration as a desert plant, movement of the metal from the roots to the stems and leaves (harvestable areas) is not as rapid but still remains significant. Provided adequate time, stem and leaf concentrations may achieve greater concentration with improved uptake. Furthermore, in desert environments, a plant that can accumulate toxic metal ions from contaminated soils through its root systems and can release these metals during transpiration facilitates the removal of metals ions from contaminated soils [15,16]. Therefore, creosote bush has shown good potential to be used for phytoremediation, especially in arid desert climates.

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REFERENCES


Table 2. Compilation of structural parameters extracted from extended x-ray absorption fine structure spectra of plants exposed to 63.5 ppm of copper(II)

<table>
<thead>
<tr>
<th>Neighboring atom</th>
<th>Coordination no.</th>
<th>R Å²</th>
<th>S²b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roots</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu-O</td>
<td>6</td>
<td>1.9415</td>
<td>0.0076</td>
</tr>
<tr>
<td>Cu-O</td>
<td>2</td>
<td>2.2607</td>
<td>0.0076</td>
</tr>
<tr>
<td>Cu-S</td>
<td>1</td>
<td>2.9409</td>
<td>0.0076</td>
</tr>
<tr>
<td>Cu-Cu</td>
<td>1</td>
<td>3.7196</td>
<td>0.0076</td>
</tr>
<tr>
<td>Stems</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu-O</td>
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<td>1.9252</td>
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<tr>
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<tr>
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<td>4.0809</td>
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<tr>
<td>Leaves</td>
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<td></td>
</tr>
<tr>
<td>Cu-O</td>
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</tr>
<tr>
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</tr>
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<td>Cu-Cu</td>
<td>1</td>
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</tr>
</tbody>
</table>

* R Å is the distance in angstroms from the metal to the neighboring atom.  
* S² is the squared standard deviation.


