SPECTROSCOPIC CONFIRMATION OF CHROMIUM UPTAKE BY CREOSOTE BUSH (*Larrea tridentata*) USING HYDRO-PONICS

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ABSTRACT

Larrea tridentata (creosote bush), shows the ability to uptake chromium(VI) ions from solution. EPA method 200.3 was followed to digest the plant samples, and flame atomic absorption spectroscopy (FAAS) was used to determine the amount of chromium taken up in different parts of the plant. The amount of chromium found within the roots, stems, and leaves was 57.4 mg/g, 14.2 mg/g, and 19.3 mg/g, respectively, after the creosote bush was exposed to a 520-ppm chromium(VI) solution. In addition to FAAS analysis, X-ray microfluorescence (XRMF) analysis of the plant samples provided *in situ* documentation of chromium absorption by the various plant parts. X-ray absorption spectroscopy (XAS) elucidated the oxidation state of the chromium as Cr(III) absorbed by the plant: The chromium(VI) absorbed from solution was partially reduced to chromium(III) in the roots. Some chromium(VI) and the reduced chromium(III) were transported through the stems, and finally accumulated as chromium(III) in the leaves of the plant. The metal was bound to oxygen-containing ligands within the plant samples. Scanning electron microscopy confirmed the presence of chromium inside the tissues of the plant. Creosote bush may provide a useful method of removing chromium(VI) from contaminated soils.

Keywords: XAS, XANES, XRMF, EXAFS, chromium(VI), absorption, creosote bush.

INTRODUCTION

Soils and waters polluted with toxic metals are a major environmental and human health problem that demand an effective and affordable solution. Concerns associated with the heavy metal contamination of soils have been well documented (Brown et al., 1994). Current methodologies to clean toxic metal-polluted soils consist mainly of excavation and burial of the hazardous waste. This method has an average cost of $1 million per acre of soil cleaned (Raskin, 2000). Many sites remain contaminated simply because it is too expensive to clean them up with the current technologies. Many industrialized countries are faced with the challenge of cleaning sites polluted with toxic heavy metals and they are turning to cost-effective methods such as phytoremediation, the use of plants to alleviate contamination problems. Phytoremedation takes advantage of the plant's natural capability to take up nutrients from the soil and the ability of the plant's cellular components to store metal ions (Raskin, 1996). The benefit of using plants over methods such as soil excavation or chemical extraction is cost. After growth and accumulation of contaminants, plants can be harvested in an inexpensive manner, which allows for a less intrusive means of contaminant removal. Therefore, the use of plants to decontaminate hazardous waste holds much promise as an inexpensive and effective way to clean hazardous waste and recover toxic metal ions from soils. Several different factors

control the use of plants in the phytoremedation process, e.g., the ability to grow fast thereby producing large amounts of biomass, and the ability of plants to accumulate large amounts of contaminant in their tissues (Blaylock et al., 1997). Many different species of plants have been studied for their potential application in removal of toxic waste from soils (Glass, 1998; Drake et al.,1996).

Non-living tissues of alfalfa are able to bind copper(II), nickel(II), chromium(III), and iron(II) and (III) from aqueous solution (Gardea-Torresdey et al., 1997; Tiemann et al., 1999; Tiemann et al., 2000). These studies suggest that alfalfa may have the ability to extract metal ions from contaminated soils. Another example of a plant that has shown much promise in the treatment of polluted environments is sunflower, which has the ability to take up uranium from contaminated water (Riordan et al., 1997; Gonzalez-Muñoz et al., 1997;Yang and Volesky, 1999). The uptake of cadmium and zinc by *Thlaspi caerulescens* has been studied. This plant has the potential to take up large amounts of these ions from soils and store the metals in its stem system (Watanabe, 1997).

Larrea tridentata, or creosote bush, is a plant that naturally grows in the Chihuahuan desert, which is found mainly in the arid Southwest regions of the United States. This plant has the capability to grow in soils contaminated with heavy metals. Previous studies revealed its ability to uptake and accumulate metals from the soil in which the plant grows (Gardea-Torresdey et al., 1996).

The accumulation of metal ions from soils and water by plants has been well documented, but the actual biological mechanism of metal uptake is not well understood. Discovering the actual ligand(s) responsible for the binding mechanism(s) would allow for the selection of specific plant species for phytoremedation processes.

The objective of this study was to gain a better understanding of the processes through which creosote bush accumulates chromium(VI) and chromium(III) ions, and ascertain the functional chemical groups responsible for chromium binding. Several spectroscopic techniques were employed in this study and they include flame atomic absorption spectroscopy (FAAS), X-ray microfluorescence (XRMF), and X-ray absorption spectroscopy (XAS). Scanning electron microscopy techniques (SEM) were also used to determine the presence of chromium inside creosote bush tissues.

METHODOLOGY

Seeds of *Larrea tridentata* (creosote bush) were obtained from a local vendor in Las Cruces, N.M. (Dr. Daniel Manuchia, Tel (505) 527-9820). The seeds were planted in 250-cm^3 polyethylene pots containing soil collected from a point located east of El Paso, Texas, near the intersection of I-10 and Avenue of the Americas. The plants were maintained under controlled greenhouse conditions at a temperature of 29°C. The chromium-free control soil used to germinate the seedlings was tested for chromium content using EPA method 3150

(acid digestion) and flame atomic absorption spectroscopy.

After the creosote plants were approximately 15 cm high (12 weeks old), every plant was transplanted to a 50-mL centrifuge tube and the roots were covered with glass beads. A 3-mm-wide hole was made at the bottom of the centrifuge tube so that a potassium dichromate (520 parts per million Cr(VI)) solution, adjusted to a pH of 5, could be passed through the glass beads, which supported the creosote plants. The tubes containing the plants were then set inside a plastic tray and exposed to the Cr(VI) solution. The plants were exposed to the solution for a 48-hr time period at a flow rate of 2mL/hr, with fluorescent light at room temperature and an average relative humidity of 60%. The experiments were performed in triplicate for quality assurance. After 48 hours, the plants were harvested from the glass beads and rinsed using tap water for 15 minutes, followed by three separate rinses with de-ionized water.

The plant samples were dried at constant temperature (59°C) in an oven for two days. The samples were then separated into three different sections: roots, stems, and leaves. The different plants' sections were digested following EPA Method 200.3. The following is a brief summary of the digestion method: first, concentrated nitric acid (Trace Pure) was added to a digestion flask containing the sample, followed by 30% hydrogen peroxide; finally, concentrated hydrochloric acid (Trace Pure) was added. Control digestions were also performed using empty digestion flasks to ensure quality control. Certified chromium standards were used in all the digestions (sample spikes).

Flame Atomic Absorption Spectroscopy

The digested plant material was analyzed for chromium content using a Perkin Elmer 3110 flame atomic absorption spectrometer with a deuterium background correction (FAAS). To maximize the sensitivity of the instrument for chromium analysis, an impact bead was used. The chromium analysis was performed at a wavelength of 359.8 nm (recommended analytical line). Calibration of the instrument was performed in the linear working range for chromium, and a correlation coefficient of 0.98 or greater was obtained for all calibrations. The instrument response for chromium was checked periodically with a known chromium standard. All the samples were analyzed in triplicate, and the mean value of the analysis was calculated.

X-ray Microfluorescence

The dried plant tissues were placed on a glass slide and held in place using Kapton tape. The instrumental parameters on the Kevex Omicron X-ray Microfluorescence Spectrometer were as follows: a 100-mm-diameter beam, a molybdenum X-ray source, and an energy dispersive solid-state detector (EDS). The operating parameters for the analysis were 40 KeV energy source, 5-minute count time, 8-10% dead time, and an operating current of 1.47 mA. The chromium analysis was performed on the different plant sections for both the control and exposed plants.

Synchrotron XAS Analysis

All XAS measurements were performed at Stanford Synchrotron Research Laboratory

(SSRL), on beam line 7-3 with the storage ring operating at 3 GeV and currents of 50-100 mA. A double-crystal silicon monochromator (Si 110) was used in conjunction with a 1.0-mm upstream aperture. The crystals of the monochromator were detuned by 50% at the absorption edge to reject higher harmonics. The Chromium-K alpha fluorescence excitation spectrum of the different samples was collected with a Canberra 13- element germanium array detector and the K-edge of the model compounds was measured using X-ray transmittance in nitrogen ionization chambers. The samples were kept at a temperature of 10 K for the analysis using liquid helium. Instrumental calibration at the chromium K-edge was achieved using a chromium(0) foil which has an inflection in the K-edge at 5997 eV. The spectra for both the model compounds and samples were obtained by averaging four scans.

XAS Data Analysis

XAS analytical software used was EXAFSPAK, a suite of computer programs commonly used for EXAFS data analysis (George, 1995). All the XAS data were processed using standard procedures for pre-edge subtraction, spline fit, spline removal, and Fourier filtering. The pre-edge subtraction on all the data was performed using a Gaussian function. To normalize the data, 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 (DW).

Model Compounds

The model compounds were obtained from Fisher Scientific (as pure reagents) to compare with the chromium (III) and chromium(VI) absorbed by the plants. The model compounds included potassium dichromate, chromium(III) nitrate, and an ion exchange resin (composed mainly of weak carboxilic group acids).

Scanning Electron Microscopy

Scanning electron microscopy images of the three different section (roots, stems, and leaves) of creosote bush were obtained in the Instituto Nacional de Investigaciones Nucleares in Mexico (ININ). A Philips XL30 Electroscan, with energy-dispersive spectroscopy (EDS), and an analytical high-vacuum microscope was employed.

RESULTS AND DISCUSSION

There was no evidence of chromium toxicity during the growth and treatment of the creosote plants. The plants were exposed for a period of 48 hours to a solution of 520 ppm of chromium(VI). The digestion procedure used on the plants was a standard EPA method for biological samples. The results collected from the sample and subsequent chromium FAAS analysis are displayed in Table 1. These results show the potential capacity of creosote bush to accumulate and store chromium from solution. The observed chromium uptake in the roots was 57.4, 14.2, and 19.3 mg of chromium per gram
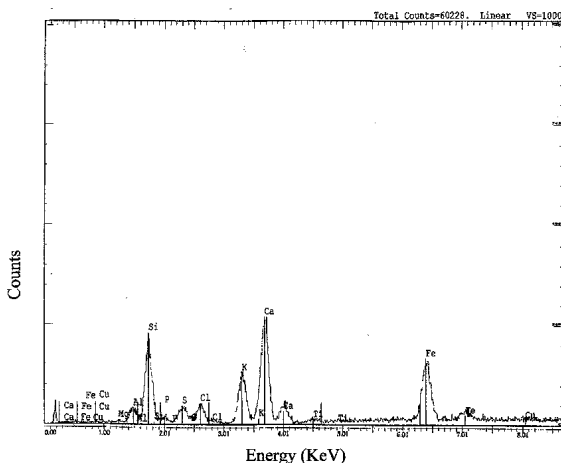
Figure 1. XRMF spectrum of the control creosote bush root samples. The x axis corresponds to the x-ray energy, and the y-axis corresponds to the x-ray counts.
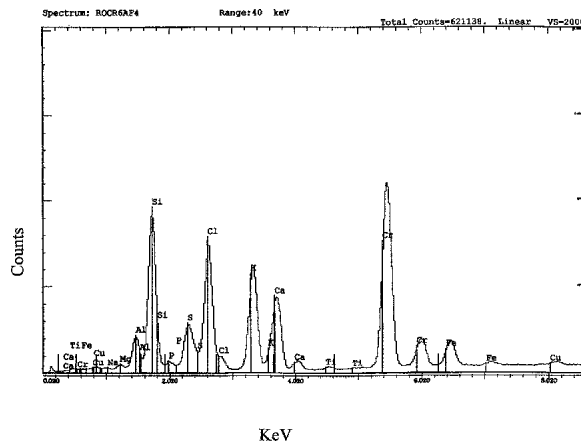

Figure 2. XRMF spectrum of the creosote bush roots of samples exposed to a 520- ppm solution of chromium(VI). The x axis corresponds to the x-ray energy, and the y-axis corresponds to the x-ray counts.

of dried biomass for, respectively, the roots, stems, and leaves of creosote bush. No chromium was detected in the control plants. The data show that the roots of the creosote bush accumulated the highest concentration of chromium from solution, followed by the leaves, and finally the stems. This trend in chromium absorption and storage by the plant reflects the time required for the chromium to be transported from the roots and stems to be accumulated in the leaves of the plant.

The three different plant sections were analyzed for chromium content using X-ray microfluorescence (XRMF). Figure 1 displays the X-ray microfluorescence spectrum for the roots of the control plants. In this figure, there is no evidence of chromium. X-ray microfluorescence spectra were also obtained for the stems and leaves of the same control creosote bush, showing no evidence of chromium (data not shown). Figure 2 shows the X-ray microfluorescence spectrum for the roots of the plants exposed to 520 ppm of chromium(VI). This figure shows a distinct chromium peak (at about 5.5 keV). Figure 3 shows the X-ray microfluorescence spectrum for the stems of the plants exposed to a 520-ppm solution of chromium(VI). In Figure 3 the chromium peak has decreased significantly as compared to the peak observed in Figure 2. Similarly,

Table 1. FAAS results of the digestion for chromium found in the roots, stems, and leaves of the creosote bush samples exposed to the 520-ppm chromium(VI) solution.

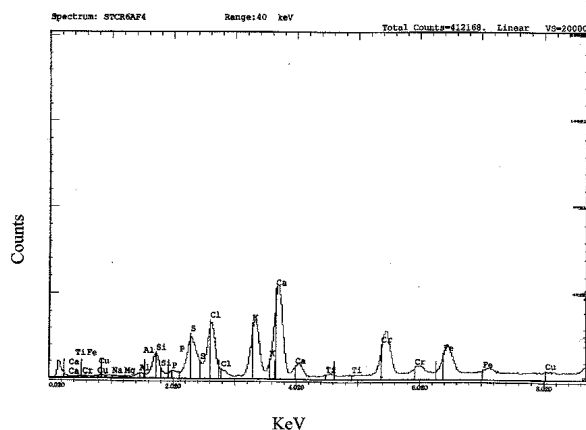| Sample Type	Concentration of Chromium in Sample	+/- 95% Confidence
Leaves	19.3 mg/g	11.0
Stems	14.2 mg/g	6.4
Roots	57.4 mg/g	6.5

Figure 3. XRMF spectrum of the creosote bush stems of samples exposed to a 520-ppm solution of chromium(VI). The x axis corresponds to the x-ray energy, and the y-axis corresponds to the x-ray counts.
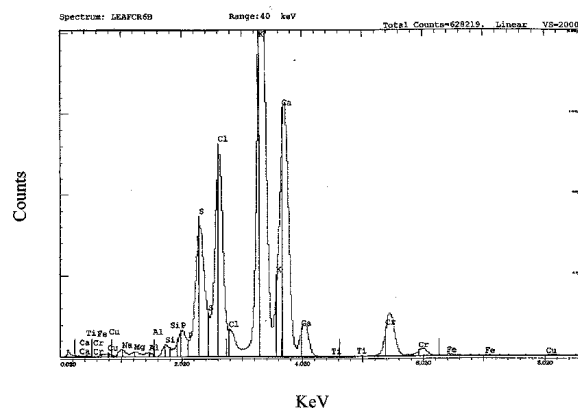

Figure 4. XRMF spectrum of the creosote bush leaves of samples exposed to a 520-ppm solution of chromium(VI). The x axis corresponds to the x-ray energy, and the y-axis corresponds to the x-ray counts.

Figure 4 shows a chromium peak for the leaves of the plant exposed to the same chromium concentration. Thus, it can be concluded from the XRMF data that the uptake of chromium from solution decreases from the roots, but stems and leaves show similar chromium uptake.

The XANES of the plant samples exposed to the chromium(VI) solution are shown in Figure 5, which displays a comparison between chromium(VI) (potassium dichromate), with an absorption energy of 5998 eV, and chromium(III) (chromium(III) nitrate), with an observed absorption energy of 6008 eV. In this figure, it is evident that the chromium in the plants and resin is mainly sorbed as Cr(III). The 1s to 3d transition is apparent in the model compound chromium(III) nitrate, the resin exposed to Cr(VI), and the plant samples (roots, stems, and leaves). Also, by comparing the energy of the edge for chromium(III), the exposed resin and the plant samples (which is at about 6008 eV), and to the edge position for the chromium(III) nitrate, one can suggest that

the coordination environment is similar in the plants and the resin. The inflection points in the figure indicate that the roots absorbed chromium(VI), but it was partially reduced to chromium(III). The stems transported some of the chromium(VI) and chromium(III), but the inflection point observed on the leaves corresponds only to chromium(III), according to the model compound chromium(III) nitrate.

The actual ligand that is binding the chromium(III) to the plant can be determined by looking at the EXAFS (extended x-ray absorption fine structure). The Fourier-transformed magnitudes for the EXAFS for the weak cation exchange resin reacted with chromium(VI) and the different sections of the plants are seen in Figure 6. Several investigators have shown that the chromium-oxygen binding distance in biomass-chromium(III) is about 2.0 Angstroms (Tiemann et al., 2000). The bond lengths obtained from the EXAFS fittings are given in Table 2 for the 520-ppm chromium(VI) exposed samples and resin. On the other hand, as
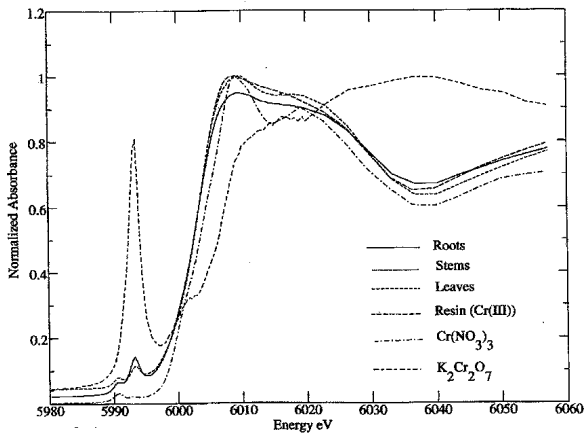
Figure 5. XANES for chromium bound to the resin, creosote bush roots, stems, and leaves of samples exposed to 520-ppm of chromium(VI) compared to chromium(VI) and chromium(III).

seen in Figure 6, a comparison of the plant samples and the resin shows that the bonding radius between chromium(III) and the nearest neighbor is very similar. The EXAFS data further strengthen the argument that some of the chromium(VI) absorbed by the plant in the roots remains in the plant as chromium(VI), but most of the chromium(VI) is reduced to chromium(III); then it is transported through the stems to the leaves, and finally it is accumulated as chromium(III) in the leaves of creosote bush. Also, the chromium(III) is coordinated to oxygen ligands. It can be seen from Table 2

Table 2. Compilation of structural parameters extracted from EXAFS spectra of plants exposed to 520-ppm of chromium(VI). R(Angstroms) is the distance in angstroms from the metal to the neighboring atom. S^2 is the squared standard deviation.

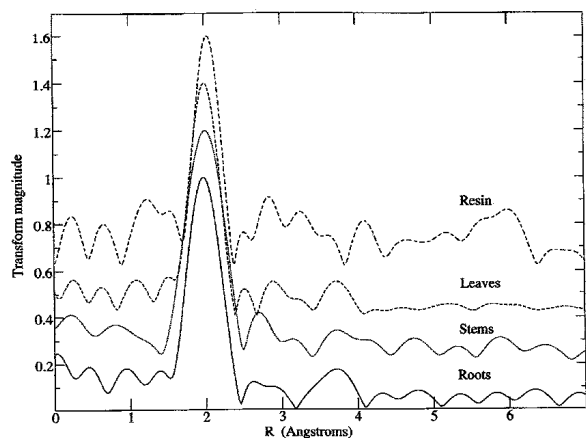| Roots			
Neighboring Atom	Coordination #	R (Angstroms)	S^2
Cr-O	4	1.965	.00275
Cr-O	1	2.3587	.00275
Cr-S	1	3.1186	.00275
Stems			
Neighboring Atom	Coordination #	R (Angstroms)	S^2
Cr-O	4	1.9636	.00035
Cr-O	1	2.3546	.00035
Cr-S	1	3.084	.00035
Leaves			
Neighboring Atom	Coordination #	R (Angstroms)	S^2
Cr-O	4	1.9619	.00146
Cr-O	1	2.3812	.00146
Cr-S	1	3.0825	.00146

Figure 6. EXAFS spectra for the chromium bound to the creosote bush roots, stems, and leaves of samples exposed to 520-ppm Cr(VI), compared with resin bound to Cr(III).
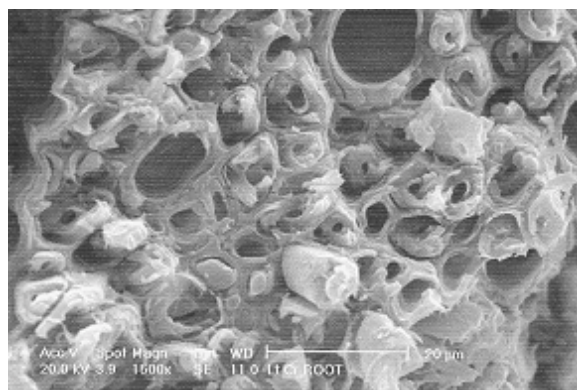

Figure 7. SEM image of the root of creosote bush exposed to 520-ppm chromium(VI).

that for a system with four oxygen atoms coordinated to the chromium, the distance to the the first shell ranges between 1.96 and 1.97 Angstroms.

The detailed photographs that provided important information about the surface structure and morphology from the control sections of creosote bush (not shown) revealed a clear structure of the roots, stems, and leaves that were free of the metal. The image of the scanning electron microscopy in Figure 7 clearly shows the presence of chromium metal accumulated inside the tubes or xylem of the roots of creosote bush of the tissue, represented as a dense clear mass. Figure 8 is an energy-dispersive spectroscopy analysis that corroborates the presence of the metal *in situ*. Figure 9 clearly documents the presence of chromium inside the leaves of creosote bush, which appears as fuzzy material inside the hairy parts of the leaf, when compared with the photograph of the control leaves displayed in Figure 10.

CONCLUSIONS

The data obtained from the FAAS shows that the roots of the creosote bush accumulated the highest concentration of chromium from solution, followed by the leaves, and finally by the stems. This trend in chromium absorption and storage by the plant indicates its ability to uptake the metal to be absorbed, partially reduced, and finally transported from the roots to be accumulated mainly in the leaves.

Examining the spectra obtained from the X-ray microfluorescence analyses, it can be concluded that the uptake of chromium from solution is higher in the roots, and the uptake between stems and leaves appears similar.

The data presented in this study show that chromium(VI) was reduced to chromium(III) by the creosote bush plants and may be bound to carboxyl groups. The XANES comparisons of chromium(VI), chromium(III), and the samples of the creosote bush show that the chromium absorbed by the different parts of the plant is in
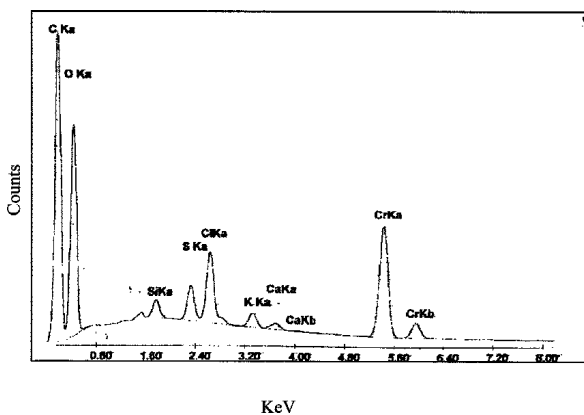
Figure 8. EDS of the root that demonstrates the presence of chromium inside the root of creosote bush exposed to a 520-ppm chromium solution. The x axis corresponds to the energy, and the y-axis corresponds to the x-ray counts.

Figure 9. SEM image of the leaves of creosote bush exposed to 520-ppm chromium(VI) .

a similar oxidation state as chromium(VI) absorbed by resin exposed to the same solution. Data obtained from the EXAFS fittings suggest that chromium(VI) bound by creosote bush starts as chromium(VI) in the roots, and then it is partially reduced to chromium(III), but the metal forms a bond with oxygen atoms that may be part of carboxyl groups. This process is suggested as part of the plant metal-binding mechanism. This study, accomplished under controlled conditions, clearly demonstrates that chromium uptake starts at the roots and is transported to stems and leaves.

AKCNOWLEDGMENTS

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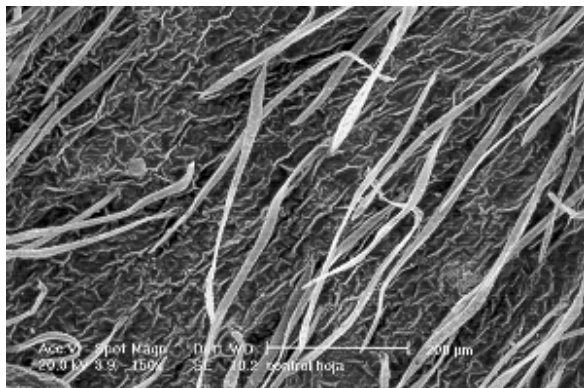Gardea-Torresdey, J. L., K. J. Tiemann, J. H. Gonzales, and O. Rodriguez. Phytofiltration of Hazardous Metals Ions

Figure 10. SEM image of the control leaf of creosote bush.

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