DETERMINATION OF THE CONTENT OF HAZARDOUS HEAVY METALS ON *LARREA TRIDENTATA* GROWN AROUND A CONTAMINATED AREA

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ABSTRACT The content of copper, lead, cadmium, and nickel on tissues of *Larrea tridentata* grown around a contaminated area was determined by atomic absorption spectroscopy. The area was divided into six sections, and each section was studied. Analyses were performed on sample roots, stems, leaves, as well as the soil where the plant grew. Roots showed a high content of the metals, followed by the leaves, and finally the stems, which had the lowest content of the metals. Lead concentrations in roots, leaves, and stems were 650 mg/Kg, 150 mg/Kg, and 110 mg/Kg, respectively, while copper concentrations were 953 mg/Kg, 493 mg/Kg, and 370 mg/Kg, respectively. In contrast, cadmium and nickel concentrations were lower and varied from 30 mg/Kg on roots, 37 mg/Kg on leaves, and 10 mg/Kg on stems for cadmium, and the content of nickel found ranged from 27 mg/Kg on roots, 23 mg/Kg on leaves, and 10 mg/Kg on stems. Soil concentrations were high in site 4 for lead and copper, 5,067 mg/Kg and 4,933 mg/Kg, respectively; lower concentrations were found for cadmium and nickel, 117 mg/Kg and 17 mg/Kg, respectively. The heavy metal content of the soils indicates the degree of pollution in the area. As expected, those sections which contained higher levels of heavy metals in the soil also showed to have higher heavy metal uptake by various parts of *Larrea tridentata*. These data demonstrate *Larrea tridentata's* ability to uptake copper and lead, and to some extent cadmium and nickel, from heavy metal contaminated soils. Analyses of other heavy metals will also be examined.

KEYWORDS: *Larrea tridentata*, hazardous heavy metals, environmental analyses, pollution, atomic absorption spectroscopy

INTRODUCTION

There has been an increasing concern regarding the accumulation of toxic heavy metals in our environment which pose a threat to both public health and the natural ecosystem. The bio-accumulation of heavy metals presents a problem both from the standpoint of how the metals migrate in the environment and how the metals can be effectively removed from contaminated sites. Unlike many substances, metals are not biodegradable, and thus they accumulate in the environment. Many studies have indicated that the accumulation of heavy metals in soil has had an adverse affect on the growth and development of a wide variety of plant species. Although low concentrations of some heavy metals, such as copper and zinc, are necessary for the proper functioning of most plant systems, higher concentrations of copper and zinc have been found to be responsible for metabolic disturbances and growth inhibition of some plants [1-3]. Other studies have demonstrated that the uptake of such metals as lead, nickel, and cadmium can damage the integrity of cell membranes in certain plants [4]. For example, excess concentrations of lead, cadmium, copper,

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and zinc significantly affected the plant water status of sunflowers, causing water deficit and subsequent changes in the plant [5]. Although the uptake of heavy metals is antagonistic to a myriad number of plant systems, other studies have shown that some plants are able to absorb heavy metals, adapt to them, and thrive. Currently, those sites that contain the highest concentrations of heavy metals are situated near industrial sources such as smelters and steel refineries [6-9]. Even in locations such as these, certain plant species have been able to adapt to heavy metal ions [10, 11]. Both the mechanisms that the plants use for adaptation and the specific effects of the metals on the plants' bio-systems, however, remain unclear [12, 13]. Presently, a number of researchers are investigating the manner in which plants absorb heavy metal ions and are conducting experiments with these plants to see if they can be used to alleviate the metal contamination problem. The solution, a technique known as phytoremediation, holds the promise of being both feasible and effective. Because of these potentials, phytoremediation research is exploding [14-16].

Larrea tridentata, referred to as the creosote bush, is the most common desert shrub of the Southwest, covering roughly 20 million acres from western Texas to California [17]. Creosote bushes are found naturally growing in the heavy metal-contaminated soils near a copper smelting operation in El Paso, Texas. The industrial activity in the area has resulted in the accumulation of many heavy metals in the soil including copper, lead, cadmium, and zinc [18, 19]. The accumulation of these heavy metals in the soils, however, may have lessened over time as a result of the metals being uptaken by creosote bushes. To date, no studies have been performed demonstrating creosote bushes as metal scavengers. Because creosote bushes are so prevalent throughout the southwest and because they are able to grow in such contaminated soil environments, they possess the qualities necessary for utilization as phytoremediation resources.

In an effort to begin investigating the potential phytoremediation characteristics of the creosote bush, we determined the amount of heavy metal present in the individual tissues including the roots, leaves, stems, and the soils from which the plants grew. Three replicate samples were collected from various locations on the University of Texas at El Paso (UTEP) property. The samples were oven dried and then the tissues of leaves, roots, and stems were separated. The soil and tissue samples were acid-digested and analyzed via flame atomic absorption spectroscopy (FAA) for the determination of the content of copper, lead, cadmium, and nickel. The objective of this study was to determine the amount of metal ions present in each of the tissues of the creosote bush.

PROCEDURES

Collection of Larrea tridentata (creosote bushes)

A map of the UTEP property and of the outlying vicinity of El Paso was obtained using a computer software ATLAS program by DeLorme©. Six locations were arbitrarily chosen to represent creosote bushes in various locations around the copper smelting industry. A seventh site, located 25 miles from sites 1-6, was chosen for control samples of the bushes. An engineering survey wheel was used to measure the exact distances between the different collection sites. During the summer season of 1995, three replicate samples of creosote bushes, approximately 3 ft. high and of the same stage of maturity, were collected using a shovel. A diameter of approximately 2 ft. was dug around each bush. The bushes were pulled up by the roots, and the residual soils from the roots were shaken off the bushes and collected in plastic containers for later analysis. All three bushes were combined for representative sampling purposes.

Preparation of Larrea tridentata and soils

Samples of creosote bushes from each site were oven dried at a temperature of 90ºC for four days. This mild temperature was chosen to avoid vapor loss of the metals or of their salts. After drying, the leaves, roots, and stems were separated and ground. Only -100 Tyler mesh material was used in the experiment. Soils were also sieved to -100 Tyler mesh to remove unwanted rock and sediment materials from the matrix. After the tissues were obtained, three replicate one gram samples of each tissue were acid-digested according to EPA method 3050 with the exception that no hydrochloric acid was used [20]. A fourth replicate sample was also prepared and digested but was spiked with either 1.0 or 5.0 ppm each of copper, lead, cadmium, and nickel, which were the metals of interest. A blank was also prepared to ensure the integrity of the analytical procedure. The EPA protocol that was adopted for the acid digestion of the soil and tissue samples here can be described as follows: A 1.00-2.00 gram homogenous representative sample was obtained and placed in conical beakers. Sample slurries were prepared by adding 10 ml of 1:1 nitric acid (HNO_3). The slurries were then covered with watch glasses, heated to near boiling, and refluxed for 15 min. After refluxing, the slurries were cooled and then 5 ml of concentrated HNO_3 were added and the solution was again allowed to reflux for an additional 30 min. This last step was repeated to ensure complete oxidation of the metals. After the third refluxing period, the sample was cooled to room temperature and 2 ml of deionized water and up to 10 ml of 30% hydrogen peroxide were added. The samples were then filtered to remove any particulates which might interfere with FAA analysis. The filtrates were collected in 100 ml volumetric flasks and were diluted with deionized water to volume. The samples, which were approximately 5.0% (v/v) nitric acid, were now ready for FAA analysis.

Flame atomic absorption analysis of metals uptaken by Larrea tridentata

Analysis for the metals of interest were performed using a Perkin Elmer model 3110 atomic absorption spectrometer with deuterium background subtraction. Impact bead was utilized to improve the sensitivity. Wavelengths used for the FAA analysis of copper, lead, cadmium, and nickel were 327.4 nm, 283.3 nm, 228.8 nm, and 352.5 nm, respectively. Samples were read three times, and a mean value and relative standard deviation were computed. Calibrations were performed in the range of analysis, and a correlation coefficient for the calibration curve of 0.98 or greater was obtained. The instrument response was periodically checked with known standards.

Data analysis

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

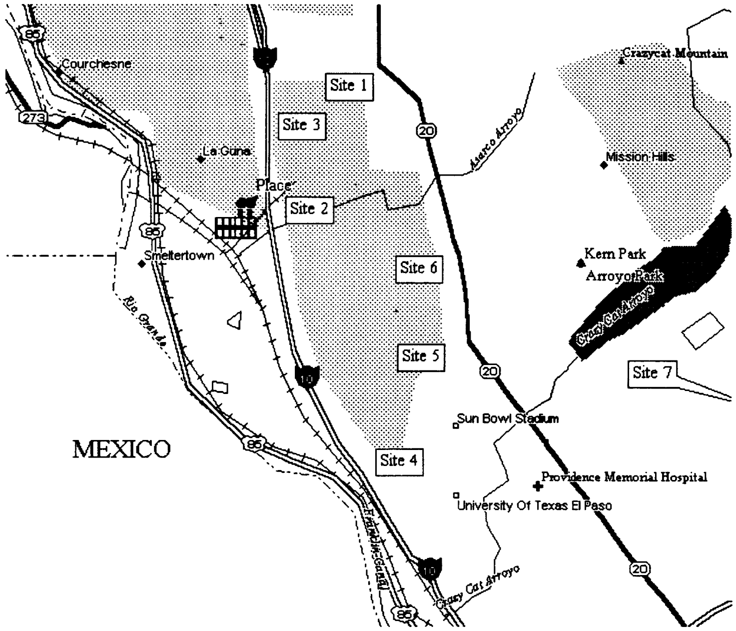

FIGURE 1. MAP OF UTEP PROPERTY AND OUTLYING VICINITY, SHOWING SAMPLING SITES.

RESULTS AND DISCUSSION

The sampling sites selected are shown in Figure 1. The highest concentrations of heavy metals were found in samples from site 4 (Figure 1). Site 4 lead concentrations in roots, leaves, stems, and soil were 650 mg/kg, 150 mg/kg, 110 mg/kg, and 5,067 mg/kg, respectively, while copper concentrations were 953 mg/kg, 493 mg/kg, 370 mg/kg, and 4,933 mg/kg respectively. In contrast, cadmium concentrations from site 4 were lower and varied from 30 mg/kg in the roots, 20 mg/kg in the leaves, 10 mg/kg in the stems, and 117 mg/kg in the soil. The content of nickel determined in creosote from site 4 ranged from 10 mg/kg in the roots, 10 mg/kg in the leaves,10 mg/kg in the stems, and 17 mg/kg in the soil. In all of the samples, copper concentrations were the highest, followed by lead. Cadmium and nickel concentrations were extremely low. Heavy metal concentrations for the soil from which *Larrea tridentata* grew are shown in Figure 2. Plant tissue metal contents for roots, leaves, and stems are reported in Figures 3, 4, and 5, respectively. The total metal concentrations in soils and plants from each site were all nearly 100-fold higher than in the control samples (site 7). The percent recoveries of the spiked metals from the creosote bush tissues and from the soils from which the plants grew is shown in Table 1. The percent recovery data is within the acceptable range, exemplifying that our quality control and assurance of laboratory methodology was performed under the optimum conditions.

The heavy metal content of the soils and plant tissues indicates the degree of pollution in the area. These data also demonstrate *Larrea tridentata*’s ability to uptake copper and lead, and to some extent cadmium and nickel, from heavy metal-contaminated soils. As expected, those sections which contained higher levels of heavy metals in the soil also showed to have higher heavy metal uptake by the various parts of *Larrea tridentata.* The highest levels of heavy metals found in

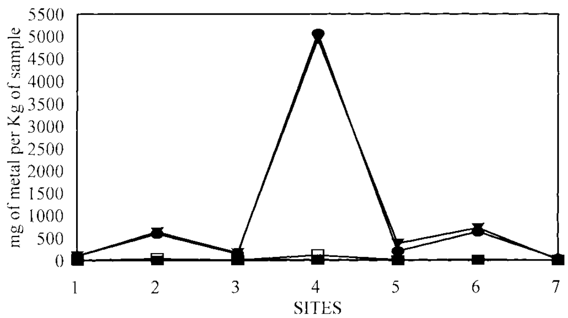

FIGURE 2. COPPER ▼, LEAD ●, NICKEL ■, AND CADMIUM □ CONCENTRATIONS IN SOILS WHERE *LARREA TRIDENTATA* GREW (EVERY DATA POINT REPRESENTS THE MEAN VALUE OF THREE REPLICATE SAMPLES).

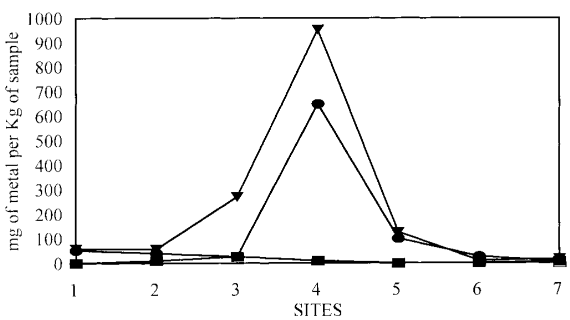

FIGURE 3. COPPER ▼, LEAD ●, NICKEL ■, AND CADMIUM □ CONCENTRATIONS IN ROOTS OF *LARREA TRIDENTATA* (EVERY DATA POINT REPRESENTS THE MEAN VALUE OF THREE REPLICATE SAMPLES).

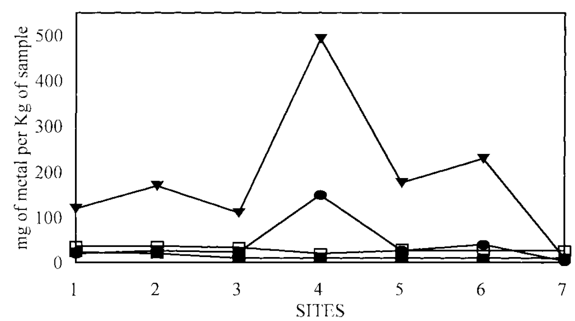

FIGURE 4. COPPER ▼, LEAD ●, NICKEL ■, AND CADMIUM □ CONCENTRATIONS IN LEAVES OF *LARREA TRIDENTATA* (EVERY DATA POINT REPRESENTS THE MEAN VALUE OF THREE REPLICATE SAMPLES).

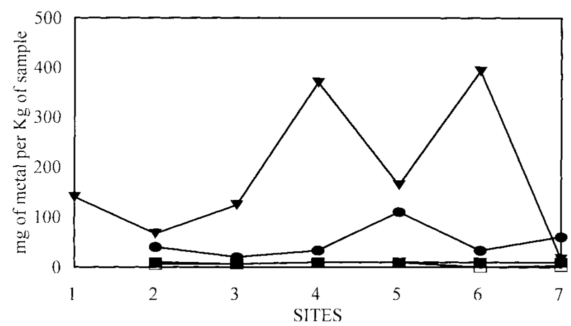

FIGURE 5. COPPER ▼, LEAD ●, NICKEL ■, AND CADMIUM □ CONCENTRATIONS IN STEMS OF *LARREA TRIDENTATA* (EVERY DATA POINT REPRESENTS THE MEAN VALUE OF THREE REPLICATE SAMPLES).

various tissues of creosote were in conflict with the expected values. Particularly, because site 2 was located within the closest proximity to the suspected contamination source, the anticipated data should have been much higher at that site.

At least three factors may be responsible for explaining the unexpectedly low levels of heavy metal concentrations in the samples from site 2. First, the Franklin mountain range which lies in the southeast portion of the Basin and Range province affects the air mass and wind velocity movements in the El Paso region. The air currents could be a possible transportation system for the aerial dispersion of some of the heavy metals. Due to the existing air shed between the mountain ranges of El Paso, New Mexico, and Mexico, southeasterly winds could push any aerial metals away from site 2. Second, the heights of the smelting tower stacks may have been increased throughout the years, resulting in expanded dispersion of the metals throughout the area. Additionally, the area under study experiences heavy thunderstorms in July and August (National Weather Bureau) which could provide the impetus for the drainage or leaching of the metals away from site 2, which is located on a slope, and into the arroyos which are prevalent in the El Paso region. The creosote bushes sampled from site 7 (control site), conversely, do not experience the same environmental conditions. Although site 7 receives more rainfall and less wind shed than do sites 1-6, it is far enough from the suspected point source of contamination that the plants or soil heavy metal concentrations are at infinitesimally low levels. A more effective and desirable method for determining the effect of heavy metal ions on creosote would be to sample from areas closer to the contamination source. One is restricted, however, due to the privately-owned property of the possible contamination site.

The United States is investing billions of dollars in cleaning up polluted ground water and soils, yet this large investment may not be producing the benefits that citizens expect [21]. Most of the current methods for this type of cleanup are very expensive and only marginally effective. Furthermore, the technologies typically used to decontaminate the soils can increase contaminant exposure to cleanup crews and to nearby residents. Because our data demonstrate the ability of creosote bushes to sequester heavy metals from the soils, phytoremediation of contaminated soils using creosote bushes may have an enormous economic value. Additionally, previous methods described using phytoremediation focus merely on water purification or on adsorption by roots, requiring harvesting of the entire plant and replanting. Creosote bushes, which are perennial, could be planted in contaminated soils and allowed to grow while scavenging the metals from the soil. At appropriate intervals, the leaves and branches could be removed from the plant, while allowing the remaining plant tissues to survive. The leaves and branches could then be properly disposed of. This process is continued until the soil is contaminant free. Our data already suggests that creosote bushes are able to uptake heavy metals from soil, but to ensure the success of creosote in commercial-scale phytoremediation, additional field tests are necessary. Additional data must be obtained which more fully describes the if and how

TABLE 1. PERCENT RECOVERIES OF HEAVY METALS.

Sample	Site	Copper	Lead	Nickel	Cadmium
Soil	1	103%	93%	80%	110%
Soil	4	150%	100%	83%	93%
Soil	7	82%	87%	96%	98%
Roots	1	100%	97%	90%	100%
Roots	4	167%	110%	100%	90%
Roots	7	119%	97%	75%	68%
Leaves	1	80%	90%	87%	93%
Leaves	4	87%	110%	100%	120%
Leaves	7	91%	88%	79%	87%
Stems	1	90%	90%	90%	93%
Stems	4	100%	100%	90%	100%
Stems	7	79%	79%	74%	81%

the bushes have adapted to the harsh growth environments. For example, the amount of heavy metal contamination due to aerial deposition on the foliage in comparison to the uptake of metals by the roots from the soil is not clearly understood. Many factors can affect either of these properties. For example, Kohut and coworkers found that soil pH's can significantly affect the mobility of some metals, thereby possibly inhibiting the availability of metals for root uptake [22]. Regarding the aerial deposition, one must also consider foliage properties such as size, and surface characteristics. Also, the entry of the metals through the leaf surfaces is influenced by both the solubility of the metals and by the acidity of the rain.

Clearly, *Larrea tridentata* takes up heavy metals from the soil. It is unclear though whether the plant has evolved to adapt to the high concentrations of heavy metals or if the bushes already possess the capability of being planted under these contaminated conditions and still grow to maturity. Plants are known to have at least two defense mechanisms whereby they are able to incorporate metals into their tissues and continue to survive. In response to the toxic elements, plants can synthesize metal-chelating proteins called metallothioneins [23, 24]. Another mechanism which may account for the accumulation of heavy metals in plants is the synthesis of phytochelatins, as suggested by Rauser [23]. Various other mechanisms have also been suggested [25-28] regarding where the heavy metals are compartmentalized. Plants can also tolerate heavy metal contaminates by excluding the metals from sensitive sites, changing the metabolic pathways to prevent damage, or by synthesizing enzymes that would detoxify the heavy metals [29]. Detrimental effects of heavy metals on plants have also been found to prevent the uptake of valuable nutrients such as potassium and phosphorus [30, 31]. All of these parameters regarding the effects of heavy metals in relation to *Larrea tridentata* will require further investigation.

Further studies will involve utilizing appropriate analytical techniques to investigate the nature of the binding mechanism as well as the chemical functional groups responsible for the binding or uptake. For example, Environmental Scanning Electron Microscopy (ESEM) studies, in conjunction with X-ray Energy Dispersive Spectroscopy (EDS), will be undertaken to examine any noticeable morphological changes as a result of the incorporation of heavy metals in the tissues of the creosote bush. The future investigation of examining the molecular and structural characteristics which control the metal ion affinity and specificity in creosote bushes will ultimately aid in establishing our ability to selectively modify the metal binding properties. As a result, the biomaterials' metal scavenging abilities can be substantially enhanced.

CONCLUSIONS

Creosote bush plants have succeeded in demonstrating the ability to uptake heavy metals such as copper and lead from contaminated soils. The metal concentrations in the plant are dispersed throughout the roots, leaves, and stems with the highest concentration found in the roots. As expected, the higher the contaminant soil concentration, the higher the metal uptake by the various parts of *Larrea tridentata*. These data demonstrate the potential of phytoremediation via creosote bush as a low-cost, effective means of removing heavy metals from contaminated soils. The nature of the chemical groups that are responsible for the binding of the metals, however, is not fully understood and will be the purpose of further investigation.

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