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Uptake of Trace Elements in Leaves of the *Larrea Tridentata* (DC.) Coville in Desert Washes of an Arid Environment

Douglas B. Sims^{1,*}, Christopher J. Collumb², Amanda C. Hudson¹ and Douglas J. Walton¹

- ¹ Department of Physical Sciences, College of Southern Nevada, Las Vegas, NV 89141, USA; amanda.hudson@csn.edu (A.C.H.); douglas.walton@csn.edu (D.J.W.)
- ² Department of Biological Sciences, College of Southern Nevada, Las Vegas, NV 89141, USA; christopher.collumb@csn.edu
- * Correspondence: douglas.sims@csn.edu; Tel.: +1-702-651-4840

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Abstract: Trace elements (As, Cd, Cr, Pb, Ba, Fe, Al, Mn and Ba) were uptaken by the leaves of the creosote bush (*Larrea tridentata* (DC.) Coville) in Nelson, Nevada, although at low concentrations. Samples were collected up-gradient of the mine tailings, the tailings, and down gradient from the source to measure spatial distribution. Data show that trace elements (As, Ba, Cr, Hg, Se) enter *L. tridentata* through root tissues, migrating to leaf tissue, but at significantly lower levels than that of the source sediments. Metalloid (As and Se) concentrations in the leaf tissues ranged from non-detect to greater than 44 mg kg⁻¹ As and non-detect to over 34 mg kg⁻¹ Se. For trace metals, Hg ranged from non-detect to 0.14 mg kg⁻¹; Ba from 1.74 to 4.12 mg kg⁻¹; and Cr from non-detect to 6.18 mg kg⁻¹ while Ag, Cd, and Pb were not detected in the plant leaves. When comparing the ratio of sediment metal concentration to plant metal concentrations, the Techatticup Wash contained the highest levels of trace elements in the leaves of the *L. tridentata*, followed by the Carnation Wash, with the Eagle Wash containing the lowest concentrations of trace elements.

Keywords: tailings; Nevada; trace elements; metals; plant uptake; arid; creosote

1. Introduction

Impacts of abandoned mine waste extend beyond potential contamination of water and sediments. The transport of trace metals and metalloids ("elements") in the environment and into the food chain can pose environmental and human health risks. Such risks depend on the bioavailability of trace elements for plants to uptake from sediment [1]. Biological impacts include the health and wellbeing of plants, animals, and humans exposed to contaminated water and food supplies [2,3]. Abandoned mining areas may pose potential risks to flora, fauna, and ultimately human health both locally and at distance from sources. Furthermore, the biological uptake by plants can affect the fate and transport of trace elements into the food chain [3,4].

Plant uptake of trace elements is a complex process involving transfer of chemical species from sediments across the root membranes to plant cells [1,5]. Morel and Hering [5] explain that the principal limiting factor for plant uptake of trace elements is the availability of sediment moisture, not simply the solubility of the trace element. Hooda [1] also states that the bioavailability of trace elements for plants to uptake from sediment is the key to understanding its environmental effect. Plant materials around mining sites were found to contain higher levels of trace elements such as Pb and As, correlating to higher concentrations in sediments [3]. Studies of base metal and uranium contaminants have proven the potential for uptake by local vegetation for remediation of soil contamination [6]. Studies of the

uptake of Cu, Ni, Fe, Co, Zn, U, and Pb in vegetation and animal tissues near mining activities have shown biological impacts beyond the immediate vicinity of mine sites [3,6,7].

Researchers have shown that the bioavailability and uptake of trace elements from sediment to plant is controlled by sediment moisture, pH, presence of mycorrhiza, and plant physiology [1,8]. Studies have determined sediment pH is a key factor influencing solubility and bioavailability of metals in plants. The species of the plant present determines which trace elements are utilized by the plant or blocked from uptake [8]. Some vascular plant species are capable of utilizing metals as a self-defense mechanism from insects, while others are capable of blocking certain trace elements due to their adverse effect on plant growth [4,9]. The arbuscular *mycorrhiza* fungi found in mutualistic association with the *L. tridentata* (DC.) Coville are also capable of protecting plants from adverse trace element exposure [10–12].

Reglero et al. [6] demonstrated that certain trace elements (metals and metalloids) such as Pb and As can be absorbed at high levels in plant tissues when present in contaminated surficial sediments. At lower levels, plants like the Elmleaf blackberry (*Rubus ulmifolius*) is known to bioaccumulate trace elements over time [6]. Studies have shown that plants with the highest levels of Pb tended to have broader leaves [3].

Higher levels of trace elements can be found in certain grasses surrounding mining contaminated sites [3,6]. Near the Pb mines in the Sierra Madrona Mountains in Southern Spain, plants showed higher concentrations of metals (Pb, Zn, Cd, Cu, As, and Se) when trace elements were highly concentrated in sediments due to the mining and milling process. High trace element concentrations were more prevalent in vegetation in or near stream sediments adjacent to tailing dumps [6].

In arid regions, limited sediment moisture reduces trace element uptake and might reduce the toxicity of contaminants in the soil [13]. Research by Gardea-Torresdey et al. [14] focused on trace element uptake by the *L*. in contaminated sediments of El Paso, Texas (USA). They determined that sediments with Pb and Ni containing roughly 5000 mg kg⁻¹, and Cu and Cd containing >100 mg kg⁻¹, had a 0.17:1 transfer ratio (plant:sediment) in the leaves of the *L. tridentata*. Other authors determined that the roots, stem and leaves of the *L. tridentata* with Ni²⁺, Cd²⁺, Pb²⁺, Zn²⁺, Cr²⁺ and Cr⁶⁺ due to an ion-exchange process and sediment pH rather than moisture [15]. Gardea-Torresdey et al. [14] illustrated that the sediment pH controls the transfer of metals from sediment solution to plant, a lower pH (<7.5) will allow trace elements such as Ni to uptake more readily then at pH above 8.0. Finally, Peralta-Videa et al. [8] demonstrated that Cd and Cr are more susceptible to plant uptake when the pH is <7.1.

Studies have shown that the uptake of trace elements in plant tissues is a potential source of bio-remediation [14]. Certain plants can tolerate a higher level of metals due to detoxification based on chelation and subcellular compartmentalization of trace elements [16,17]. Other plants block or limit uptake of certain trace elements that would be toxic to the plant under the environmental conditions of the site [17,18].

L. tridentata is a recent addition to the Mojave Desert and the Great Basin dating back to approximately 10,000 years before present (BP) [19]. It began its migration northward at the end of the Last Glacial Maximum (Pleistocene) as the climate began to warm [19,20]. McAuliffe [19] showed that a single *L. tridentata* plant can survive more than 1000 years by cloning itself when the inner stems die following new stems appearing on the fringe of the plant.

The *L. tridentata* is the dominant plant species in the study area due to its ability to survive in arid to hyper-arid climates. *L. tridentata* is a perennial plant that can preserve its leaf structure for extended periods of time and extend its main root (tap root) up to 25 m below the surface in search of water resources [7,21,22]. *L. tridentata* can absorb and accumulate trace elements and metalloids from sediments in tissues, even in hyper-arid desert climates [14,23,24]. It is known that the *L. tridentata* has a high tolerance to environmental stresses such as extreme droughts [24].

Research goals of this study were to evaluate the ability of *L. tridentata* biomass grown in contaminated soils to adsorb or, uptake, trace elements (Al, As, Ag, Ba, Cd, Cr, Hg, Fe, Mn, Pb, and Se) in an arid environment. In order to help understand trace elements uptake, the leaves of the

L. tridentata growing in desert washes of historic Nelson, Nevada, a mining area, were collected and evaluated (Figure 1). Plants chosen for sampling had to be at least 120 cm high and 120 cm wide for appropriate maturity. This area is characterized as having little rainfall, low sediment moisture, and high summer temperatures. The *L. tridentata* leaves were chosen for sampling as it has been documented to absorb metal (e.g., Al, Ag, Ba, Cd, Cr, Hg, Fe, Mn, Pb) and metalloid (e.g., As, Se) contaminants from sediments [15,16,23–26]. The *L. tridentata* is the dominate plant species at all sampling sites; no other plants or shallow-rooted grasses were commonly present. Objectives were to (1) evaluate the uptake of trace elements in the biomass of the leaves, (2) their element accumulation, (3) transfer of trace elements from sediment to leaf tissue, and (4) compare data on the transfer of trace elements from sediment to roots of the *L. tridentata*.



Figure 1. Creosote Bush (*Larrea tridentata* (DC.) Coville) in the Mojave Desert, approximately 1.25 meters in height.

2. Environmental Setting

As stated in Sims [27], igneous and metamorphic rocks comprise most of the bedrock geology across the region. In the Nelson, NV area, materials are comprised of fine-grained quartz monzonites. The sediments are composed of sandy gravel and sediments of volcanic origins. Sediments are fine, aeolian silts deposited at the surface to a depth of approximately 15–25 cm. Wind-blown materials are covered by stage-2 desert pavement atop alluvial fan deposits and bedrock out croppings, with exposed sediments in east-west trending ephemeral washes. Below the wind-blown deposits are layers of alluvial gravels and cobbles with cemented layers ranging from a few centimeters to several meters in thickness. The area has exposed bedrock in deeply cut, dry washes oriented east to west with a constant 3–5% downward slope towards Lake Mohave. Sediments are described as containing 1% to 7% calcium-carbonate deposits because of the low precipitation and therefore low potential for transport in the area [27].

The annual precipitation across southern Nevada averages approximately 10 cm; however, it can be as little as 7.6 cm in Nelson, Nevada and in 2008, less than 2 cm of precipitation was measured. The summer precipitation occurs as major convectional storms while winter rains are known to produce less than 1 cm per event. Approximately 35% to 40% of precipitation events occur as 1 cm or greater from a single summer thunderstorm [27].

3. Methods and Materials

3.1. Metals in Sediments

Sediment samples were collected during the summer of 2008 to investigate the transport of trace elements in surface sediments of the three ephemeral washes of Techatticup Wash, Eagle Wash,

and Carnation Wash. Samples were collected from three washes (n = 30) to evaluate the relationship between contaminant distributions in sediments and the *L. tridentata*: 10 samples each from the Techatticup, Eagle, and Carnation washes (Figures 2 and 3. Accordingly, composite sediment were collected in the Techatticup Wash, Eagle Wash Carnation Wash. Samples were collected in the following manner: -60 m, -120 m and -180 m in the respective washes, above the source area, and at several locations down from each wash (50 m, 100 m, 200 m, 400 m, 1000 m, 2000 m, 4000 m and 6000 m) starting from source areas in each selected wash system. Sampling distances were largely necessitated by field conditions and natural variations in the washes. However, the chosen distances and locations make it possible to evaluate a larger area of tailings distributions with a limited number of locations.

Sediment sampling consisted of a thoroughly mixed composite of three subsamples collected from each sampling location. Field procedures for sampling washes involved the following: (1) three equally spaced locations were selected in a linear fashion across a wash area; (2) one sample was taken from each boundary of the wash and one from the center of the wash; and (3) each subsample was taken from a 10 cm by 10 cm square excavated to a depth of 5 cm below the surface.

Sediment analysis for metals and metalloids was accomplished using the United States Environmental Protection Agency (USEPA) preparation method 3050B followed by USEPA method 6010B on a Perkin Elmer Optima 3000 Dual View (DV) inductively coupled plasma-optical emission spectrometry instrument [26]. Mercury analysis was performed per USEPA method 7471 [26] on a Leeman Labs Hydra AA Mercury Analyzer HA-1020 W/ Hydra Pump HP-1007, Cold Vapor Atomic Adsorption instrument. A more detailed description on sediment processing and data is available in Sims [27].



Figure 2. Map of sampling areas in Nelson, Nevada (USA). 2a: Techatticup Wash sampling insert; 2b: Carnation Wash sampling insert; and 2c: Eagle Wash sampling insert.



Figure 3. (**a**): Techatticup Wash sampling locations; (**b**): Carnation Wash sampling locations; and (**c**): Eagle Wash sampling locations.

3.2. Metals in Leaves of the Larrea Tridentata (DC.) Coville

Plant samples were collected from three washes (n = 30) to evaluate the relationship between contaminant distributions in sediments and uptake by the *L. tridentata*: 10 samples each from the Techatticup, Eagle, and Carnation washes (Figure 2). Leaves were gathered from *L. tridentata* closest to sediment sampling (sediment n = 30) points in the wash transects as outlined in Sims [27] (Figure 3). *L. tridentata* leaves were collected with new disposable gloves, placed in a plastic bag and labeled for later reference in the laboratory. *L. tridentata* was chosen for sampling as it has been documented to be able to absorb metal and metalloid contaminants from sediments in arid climates [16,23].

Leaf samples were analyzed for trace elements per USEPA methods by a Perkin Elmer Optima 3000 Dual View (DV) inductively coupled plasma-optical emission spectrometry techniques. Mercury was analyzed by a Leeman Labs Hydra AA Mercury Analyzer HA-1020 W/ Hydra Pump HP-1007, Cold Vapor Atomic Adsorption techniques [26]. While there is an elevated level of cyanide (CN^-) in this area as a result of mining, CN^- in the *L. tridentata* was not one of the target analytes because of its toxicity to plants [23]. Moreover, while CN^- was part of the sediment evaluation [27], vascular plants possess the ability to convert CN^- to amino acid asparagine where free CN^- is less

than 0.4 mg L⁻¹. When CN⁻ concentration was greater than 0.4 mg L⁻¹, plants including *L. tridentata* died [28].

Plant samples were washed with hot (95 °C) deionized water to remove any wind and/or atmospherical deposition from leaf materials. After washing, plant samples were dried at 30 °C for 48 h to remove all moisture. After drying, plants leaves were analyzed using USEPA preparation method 3050B (hot aqua-regia acid) followed by USEPA analytical method 6010B (inductively coupled plasma-optical emission spectrometry) for metals and metalloids (As, Cd, Cr, Pb, Ba, Fe, Al, Mn and Ba) analysis.

Briefly, USEPA method 3050B encompasses 1.0 g of dried plant material digested with 6 mL of concentrated hot aqua-regia acid for two hours on a hot plate heated to 100 °C. Samples were observed constantly to ensure that they were not evaporated to dryness by adding deionized reagent water. After the initial two hours heating, 3 mL of 30% H_2O_2 was added to each sample and reheated for an additional 30 min. After the second heating with an addition of H_2O_2 , samples were brought to a final volume of 100 mL with deionized reagent water. Each sample was filtered through a Whatman 541 (2 µm) filter paper prior to analysis by inductively coupled plasma-optical emission spectrometry (ICP-OES) with vertical and axial measurement.

Mercury analysis was performed according to USEPA method 7471 [26]. Briefly, this involved heating 0.2 g of dried sample mass with 5 mL of double deionized water and 5 mL of aqua regia heated for 2 min in a 95 °C water bath before adding 50 mL of double deionized reagent water and 15 mL of a 10% solution of KMnO₄. After 30 min the samples were removed from the bath, cooled and 6 mL of a 10% solution of sodium chloride-hydroxylamine sulfate were added to reduce the KMnO₄. Digestates were analyzed on a cold vapor atomic adsorption (CVAA) instrument per USEPA method 7471.

During sample preparation, samples were processed in duplicate and included field duplicates, one laboratory duplicate, matrix spike duplicates of one field sample, a fortified sample (certified reference material was not available for this research), and a reagent blank. All samples were processed in the same fashion as stated above. All reagents used were of ultra-pure quality, National Institute of Standards and Technology (NIST) traceable.

3.3. Correlation Plots, Box Plots, Transfer Ratios

Correlation plots were utilized to identify relationship between As, Hg, Ba, Cr, and Se in the leaves of the *L. tridentata* and sediment across the Carnation, Eagle, and Techatticup washes. Furthermore, Box plots showing sediment trace metals to plant transfer ratios across the three washes was also utilized to identify association and presented below with a detailed statistical evaluation located in Sims [27].

3.4. Quality Control Measure

Plant samples were processed according to USEPA Solid Waste 846 (SW-846) protocols, including quality control [26]. Prior to digestion, plant samples were washed with 90 °C deionized water to remove dust and debris. Samples were processed in duplicate and included field duplicates, one laboratory duplicate, matrix spike duplicates of one field sample, a fortified sample (certified reference material was not available for this research), and a reagent blank. Analytical integrity was further verified with a USEPA certified reference sample (RTC Corporation: CRM022-020, Sample 5, lot D522) matrix of non-plant material. Instrument calibration consisted of six points across a concentration range (including a blank) and was fitted by linear regression (R2) > 0.995 [26]. The quality control sample measures within specified windows (\pm 15%) and exceeded specified requirements. Samples were analyzed in duplicate with a required relative standard deviation (RSD) of <20% to qualify as acceptable per USEPA protocols [26]. Statistics for mean, standard deviation, and duplicate for each sample location can be found in Sims [27]. Statistics for data across the 30 samples can be found in tables of each wash presented below (Tables 1–3).

4. Results

This study evaluated the transport of trace elements (Ag, As, Ba, Cd, Cr, Hg, Pb, and Se) in leaf tissue of *L. tridentata* in three desert washes (e.g., Carnation, Eagle and Techatticup) near the historic mining town of Nelson; an arid region of southern Nevada (USA). Sediment data of the three washes is available in Sims [27] were used for comparing of trace element concentration in leaf tissues. That study [27] provided evidence of the transport of contaminants in this arid region, contrary to the generally held belief of such materials are stationary due to arid environmental conditions [29]. They found that trace elements were transported as far as 6000 m down gradient from source to areas not directly impacted by mining activities.

While Ag, Cd, and Pb were detected in sediments as discussed in Sims [27], they were not detected in plant leaves from the wash or background areas. It is likely that Ag, Cd, and Pb are not present in the leaves of the *L. tridentata* due to selective uptake by the plant root as described by Prasad [30]. Studies have found that certain plants are averse to metals such as Cd, Cu, and Pb in water poor environments [17,31,32]. Mackey et al. [32] found that reduction of transport of trace elements across the cell membranes has evolved as survival strategies of some plants in water poor environments. For example, Cd can reduce water uptake in plant roots, reducing the uptake of toxic trace elements. Research has shown that Ag, Cd, and Pb are not susceptible to sediment-to-plant transfer in a system with a pH greater than 8.0 [8,33–35]. Sediment pH in the Nelson, NV areas is consistently greater than 8.0, the reason these three metals are not detected in plant tissues.

4.1. Carnation Wash System

Concentrations of trace elements in the *L. tridentata* leaves from Carnation Wash are provided in Table 1. Background concentrations (up-gradient from the source) of Hg in leaf material were below the detection limit of 0.01 mg kg⁻¹. The mean concentration of Hg in leaf material (down ward from the source) was 0.09 mg kg⁻¹, ranging from <0.01 to 0.14 mg kg⁻¹. Background concentration of As in leaf material was relatively high at 37.68 mg kg⁻¹. The mean concentration of As in leaf material was 16 mg kg⁻¹ and ranged from below detection limit (<0.05) to 44 mg kg⁻¹. Barium in leaf material was 6.9 mg kg⁻¹ with a mean concentration of 11.86 mg kg⁻¹ ranging from 1.74 to 37.5 mg kg⁻¹. Chromium in leaf material was 0.629 mg kg⁻¹ with a mean concentration of 1.94 mg kg⁻¹ ranging between <0.01 and 6.18 mg kg⁻¹. Background concentration of Se in leaf material was 6.14 mg kg⁻¹ with a mean of 9.79 mg kg⁻¹ and range of 0.36 to 21.1 mg kg⁻¹.

Sample Location (meter)	pН	Hg	Ag	As	Ba	Cd	Cr	Pb	Se
Background	8.5	< 0.01	< 0.01	37	6.88	< 0.01	0.629	< 0.05	6.14
Source	8.5	0.11	< 0.01	31	18.4	< 0.01	6.18	< 0.05	0.514
50	8.5	< 0.01	< 0.01	6.2	15	< 0.01	6.07	< 0.05	0.359
100	8.2	0.10	< 0.01	44	4.5	< 0.01	0.656	< 0.05	21.1
200	8.4	0.11	< 0.01	17.9	4.18	< 0.01	0.388	< 0.05	17.3
400	8.6	0.09	< 0.01	16.8	4.20	< 0.01	0.394	< 0.05	16.7
1000	8.5	< 0.01	< 0.01	4.97	37.5	< 0.01	0.445	< 0.05	13.1
2000	8.4	0.10	< 0.01	< 0.05	1.74	< 0.01	0.741	< 0.05	7.47
4000	8.4	0.14	< 0.01	3.32	5.94	< 0.01	< 0.01	< 0.05	8.11
6000	8.5	0.13	< 0.01	0.08	7.62	< 0.01	1.06	< 0.05	10.4
STDV	-	0.06	-	15.6	11.8	-	2.6	-	7.36
Mean	-	0.09	-	14.1	11.9	-	1.94	-	9.79
Max	8.6	0.14	-	44.3	37.5	-	6.18	-	21.1
Min	8.2	-	-	-	1.74	-	-	-	0.36

Table 1. Carnation Wash trace metals concentrations (mg kg⁻¹) in *L. tridentata* (DC.) Coville leaves (n = 10).

Background is an average of three points (-60, -120 and -180 meters [m]) up-gradient from source material. < is below detection limits; background data not used for summary statistics.

4.2. Eagle Wash System

Trace elements in the Eagle Wash *L. tridentata* leaves are provided in Table 2. Background Hg in leaf material was below the detection limit of 0.01 mg kg⁻¹. The mean concentration of Hg in leaf material was 0.08 mg kg⁻¹ and ranged from <0.01 to 0.121 mg kg⁻¹. Background concentration of As in leaf material was <0.05 mg kg⁻¹ with a mean equaling background levels in *L. tridentata* leaves. Background concentration of Ba in leaf material was 2.73 mg kg⁻¹ with a mean concentration of Ba in leaf material of 9.04 mg kg⁻¹ and ranged from 4.12 to 14.5 mg kg⁻¹. Background of Cr in leaf material was <0.01 with a mean of 0.63 mg kg⁻¹ and ranged from 0.198 to 1.01 mg kg⁻¹ in the *L. tridentata* leaves. Background concentration of Se in leaf material was 6.14 mg kg⁻¹ with a mean concentration of Se in *L. tridentata* leaves of 9.78 mg kg⁻¹ with a maximum level of 21.1 mg kg⁻¹.

Sample Location (meter)	pН	Hg	Ag	As	Ba	Cd	Cr	Pb	Se
Background	8.5	< 0.01	< 0.01	< 0.05	2.73	< 0.01	0.198	< 0.05	9.7
Source	8.4	< 0.01	< 0.01	< 0.05	4.12	< 0.01	0.875	< 0.05	12.1
50	8.5	0.09	< 0.01	< 0.05	4.52	< 0.01	1.01	< 0.05	11.4
100	8.3	0.08	< 0.01	< 0.05	5.56	< 0.01	0.241	< 0.05	21.5
200	8.5	0.09	< 0.01	< 0.05	11.0	< 0.01	0.545	< 0.05	12.4
400	8.4	< 0.01	< 0.01	< 0.05	9.72	< 0.01	0.909	< 0.05	34.7
1000	8.5	0.13	< 0.01	< 0.05	10.9	< 0.01	0.203	< 0.05	11.3
2000	8.5	0.11	< 0.01	< 0.05	12.2	< 0.01	0.535	< 0.05	11.4
4000	8.2	0.121	< 0.01	0.103	14.5	< 0.01	0.589	< 0.05	18.6
6000	8.5	0.12	< 0.01	0.152	13.8	< 0.01	0.503	< 0.05	12.21
STDV	-	0.05	-	0.05	4.10	-	0.28	-	7.46
Mean	-	0.08	-	0.03	9.04	-	0.63	-	15.88
Max	8.5	0.121	-	0.152	14.50	-	1.01	-	34.7
Min	8.2	-	-	-	4.12	-	0.20	-	11.3

Table 2. Eagle Wash trace element concentrations (mg kg⁻¹) in *L. tridentata* (DC.) Coville leaves (n = 10).

Background is an average of three points (-60, -120 and -180 m) up-gradient from source material. < signifies below detection limits; background data not used for statistics.

4.3. Techatticup Wash System

Trace element concentrations in the Techatticup wash *L. tridentata* leaves are provided in Table 3. Background concentration of Hg in leaf material was below the detection limit of 0.01 mg kg⁻¹. The mean concentration of Hg in leaf material was 0.08 mg kg⁻¹ and ranged from below detection limit of 0.01 to 0.13 mg kg⁻¹. Background concentration of As in leaf material was below the detection limit of 0.05 mg kg⁻¹. The mean concentration of As in leaf material was 10.44 mg kg⁻¹ and ranged from below detection limit of 0.05 to 33.16 mg kg⁻¹.

Barium in leaf material was measured at 2.83 mg kg⁻¹ with a mean of in leaf material of 3.16 mg kg⁻¹; ranging from 1.88 to 3.78 mg kg⁻¹. Background concentration for Cr in *L. tridentata* leaves was 0.191 mg kg⁻¹; mean concentration was 0.27 mg kg⁻¹ and ranged from below detection limit of 0.01 to 0.92 mg kg⁻¹. Background levels of Se in *L. tridentata* leaves was measured at 13.4 mg kg⁻¹; mean concentration of Se was 9.03 mg kg⁻¹ and ranged from below detection limit of 0.05 to 15.4 mg kg⁻¹.

Sample Location (meter)	pН	Hg	Ag	As	Ba	Cd	Cr	Pb	Se
Background	8.4	< 0.01	< 0.01	< 0.05	2.83	< 0.01	0.191	< 0.05	13.4
Source	8.4	0.118	< 0.01	0.144	3.48	< 0.01	0.842	< 0.05	13.9
50	8.3	0.089	< 0.01	13.8	2.59	< 0.01	< 0.01	< 0.05	13.9
100	8.6	0.104	< 0.01	33	3.78	< 0.01	< 0.01	< 0.05	15.4
200	8.5	< 0.01	< 0.01	10.1	1.88	< 0.01	0.291	< 0.05	5.35
400	8.5	0.115	< 0.01	< 0.05	3.1	< 0.01	0.131	< 0.05	2.76
1000	8.5	0.125	< 0.01	11.0	3.35	< 0.01	0.922	< 0.05	< 0.05
2000	8.3	0.077	< 0.01	9.12	3.7	< 0.01	0.081	< 0.05	10.8
4000	8.2	0.051	< 0.01	8.41	3.21	< 0.01	0.078	< 0.05	9.75
6000	8.5	0.054	< 0.01	8.21	3.31	< 0.01	0.071	< 0.05	9.41
STDV	-	0.04	-	9.71	0.59	-	0.36	-	5.32
Mean	-	0.08	-	10.44	3.16	-	0.27	-	9.03
Max	8.6	0.13	-	33.16	3.78	-	0.92	-	15.4
Min	8.2	-	-	-	1.88	-	-	-	-

Table 3. Techatticup Wash trace element concentrations (mg kg⁻¹) in *L. tridentata* (DC.) Coville leaves (n = 10).

Background is an average of three points (-60, -120 and -180 m) up-gradient from source material. < signifies below detection limits; background data not used for statistics.

5. Discussion

Previous studies have shown that *L. tridentata* can survive in sediments containing significantly high levels of trace elements (>5000 mg kg⁻¹ Pb, >100 mg kg⁻¹ Cd, >4900 mg kg⁻¹ Cr, >10 mg kg⁻¹ Hg and >600 mg kg⁻¹ Se) with little impact on its physiology [4,14,33]. Trace elements in the sediments of the three washes in Nelson, NV were found to be at lower levels than those previously referenced studies and are therefore not likely to adversely impact *L. tridentata* physiology or growth. Concentrations of trace elements measured in *L. tridentata* leaves across the three desert washes are similar to those found by Gardea-Torresdey et al. [14,16]. Some trace elements, while present in sediments, might not be available for transfer from sediment to the *L. tridentata* due to low sediment moisture and high pH, two key factors that are critical uptake according to Hooda [1]. Thus, sediment trace elements uptake.

5.1. Comparisons of Trace Elements in L. Tridentata (DC.) Coville Leaves and Sediments in the Three Washes

Sample data comparison of trace elements in sediments; see Sims et al. [36], and the leaves of the *L. tridentata* was analyzed by regression analysis to determine if relations existed between the two media. Results of As, Hg, Ba, Cr, and Se in sediments vs. *L. tridentata* leaves are shown in Figure 4.

Correlation analyses showed trace element concentrations in sediments have little to no association with trace element concentrations in the leaves of *L. tridentata* (Figure 4). While low sediment pH (<7.0) would increase bioavailability of metals for plant uptake [35], the pH in the three washes was consistently >8.0, indicating low bioavailability of certain metals such as Cd, Cu, Ni, and Zn [8]. Moreover, low precipitation in the Nelson area (<7cm per year) would greatly reduce root uptake of As, Ba, Cd, Cr, Pb, and Se [33,34].

Concentrations of trace elements in sediments can have a significant impact on plants' uptake according to Gardea-Torresdey et al. [16]. Plants collected during this study contained low levels of trace elements, many of which were <0.1 mg kg⁻¹; whereas, in studies by Gardea-Torresdey et al. [16] and Polette et al. [4], trace elements detected in leaves were >100 mg kg⁻¹. This is likely the result of the levels present in the sediments being lower than those reported by those authors. The lower concentrations of metals, the high pH, and low moisture in the soil would all lessen uptake of trace elements by the *L. tridentata*.

In a separate study, Sims et al. [37] showed that some trace elements entering the plant system through the root that certain metals (e.g., Cu, Pb, Zn, Co, Fe, and Li) are lower in concentration when compared to levels in the contaminated tailings. Their research suggested that the outer root tissue is blocking metals from entering the vascular system of the *L. tridentata* (inner root tissue), reducing their transfer to and presence in leaf tissue. The *L. tridentata* has likely evolved several methods of mitigating the toxic effects of these trace elements. When toxic metals enter the root, they are likely blocked from entering the vascular system of the plant (i.e., inner root tissue), reducing trace elements in the leaf tissue.



Figure 4. Correlation plots for As (a), Hg (b), Ba (c), Cr (d) and Se (e) in *L. tridentata* (DC.) Coville leaves compared to As, Hg, Ba, Cr, and Se in sediment (n = 30).

5.2. Interwash Comparison

Trace element uptake ratios for *L. tridentata* leaves to sediments are shown for Hg, As, Ba, Cr, and Se. (Figure 5); full summary data are available in Sims [27]. Data below detection limits were not used for the summary statistics or the ratios (Table 4). Transfer ratios for Hg in the three washes ranged from 0.07 to 1.41. The ratios of As in the Carnation and Techatticup washes ranged from 0.01 to 34.43. The mean transfer ratios for Ba in the three washes ranged from 0.01 to 32.6; Cr in the three washes ranged from 0.03 to 177; and Se ranged from 0.0 to 24.51. The highest mean transfer ratios for Hg, As, Ba, and Cr were in the Techatticup Wash (Figure 5) with the Eagle Wash having the lowest transfer ratios for trace elements; only one sample contained As in detectable amounts. Selenium however contained the highest transfer ratio in the Eagle Wash sediments.



Figure 5. Box plots of sediment to *L. tridentata* (DC.) Coville plant transfer ratios of trace metals across (a) Carnation, (b) Eagle and (c) Techatticup washes (n = 30). Data are mean values with upper and lower quartiles shown by the boxes. Arsenic was only detected in one leaf location of the Eagle Wash and therefore, this single point was omitted from the figure. The solid black line represents a transfer ratios > 2.5.

Table 4. Standard deviation (STDV) and mean for plant sediment transfer ratios across the Carnation, Eagle and Techatticup washes (n = 30).

Element	STDV	Mean
Hg	0.29	0.29
As^{1}	3.01	1.28
Ba	0.07	0.10
Cr	0.80	0.23
Se	6.56	3.66

¹: Data is without the single outlier location in the Eagle Wash.

Studies have shown transfer ratios ranging between 0.9 and 9.4 for plant uptake to sediment concentrations [33,34] and therefore, data means fall within previous established windows with a few outliers that are likely due to spatial variability of sampling and are not considered an issue environmentally. The relationship between concentrations in sediments and plants were often non-linear, similar to that observed by Sheppard and Evenden [33] and Vandenhove et al. [35]. Sheppard and Evenden [34] speculated that the non-linear relations could be caused by environmental conditions considering that their study was performed during the summer months when sediment moisture was at its lowest due to a lack of precipitation.

Transfer ratios above 2.5 may indicate consistent plant uptake of metals from sediments with significant levels of contamination according to Polette, et al. [4]. It was also established that below the 2.5 threshold uptake is likely from spatial variability of trace element in sediments, resulting in greater variability among the uptake ratios [4,34]. However, authors Vandenhove et al. [35] recommend that transfer ratios above 10 are needed to show consistent plant uptake.

Mercury transfer ratios were not above 2.5 in the three washes and could be the result of spatial variability of sampling, sediment conditions, or random error as discussed by Polette et al. [4]. Arsenic present at the Carnation, Eagle and Techatticup washes had a transfer ratio >2.5. These locations were random and at different locations along the sampling transect and likely the result of spatial variability of sampling as was Hg. Barium and Cr were similar to Hg ratios with all locations <2.5 except for one at the end of the Carnation Wash. This single Cr ratio > 2.5 in the Carnation Wash could also be from spatial variability as suggested by Polette et al. [4]. Transfer ratios > 2.5 indicate considerable uptake by plants; however, Se is more susceptible to transfer from sediment to plants owing to its ubiquitous nature in arid regions and therefore, not environmentally significant [38].

Generally, trace elements expressed as plant:sediment transfer ratios had similar means among the three washes with the exceptions of the lack of As in leaves in the Eagle; and a high mean transfer ratios for Cr in the Techatticup. One possible explanation for these extremes could be low bioavailability of As in the Eagle sediments and a low bioavailability of Cr in the Techatticup.

Means and standard deviations for transfer ratios of trace elements in sediment to the leaves of the *L. tridentata* for the Carnation, Eagle, and Techatticup washes are provided in Table 4. Transfer ratios in the Carnation Wash ranged between 0.008 and 0.10, with an average transfer ratio for Cr 0.027, significantly lower compared to Sheppard and Evenden [34]. The Eagle Wash had one sample with a transfer ratio of Se > 2.5, and is likely an outlier with other locations and metals being significantly lower. In the As calculations, the Eagle Wash contained one transfer ratio of 34.4, which was deemed an outlier and omitted as was one Se value (24.5) in the Eagle Wash. Transfer ratios for the three washes were comparable to those reported by Polette et al. [4] where trace element uptake by the *L. tridentata* leaves showed ratios ranging between 0.08 and 0.19 for sediment to plant transfers. Summary statistics in Table 4 indicate that there is little uptake and, transfer to the leave of the *L. tridentata*. The average uptake ratio is below 2.5, per Polette et al. [4], for all metals accept Se, a metal know to be ubiquitous in arid environments. Data show that the *L. tridentata* physiology is blocking the uptake of metals to leaf biomass.

Gardea-Torresdey [14,39] determined that transfer ratios from sediment to plant ranged between 0.03 and 0.18 in the semi-arid region of El Paso, Texas. This data is similar to Gardea-Torresdey [16] in the El Paso, TX, desert and likely not environmental issues. The mean values of transfer ratios for Hg, As, Ba, Cr, and Se for data from all three washes is presented in Table 4, showing similar ratios to those reported by Polette et al. [4], Gardea-Torresdey [16] and Vandenhove et. al. [35]. It is likely that the ratios in the three washes are affected by spatial variability caused by environmental factors such as sediment moisture, pH, and root zone (i.e., mycorrhiza and the plant root systems) leading to no significant environmental issues.

6. Conclusions

Findings showed that Ag, Cd, and Pb were not uptaken by the *L. tridentata* (DC.) Coville. In addition, Ag, Cd, and Pb are not present in the leaves likely due to selective uptake by the plant root due to an exclusion to metals such as Cd, Cu, and Pb in water-poor environments. Data also showed that leaves of the *L. tridentata* contained measurable Hg, As, Ba, Cr, and Se despite low sediment moisture and high pH, but data show little relation to the occurrence of metals in the leaves of the *L. tridentata* roots being able to penetrate up to 5 meters in depth to gain water from sediment moisture, perched water, or groundwater sources. This could result in bioaccumulation of trace elements in the leaves of the *L. tridentata* over time at higher levels than present in the surficial sediments of the Carnation, Eagle,

or Techatticup washes. Moreover, variability of environmental conditions, including low soil moisture, high sediment pH, plant physiology, and deep growth of *L. tridentata* root systems are likely the driving factors in the bioavailability of trace elements in its leaves.

In conclusion, additional research of trace element movement through the outer root tissue (i.e., epidermis) is needed to better understand the transfer of trace elements from sediment to plant. It would be valuable to understand how the *L. tridentata* is preventing trace elements from entering the cortex, and isolating the metals from the vascular tissue by the casparian strip, preventing bulk movement through the plant.

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References

- 1. Hooda, P.S. Assessing Bioavailability of Soil Trace Elements. In *Trace Elements in Soils*; Hooda, P.S., Ed.; Wiley: West Sussex, UK, 2010; pp. 229–265.
- 2. Appanna, V. Impact of heavy metals on an arctic Rhizobium. *Bulletin Environ. Contam. Tox.* **1991**, *46*, 450–455. [CrossRef]
- 3. Nicholson, F.A.; Smith, S.R.; Alloway, B.J.; Carlton-Smith, C.; Chambers, B.J. An inventory of heavy metals inputs to agricultural soils in England and Wales. *Sci. Total Environ.* **2003**, *311*, 205–219. [CrossRef]
- 4. Polette, L.A.; Gardea-Torresdey, J.L.; Chianelli, R.R.; George, G.N.; Pickering, I.J.; Arenas, J. XAS and Microscopy Studies of the Uptake and Bio-transformation of Copper in *Larrea tridentata* (Creosote Bush). *Microchem. J.* **1998**, 65, 227–236. [CrossRef]
- 5. Morel, F.; Hering, J. *Principles and Applications of Aquatic Chemistry*, 1st ed.; John Wiley & Sons, Inc.: New York, NY, USA, 1993; Volume 1.
- 6. Reglero, M.M.; Monsalve-González, L.; Taggart, M.A.; Mateo, R. Transfer of metals to plants and red deer in an old lead mining area in Spain. *Sci. Total Environ.* **2008**, 406, 287–297. [CrossRef]
- 7. Ullah, A.; Heng, S.; Munis, M.F.H.; Fahad, S.; Yang, X. Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: A review. *Environ. Exper. Bot.* **2015**, *117*, 28–40. [CrossRef]
- Peralta-Videa, J.R.; Torresdey, G.; Gomez, E.; Tiemann, K.J.; Parsons, J.G.; Carrillo, G. Effect of mixed cadmium, copper, nickel, and zinc at different pHs upon alfalfa growth and heavy metal uptake. *Environ. Poll.* 2002, *119*, 291–301. [CrossRef]
- 9. Haque, N.; Peralta-Videa, J.R.; Jones, G.L.; Gill, T.E.; Gardea-Torresdey, J.L. Screening the Phytoremediation Potential of Desert Broom (Baccharis sarothroides Gray) Growing on Mine Tailings in Arizona, USA. *Environ. Poll.* **2008**, *153*, 362–368. [CrossRef]
- 10. Andrade, S.A.L.; Silveira, A.P.D.; Mazzafera, P. Abruscular mycorrhiza alters metal uptake and the physiological response of Coffea arbica seedlings to increasing Zn and Cu concentrations in soil. *Sci. Total Environ.* **2010**, *408*, 5381–5391. [CrossRef] [PubMed]
- 11. Apple, M.E.; Thee, C.I.; Smith-Longozo, V.L.; Cogar, C.R.; Wells, C.E.; Nowak, R.S. *Arbuscular Mycorrhizal Colonization of Larrea Tridentata and Ambrosia Dumosa Roots Varies with Precipitation and Season in the Mojave Desert Symbiosis*; University of Nevada: Las Vegas, NV, USA, 2004.
- 12. Hildebrandt, U.; Regvar, M.; Bothe, H. Arbuscular mycorrhiza and heavy tolerance. *Phytochemistry* **2007**, *68*, 139–146. [CrossRef]
- 13. Hancock, G.R.; Grabham, M.K.; Martin, P.; Evans, K.G.; Bollhöfer, A. A methodology for the assessments of rehabilitation success of post mining landscapes-sediment and radionuclide transport at the former Nabarlek uranium mine, Northern Territory, Australia. *Sci. Total Environ.* **2006**, *354*, 103–119. [CrossRef]

- Gardea-Torresdey, J.L.; Polette, L.; Arteaga, S.; Tiemann, K.J.; Bibb, J.; Gonzalez, J.H. Determination of the content of hazardous heavy metals on *Larrea tridentata* grown around a contaminated area. In Proceedings of the Eleventh Annual EPA Conf. on Hazardous Waste Research, (HSRC/WERC) Joint Conference on the Environment, Albuquerque, NM, USA, 21–23 May 1996; Erickson, L.R., Tillison, D.L., Grant, S.C., McDonald, J.P., Eds.; pp. 660–669.
- 15. Gardea-Torresdey, J.L.; Hernandez, A.; Tiemann, K.J.; Bibb, J.; Rodriguez, O. Adsorption of toxic metal ions from solution by inactivated cells of *Larrea tridentata* (creosote bush). *J. Hazard Subst. Res.* **1998**, *1*, 3. [CrossRef]
- Gardea-Torresdey, J.L.; Bibb, J.; Tiemann, K.J.; Gonzalez, J.H.; Arenas, J. Adsorption of Copper Ions from Solution by Heavy Metal Stressed *Larrea Tridentata* (Creosote Bush) Biomass. In Proceedings of the Eleventh Annual EPA Conf. on Hazardous Waste Research (HSRC/WERC) Joint Conference on the Environment, Albuquerque, NM, USA, 21–23 May 1996; Erickson, L.R., Tillison, D.L., Grant, S.C., McDonald, J.P., Eds.; pp. 638–648.
- 17. Yadav, S.K. Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. *S. African J. Bot.* **2010**, *76*, 167–179. [CrossRef]
- 18. Briat, J.F.; Labrun, M. Plant responses to metal toxicity. *Comptes Rendus de l'Académie des Sci.-Series III-Sci. de la Vie* 1999, 322, 43–54. [CrossRef]
- 19. McAuliffe, J.R.; Hamerlynck, E.P.; Eppes, M.C. Landscape dynamics fostering the development and persistence of long-lived creosote bush (*Larrea tridentata*) clones in the Mojave Desert. *J. Arid Environ.* **2007**, *69*, 96–126. [CrossRef]
- 20. Schafer, J.L.; Mudrak, E.L.; Haines, C.E.; Parag, H.A.; Moloney, K.A.; Holzapfel, C. The association of native and non-native annual plants with *Larrea tridentata* (creosote bush) in the Mojave and Sonoran Deserts. *J. Arid Environ.* **2012**, *87*, 129–135. [CrossRef]
- 21. Kim, S.J.; Vassão, D.G.; Moinuddin, S.G.A.; Bedgar, D.L.; Davin, L.B.; Lewis, N.G. Allyl/propenyl phenol synthases from the creosote bush and engineering production of specialty/commodity chemicals, eugenol/isoeugenol, in *Escherichia coli. Arch. Biochem. Biophys.* **2014**, *541*, 37–46. [CrossRef]
- 22. Visioli1, G.; Vamerali, T.; Mattarozzi, M.; Dramis, L.; Sanangelantoni, A.M. Combined endophytic inoculants enhance nickel phytoextraction from serpentine soil in the hyperaccumulator Noccaea caerulescens. *Front. Plant Sci.* **2015**, *6*, 638. [CrossRef]
- 23. Eilitta, M.; Mureithi, J.; Derpsch, R. *Green Manure/Cover Crop Systems of Smallholder Farmers: Experiences from Tropical and Subtropical Regions;* Springer Netherlands: Heidelberg, Germany, 2004; p. 355.
- 24. Naumburg, E.; Loik, M.E.; Smith, S.D. Photosynthetic responses of *Larrea tridentata* to seasonal temperature extremes under elevated CO₂. *New Phytol.* **2004**, *162*, 323–330. [CrossRef]
- 25. Runyon, E.H. The Organization of the Creosote Bush with Respect to Drought. *Ecology* **1934**, *15*, 128–138. [CrossRef]
- 26. USEPA. *Test Methods for Evaluating Solids and Wastes—Physical/Chemical Methods;* U.S. Environmental Protection Agency: Washington, DC, USA, 1986.
- 27. Sims, D.B. Fate of Contaminants at an Abandoned Mining Site in an Arid Environment. Ph.D. Thesis, Kingston University, London, UK, 2011.
- 28. Larsen, M.; Trapp, S.; Pirandello, A. Removal of cyanide by woody plants. *Chemosphere* **2004**, *54*, 325–333. [CrossRef]
- 29. Ross, C. Preserving the Culture While Closing the Holes-Abandoned Mine Reclamation in Nevada. In Proceedings of the 30th annual National Association of Abandoned Mine Land Program Conference, Durango, CO, USA, 26–29 October 2008.
- 30. Prasad, M.N.V. Cadmium Toxicity and Tolerance in Vascular Plants. *Environ. Exper. Bot.* **1995**, *35*, 525–545. [CrossRef]
- 31. An, Y.-J. Assessment of comparative toxicities of lead and copper using plant Assay. *Chemosphere* **2006**, *62*, 1359–1365. [CrossRef] [PubMed]
- 32. Mackay, W.P.; Mena, R.; Pingitore, N.E.; Redetzke, K.; Freeman, C.E.; Newman, H.; Gardea, J.; Navarro, H. Seasonal changes in concentration and distribution of heavy metals in creosote bush, *Larrea tridentata* (Zygophyllaceae), tissues in the El Paso, TX/Ciudad Juarez, Mexico area. *SIDA Contrib. Bot.* **1998**, *18*, 287–296.

- 33. Sheppard, S.D.; Evenden, W.G. Critical compilation and review of plant/soil concentration ratios for uranium, thorium and lead. *J. Environ. Radio.* **1988**, *8*, 255–285. [CrossRef]
- 34. Sheppard, S.C.; Evenden, W.G. Characteristics of plant concentration ratios assessed in a 64-site field survey of 23 elements. *J. Environ. Radio.* **1990**, *11*, 15–36. [CrossRef]
- Vandenhove, H.; Olyslaegers, G.; Sanzharova, N.; Shubina, O.; Reed, E.; Shang, Z.; Velasco, H. Proposal for new best estimates of the soil-to-plant transfer factor U, Th, Ra, and Po. J. Environ. Radio. 2009, 100, 721–732. [CrossRef]
- Sims, D.B.; Hooda, P.S.; Gillmore, G.K. Sediment Contamination along Desert Wash Systems from Historic Mining Sites in a Hyperarid Region of Southern Nevada, USA. *Soil Sed. Contam.* 2013, 22, 737–752. [CrossRef]
- 37. Sims, D.B.; Collumb, C.; Hodge, V.F.; Vatalis, K.I.; George, V. Larrea Tridentata Uptake of Trace Metals in Mine Tailings of an Arid Region. *Int. J. Plant Soil Sci.* **2015**, *9*, 1–11. [CrossRef]
- 38. Bañuelos, G.S.; Lin, Z.-Q.; Arroyo, I.; Terry, N. Selenium volatilization in vegetated agricultural drainage sediment from the San Luis Drain, Central California. *Chemosphere* **2005**, *60*, 1203–1213. [CrossRef]
- 39. Gardea-Torresdey, J.L.; Peralta-Videa, J.R.; Montes, M.; de la Rosa, G.; Corral-Diaz, B. Bioaccumulation of cadmium, chromium and copper by *Convolvulus arvensis L*.: Impact on plant growth and uptake of nutritional elements. *Bioresour. Tech.* **2004**, *92*, 229–235. [CrossRef] [PubMed]



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