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Larrea tridentata Uptake of Trace Metals in Mine Tailings within an Arid Region of Nevada, USA

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Authors' contributions

This work was carried out in collaboration between all authors. Authors DBS, CJC and VFH designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors DBS and CJC managed the literature searches, analyses of the study samples and ICP-OES analysis. Authors KIV and GV performed the statistical analysis. Authors DBS and CJC performed the experimental process and identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Larrea tridentata (creosote bush) growing in contaminated tailings of the Techatticup Mine in Nelson, Nevada were sampled and analyzed for trace metals. Samples were also collected outside the mine tailings to measure geogenic trace metal levels. These data show that some trace metals (Cu, Pb, Zn, Co, Fe, Li) enter *L. tridentata* through root tissues, but at significantly lower levels than in the tailings area. Trace metals that enter the root are higher in concentration in the outer than in the inner root tissue, possibly due to *L. tridentata* blocking their absorption at the outer root surface.

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Data further show the plant's ability to block the intake of these toxic trace metals that may adversely affect the plant. Statistical analysis suggests that certain metals, while not in high abundance, may be utilized by the plant for self-defense mechanisms or to aid in plant development. Finally, differences between plant components may be the result of hyper-accumulation of useful trace metals (e.g. B, Cr, Zn) and a blockage of potentially toxic trace metals (e.g. Cd, Pb, V).

Keywords: Mining; Nevada; trace metals; arid; Larrea tridentata; creosote bush.

1. INTRODUCTION

Waste from abandoned mine sites has the potential to impact more than just water and sediment resources. The processes and pathways that affect transport of trace elements in any ecosystem can pose environmental and human health risks, even beyond the vicinity of the site. Hooda [1] states that such risks are contingent on the bioavailability of trace elements, not just on their presence in the environment. Biological impacts include the health and wellbeing of flora, fauna and humans exposed to contaminated water and food resources [2-4]. The possible incorporation of trace metals from abandoned mining areas adjacent to population centers into biomass poses unknown hazards. Tracking how a contaminant moves from sediments to biomass of local plant species, thus potentially entering the food web, is of great importance.

Mining site wastes have been shown to have effects on the abundance of trace metals (e.g. Cu, Ni, Fe, Co, Zn, U, and Pb) in plant and animal tissues in the vicinity of the mining site [5,6]. However, the uptake of trace metals is a complex process of metal-ligand binding and chemical conversions (biocatalysts) involving transfer of chemical species across root membranes to plant cells. In addition to the bioavailability of trace metals, biomass uptake can be limited by other factors such as moisture, nutrients (e.g. N and P), and organic acids [7-10]. The mycorrhiza associated to the plant root systems have been shown to reduce plant tissue exposure to trace metals in contaminated sediments [11,12]. Other studies of trace metal abundance in vegetation have focused on the impact to plants in stream sediments and farming adjacent to tailings dumps [13]. In arid regions, however, contaminated sediments may be redistributed by short duration and high intensity storm-water transport events, exposing drought resistant plants to substantial trace metal loads.

Researchers have shown that the bioavailability and transfer of trace metals from sediment to plant is controlled by sediment moisture, pH, presence of mycorrhiza, and plant physiology [1,14]. Studies have determined sediment pH is a key factor influencing solubility and resultant bioavailability of metals to plants. The presence of certain plant species also plays an important part in determining which trace metals are used or blocked [14]. Some vascular plant species are capable of utilizing metals as a self-defense mechanism from insects, while others are capable of blocking certain trace metals due to their adverse effect on a plant growth [15,16]. A symbiotic relationship of plants with mycorrhiza fungi has proven to be a benefit to both the mycorrhiza fungi and the plant itself, and in particular it has been shown that arbuscular mycorrhiza fungi found in association with the creosote bush (*L. tridentata*) are also capable of protecting plants from adverse trace metal exposure [17,11,12].

This study was performed at the area of the Techatticup Mine, a large-scale mining operation that functioned between 1850 and 1960 located close to Nelson, Nevada [18]. This mine area was chosen due to the presence of heavily contaminated tailings exposed to the open environment [19]. A more detailed overview of the operation of this mine, its ore and tailing processing can be found in Sims [20]. This area is located within the southern edge of the Great Basin, where topography controls regional hydrologic processes resulting in closed valley systems with no significant external drainage [21]. Ephemeral streams in the area run east-west towards Lake Mohave, a regional reservoir. Mine waste (tailings) were dumped into the ephemeral washes during operation. Tailings mounds currently have a variety of sparse desert vegetation growth including *L. tridentata*, *Ambrosia dumosa,* and *Eriogonum inflatum*.

Among these, the dominant species, *L. tridentata*, was chosen for sampling. This is a perennial plant that can accumulate trace elements and metalloids in its tissues, preserving its leaf structure for extended periods of time, and can extend its main root (tap root) up to 25 m below the surface in search of water resources [22,23]. Studies have documented its uptake of trace metals and metalloids by *L. tridentata* from sediments, even in desert climates [22,24,25]. This research evaluates the uptake, accumulation, and transfer of trace elements and metalloids into particular tissues of *L. tridentata.*

2. MATERIALS AND METHODS

2.1 Sampling Methodologies

Seven (7) plants were sampled within a 20 by 20 meter area of the Techatticup tailings dump (Fig. 1). Three (3) separate *L. tridentata* samples were collected outside of the Techatticup Mine area for background analysis to measure geogenic levels of trace metals. At each sampling location, 60-cm long stems containing leaves were cut from a mature *L. tridentata* that was at least 120 cm in height. Additionally, the main root (tap root) was removed at each bush location for analysis. Sediment samples were collected from the contaminated tailings of the seven bush locations to 30 cm below the surface, composited, and analyzed for trace metals. In addition, dust on the leaves and branches was collected for analysis.

2.2 Sample Analytical Procedures

Plant and tailing samples were analyzed for trace metals per USEPA method 6010B by inductively coupled plasma-optical emission spectrometry (ICP-OES) techniques [26]. Plants were processed by cutting a 30-cm length of branch that was washed with 100 ml of 90ºC deionized water to remove particulate dust from tailings off the leaves and branches [27-30]. The wash rinsate was acidified with 10 ml of concentrated nitric acid (HNO_3), evaporated to near-dryness on a hotplate, and cooled. The residual was weighed and treated with 10 ml of concentrated nitric acid and brought to 100 ml with deionized water to evaluate trace metals in the dust accumulated on leaves and branches.

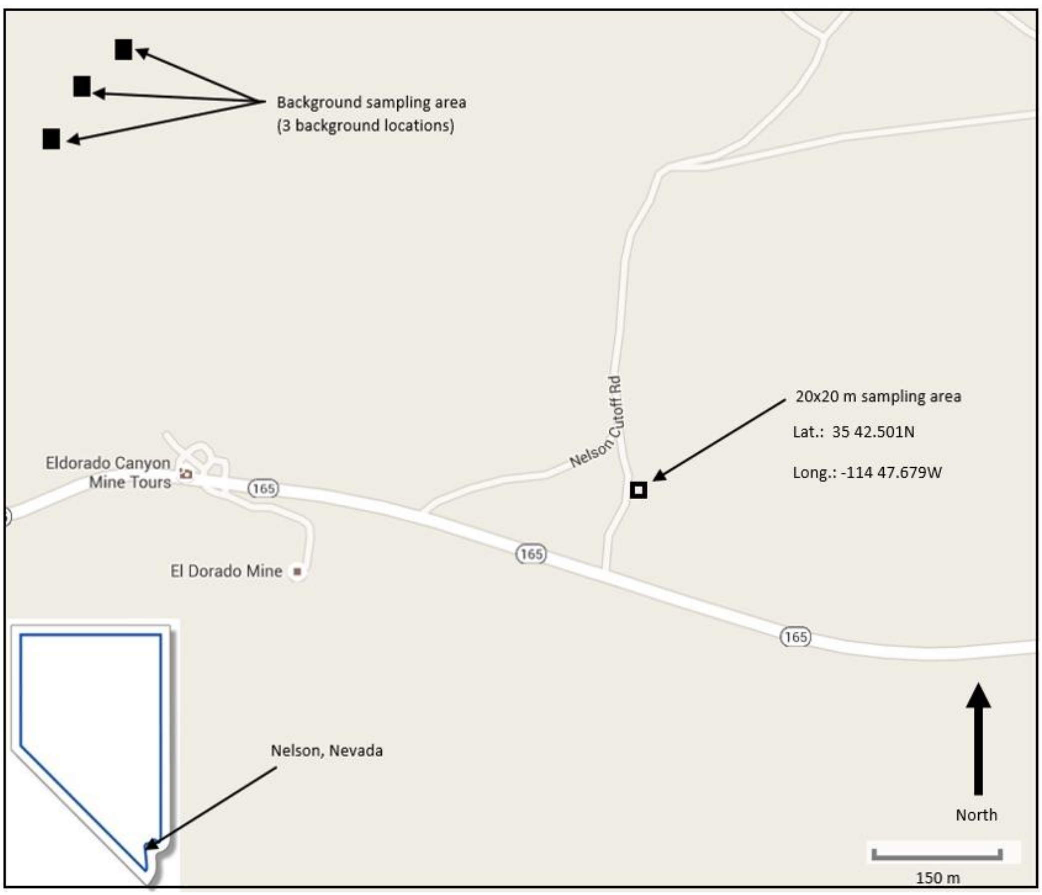

Fig. 1. Sampling locations at Techatticup Mine and Mine, Nelson, Nevada [48]

After washing the 30 cm long branches, approximately 1 gram of leaves was cut from each branch and digested per USEPA method 3050 B (hot aqua-regia digestion) and filtered through a Whatman® 541 (2 µm) to evaluate trace metals in the leaves of the *L. tridentata* [26]. The main root was cleaned with a soft bristle bush and ambient temperature deionized water to remove tailing debris. After brushing, the outer root tissue (epidermis layer and cortex) was removed with a clean plastic knife. The outer and inner root (stele) tissues were then digested (1 gram) per USEPA method 3050B and filtered through a Whatman® 541 filter (2 µm). Finally, 1 gram of mine tailings was processed per USEPA method 3050B and analyzed by USEPA method 6010B; ICP-OES [26].

2.3 Principal Components Analysis

Principal Components Analysis (PCA) was utilized to describe clustering of trace metals between sediments and plant components after log transformation, since data were not homoscedastic [31].

2.4 Quality Assurance/Quality Control

All samples analyzed for trace metals were processed per USEPA method 6010B for inductively coupled plasma-optical emission spectrometer [26]. Reagents were of ultra-pure quality, NIST traceable. Analytical integrity was confirmed with a USEPA certified reference sample (RTC Corporation: CRM022-020, Sample 5, lot D522). Quality control was further maintained by performing all analyses in triplicate.

3. RESULTS AND DISCUSSION

Metal contents varied widely within each part of the plant analyzed, and data show that there were significant differences between plant regions (Table 1). Typically, while both the sediment and dust showed high levels of metal, there was a significant reduction of metal concentrations in the biomass of the plants, particularly in the inner root and leaf tissues. Data show Cu, Pb and Zn reductions greater that 50% between sediment and outer root tissue. Cobalt (35%), Fe (35%) and Li (44%) also showed large decreases in concentration between sediment and outer root tissue (Table 1). As shown by other authors, trace metals are blocked at the outer root, possibly due to their toxicity or dehydrating nature [15,32].

It has been shown that the *L. tridentata* and analogous plants have similar patterns of trace metal uptake [32], and that similar plants block potentially toxic trace metals at the outer root [15,16]. For example, Prasad [33] demonstrated that some plants will block trace metal cations (e.g. Cd^{2+}, Cr^{3+}, Pb^{2+}, V^{3+}) and alkali metals (e.g. Li) at the outer root by affecting the water balance, and explained that when plants grow in metal cation laden sediments, Cd tended to bind to the cell membrane reducing its intake of water. Studies further suggest that the *L. tridentata* blocks trace metals in the outer root as a self-defense mechanism from certain trace metals [34,35]. For example, while B is not a metal cation, it is well documented to inhibit a plant's ability to intake water [36], and Table 1 shows that it was blocked at the outer root and absent in the inner root, although it was present at low levels in leaf tissues. Other authors suggest that in a plant's vascular system some trace metals (Cu, Cr, Mo, Zn) are not blocked and move through the tissue of the plant, most likely due to the benefit of the trace metal to the plant [16].

While the outer root tissue shows reductions in many of the metals analyzed, nearly all of the metal concentrations are significantly lower in the inner root and leaf tissues (Fig. 2). Higher concentrations of metals in the outer root compared to inner root tissue (except Mo, and leaf tissues compared to dust; except for Al and Ti) could be the result of sequestering of metal ions in the cells of the outer root, therefore preventing transport of metal ions by the xylem and phloem to other parts of the plant [37,38].

Dust on the leaves showed significantly higher levels of trace metals (Table 1). This is not unexpected, since *L. tridentata* has a resin coating on the leaves that can easily retain dust and other contaminants present in the tailing area [39]. While trace metals were present in the dust, studies show that surface dust on plant leaves, does not impact the levels of trace metals within the tissues of the plant as metals are absorbed by the roots *not* through the leaves [6,10,32,40]. Due to a non-transdermal nature of trace metals from the dust to the *L. tridentata*, its presence on the leaves has little impact on the overall health of the plant. The presence of contaminated dust on the leaves however, can impact the food-web if consumed by an animal or human.

Scatter plots and Principle Components Analysis (PCA) were utilized to identify trends and relationships between trace metals, sediments

Table 1. Average concentration of trace metals in tailings, outer and inner root, and leaf tissue and dust on the leaf of the *L. tridentata*

	Mine tailings	Outer root	Inner root	Leaf tissue	Leaf dust
Al	1416	1345	83.7	110	89
As	3.2	6.08	0.70	0.53	7.57
Ba	11.1	17.1	7.40	2.58	13.7
B	-	64.8	-	20.0	219
Cd	5.55	7.28	-	-	-
Cr	3.70	2.64	-	-	0.50
Co	2.53	1.65	0.30	0.19	0.56
Cu	74	33.7	6.43	4.84	23.6
Fe	3000	1937	129	159	1388
K	2466	13582	2759	4518	42610
Pb	264	42.9	1.05	1.29	2.15
Li	3.95	2.21	0.04	0.48	3.89
Mn	296	248	23.1	29.5	84.8
Mo	-	4.55	4.97	0.22	0.23
Ni	4.98	6.50	0.70	1.03	18.6
Ag	0.43	15.6	1.42	1.14	37.4
Ti	12.7	39.0	2.54	4.06	3.90
V	2.83	4.49	0.12	-	-
Zn	675	114	31.5	24.2	79.5

(-) indicates concentrations below detection levels, sample size was n=7, standard deviations (SD) are shown in Fig. 2, units are in mg kg^{-1}

and selected plant components *L. tridentata*. Results indicate a relationship between trace metals in the sediment, outer and inner root, but there is a diminutive relationship between the plant and dust present on the leaves (Figs. 3-4). Fig. 3 shows a significant relationship between the outer root and sediments. Fig. 4, also shows a similar relationship between sediment and inner root. There was no relationship between sediments and leaf tissue or dust on the leaf and is therefore not presented.

Figs. 3 and 4 show distinct relationships between mine tailings and the outer and inner root components; however, PCA (Figs. 5a through d) displays clustering of trace metals in plant components and mine tailings. For example, Fig. 5a through 5d shows clustering (relationships) between trace metals in the outer and inner roots and leaf tissues when compared to tailings. Data from the rinsate (dust); however, displayed no common distribution between outer and inner roots and leaf tissues. PCA clustering further exhibits a similar distribution with trace metals in plant components when compared to tailings, suggesting possible relationships with the movement through the plant's system.

Figs. 5a, b and d show that the outer root contains trace metals known to be water inhibiters (i.e. Cd), findings similar to Franzle et al. [10] and Haque et al. [16]. There is a dissimilar distribution among clusters A shown in Fig. 5a and b. Data show cluster A has little relationship between the outer and inner root; however, cluster B shows that there is a possible relationship between the inner and outer root with Al, Co, Fe, Mn, Pb and Ti (Figs. 5a and b). These finding are similar to other researchers who found that certain trace metals can move through the outer root to the inner root, but in limited concentrations due to adverse effects to the plant [10,41,42]. Fig. 5c and d display clustering with limited relationships between trace metals (i.e. Al, K and Li). PCA plots show little association between dust, leaf tissue and trace metal concentration in the leaves, these findings are similar to those of Smolders et al. [43].

The difference between the outer and inner root components may be the result of hyper-accumulation of trace metals in the outer root due to the plant's ability to block particular trace metals as suggested by Franzle et al. [10]. Trace metals that showed commonality between all three plant components were Al, Fe, Mn, Pb and Ti (Figs. 4a, b and d). While Al, Fe and Mn are within normal levels for the *L. tridentata*, Pb was also well below suggested toxic levels as reported by Smolders et al. [43]. Cluster B illustrates descending ratios for tailing-inner root transfer (Al having 16:1 and 13:1; Fe 23:1 and 18:1; Pb 26:1 and 24:1, Mn 12:1 and 10:1, and Ti 5:1 and 3:1). Although Pb is known to be toxic to plants, it was detected in plant components at approximately 1 mg kg^{-1}, lower by known standards for *L. tridentata* [32,40]. Other trace metals present (Al, Fe, Mn and Ti) are known to be beneficial for plant growth, although at significantly lower concentrations than those detected in the tailings [6].

Further analysis indicated there were some groupings of trace metals with plant components and tailings. Data show that Al, Fe, Mn, Pb and Ti were the only trace metals clustering (cluster B) across Figs. 5a, b and d; there was no commonalities with Fig. 5c. These trace metals (Al, Fe, Mn, Pb and Ti) are both beneficial and harmful to the *L. tridentata*, depending on the level of absorption [43]. Dust on the leaves of the *L. tridentata* contained no clustering between the plant, dust or tailings.

It is possible that the *L. tridentata* absorbs specific trace metals (e.g. Al, Fe, Mn, Ti) for specific reasons, such as growth [15]. It has been demonstrated that certain trace metals at high concentrations in the inner and outer root tissues of vascular plants (e.g. Al, Fe, Ni, Co, Mn) are related to a plant's selectivity [44]. Researchers have reported that Zn present in vascular plants for example, may be a defense mechanism [15,16]. This study found similar findings to Badri et al. [44] with Zn in the leaf tissues having similar concentrations to root components. The difference between leaf tissue and root tissues may be the result of hyper-accumulation of B, Cr, and Zn, and to a lesser extent Cd and V, findings similar to those reported by Franzle et al. [10].

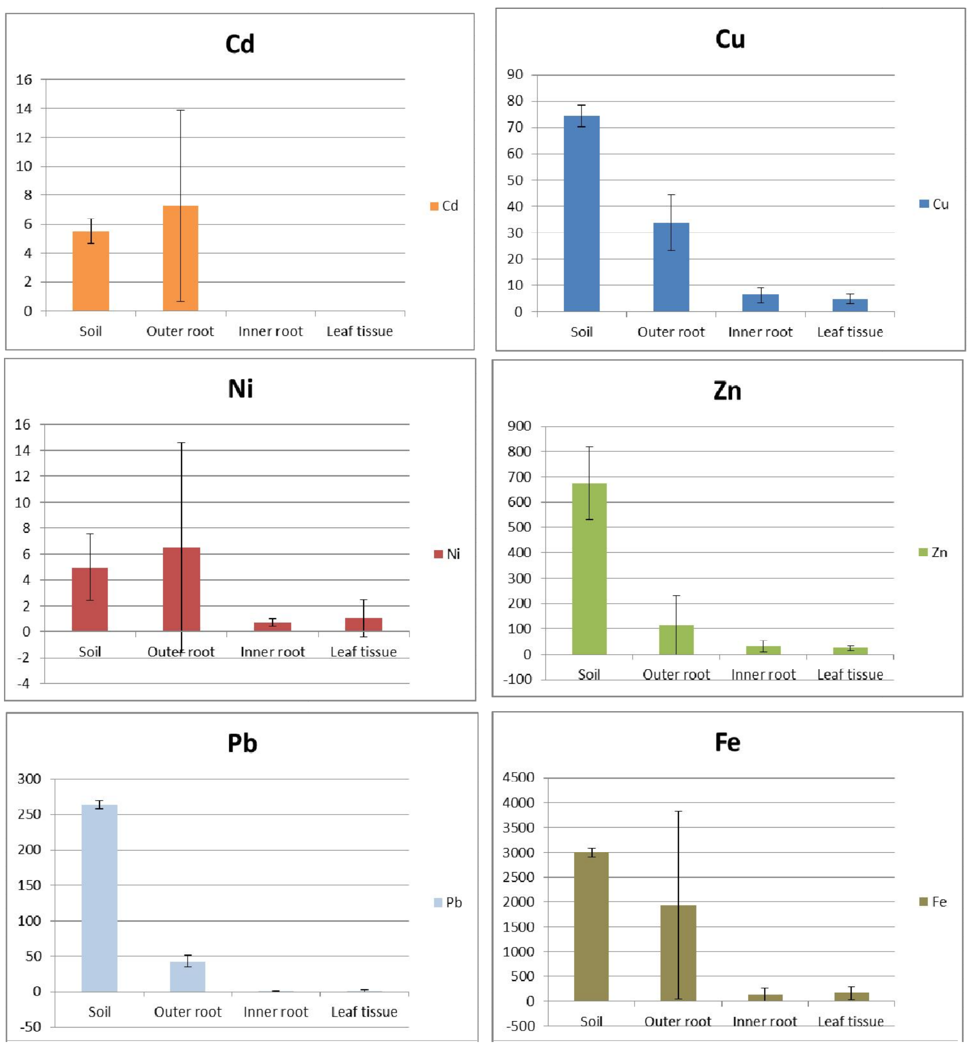

Fig. 2. Concentration of trace metals in various part of *L. tridentata,* +/- 1 standard deviation (SD), y-axis is concentration; x-axis is plant component, concentration in mg kg^{-1}

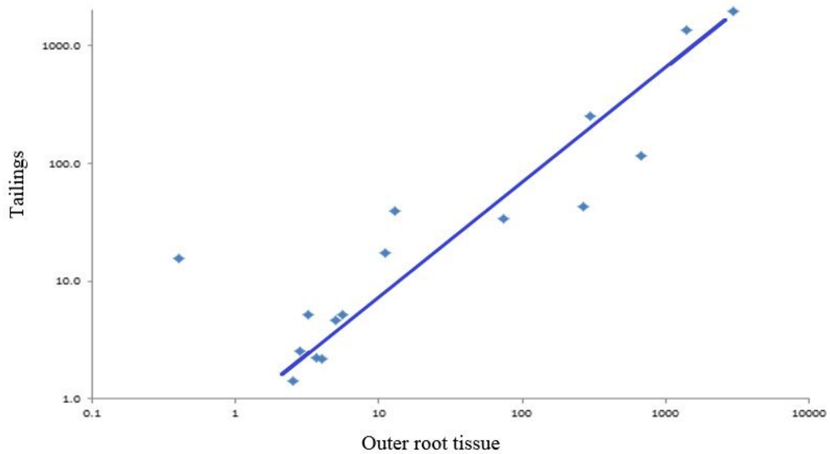

Fig. 3. Trace metal (mg kg^{-1}) relationship between tailings (y-axis) and outer root tissue (x-axis); R^2 = 0.9879

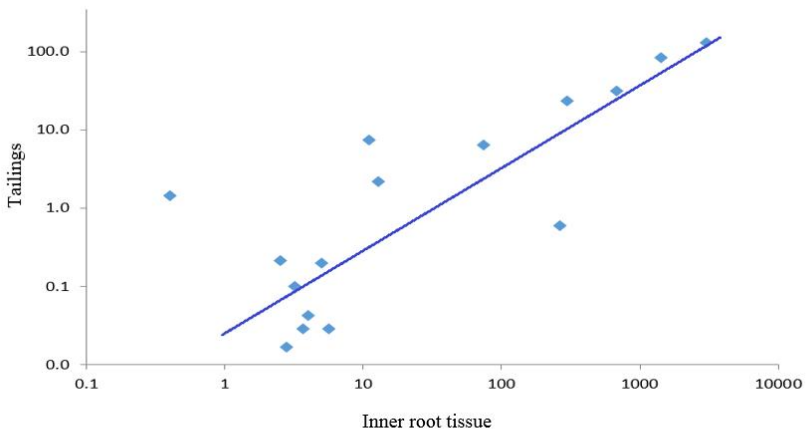

Fig. 4. Trace metal (mg kg^{-1}) relationship between tailings (y-axis) and inner root tissue (x-axis), R^2 = 0.9655

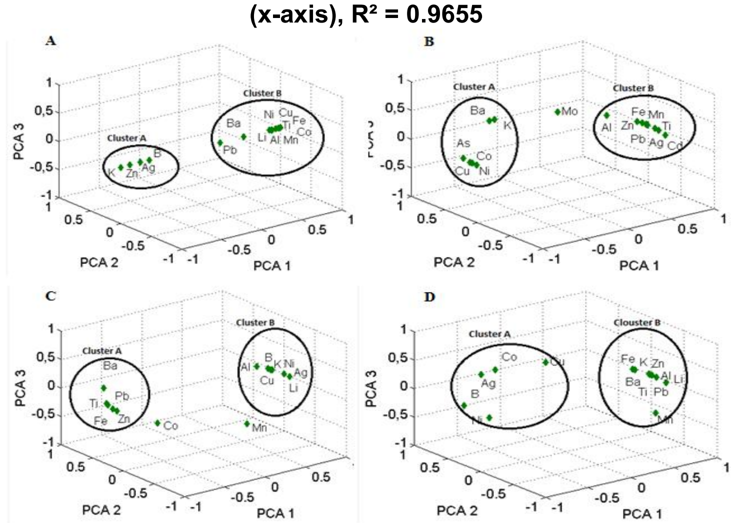

Fig. 5. PCA showing relationship of tailings to outer root (A), Inner root (B), Rinsate (C) and Leaves (D)

L. tridentata is a recent addition to the Mojave Desert and the Great Basin dating back to approximately 10,000 years B.P. [45,46]. Studies have shown that it began its migration northward at the end of the Last Glacial Maximum (Pleistocene) as the climate began to warm [45,47]. McAuliffe [45] further showed that a single *L. tridentata* plant can live more than 1000 years by cloning itself. It is possible that some of its early physiological traits, such as trace metals uptake for self-defense, persist today. *L. tridentata* may have preserved some of its physiological traits that give it no advantage in today's environment.

4. CONCLUSION

L. tridentata growing in mining regions are exposed to high levels of potentially toxic trace metals. Plants have evolved several methods of mitigating the toxic effects of these trace metals. This study shows that while some trace metals are entering the plant system through the root, levels are greatly reduced for Cu, Pb, Zn, Co, Fe, and Li when compared to levels in the contaminated tailings. Metals that enter the root and are found at high concentrations in the outer root tissue are likely blocked from entering the vascular system of the plant (inner root tissue), reducing their presence in the leaf tissue. Certain metals, while not absorbed at high concentrations, may be taken up by the plant for self-defense mechanisms or as aids in plant's growth. This lack of accumulation in tissues is of biological importance and shows the plant's ability to block intake of toxic metals. Finally, it is possible that *L. tridentata* physiological qualities remain as a result of its developmental stages, although they may not be of importance today as a result of environment change. Further research of trace metal movement through the outer root tissue and into the plant, and if possible, isolating it from mycorrhiza tissue would provide valuable information on how *L. tridentata* utilizes, or blocks trace metals in hyperarid regions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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