Phytostabilization Potential of Quailbush for Mine Tailings: Growth, Metal Accumulation, and Microbial Community Changes

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ABSTRACT

Abandoned mine tailings sites in semiarid regions remain unvegetated for extended periods of time and are subject to eolian dispersion and water erosion. This study examines the potential phytostabilization of a lead-zinc mine tailings site using a native, drought-tolerant halophyte, quailbush [*Atriplex lentiformis* (Torr.) S. Wats.]. In a greenhouse study germination, growth, and metal uptake was evaluated in two compost-amended mine tailings samples, K4 (pH 3) and K6 (pH 6) at 75, 85, 90, 95, and 100% mine tailings, and two controls, off-site and compost. Microbial community changes were monitored by performing MPN analysis of iron- and sulfur-oxidizing bacteria as well as heterotrophic plate counts. Results demonstrate that germination is not a good indicator for phytostabilization since it was only inhibited in the unamended K4 treatment. Plant growth was significantly reduced in 95 and 100% mine tailings, while growth in 75, 85, and 90% treatments was similar to the off-site control. Quailbush accumulated elevated levels of the nutrient metals Na, K, Mn, and Zn in the shoot tissues; however, metal accumulation was generally below the domestic animal toxicity limit. Initially, autotrophic population estimates were four to six logs higher than heterotrophic counts, indicating extremely stressed conditions. However, post-harvest, heterotrophic bacterial counts increased to normal levels (~10^6 CFU g^{-1} dry tailings) and dominated the rhizosphere. Therefore, with compost amendment, quailbush has good potential as a native species candidate for phytostabilization of mine tailings in semiarid environments.

Mine tailings sites are extensive throughout semiarid regions of the world including South Africa, Australia, North America, and Mexico (Munshower, 1993). Historically, waste products from ore processing were returned to the mine, placed into streams or lakes adjacent to the site, or discharged into a receiving pond. These practices have resulted in an emerging and extensive problem for the following reasons. Metals such as Cu, Fe, Zn, Ni, Pb, Cd, and the metalloid As are present in tailings material at concentrations that range from as low as 1 g kg^{-1} to 50 g kg^{-1} in older sites (Bradshaw et al., 1978; Walder and Chavez, 1995; Boulet and Larocque, 1998). Mine tailings are usually characterized by the absence of organic matter, nitrogen, and phosphorus, and by their neutral to low pH and high acid-producing potential (Wong et al., 1998; Krzaklewski and Pietrzykowski, 2002; Ye et al., 2002). Additionally, tailings piles are generally devoid of vegetation and have no soil structure (Munshower, 1993; Krzaklewski and Pietrzykowski, 2002). In semiarid areas these problems are exacerbated by high salt content resulting from conditions wherein a high proportion of rainfall undergoes evaporation rather than infiltration into the tailings (Munshower, 1993). Finally, tailings support severely stressed microbial communities. Heterotrophic populations are low in number, while acidophilic autotrophic bacteria such as *Acidothiobacillus* spp. and *Leptospirillum ferrooxidans* thrive (Southam and Beveridge, 1992). In general, the physical, chemical, and biological characteristics of mine tailings interact to almost completely suppress seed germination and plant growth (Yang et al., 1997). As a result, tailings are spread throughout the environment via eolian dispersion, water erosion, and leaching which can result in the formation of acid mine drainage.

The establishment of a permanent vegetative cap is recognized as a potentially cost-effective and ecologically sound approach to containment of mine tailings and for initiation of soil formation processes (Munshower, 1993; Brooks, 1998). In particular, there is interest in phytostabilization, a process wherein plants are established and function primarily to accumulate metals into root tissue or aid in their precipitation in the root zone (Cunningham et al., 1995). Use of native plants is a focus of this technology because they often demonstrate tolerance for local environmental conditions and provide a foundation for natural ecological succession. One of the largest cost factors associated with revegetation is the requirement for large amounts of organic amendments, e.g., compost or biosolids. These amendments mitigate the toxicity of the tailings and plants fail to grow in their absence (Sabey et al., 1990; Ye et al., 2001; Brown et al., 2003).

In this study we evaluated quailbush [*Atriplex lentiformis* (Torr.) S. Wats.]for its ability to establish in extremely and moderately acidic lead-zinc mine tailings typically found in semiarid areas. Quailbush is a perennial halophytic subshrub that is native to Arizona, California, Nevada, and Utah (USDA-NRCS, 2005) and has been examined for use in the reclamation of salt-affected lands (Malik et al., 1991; Blank et al., 1998; Malcolm et al., 2003). Quailbush is considered drought-tolerant and has previously been observed encroaching into historical mine tailings sites (USDA-SCS, 1977; Booth et al., 1999; Arunachalam et al., 2004; Jefferson,

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Abbreviations: BNM, basal nutrient medium; CC, compost control; CEC, cation exchange capacity; CFU, colony forming units; DTPA, diethylenetriaminepentaacetic acid; EC, electrical conductivity; ICP–MS, inductively coupled plasma mass spectrometry; MPN, most probable number; MSM, minimal salts medium; OS, off-site control sample; SBRP-HIC, Superfund Basic Research Program-Hazard Identification Core; SPL, soil plant toxicity level; SRL, soil remediation level; TOC, total organic carbon; TRTMT, treatment; Tukey's HSD, Tukey's Honestly Significant difference; WQARF, Water Quality Assurance Revolving Fund; WQC, Water Quality Center.

2004). In addition, *Atriplex* spp. have demonstrated accumulation of metals primarily in the roots, which is favorable for phytostabilization strategies (Williams et al., 1994; Jordan et al., 2002). The objectives of this study were to determine (i) the minimum level of compost required for establishment of quailbush in lead-zinc tailings by evaluating seed germination and seedling growth; (ii) metal accumulation in shoot tissue during growth of quailbush; and (iii) the impact of plant establishment on the microbial community as measured by enumeration of autotrophic and heterotrophic bacterial community before and after planting.

MATERIALS AND METHODS

Mine Tailings Site

The Klondyke mill site is located on the eastern bank of Aravaipa Creek in the transition zone between the riparian corridor and the semiarid uplands in the Aravaipa Valley, Graham County, Arizona. The riparian corridor is classified as a broadleaf riparian forest community with mesquite in the uplands and cotton wood, ash, sycamore, and alder in the riparian corridor. From 1948 to 1958, the Klondyke site was primarily a Pb and Zn ore processing operation that disposed of approximately 100 000 metric tons of flotation tailings that have remained devoid of vegetation (Wilson, 1959). In 1993, erosion and runoff from the tailings piles was observed and the Arizona Department of Environmental Quality (ADEQ) found that levels of arsenic and lead in the tailings exceeded the Arizona Non-Residential Soil Remediation Levels (SRLs) of 10 and 1200 mg kg^{-1}, respectively (ADEQ, 2001b, 2002). In addition, elevated levels of Cd and Pb were found in fish sampled from Aravaipa Creek downstream from the site (King and Martinez, 1998). As a result, the site was placed on the Arizona Water Quality Assurance Revolving Fund (WQARF) Registry in 1998.

Sampling

Two samples (80 L) were collected from the Klondyke upper tailings pile: K4 from 28 to 53 cm below the surface (32°51′0″ N; 110°20′34″ W) and K6 from 21 to 42 cm (35°51′1″ N; 110°20′33″ W). An off-site control sample (OS) was taken from 17 to 28 cm below the surface of a vegetated area adjacent to the tailings pile at 32°51′3″ N and 110°20′32″ W. The compost (EKO Compost, Richland Turf Food, Plateville, CO) used in this study was a mixture of poultry manure, forest products, and recycled wood products. All materials were stored at 4°C and thoroughly mixed before use. The off-site control sample and compost were sieved with a 5 × 5 mm mesh screen.

Mine Tailings and Compost Analysis

For pH analysis, triplicate samples were air-dried for 2 d and sieved through a 2-mm mesh screen. A 10-g aliquot was placed into a 50-mL centrifuge tube and 20 mL deionized water was added to achieve a 2:1 ratio of deionized water to soil (v/w). Each solution was shaken for 1 h, centrifuged for 5 min at 15 000 × *g*, and the pH of the supernatant was determined. Plant-available metals were analyzed using a DTPA (diethylenetriaminepentaacetic acid) extracting solution as described by Lindsay and Norvell (1978). The DTPA extraction was chosen based on its effective chelation of a mixture of plant micronutrients such as Fe, Zn, Cu, and Mn. In triplicate, an air-dried 10-g aliquot of each sample was mixed with 20 mL of DTPA extracting solution at pH 7.3. The solution was shaken in a 125-mL flask at 150 rpm for 2 h, vacuum filtered through a Whatman no. 42 filter paper, and finally filtered through a 0.45-μm hydrophilic polyethersulfone membrane (Supor-450, Pall Life Sciences, East Hills, NY). Samples were analyzed by inductively coupled plasma mass spectrometry (ICP–MS) for As, Cd, Cu, Fe, Mn, Pb, and Zn by The University of Arizona Superfund Basic Research Program's Hazard Identification Core (SBRP-HIC) using USEPA Method 6020 (USEPA, 2004). For the remaining analyses, single composite samples of K4, K6, OS, and compost were oven-dried at 105°C and sieved through a 2-mm mesh screen. These samples were then analyzed for texture by the hydrometer method; electrical conductivity (EC; 1:1 H_2O extraction); cation exchange capacity (CEC) by the sodium acetate method (Chapman, 1965); plant-available PO_4–P by the ammonium bicarbonate method (Olsen et al., 1954); total organic carbon (TOC), total carbon, and total nitrogen by high temperature combustion; and total elements (As, Cd, Cu, Fe, K, Mn, Na, Pb, Zn). These analyses were conducted by the Water Quality Center (WQC) Laboratory (University of Arizona, Tucson, AZ) except the total elemental analysis for which samples were prepared by microwave acid digestion (EPA 3051; USEPA, 2004) by the WQC Laboratory and then analyzed by ICP–MS by the SBRP-HIC. For the compost sample the C/N ratio and total nitrogen values were provided by Richland Turf Food, and used to calculate the TOC.

Germination and Plant Growth Study

Two experiments were performed to determine the minimum amount of compost required for growth of quailbush. Compost was selected to serve as a slow-release fertilizer and an organic amendment for both reduction of metal bioavailability and enhancement of heterotrophic microbial growth. Quailbush was selected because *Atriplex* spp. have performed well in disturbed and contaminated sites (Williams et al., 1994; Booth et al., 1999; Jordan et al., 2002; Arunachalam et al., 2004; Jefferson, 2004), it is native to the area as well as salt-tolerant (Blank et al., 1998; USDA-NRCS, 2005), and it performed similarly or better compared with other native species in a preliminary screening study.

In the first experiment, seed germination and plant growth were examined in four K4 and K6 tailings-compost mixtures (25, 50, 75, and 100% tailings by mass). Results of this study indicated equally good germination and growth of quailbush at all three compost levels tested. Therefore, a second experiment was performed to further titrate the level of compost required. In this experiment seed germination and plant growth were determined in five K4 and K6 tailings-compost mixtures (75, 85, 90, 95, and 100% tailings), as well as compost (100% compost control, CC) and the off-site sample (100% OS) alone.

For each experiment, quailbush seeds (Mistletoe-Carter Wholesale Seeds, Goleta, CA, USA) were sown in 12 × 8.5 × 3 cm plastic pots (with six 1.6-mm drainage holes) containing the various tailings or control treatments. Each treatment contained five replicates with 20 seeds per replicate (100 seeds per treatment). Seeds were irrigated with approximately 84 mL of tap water d^{-1} in a fiberglass greenhouse with temperatures ranging from 24 to 38°C. On Day 23, germination was quantified, and seedlings of similar size were transferred into 3.8-L pots (15.2 cm top diameter × 17.8 cm height × 12.7 cm bottom diameter) lined with fiberglass screen and containing the same tailings or control treatment. Fiberglass screen material prevented tailings from leaking and allowed water to drain. All pots were prepared and wetted 3 d before seedling transfer. Following transplantation, pots were irrigated with 360 mL of tap water d^{-1}. Each treatment was comprised of five replicates with one plant per pot. During the study, seedling height, number of leaves, and basal diameter of each plant were measured every 7 d. Plants were harvested 68 d after transplantation (Day 91 of the experiment) for determination of shoot and root dry mass and metal content.

Plant Dry Mass and Metal Analysis

At the end of the experiment (Day 91), plants were harvested for shoot and root dry mass measurements. The shoots were separated from the roots and placed in a preweighed paper bag. Root tissues were washed with tap water followed by a thorough rinse with distilled water to remove soil and particulate matter and then blotted with a paper towel and wrapped in a preweighed piece of aluminum foil. All samples were dried in a forced air oven at 65°C and weighed after 3 d to obtain the shoot and root dry mass.

Quailbush shoot tissue was analyzed for total metal (Na, K, Mn, Fe, Cu, Zn, As, Cd, and Pb) concentrations. Three plants from each treatment were selected for metal analysis. Plant material was dried at 65°C, ground with a Wiley Mill, and passed through a 40-mesh (0.419 mm) screen. Shoot tissue was prepared by microwave acid digestion by the WQC Lab and analyzed by ICP–MS by the SBRP-HIC.

Enumeration of Heterotrophic Bacteria

Initial (before seed germination) and final (post-harvest) heterotrophic bacterial counts were measured for all treatments. Ten grams of each treatment were placed in a 250-mL jar containing 95 mL of Zwittergent extractant (8.5 g NaCl and 200 μL of 1% Zwittergent solution per liter), shaken vigorously for 2 min, serially diluted in triplicate, and then plated on R2A agar (Becton, Dickinson and Company, Sparks, MD) amended with 200 mg L^{-1} of cycloheximide to inhibit fungal growth. Plates were incubated for 5 d at 23°C and then enumerated. Counts are reported as colony forming units (CFU) per gram dry weight of each sample.

For planted treatments, heterotrophic bacterial counts were also performed on rhizosphere samples at the end of the experiment. Three plants from each treatment were removed from the pots and all loose soil or tailings material was shaken off the roots. Roots along with adhering soil were immediately stored at 4°C until processed. For each plant, 0.1 g of fresh root material was consolidated from three separate 2-cm root tip sections, 0.5 cm of the root-shoot transition region, and 1.5 cm of the main root axis. The roots were placed in 9.9 mL of 1X PBS, sonicated twice for 30 s, and serially diluted in 1X PBS (Ausubel et al., 1995). All treatments were plated in triplicate on R2A agar amended with 200 mg L^{-1} of cycloheximide, incubated for 5 d at 23°C, and enumerated.

Enumeration of Autotrophic Bacteria

Initial and final counts of autotrophic bacteria, specifically iron- and sulfur-oxidizing bacteria, were assessed using a modification of the most probable number technique (Cochran, 1950; Woomer, 1994). Ten-gram samples of each treatment were placed into 250-mL jars containing 95 mL of Zwittergent extractant and shaken vigorously for 2 min. Each slurry was serially diluted from 10^{-2} to 10^{-8} in 4.5 mL with five replicates for each dilution. For the rhizosphere samples, the initial 10^{-1} dilution for each plant within a treatment was consolidated and serially diluted from 10^{-3} to 10^{-6} in 4.5 mL with five replicates for each dilution. All samples were inoculated into both iron and sulfur oxidizer enrichment media. Iron oxidizers were grown in modified 9K minimal salts medium (MSM) containing per liter: 3.0 g $(NH_4)_2SO_4$, 0.1 g KCl, 0.5 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.01 g $Ca(NO_3)_2 \cdot 4H_2O$, and adjusted to a pH of 2.3 with 10 N H_2SO_4. The autoclaved 9K MSM was amended with filter-sterilized $FeSO_4 \cdot 7H_2O$ at a final concentration of 33.3 g L^{-1} (Silverman and Lundgren, 1959; Southam and Beveridge, 1992). Sulfur oxidizers were grown in modified Starkey's medium (pH 4.5) consisting of a basal nutrient medium (BNM) and a thiosulfate solution (Starkey, 1925; Knickerbocker et al., 2000). The BNM (900 mL) contained 0.3 g of $(NH_4)_2SO_4$, 3.5 g of KH_2PO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.33 g of $CaCl_2 \cdot 2H_2O$, and 180 μL of a 1% solution of $FeSO_4 \cdot 7H_2O$ adjusted to pH 2.3 with H_2SO_4. A 100-mL solution of sodium thiosulfate with 200 m*M* Na_2SO_3 (20 m*M* Na_2SO_3 L^{-1}) was autoclaved separately and added to the BNM.

After 47 d of incubation on a shaker at 180 rpm, positive and negative results for growth were documented. Positive results were based on a color change from yellow to orange for the iron oxidizers and a change in turbidity as well as decrease in pH for the sulfur oxidizers. Population estimates were calculated as described by Briones and Reichardt (1999) and reported as most probable number (MPN) g^{-1}.

Statistics

Statistical analyses were generated using SAS Version 9.0 of the SAS System for Windows (SAS Institute, 2002). All data were tested for normality. For cases of nonhomogeneity of variances, data were log-transformed before analyses. Due to plant mortality within some of the treatments, the procedure for unequal sample size was used. The effect of compost addition on mean pH was examined within each mine tailings sample by employing a one-way ANOVA. For plant shoot metal concentrations, values were averaged over all treatments within a sample source and analyzed by a one-way ANOVA. Significant factor effects for both sample source and mine tailings concentration were determined using a two-way ANOVA followed by a one-way ANOVA to compare means for plant growth and microbial counts. For all analyses, significant differences between means at the $p < 0.05$ level were determined by employing the Tukey's studentized range test (Tukey's Honestly Significant Difference [Tukey's HSD]).

RESULTS

Mine Tailings Analysis

The K4 and K6 tailings samples had sandy loam and silt loam textures, respectively, with either an extremely acidic (K4, pH 2.7) or a moderately acidic pH (K6, pH 5.7) (Table 1). Both tailings samples exhibited a low CEC, and had minimal inorganic nutrient content, little organic matter, and a low C/N ratio compared with the OS and CC samples. Compost addition generally improved the soil properties of the tailings by increasing the CEC, TOC, total N, and the C/N ratio (Table 1). The EC increased slightly as well but not out of an acceptable range for quailbush. Additionally, pH significantly increased in the K4 mine tailings with each increasing level of compost ($F_{4,\,10} = 1149$; $p < 0.0001$), while the pH of the K6 mine tailings significantly increased at the highest compost levels (K6–75 and K6–85; $F_{4,\,10} = 9.74$; $p = 0.0018$).

Total As, Cu, Pb, and Zn concentrations were elevated in both tailings samples in comparison to background levels normally found in soil while Mn was higher than background only in the K6 sample (Table 2).

Table 1. Physicochemical characteristics of Klondyke mine tailings samples and compost.

	Soil texture									
TRTMT†	Sand	Silt	Clay	pH‡	EC§	CEC	PO_4–P	TOC	Total N	C/N
	%				dS m^{-1}	$cmol_c$ kg^{-1}	g kg^{-1}			
K4										
K4–75	69	21	11	6.3a	7.4	11	0.10	19	2.2	9
K4–85	65	23	12	4.4b	6.5	13	0.11	15	1.8	9
K4–90	64	25	12	4.2c	5.2	10	0.08	9.5	1.1	9
K4–95	62	26	13	3.4d	5.1	8	0.05	5.3	0.6	9
K4–100	58	31	11	2.7e	5.3	6	<0.01¶	0.4	<0.2	<2
K6										
K6–75	60	25	15	6.5a	8.6	14	0.09	20	2.1	10
K6–85	59	27	14	6.3ab	8.9	12	0.09	11	1.3	9
K6–90	64	21	15	5.8abc	5.6	8	0.08	9.2	1.0	9
K6–95	66	22	12	5.7bc	4.7	7	0.03	4.4	0.5	9
K6–100	39	51	10	5.7c	3.5	5	<0.01	0.3	<0.2	<2
Controls										
OS	38	45	17	7.7	1.2	25	0.03	18	1.8	10
CC	ND#	ND	ND	8.3	7.5	54	<0.01	270	11	25

† TRTMT, treatment. Treatment designations are as follows: K4 (pH 2.7); K6 (pH 5.7); OS (offsite control); CC (compost control); number following K4 and K6 are percentage mine tailings.
‡ Within the pH column and mine tailings sample, different letters for each mean (n = 3) represent a significant difference at $p < 0.05$ (Tukey's HSD).
§ EC, electrical conductivity; CEC, cation exchange capacity; TOC, total organic carbon.
¶ Values preceded by " < " indicate detection limits.
# ND, not determined.

Lead and zinc were also elevated in the off-site control sample likely due to eolian dispersion from the adjacent tailings piles. Plant-available metals were extremely low for As, Cd, Fe, and Pb in comparison to the total metal concentrations (0.01 to 2% of total metals) while Cu (6% of total metals) and Zn (13 to 18% of total metals) were slightly higher (data not shown). These values are similar to those reported for other lead-zinc mine tailings sites (Zhu et al., 1999; Ye et al., 2002). Manganese exhibited high availability in K4 (70% of total Mn) but only moderate availability in the K6 (5%) even though total Mn in K6 was 25-fold higher. In general, plant-available metals increased in the order (mg kg^{-1}): Cd < As < Pb < Cu < Fe < Zn < Mn in the K4 tailings, and As < Cd = Fe = Pb < Cu << Mn << Zn in the K6 tailings. Compost addition had little impact on the plant availability of any metal except Mn (data not shown) which decreased from 70 to 40% of total Mn as compost levels increased in the K4 tailings and from 5.4 to 4.6% as compost was added to K6 tailings.

Germination

Germination of quailbush seeds (data not shown) was evaluated to help determine the stage at which mine tailings inhibit plant growth. Results demonstrated minimal difference in germination among all treatments with the exception of the unamended K4 sample where percentage seed germination was significantly inhibited (16%, $F_{11,\ 48}$ = 3.37, p = 0.0017) compared with amended treatments (K4–75, K4–85, K4–90, K6–85, and K6–95; 60 to 72%) or compost alone (57%).

Table 2. Total metal concentrations in the Klondyke mine tailings and compost samples.

Samples†	As	Cd	Cu	Fe	Mn	Pb	Zn
	mg kg^{-1}						
K4	62	<0.1‡	671	38 100	185	5300	366
K6	72	4	792	29 300	4840	5010	3760
OS	<0.1	<0.1	140	21 800	1010	781	844
CC	<0.1	<0.1	35	5650	411	18	209
Background§	1–50	0.01–0.7	2–200		20–3000	2–200	10–300

† Sample designations are as follows: K4 (pH 2.7); K6 (pH 5.7); OS (offsite control); CC (compost control).
‡ Values preceded by " < " indicate detection limits.
§ Background ranges from Swaine (1955).

Plant Growth and Dry Mass

Following germination, five seedlings of similar size from each treatment were transplanted into larger pots containing the same treatment and evaluated for plant growth. Results for mean height, mean number of leaves, and mean basal diameter (data not shown) were similar, collectively demonstrating that the mine tailings alone treatment significantly reduced the growth of quailbush ($F_{4,\ 40} > 16, p < 0.0001$). In fact, all plants in the K4–100 treatment died 2 wk after being transplanted, while plants were severely stunted in the K6–100 treatment. In contrast to seed germination, significant differences in growth of quailbush could be attributed to both sample source (K4, K6, OS, or CC) ($F_{3,\ 40} > 12, p < 0.0001$) and mine tailings concentration ($F_{4,\ 40} > 34$, $p < 0.0001$). However, there was no significant interaction between these factors ($F_{4,\ 40} < 2, p > 0.5$).

At the end of the experiment, plants were harvested and dried to determine the effect of mine tailings concentrations on the total dry mass of quailbush (Fig. 1). Both tailings materials significantly inhibited mean total dry mass of quailbush in the 95% and 100% tailings treatments. Plants in the K4–100 treatment all died within 2 wk, while those surviving the K6–100 treatment produced extremely low plant mass. For the 95% treatment, the total dry mass in K4 and K6 was 6 and 13% of the OS dry mass, respectively. Growth of quailbush was similar to the off-site control at 75, 85, and 90% mine tailings concentration. Furthermore, growth was enhanced in some of the 75 and 85% mine tailings treatments compared with the OS. Although not significant, there was a 14 to 27% increase in total dry mass when

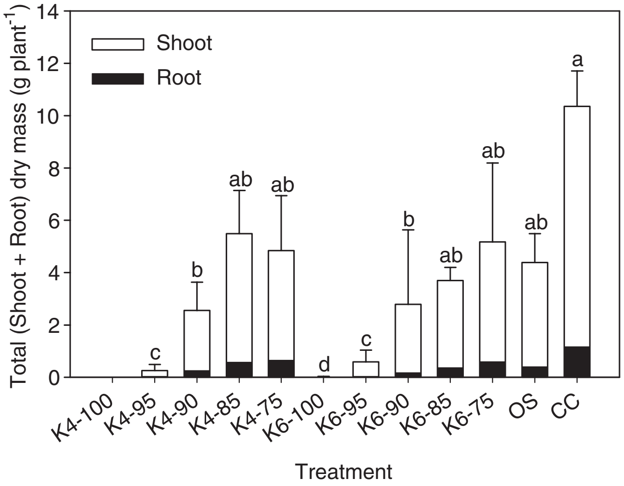

Fig. 1. Final mean total (shoot + root) dry mass of quailbush grown in mine tailings-compost mixtures (mean + 1 SD). Treatment designations are as follows: K4 (pH 2.7); K6 (pH 5.7); OS (offsite control); CC (compost control); number following K4 and K6 are percentage mine tailings. A one-way ANOVA determined there were significant differences between treatments ($p < 0.0001$). All plants in the K4–100 treatment died; therefore, this data was not included in the analysis. Means with different letters are significantly different at $p < 0.05$ (Tukey's HSD test).

quailbush was grown in K4–75, K4–85, and K6–75 compared with the OS sample.

Plant Metal Analysis

Plant metal accumulation in quailbush was examined to assess the metal tolerance and phytostabilization potential of this species. The monovalent cations K^+ and Na^+ were both taken up extensively into shoot tissues as is normal for this halophyte (Table 3). Lead, despite its high concentrations in the tailings was accumulated at very low levels. Patterns for accumulation in shoot tissues were examined in K4 and K6 treatments and generally followed the order (excluding K and Na): Zn $\geq$ Fe $\geq$ Mn $>$ Pb $>$ Cu $>$ As $>$ Cd on a mass basis (Table 3). Thus, accumulation in shoots was selective with nutrient metals, particularly Mn and Zn, taken up preferentially over the three nonessential metals As, Cd, and Pb, as well as Cu. Additionally, we observed a trend suggesting that as compost increased, shoot metal accumulation decreased (data not shown).

Microbial Counts

To assess the level of stress in the mine tailings treatments, the bacterial community was enumerated and compared. Microbial counts were conducted for autotrophic iron- and sulfur-oxidizing bacteria as well as heterotrophic bacteria in bulk samples before planting (initial) and in post-harvest (final) bulk and rhizosphere samples.

For both autotrophic iron and sulfur oxidizers, initial bulk counts in K4 and K6 tailings were between 10^5 and 10^6 MPN g^{-1} dry material. These counts were not impacted by the addition of compost to the tailings (Fig. 2). Comparing the K4 and K6 treatments, initial counts for iron oxidizers were generally one log higher in the K6 treatments, whereas sulfur oxidizers were ~0.5 log greater in the K4 treatments. No iron or sulfur oxidizers were present in the OS or CC samples at the detection limit of 10^2 MPN g^{-1} dry material. Post-harvest autotrophic counts showed a 1 to 5 log reduction in iron oxidizers and a 0.5 to 2 log reduction for sulfur-oxidizers across all samples. For post-harvest counts, compost addition further decreased autotrophic population estimates, particularly for iron-oxidizers which were not detected in either the K4–75 or K6–75 treatments. Iron and sulfur oxidizers were not detected in any rhizosphere samples (data not shown).

Heterotrophic bacterial counts in initial bulk samples were significantly different ($F_{11,\,24} = 4059$, $p < 0.0001$) among treatments (Fig. 3A). The unamended K4 and K6 tailings had low initial heterotrophic counts, 10 and 75 CFU g^{-1}, respectively, that were significantly different from each other and all other treatments ($p < 0.05$). The highest heterotrophic count was observed in the CC treatment (1.4×10^8 CFU g^{-1}) while the OS had 8.0×10^4 CFU g^{-1}. The addition of compost significantly increased heterotrophic counts in all compost-tailings treatments to a level higher, in most cases, than the OS. For the initial bulk treatments, significant differences can be attributed to sample source ($F_{3,\,24} = 4936$, $p < 0.0001$) and tailings concentration $F_{5,\,24} = 6576$, $p < 0.0001$), but there was no significant interaction between these factors ($F_{3,\,24} = 0$, $p = 1.000$).

For post-harvest bulk samples, heterotrophic counts were comparable to OS levels in all compost treatments (Fig. 3B). Specifically, final bulk heterotrophic counts in the 75 to 95% K4 and K6 tailings treatments averaged 2.6×10^6 CFU g^{-1} in comparison to 1.1×10^6 CFU g^{-1} for the OS treatment. For tailings alone, heterotrophic numbers remained significantly lower than all compost treatments ($F_{11,\,24} = 67.03$, $p < 0.0001$) with 131 CFU g^{-1} in the K4 treatment (no plants survived) and 6.3×10^4 CFU g^{-1} in the K6 treatment (plants were severely stunted). Sample source ($F_{3,\,24} = 25.16$, $p < 0.0001$) as well as tailings concentration ($F_{5,\,24} = 113.5$, $p < 0.0001$) had a significant effect on final bulk-planted heterotro-

Table 3. Total metal concentrations of quailbush shoot tissues when grown in mine tailings-compost mixtures.

TRTMT†	As‡	Cd	Cu	Fe	K	Mn	Na	Pb	Zn
	mg kg^{-1}								
K4	1.5 ± 0.4a	0.33 ± 0.37a	28 ± 9a	330 ± 171a	55 700 ± 18 000a	395 ± 439a	16 800 ± 8890a	44 ± 31a	550 ± 287a
K6	0.5 ± 0.14b	0.49 ± 0.45a	23 ± 8a	251 ± 192ab	52 000 ± 18 400a	171 ± 149a	20 200 ± 10 000a	28 ± 31ab	637 ± 305a
OS	0.1 ± 0.0b	0.03 ± 0.04a	17 ± 0a	103 ± 22b	57 600 ± 9190a	94 ± 7a	10 100 ± 953a	7 ± 1b	126 ± 20b

† TRTMT, Treatment. Treatment designations are as follows: K4 (pH 2.7); K6 (pH 5.7); OS (offsite control).

‡ Within columns, different letters for each mean ± SD ($n = 3$) represent a significant difference at $p < 0.05$ between means for all treatments within each sample source.

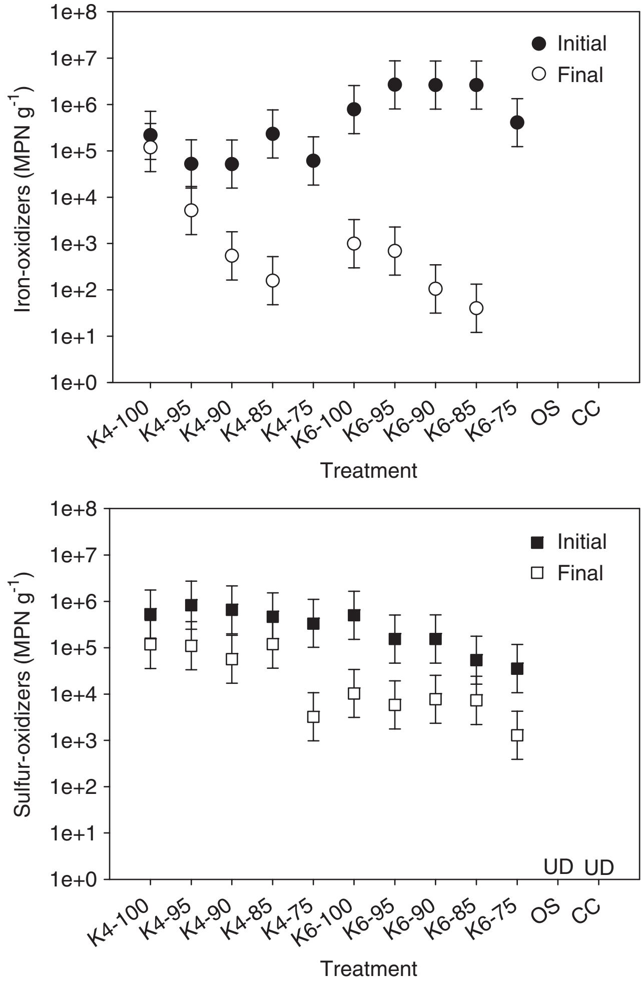

Fig. 2. Initial and final population estimates (most probable number [MPN] g^{-1}) of autotrophic iron and sulfur oxidizers in bulk mine tailings-compost mixtures. Treatment designations are as follows: K4 (pH 2.7); K6 (pH 5.7); OS (offsite control); CC (compost control); number following K4 and K6 are percentage mine tailings. Several treatments were undetectable (UD) at the 10^{-2} dilution level. Bars represent the upper and lower limit at a 95% confidence limit ($p = 0.05$).

phic counts with a significant sample source × tailings concentration interaction ($F_{4,\,24} = 31.45$, $p < 0.0001$).

Finally, as expected, heterotrophic rhizosphere counts (Fig. 3C) were higher than both initial and final bulk heterotrophic counts ranging from 2.4×10^9 to 2.0×10^{10} CFU g^{-1} with no significant differences among treatments with surviving plants ($F_{11,\,23} = 0.64$, $p = 0.7607$).

DISCUSSION

The Klondyke tailings are similar in physicochemical properties to other lead-zinc mine tailings that have been studied for their impedance of plant growth (Wong et al., 1998; Krzaklewski and Pietrzykowski, 2002; Ye et al., 2002). The unamended K4 and K6 mine tailings samples did not support plant growth which was expected since the Klondyke tailings site has remained unvegetated for 48 yr. There are many possible reasons why plant growth is suppressed. The K4 sample is extremely acidic and below the optimal plant growth range of pH 5.0 to 7.5 (Marschner, 1995). Although the K6 tailings sample is moderately acidic, it shares the following properties with the K4 sample that contributes to the unsuitability of the Klondyke site for plant growth: low CEC, low organic carbon content, almost undetectable phosphate and nitrogen content, high metal content, moisture stress, low heterotrophic counts, and high iron and sulfur oxidizer counts (Stevenson and Cole, 1999). Thus, it was not surprising that even under greenhouse conditions with sufficient water, plants did not survive in unamended K4 tailings and only severely stunted plants survived in the unamended K6 tailings.

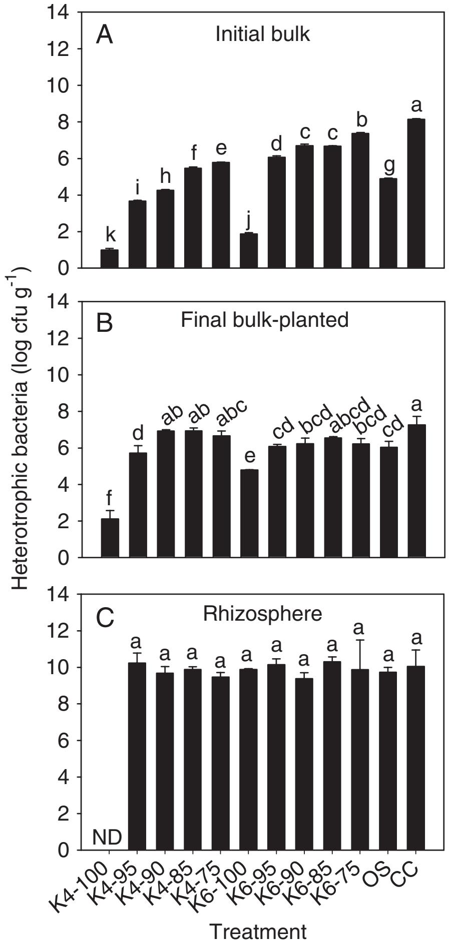

Fig. 3. (A) Initial bulk, (B) final bulk-planted, and (C) rhizosphere heterotrophic bacterial counts (colony forming units [CFU] g^{-1}) in mine tailings-compost mixtures (mean + 1 SD). Treatment designations are as follows: K4 (pH 2.7); K6 (pH 5.7); OS (offsite control); CC (compost control); number following K4 and K6 are percentage mine tailings. A one way ANOVA determined there were significant differences between treatments for initial mean bulk and final mean bulk-planted heterotrophic counts ($p < 0.0001$), but there were no significant differences for mean rhizosphere heterotrophic counts ($p = 0.7607$). All plants in the K4–100 treatment died; therefore, rhizosphere counts were not determined (ND) for this treatment. Means with different letters are significantly different at $p < 0.05$ (Tukey's HSD test).

In terms of metals, both tailings samples contain total As and Pb that exceed the limits for remedial action in Arizona nonresidential areas (10 and 1200 mg kg^{-1} respectively), and thus are considered to be hazardous waste (ADEQ, 2002). In regard to phytotoxicity, several metals at the Klondyke site exceed reported soil plant toxicity levels (SPL). This is true for Pb (SPL = 100 to 500 mg kg^{-1}) in the K4, K6, and OS samples, for As and Cu (SPL = 15 mg kg^{-1} and 200 mg kg^{-1}, respectively) in the K4 and K6 samples, for Zn (SPL = 400 mg kg^{-1}) in the K6 and OS samples, and for Cd and Mn (SPL = 3 and 3000 mg kg^{-1} respectively) in the K6 sample (Munshower, 1993; Mulvey and Elliott, 2000; Kataba-Pendias and Pendias, 2001). The reported values are not specific for quailbush but provide a general reference for plant health.

Establishment of Quailbush in Mine Tailings

This study suggests that germination is not a good indicator for quailbush establishment in mine tailings since germination results were similar for all treatments except the extremely acidic unamended K4 sample. These results are similar to the few studies that have examined seed germination in lead-zinc mine tailings. While none of the studies used *Atriplex* spp., they showed that germination in mine tailings is related to a threshold pH of 3.0 (Yang et al., 1997; Ye et al., 2000; Ye et al., 2001, 2002). Specifically, in the absence of an amendment, germination of Bermuda grass (*Cynodon dactylon* [L.] Pers.) and tall wheatgrass (*Agropyron elongatum* [Host] Beauv.) was not significantly inhibited if the mine tailings had a pH > 3 (Ye et al., 2000). Taken together, these results suggest that seed germination is dependent on the pH of the mine tailings sample and is impacted only at very low pH.

In contrast, growth data indicate that establishment of quailbush in the Klondyke mine tailings requires an organic amendment such as the compost used in this study. Although establishment occurred with 5% compost, quailbush required at least 10% compost to produce growth statistically similar to that in the off-site control. Other studies have similarly demonstrated stunted plant growth of grasses and shrubs in lead-zinc tailings alone compared with amended tailings material and minimal survival past the seedling stage (Yang et al., 1997; Ye et al., 2000; Ye et al., 2001; Shu et al., 2002). For example, the height and biomass of four-wing saltbush (*Atriplex canescens* [Pursh] Nutt.) grown in an acidic copper mine spoil sample alone was stunted compared with sludge-amended spoil (Sabey et al., 1990). Similarly, Hennessy (1985) found that four-wing saltbush grown in an amended mine spoil sample at 50% v/v produced a normal height compared with plants grown in topsoil alone, and its biomass even exceeded that in the topsoil.

While this study did not identify mechanisms of establishment, compost addition increased the organic matter and nutrient content as well as CEC, pH, and heterotrophic bacteria in the tailings. In general, compost addition to mine tailings is known to increase water-holding capacity, CEC, and help to improve the structure of mine tailings by forming stable aggregates (Ye et al., 1999; Stevenson and Cole, 1999; Schippers et al., 2000; Krzaklewski and Pietrzykowski, 2002). Furthermore, added compost can sorb and stabilize metals thereby decreasing their bioavailability (Stevenson and Cole, 1999), although we observed little change in metal bioavailability as measured by DTPA extraction in this study.

Quailbush as a Candidate for Phytostabilization

Phytoremediation of metal-contaminated soils can focus on extraction (hyperaccumulation) of metals into plant tissues, phytoextraction, or the stabilization of metals in the plant rhizosphere and roots, phytostabilization. In phytostabilization shoot accumulation of metals is undesirable as these plants may eventually serve as forage material. Thus, information on plant tissue metal accumulation in mine tailings environments is important since *Atriplex* spp. are sometimes the preferred grazing food of livestock or wildlife living in the area of remediated mine tailings sites (Wood et al., 1995). Only a few studies have investigated the accumulation of metals in the shoot tissues of *Atriplex* grown on mine tailings (Sabey et al., 1990; Jordan et al., 2002). Furthermore, only a single study has observed metal accumulation trends in *Atriplex* spp. while grown in mine tailings with organic amendments (Sabey et al., 1990). From the plant metal analysis results of quailbush grown in the Klondyke mine tailings, this species can be considered metal tolerant as well as a good candidate for phytostabilization strategies.

Of the nine metals measured, quailbush accumulated high levels of K and Na into shoot tissues (Table 3). This was expected since *Atriplex* spp. are commonly found in saline soils (Osmond et al., 1980; Malik et al., 1991; Blank et al., 1998) and is not of concern for foraging animals. Quailbush also shoot-accumulated some metals to levels of concern for plant growth reaching reported plant leaf tissue toxicity limits for Mn (400 to 1000 mg kg^{-1}), Pb (30 to 100 mg kg^{-1}), and Zn (100 to 400 mg kg^{-1}) (Table 3). However, this does not seem to have impacted growth in most cases suggesting that quailbush is metal-tolerant. Shoot tissue metal concentrations are also of concern with respect to domestic animal toxicity limits. A recent National Research Council report (National Research Council, 2005) indicates that these limits are 400 to 2000 mg kg^{-1} for Mn, 30 mg kg^{-1} for Pb, and 500 mg kg^{-1} for Zn. In examining the data, it appears that quailbush metal accumulation exceeded these limits in some cases, particularly for Zn. However, it is unlikely that the mine tailings site would provide the only forage for wildlife in the area. Thus, it may not be critical (or possible) to use plants that will never exceed the domestic animal toxicity limits in shoot materials.

The Microbial Community as an Indicator of Plant Establishment

This appears to be the first study to have measured both autotrophic and heterotrophic microbial numbers in bulk soil and rhizosphere samples during the revegetation of a tailings site. Autotrophic iron and sulfur oxidizers were enumerated because of their ability to create an acidic environment in the tailings and impede revegetation (Schippers et al., 2000). In this study, the initial presence of iron and sulfur oxidizers served as an indicator of an acidic, disturbed environment. This was confirmed by the measured acid-generating potential at the site which was extremely high with an acid neutralization potential to acid generating potential ratio of 0.01 (ADEQ, 2001a). Enumeration of the heterotrophic community, which is dependent on available organic matter and is sensitive to environmental stressors, served both as an indicator of disturbance (low initial numbers) as well as an indicator of improvement of the mine tailings for plant growth (high post-harvest numbers). Although we recognize that culture techniques are limited in assessing the total microbial community, they can serve as a comparison between the treatments for inferring soil health.

Other studies have shown similar results either measuring heterotrophic numbers during revegetation or characterizing both heterotrophs and autotrophs in bulk tailings. For example, Mummey et al. (2002) and Moynahan et al. (2002) linked increased heterotrophic numbers and biomass to normal plant growth in the revegetation of mine tailings. Southam and Beveridge (1992, 1993) and Schippers et al. (2000) have shown that unamended bulk tailings contained high numbers (up to 10^6 MPN g^{-1} dry tailings) of iron- and sulfur-oxidizing bacteria while heterotrophic bacteria ranged from as low as 10^1 to 10^5 CFU g^{-1}.

The results of this study demonstrate that the composition of the microbial community in a disturbed environment like mine tailings is an important indicator of the extent of disturbance and the potential success of a remediation strategy such as phytostabilization. For highly disturbed sites, one impact of compost addition is the immediate infusion of a substantial heterotrophic microbial community that is requisite for plant growth and long-term ecosystem health. Heterotrophic bacteria, as well as fungi, are required for a number of critical functions: organic matter cycling, formation of soil aggregates, and enhanced nutrient uptake in metal-contaminated environments (Bearden and Petersen, 2000; Moynahan et al., 2002). Also, bacterial involvement in redox reactions can decrease metal availability as has been demonstrated with Pb (Blake et al., 1993). Garcia-Meza et al. (2006) demonstrated a reduction in exchangeable Cu, Mn, Pb, and Zn with direct inoculation of tailings with bacteria, as well as an increase in organic matter. In addition, increased plant biomass, enhanced nutrient uptake, and reduced metal accumulation has been documented in plants grown in inoculated mine tailings (Carrillo-Castaneda et al., 2003; Petrisor et al., 2004).

CONCLUSION

Quailbush is a good candidate for phytostabilization of mine tailings in semiarid regions of the U.S. Southwest and northern Mexico. Organic matter amendment up to 15% by mass may be required depending on the extent of pH, metal, and microbial community stress that exists in a given site. Attributes of quailbush include its status as a halophyte, its metal tolerance, and the fact that it does not hyperaccumulate toxic metals such as As, Cd, or Pb. This study demonstrates that microbial community composition can be used to indicate the potential for and success of a mine tailings revegetation. Indicators to look for include an increase in heterotrophic counts to “normal” levels of approximately 10^6 CFU g^{-1} and a decrease in iron and sulfur oxidizers to undetectable levels.

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