Published in final edited form as:
Environ Sci Technol. 2012 January 17; 46(2): 1019–1027. doi:10.1021/es202846n.

Response of Key Soil Parameters During Compost-Assisted Phytostabilization in Extremely Acidic Tailings: Effect of Plant Species

Fernando A. Solís-Dominguez, **Scott A. White**, **Travis Borrillo Hutter**, **Mary Kay Amistadi**, **Robert A. Root**, **Jon Chorover**, and **Raina M. Maier***

Abstract

Phytostabilization of mine tailings acts to mitigate both eolian dispersion and water erosion events which can disseminate barren tailings over large distances. This technology uses plants to establish a vegetative cover to permanently immobilize contaminants in the rooting zone, often requiring addition of an amendment to assist plant growth. Here we report the results of a greenhouse study that evaluated the ability of six native plant species to grow in extremely acidic (pH ~ 2.5) metalliferous (As, Pb, Zn: 2000–3000 mg kg^{-1}) mine tailings from Iron King Mine Humboldt Smelter Superfund site when amended with a range of compost concentrations. Results revealed that three of the six plant species tested (buffalo grass, mesquite, and catclaw acacia) are good candidates for phytostabilization at an optimum level of 15% compost (w/w) amendment showing good growth and minimal shoot accumulation of metal(loid)s. A fourth candidate, quailbush, also met all criteria except for exceeding the domestic animal toxicity limit for shoot accumulation of zinc. A key finding of this study was that the plant species that grew most successfully on these tailings significantly influenced key tailings parameters; direct correlations between plant biomass and both increased tailings pH and neutrophilic heterotrophic bacterial counts were observed. We also observed decreased iron oxidizer counts and decreased bioavailability of metal(loid)s mainly as a result of compost amendment. Taken together, these results suggest that the phytostabilization process reduced tailings toxicity as well as the potential for metal(loid) mobilization. This study provides practical information on plant and tailings characteristics that is critically needed for successful implementation of assisted phytostabilization on acidic, metalliferous mine tailings sites.

Keywords

assisted phytostabilization; phytoremediation; mine tailings; rhizosphere; mesquite; buffalo grass; quailbush; catclaw acacia

Introduction

The goal of phytostabilization in remediation of mine tailings sites, particularly in arid and semi-arid environments, is to create a vegetative cap using native plants that will 1) prevent wind and water erosion of the tailings, 2) stabilize metal contaminants in the rooting zone, 3) avoid shoot uptake of metal contaminants, and 4) pioneer the development of a long-term sustainable cover. Important characteristics that have been reported to prevent plants from growing on mine tailings and mine waste sites include: low pH; high levels of metal(loid)s

*Corresponding Author Raina M. Maier, University of Arizona, Department of Soil, Water and Environmental Science, 429 Shantz Building #38, Tucson, AZ 85721, Phone: 520-621-7231, FAX: 520-626-6782,rmaier@ag.arizona.edu.

and salts; low organic matter content; and low numbers of heterotrophic bacteria[1–3] . Clearly, given these constraints, the choice of the plant species used in phytostabilization is important. Considerable screening information is available for selection of suitable plant species regarding whether they are indigenous to a particular area and the functional group they represent, for example: rooting depth; canopy height and cover; or the ability to fix nitrogen. Very little, other than anecdotal, information is available concerning plants that can actively aid in the phytostabilization process through modification of the bio-chemical-physical environment. Further, there is as yet no accepted set of key system parameters to use in evaluating the potential for success of plant species in the mine tailings phytostabilization process.

Some system parameters have been discussed in the literature as important for predicting and evaluating the success of phytostabilization. One is the need for information on plant tissue metal accumulation in mine tailings environments as the plants used may serve as grazing food for livestock or wildlife[4]. Relatively few studies have investigated the accumulation of metals in the shoot tissue of native plants grown in desert mine tailings[2, 5, 6]. Mendez et al.[2] suggested that enumeration of the neutrophilic heterotrophic community, which is dependent on available organic matter and sensitive to environmental stressors, serves both as an indicator of stress (low initial numbers) as well as an indicator of improvement of the mine tailings for plant growth (high post-harvest numbers). The availability of metal(loid)s which exist in several physicochemical forms in tailings: as ionic or neutral solutes, adsorbed to surfaces, or as constituents within the crystalline structure of primary or secondary minerals, is important because it dictates toxicity and the potential for mobility. Generally, soluble forms, also often considered as the "bio-accessible" fraction, are most toxic[7]. Finally, low pH is one of the more difficult parameters to control in tailings which contain high levels of pyritic and sulfidic minerals, (e.g., arsenopyrite). A combination of chemical and biologically-mediated sulfide mineral oxidation reactions contribute to the formation of acidic conditions in the tailings, with the biological contribution increasing in importance as the pH decreases[8]. The bacteria involved in this process are chemoautotrophic iron and sulfur-oxidizers that have optimal growth in environments with pH ranging from 2 to 3. In contrast, plant growth is optimal at pH values between 5.5 and 6.5[9, 10]

The objective of this study was to evaluate a selection of six native desert plants under greenhouse conditions for their ability to grow in compost-amended mine tailings and to evaluate the effect of plant species on key system parameters including: biomass production, pH, aqueous extractable and plant shoot tissue metal(loid) concentrations, neutrophilic heterotrophic bacterial counts (NHC), and iron oxidizers. Compost was chosen as an amendment because it has an immediate influence on several of these system parameters; pH, metal(loid) solubility, and NHC. The mine tailings used in this study were collected from the Iron King Mine-Humboldt Smelter Site (IKMHSS), Dewey-Humboldt, Arizona which was placed on the National Priority List by the EPA in September 2008. A major feature of the site is the 153-acre mine tailings pile that is thought to have contributed to elevated levels of arsenic, lead and zinc found in the soil in some Dewey-Humboldt residential yards, dispersed primarily by wind. These tailings are extremely acidic (pH 2.5 to 3.5), contain high levels of metal(loid)s mainly arsenic and lead (> 3000 mg kg^{-1} each), high level of salts (EC 6.5–9 ds m^{-1}), low organic matter and nitrogen content, and low NHCs.

Materials and methods

Mine tailings and compost analysis

All tailings were collected from the IKMHSS site from a depth of 0–30 cm. The compost was a mixture of dairy manure and green waste obtained from a local farm in Dewey-Humboldt, Arizona. Initial analysis was performed on triplicate samples of tailings, compost, and tailings/compost mixtures that were air-dried at room temperature and sieved through a 2 mm mesh screen. Samples were analyzed for particle size (tailings only), pH, electrical conductivity(EC), and aqueous extractable metal(loid)s as described by Hayes et al.[11] . Total metal(loid) content (tailings and compost only) including: As, Pb, Zn, Cd, Cu, Ni, Al and Cr, was determined using microwave-assisted digestion (USEPA method 3051) and ICP-MS analysis in the University of Arizona Laboratory for Emerging Contaminants. Bulk tailings mineralogy was determined by powder XRD using both laboratory and synchrotron X-ray sources. Samples were finely ground to powders, and randomly orientated on quartz micro-slides for laboratory XRD and on X-ray transparent tape mounts for synchrotron XRD[11]. Total carbon (TC), total inorganic carbon (TIC), and total nitrogen were measured for tailings and compost only samples using a Shimadzu TOC-VCSH analyzer (Columbia, MD) with a solid state module (SSM-5000A). Total organic carbon (TOC) was determined from the difference between TC and TIC. Acid neutralizing capacity (ANC) of the compost was determined titrimetrically[12]. The acid potential (AP) of the tailings was calculated from the amount of pyritic sulfur in the tailings[13]: the pyretic fraction of the total S (determined by total digestion) was determined by synchrotron near edge X-ray absorption spectroscopy (NEXAFS)[14].

Greenhouse experiment

A greenhouse experiment was performed to determine the minimum compost amendment rate required to establish plants in the Iron King tailings and evaluate the influence of plant establishment on key tailings parameters. Two representative drought and salt-tolerant native species of the Sonoran-Arizona desert ecosystem were evaluated from each of three different plant functional groups: trees (mesquite and catclaw acacia); shrubs (quailbush and mountain mahogany); and grasses (buffalo grass and arizona fescue). For the two species tested in each functional group, the species listed first has previously been shown to grow successfully in other types of desert mine tailings [2, 15, 16] while the latter species were chosen based on their suitability to the specific elevation and climate conditions at the IKMHSS site[17]. Four treatments (compost amendment rates) were tested: 0, 10, 15 or 20% (w/w) compost in triplicate.

The experiment was conducted in a greenhouse (natural light, temperature day/night 32°C/24°C) located at the University of Arizona Controlled Environment Agriculture Center (Tucson, AZ). One gallon black plastic pots with drainage (17 cm top diameter × 18 cm height × 13.5 cm bottom diameter) were filled with the tailings-compost mixture (3 kg). Mesquite (*Prosopis juliflora*), catclaw acacia (*Acacia greggi*), buffalo grass (*Buchloe dactyloides*), Arizona fescue (*Festuca arizonica*), quailbush (*Atriplex lentiformis*), and mountain mahogany (*Cercocarpus montanus*) seeds (Desert Nursery, Phoenix, AZ) were sown in each pot (40, 30, 50, 50, 50, 50 seeds, respectively) at a depth of approximately 0.5 cm. Pots were watered with tap water using drip irrigation every other day (80 mL pot^{-1}). Germination occurred after three to eleven days depending on the plant species. Pots were thinned to two, four or ten seedlings for trees, shrubs and grasses, respectively. Plants were harvested after 60 d.

Plant and tailings sample collection and analysis

At 60 d, plants were removed from each pot and the shoots separated from the roots. Both bulk and rhizosphere-influenced tailings samples were collected. Bulk tailings were collected from the pots after the plants were removed. Rhizosphere-influenced tailings were collected by vigorously shaking the plant root system after it was removed from the pot. A portion of the rhizosphere-influenced and bulk samples were placed in sterile plastic bags and stored at 4°C for microbial analysis. Another portion of these samples was dried to room temperature and subsampled to determine pH, EC and aqueous extractable metals as described above.

Plant biomass was determined following washing and drying at 70°C for 72 h. To determine shoot metal concentrations, samples were ground with a Wiley Mill for one min, passed through a 30 mesh (0.595 mm) screen, microwave digested using EPA Method 3051 for total elements (As, Cd, Cu, Cr, Mn, Zn, Pb), and analyzed by ICP-MS.

Enumeration of heterotrophic and chemoautotrophic iron-oxidizing bacteria

Initial (before seed germination) and final (post-harvest) neutrophilic heterotrophic bacterial counts (NHCs) were assessed in bulk samples from all treatments[2]. Initial NHCs were also determined for the compost amendment alone. Iron oxidizing bacteria were assessed using the most probable number (MPN) method[2, 18].

Assay for root-induced influence on pH

The purpose of this assay was to determine whether any of the plant species had the ability to increase the rhizosphere pH. The assay was performed using germinated seedlings and agarose gel slabs amended with the pH indicator bromocresol purple adjusted to pH 5.5[19]. Seeds from each of the six plant species were disinfected with NaClO (15% commercial bleach) for 15 min, rinsed with sterile distilled water (pH 5.5), and germinated on a wet paper soaked in sterile acidic (pH 5.5) distilled water. When the seedlings roots were ~2 cm in length they were sandwiched between two agarose slabs, incubated for up to one week, and examined for color change (Fig 4).

Statistical analysis

Plant biomass, germination percentage and heavy metal concentration data were analyzed using SAS version 9.1 (SAS Institute Inc., Cary, NC). Significant differences were detected by employing either one-way or two way ANOVA ($p < 0.05$). Significant differences between many means were determined by Tukey's test ($p < 0.05$) and between two means by the Student t test ($p < 0.05$).

Results and Discussion

Tailings characteristics before and after compost amendment

The IKMHSS mine tailings had a loam texture: 34.7% sand, 44.8% silt and 20.4% clay and TOC and TN of 1.22 g kg^{-1} and 0.0423 g kg^{-1} respectively. Mineralogical analyses indicate that the tailings are comprised dominantly of quartz, albite, pyrite, gypsum, jarosite, plumbojarosite, and goethite. The AP of the tailings was 1.6 kg H_2SO_4 t^{-1} and the ANC of the compost was 1.5 kg H_2SO_4 t^{-1}. The net acid producing potential (NAPP) for the tailings-compost treatments was then calculated from the tailings AP and the compost ANC (assuming the tailings ANC and compost AP were negligible) using the equation: NAPP = ANC - AP. Values of NAPP >20 are considered potentially acid generating, values <−20 are considered non-acid generating, and the range from −20 to 20 is considered a zone of uncertainty[20]. The NAPP values for the compost-amended tailings were all in the zone of uncertainty: 1.3 for the 10%; 1.1 for the 15%; and 0.98 for the 20% compost treatments, indicating the possibility for further acid generation.

Other selected properties of the tailings are provided in Tables 1–3. Unamended tailing exhibited low pH, low NHC, elevated numbers of iron oxidizing bacteria, and high EC (Table 1). Addition of compost amendment to the mine tailings immediately increased the pH, EC, and NHC, and decreased iron oxidizer counts. All of these changes, except for increased EC, would be expected to make the tailings more suitable as a plant growth substrate[2, 15, 21]. The compost used in this study was characterized by a total organic carbon content of 297 ± 20 g kg^{-1}, and a total nitrogen content of 24.8 ± 0.39 g kg^{-1}. Total metal(loid) content in the compost was (mg kg^{-1}): As, 0.949 ± 0.267; Pb, 2.52 ± 1.09; Zn, 233 ± 30.3; Cd, 0.274 ± 0.036; Cu, 94.1 ± 10.6; Ni, 5.77 ± 1.00.

Plant biomass production

No detectable biomass was produced by any plant species in unamended tailings. In compost-amended treatments, four of the plant species (buffalo grass, mesquite, quailbush, and catclaw acacia) produced biomass ranging up to 14 g pot^{-1} (Fig. 1). These four species generally produced higher levels of biomass in the 15 and 20% compost treatments than in the 10% compost treatment (Fig 1). Plants in the 10% treatment were clearly stressed and showed signs of chlorosis and stunted growth in comparison to the plants in the 15 and 20% compost treatments which looked healthy. Biomass production was similar in the 15 and 20% treatments suggesting that 15% is the minimum measured compost amendment rate required for optimum growth of these plants in the IKMHSS tailings. The remaining two species, mountain mahogany and arizona fescue, produced low amounts of biomass and plants appeared stressed at all compost concentrations, indicating that these two species are not suitable pioneer candidates for phytostabilization of IKMHSS tailings.

Effect of plant establishment on heterotrophic and iron oxidizer counts

In unamended tailings, NHCs were low (~700 CFU g^{-1} dry tailings) at both the beginning and the end of the experiment (Fig. 2). Compost amendment immediately increased NHCs by 0.5 to 3 logs; an increase that was significant for each compost level and directly related to the amount of compost added. A calculation was performed to determine whether the compost effect on initial NHCs reflects the mass fraction added by the compost amendment which contained 3.0 ± 0.5 × CFU g^{-1} compost. This calculation suggests that the NHCs expected in the 10, 15, and 20% (w/w) compost treatments would be 3×10^6, 4.5×10^6, and 6×10^6 CFU g^{-1} , respectively. However, only 0.07%, 0.8%, and 27% of the expected NHCs were recovered for the three treatments respectively (Table 1). These results suggest that the majority of compost bacteria become compromised when mixed with mine tailings, though as the mass fraction of compost is increased, this effect becomes buffered.

Day 60 NHCs in the unplanted composted-amended controls were not significantly different from day 0 except for the 15% compost treatment (Fig. 2). In contrast, for planted treatments (only the four best plants were included in this analysis), NHCs were significantly higher than the unplanted compost-amended controls after 60 d in the 15% and 20% compost treatments (Fig. 2). This increase occurred regardless of plant species but was not directly related to compost amount; the largest increase, 2.7 logs, occurred in the 15% compost treatment. The average final 60 d counts were similar in the 15% ($1.2 \pm 1.2 \times 10^8$) and 20% ($8.1 \pm 7.3 \times 10^7$) compost planted treatments and these counts were 2.5 logs higher than counts in the 10% ($3.3 \pm 8.1 \times 10^5$) treatment. A regression analysis comparing the 60 d NHCs to plant biomass production showed a significant positive relationship between plant biomass yield and NHCs for all four plant species (Fig. 2, inset). These data strongly support the use of NHCs, a rapid and technically easy assay, to both initially assess the ability of

NIH-PA Author Manuscript

NIH-PA Author Manuscript

mine tailings to support plant growth and to evaluate the on-going success of the phytostabilization process.

Iron oxidizer counts were also enumerated to indicate the presence of bacteria that participate in the formation of acidic conditions in mine tailings. Unamended tailings had MPN counts of 1.6×10^4 iron oxidizers g dry tailings^{-1}. Compost amendment immediately decreased iron oxidizer counts by up to 1.5 logs to 5.3×10^2 (Table 1). A mass fraction calculation showed a larger reduction in iron oxidizer counts than would be expected from a simple compost dilution effect, ranging from a recovery of 3% of the expected iron oxidizers in the 20% compost treatment to 56% in the 10% compost treatment. These results suggest that compost amendment has an immediate compromising effect on the ability of mine tailings iron oxidizers to be cultured.

Iron oxidizers were enumerated in the 15% compost treatment after 60 d. Results showed a further ~0.5 log decrease in the MPN in both the planted treatments and the unplanted compost-amended control after 60 d (data not shown).

Accumulation of metal(loid)s into plant shoot tissue

Metal(loid) concentration in shoot tissues was determined for the four successful plant species grown in the 10 and 15% compost treatments (Table 2). Results show that accumulation after 60 d was lower than the domestic animal toxicity limit (DATL) for cattle[22] for all metal(loid)s tested (As, Pb, Zn, Cd, Cu and Ni) except for Zn (Table 2). In examining the Zn results more closely, only quailbush exceeded the zinc DATL at the optimal 15% compost concentration with an exceedance of 2-fold. Both quailbush and catclaw acacia exceeded the Zn DATL in the 10% compost treatment. These results suggest that three of the four plants tested, buffalo grass, mesquite, and catclaw acacia, meet the phytostabilization criterion of low shoot tissue accumulation of all the metal(loid)s tested under conditions of optimal compost amendment.

In examining metal(loid) uptake patterns of the four plant species more closely, buffalo grass accumulated more As, Pb, and Cu than the other species tested while quailbush accumulated significantly higher amounts of Zn and Cd. All plant species studied accumulated similar concentration of Ni and Cu.

Metal(loid) accumulation in the optimal 15% compost amendment treatment was compared to accumulation in the 10% treatment (where plants did not look healthy) to determine whether metal accumulation was related to compost concentration. In general, there was not a significant difference in metal(loid) accumulation between the treatments, although mesquite and catclaw acacia showed a tendency to accumulate less metal(loid)s in the 15% treatment (Table 2). The exception was catclaw acacia for which zinc accumulation in the 15% compost treatment was significantly lower than in the 10% treatment. In this case, the reduction in accumulation resulted in the plant meeting the DATL for zinc at the 15% compost level.

Aqueous extractable metal(loid)s

The impact of compost and plants on aqueous extractable metal(loid) concentrations was determined in the unamended, 10%, and 15% compost treatments (Table 3). There was a significant effect of compost amendment on metal(loid) aqueous extractable concentration with the 15% treatment generally having the lowest values. Lead was an exception to this pattern with aqueous concentrations increasing in the order 10% compost < 15% compost < unamended control. The magnitude of the reduction in aqueous concentration was relatively large; from 2 to 60-fold between the unamended and 10% compost treatment and a further reduction (except for Pb) of > 5-fold between the 10% and 15% compost treatments. Thus, the optimal 15% compost treatment resulted in metal(loid) aqueous extractability reductions ranging from 6 to 1900-fold compared to the unamended tailings. This likely substantially reduced metal(loid) toxicity in 1 n the system contributing to the increased biomass production.

There was no significant effect of plants species on aqueous extractable metal(loid) concentrations with the exception of Zn (Table 3). For zinc, catclaw acacia and quailbush were associated with higher levels of aqueous Zn in the 15% treatment. These are the species that were also associated with plant shoot tissue Zn levels higher than the DATL as described in the previous section.

Effect of plant species on pH changes in the tailings

Previous work has shown that plant roots and associated microorganisms can play an important role in determining and modifying rhizosphere pH[19, 23–25]. Acidification or alkalinization is affected by the relative ratio of protons or anions exuded by plant roots. When plants take up more cations than anions, they compensate by releasing the excess positive charge as H^+ thereby acidifying the rhizosphere. Alternatively, when more anions are taken up by plants, the excess negative charge is released as OH- alkalinizing the rhizosphere[25, 26]. In normal soils the proton:anion ratio depends on different factors including: iron deficiency or excess, nitrogen source[23], phosphorus concentration[27], organic matter[10], heavy metal stress[28, 29], and oxygen release[30]. It has been shown that different plant species can have different rhizosphere pH behavior under the same growth conditions[30].

In the present study, compost addition immediately increased the initial tailings pH from 2.6, but the effect was still sub-optimal for the 10% (pH 3.6) and 15% (pH 4.2) compost treatments, and was temporary (Table 1). Plants survived, albeit poorly, in the 10% compost treatment and thrived in the 15% compost treatment (Fig. 1). We therefore examined the pH in these treatments at the end of the experiment to determine whether any of the plant species influenced the tailings pH either in rhizosphere-influenced or bulk tailings samples (Fig. 3).

The pH of the tailings in the 10% compost treatment declined from 3.6 to 1.9 after 60 d in the absence of plants (Fig. 3A). Most of the planted treatments also showed a similar general reduction in pH in both bulk tailings and rhizosphere influenced tailings samples after 60 d (Fig 3A). The exception to this was buffalo grass which showed a significantly smaller reduction in pH than the unplanted control suggesting that this plant helped buffer the acidification process over the 60 d incubation period.

The 15% compost treatment had an initial pH of 4.2. The pH of the unplanted control returned to the initial pH of 2.6 during the 60 d incubation period (Fig. 3B). In contrast and illustrating the potential for plants to influence pH, both bulk and rhizosphere-influenced tailings samples from three of the four good biomass-producing species, buffalo grass, mesquite, and catclaw acacia, exhibited increased pH relative to both the 0 and 60 day unplanted controls. Focusing just on the 60 d results, the difference in pH between these three plant species and unplanted control samples was significant (Fig. 3). Specifically, buffalo grass (pH 6.5), mesquite (pH 6.4), and catclaw acacia (pH 4.8) dramatically prevented the reduction in pH of tailings samples that was observed in the unplanted control (pH 2.6) after 60 d. Moreover, an ANOVA analysis showed that these three species significantly increased the pH in comparison to the day 0 unplanted control with 15% compost. The pH of quailbush samples was not significantly different from either the day 0 or day 60 unplanted controls, however, even this species appeared to prevent as much of a decrease in pH as occurred in the unplanted control (Fig. 3B).

For the 20% compost treatment, the initial pH following compost amendment was 5.8, which is in the optimal plant pH growth range[10]. The pH of the unplanted control increased to 6.4 over the 60 d incubation (Fig. 3C). Intriguingly, the planted treatments generally had a reduced pH in comparison to the unplanted control at 60 d. In particular, buffalo grass and quailbush had significantly lower pH in both bulk and rhizosphere influenced samples, ranging from 3.9 to 4.8. Clearly, the plant species that engaged in alkalinization at the lower compost levels did not do so at the 20% compost level.

Overall, these results demonstrate that compost, in the absence of plants, transiently increased the tailings pH but that the ANP of the compost did not overcome the AP of the tailings. Importantly, these results suggest that some of the native plant species tested have the capacity to modify the pH in their environment. While it is well known that plant roots can modify soil pH, there are relatively few reports of the effect of initial soil pH on this process[25]. One such report was a study of barley and soybean grown in soil with an initial pH of 4.8 and in the same soil that was pH adjusted to 7.1 with lime. At the low pH these plants alkalinized the rhizosphere while acidification occurred at the higher initial pH of 7.1[31]13 . A second study, using fava bean grown in $CaSO_4$ solution, showed that the plant acidified the medium when the initial pH was > 6 while alkalinization occurred when the initial pH was < 5[32]15 . Most relevant to the present study is the report that when rape was grown in an acidic soil contaminated with copper, the plant had the capacity to alkalize the rhizosphere for initial pH below 4.8 and to acidify the rhizosphere for higher initial pH values[33].

Clearly some type of amendment, such as the organic matter used here or lime, is required to immediately increase the pH of the tailings. In the short-term this immediate increased pH allows plants to germinate and grow and also suppresses the numbers and activity of iron and sulfur oxidizers. However, in the longer-term, the tailings can continue to generate acidity, especially when exposed to a combination of moisture and oxygen, parameters that are also important for plant growth. This can result in reduced pH after a period of time and the development of an environment that is once again inhospitable to plants. Taking this into consideration, a long-term successful outcome for phytostabilization in pyritic tailings will depend on maintaining an elevated pH to prevent the acidification process from occurring. The results of this study indicate that some plants are more effective at maintaining elevated pH than others.

In order to support our observations of the distinct behavior of these different plant species in affecting tailings alkalinization and acidification, an indicator assay was performed to examine whether roots of the six plant species could directly modify the pH of their immediate environment. Seedlings were germinated and placed between two agarose gel slabs amended with bromocresol purple[19]. A control plate prior to incubation with seedlings shows the orange color of the indicator medium at pH 5.5 (Fig. 4A). Results showed that mesquite and buffalo grass strongly alkalinized the culture medium to > pH 6.8 resulting in a deep purple halo surrounding the seedling roots (Fig. 4B and 4C). Quailbush and catclaw acacia weakly increased the pH (data not shown) while mountain mahogany and arizona fescue had a yellow halo around the roots suggesting that the pH was actually decreased to < 5.2 (Fig. 4D and 4E). These results are consistent with the tailings pH results shown in Fig. 3 suggesting that these plant species actively contribute to the modification of pH observed in the tailings following plant establishment.

In this study there was a direct correlation between pH and plant biomass production for buffalo grass ($r^2 = 0.68$, $p = 0.028$), mesquite ($r^2 = 0.77$, $p = 0.028$), quailbush (r^2 21 = 0.71, $p = .0005$), and catclaw acacia (r^2 22 = 0.61, $p = 0.026$). The plant species that performed best were those that could increase - either strongly (buffalo grass) or more weakly (mesquite, quailbush and catclaw acacia) - both the bulk and rhizosphere pH. Buffalo grass, the plant with the strongest ability to modify pH, produced the most biomass while the two species that did not alkalinize the rhizosphere detectably (mountain mahogany and arizona fescue) showed poor growth in the tailings (Fig. 1). An important point here is that the ability to modify pH is plant specific. As stated earlier, there are few reports of plant modification of contaminated acidic soils and this study shows that some plants excel at pH modification while others do not. Since long-term success for phytostabilization in pyritic tailings will depend on maintaining an elevated pH to prevent the acidification process from occurring, this ability may be quite important. Thus, the capacity for pH modification may be a useful criterion for screening potential plants for their use in phytostabilization of acidic contaminated soils and mine tailings. Since the soil pH and the agarose-bromocresol purple assay were similar, this suggests that the simple agarose-based assay would be a good plant screening tool, both easy and relatively rapidly performed.

In summary, three native plant species (buffalo grass, mesquite, and catclaw acacia) met all criteria set out to define a good candidate for assisted phytostabilization of the extremely acidic IKMHSS mine tailings at an optimum level of 15% compost (w/w). These criteria are: (i) their ability to grow in the tailings with low accumulation of metal(loid)s into shoot tissues (below DATLs); and (ii) an improvement of key tailings characteristics including increased tailings pH and NHCs. In addition, compost amendment decreased aqueous extractable metal(loid) concentrations both of which suggest lower toxicity of the tailings and reduced mobility of metal(loid) contaminants. A fourth species, quailbush, also met these criteria except for exceeding the DATL for shoot accumulation of Zn. An important finding of this study is that the plant species that grew most successfully in the tailings were able to actively influence the pH as well as NHCs in the compost-amended tailings growth medium. The combination of increased NHCs and decreased iron oxidizers suggests that the ability of the tailings to continue to produce acidity has been modified and this ratio would be interesting to examine over the long-term. Finally, the results of this study confirm previous work in other acidic tailings indicating that a positive relationship exists between plant biomass yield and NHCs suggesting that simple NHC could be used by practitioners to indicate the level of stress at any given mine tailings site. In conclusion, this study provides practical information that is critically needed for successful phytostabilization planning and implementation for acidic, high metal(loid) content mine tailings sites. Also needed are well-documented field studies to examine how well greenhouse results translate to the field over the long-term.

Acknowledgments

This research was supported by Grants P42 ESO4940 and R01 ES017079 from the National Institute of Environmental Health Sciences Superfund Research Program, NIH (USA) and by a scholarship awarded to F.A.S-D. by CONACyT-Mexico and the Organization of American States for his stay at the University of Arizona. We wish to thank Steven Schuchardt, president of North American Industries for generously providing access to the IKMHSS site and for facilitating collection and subsequent disposal of the tailings used in this study.

References

(1). Gardea-Torresdey J, Peralta-Videa J, de la Rosa G, Parsons J. Phytoremediation of heavy metals and study of the metal coordination by X-ray absorption spectroscopy. Coord. Chem. Rev. 2005; 249:1797–1810.

(2). Mendez MO, Glenn ER, Maier RM. Phytostabilization potential of quailbush for mine tailings: Growth, metal accumulation, and microbial community changes. J. Environ. Qual. 2007; 36:245–253. [PubMed: 17215233]

(3). Mendez MO, Maier RM. Phytostabilization of mine tailings in arid and semiarid environments - An emerging remediation technology. Environ. Health Perspect. 2008; 116:278–283. [PubMed: 18335091]

(4). Wood M, Buchanan B, Skeet W. Shrub preference and utilization by big game on New-Mexico reclaimed mine land. J. Range Manage. 1995; 48:431–437.

(5). Sabey B, Pendleton R, Webb B. Effect of municipal sewage-sludge application on growth of two reclamation shrub species in copper mine spoils. J. Environ. Qual. 1990; 19:580–586.

(6). Jordan F, Robin-Abbott M, Maier R, Glenn E. A comparison of chelator-facilitated metal uptake by a halophyte and a glycophyte. Environ. Toxicol. Chem. 2002; 21:2698–2704. [PubMed: 12463567]

(7). Goyer, R.; Clarkson, R. Toxic effects of metals. In: Klaassen, CD.; Casarett, LJ.; Doull, J., editors. Casarett and Doull's toxicology :the basic science of poisons. 6th ed.. New York: McGraw-Hill; 2001. p. pp 811

(8). Maier, RM.; Pepper, IL.; Gerba, CP. Environmental Microbiology. Burlington, MA: Academic Press; 2009. p. 323-325.

(9). Nuruddin A, Chang M. Responses of herbaceous mimosa (Mimosa strigillosa), a new reclamation species, to soil pH. Resour. Conserv. Recy. 1999; 27:287–298.

(10). Taiz, L.; Zeiger, E. Plant Physiology. Sinauer Assoc.; Massachusetts: 2006. p. 764

(11). Hayes SM, White SA, Thompson TL, Maier RM, Chorover J. Changes in lead and zinc lability during weathering-induced acidification of desert mine tailings: Coupling chemical and micro-scale analyses. Appl. Geochem. 2009; 24:2234–2245. [PubMed: 20161492]

(12). Wong MTF, Nortcliff S, Swift RS. Method for determining the acid ameliorating capacity of plant residue compost, urban waste compost, farmyard manure, and peat applied to tropical soils. Comm. Soil Sci. Plant Anal. 1998; 29:2927–2937.

(13). Parker, GK.; Robertson, A. Acid Drainage. Melbourne: The Australian Mineral & Energy Environment Foundation; 1999. p. 101-117.

(14). Toevs GR, Morra MJ, Polizzotto ML, Strawn DG, Bostick BC, Fendorf S. Metal(loid) diagenesis in mine-impacted sediments of Lake Coeur d'Alene, Idaho. Environ. Sci. Technol. 2006; 40:2537–2543. [PubMed: 16683589]

(15). Grandlic CJ, Palmer MW, Maier RM. Optimization of plant growth-promoting bacteria-assisted phytostabilization of mine tailings. Soil Biol. Biochem. 2009; 41:1734–1740. [PubMed: 20161141]

(16). Solis-Dominguez FA, Valentin-Vargas A, Chorover J, Maier RM. Effect of arbuscular mycorrhizal fungi on plant biomass and the rhizosphere microbial community structure of mesquite grown in acidic lead/zinc mine tailings. Sci. Total Environ. 2011; 409:1009–1016. [PubMed: 21211826]

(17). USDA, NRCS. The PLANTS Database. http://plants.usda.gov, accessed 1 August 2011

(18). Southam G, Beveridge T. Enumeration of *Thiobacilli* within pH-neutral and acidic mine tailings and their role in the development of secondary mineral soil. Appl. Environ. Microbiol. 1992; 58:1904–1912. [PubMed: 16348721]

(19). Bago B, AzconAguilar C. Changes in the rhizospheric pH induced by arbuscular mycorrhiza formation in onion (Allium cepa L). Z. Pflanzen. Bodenk. 1997; 160:333–339.

(20). Hutchinson, IPG.; Ellison, RD. Mine Waste Management. Chelsea, MI: Lewis Pub.; 1992.

(21). Stuczynski T, Siebielec G, Daniels WL, McCarty G, Chaney RL. Biological aspects of metal waste reclamation with biosolids. J. Environ. Qual. 2007; 36:1154–1162. [PubMed: 17596624]

(22). National Research Council. Mineral tolerance of animals. Washington, D.C.: National Academies Press; 2005. p. 496

(23). Bashan Y, Levanony H. Effect of root environment on proton efflux in wheat roots. Plant Soil. 1989; 119:191–197.

(24). Carrillo A, Li C, Bashan Y. Increased acidification in the rhizosphere of cactus seedlings induced by Azospirillum brasilense. Naturwissenschaften. 2002; 89:428–432. [PubMed: 12435098]

(25). Hinsinger P, Plassard C, Tang C, Jaillard B. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: A review. Plant Soil. 2003; 248:43–59.

(26). Thibaud, J.; Sentenac, H.; Grignon, C. Biochemistry of metal micronutrients in the rhizosphere. Manthey, JA.; Crowley, DE.; Luster, DG., editors. Boca Raton: Lewis Publishers; 1994. p. 309-323.

(27). Grinsted M, Hedley M, White R, Nye P. Plant-induced changes in the rhizosphere of rape (Brassica-Napus var - Emerald) seedlings .1. pH change and the increase in P concentration in the soil solution. New Phytol. 1982; 91:19–29.

(28). Zeng F, Chen S, Miao Y, Wu F, Zhang G. Changes of organic acid exudation and rhizosphere pH in rice plants under chromium stress. Environ. Pollut. 2008; 155:284–289. [PubMed: 18162271]

(29). Astolfi S, Zuchi S, Chiani A, Passera C. In vivo and in vitro effects of cadmium on H(+)ATPase activity of plasma membrane vesicles from oat (A vena sativa L.) roots. J. Plant Physiol. 2003; 160:387–393. [PubMed: 12756918]

(30). Blossfeld S, Gansert D, Thiele B, Kuhn AJ, Loesch R. The dynamics of oxygen concentration, pH value, and organic acids in the rhizosphere of Juncus spp. Soil Biol. Biochem. 2011; 43:1186–1197.

(31). Youssef R, Chino M. Root-induced changes in the rhizosphere of plants .1. pH changes in relation to the bulk soil. Soil Sci. Plant Nutr. 1989; 35:461–468.

(32). Schubert S, Schubert E, Mengel K. Effect of low pH of the root medium on proton release, growth, and nutrient-uptake of field beans (Vicia-Faba). Plant Soil. 1990; 124:239–244.

(33). Chaignon V, Quesnoit M, Hinsinger P. Copper availability and bioavailability are controlled by rhizosphere pH in rape grown in an acidic Cu-contaminated soil. Environ. Pollut. 2009; 157:3363–3369. [PubMed: 19608319]

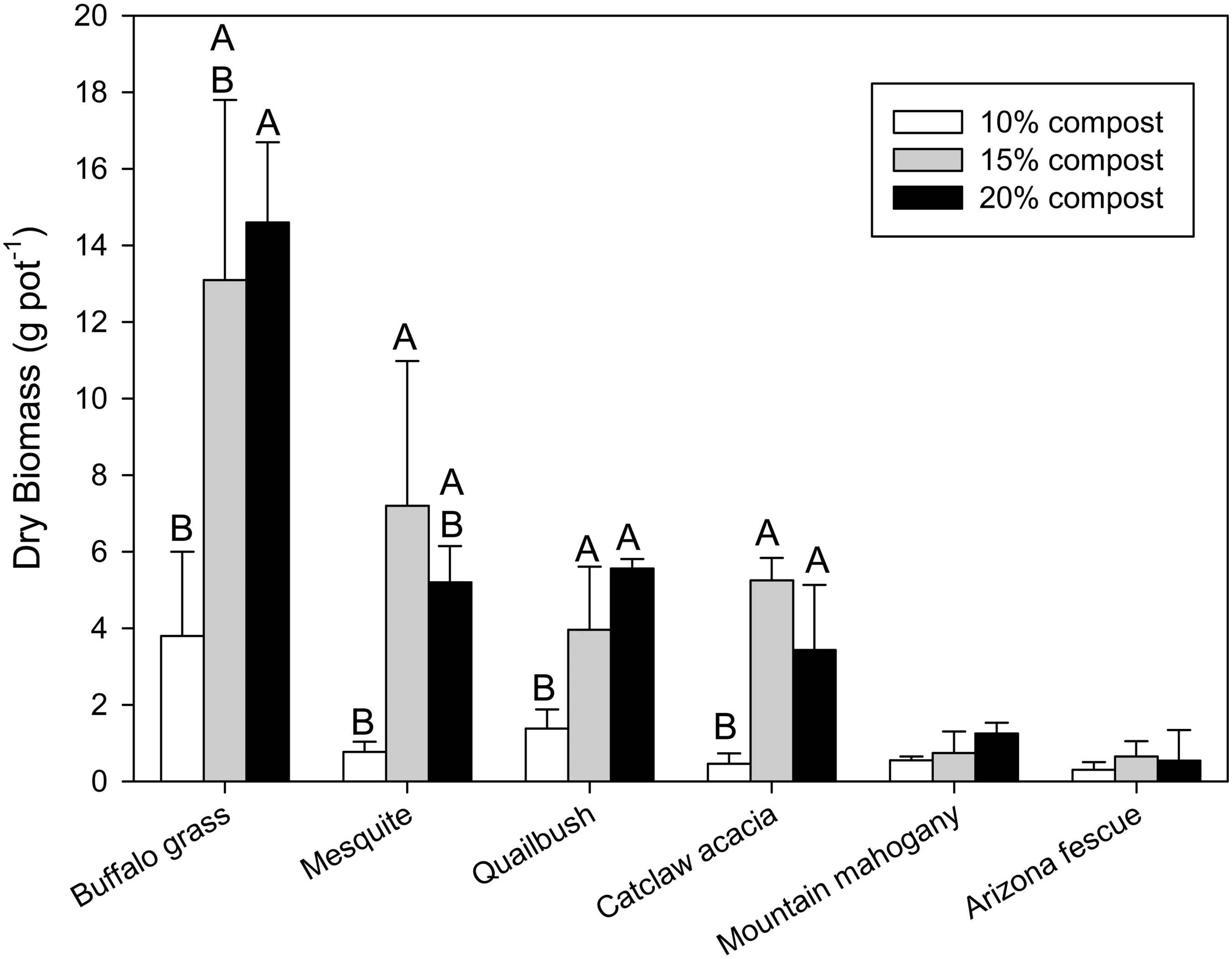

Fig. 1.

Total (root + shoot) dry biomass of desert plant species grown for 60 d in IKMHSS mine tailings amended with 10, 15, or 20% compost (mean + 1 SD). No plants survived in unamended tailings. A separate one-way ANOVA was performed for each plant species. There was no difference among the three compost treatments for mountain mahogany or Arizona fescue. For the other species, treatment means with different letters are significantly different at $p < 0.05$ (Tukey's HSD test; $n = 3$).

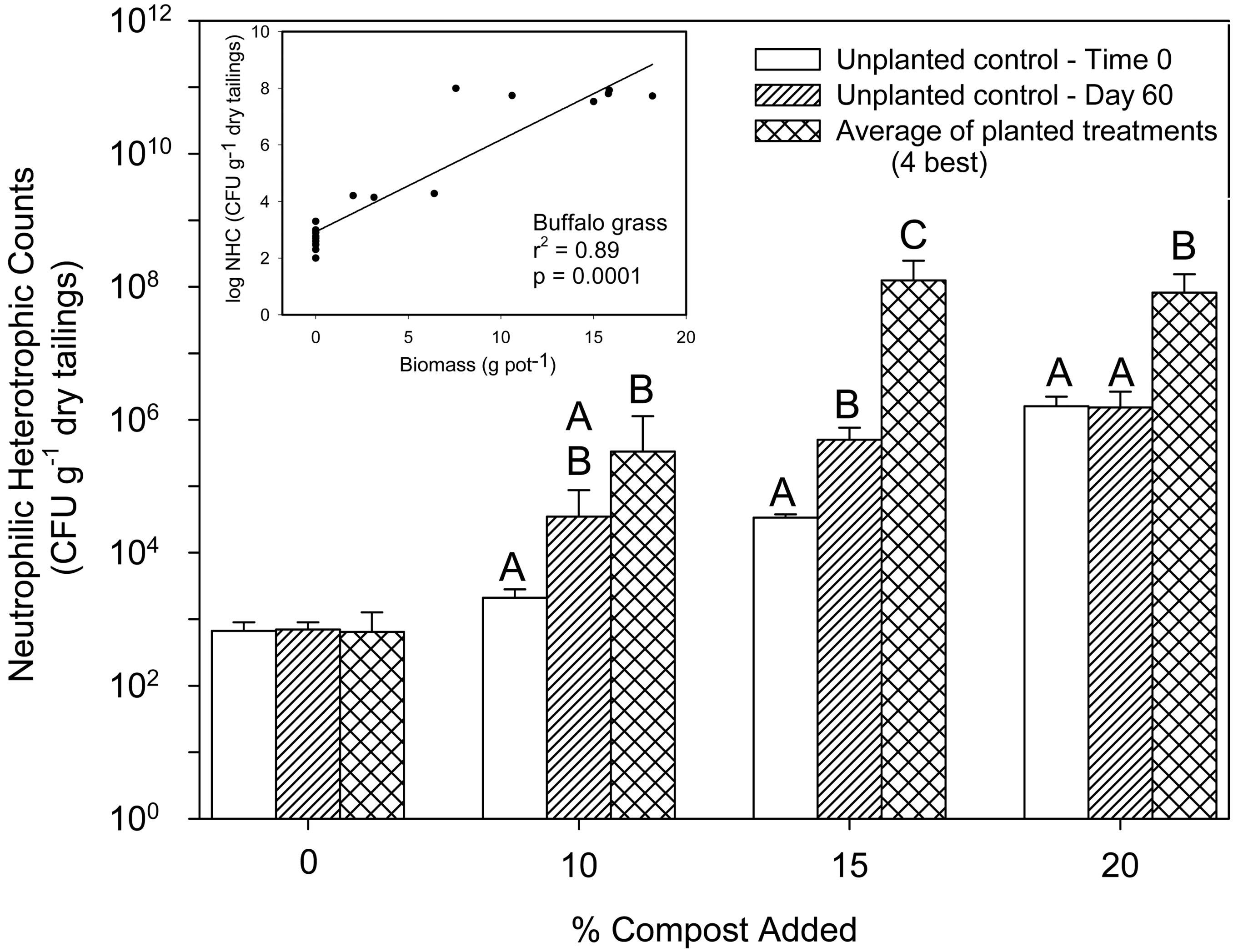

Fig. 2.

Initial (time 0), unplanted final (60 d), and planted final (60 d) NHCs in bulk IKMHSS tailings (mean + 1 SD). The planted final count represents the average of the four best-performing plant species: buffalo grass; mesquite; quailbush; and catclaw acacia. A separate one-way ANOVA was performed for each compost level. There was no difference among the three treatments in unamended tailings (0% compost). For 10, 15, and 20% compost amended tailings, treatment means with different letters are significantly different at $p < 0.05$ (Tukey's HSD test; $n = 3$). Inset: An example, using buffalo grass, of the relationship between NHCs and biomass. A regression analysis shows as significant relationship, $r^2 = 0.89$, $p = 0.0001$. Similar analyses were performed for mesquite, $r^2 = 0.78$, $p = 0.0001$; quailbush, $r^2 = 0.88$, $p = 0.0001$; and catclaw acacia, $r^2 = 0.77$, $p = 0.0001$).

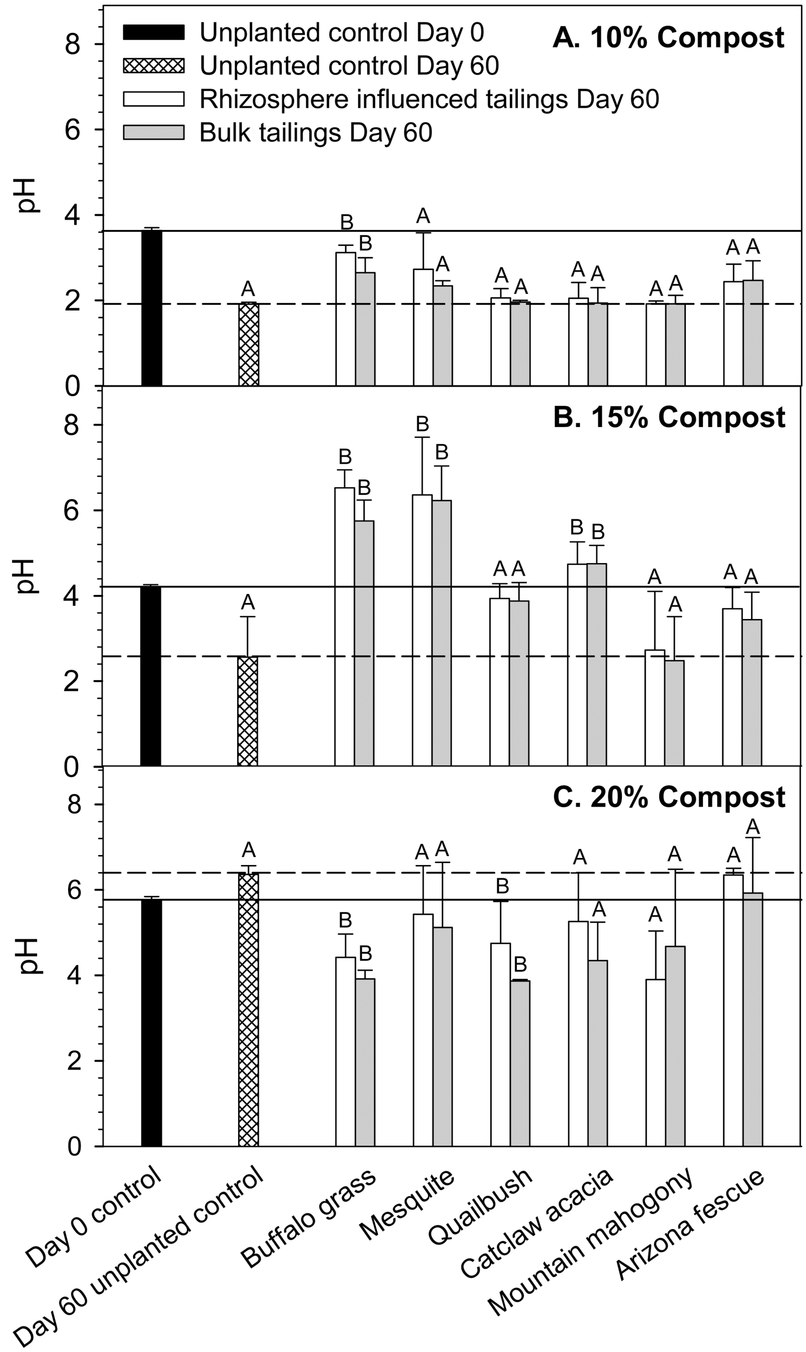

Fig. 3.

Effect of plant species on IKMHSS rhizosphere-influenced and bulk tailings pH after 60 d in (A) 10%, (B) 15%, and (C) 20% compost amended tailings (mean + 1 SD). The solid line is the pH of the bulk tailings immediately following compost amendment at time 0. The dashed line is the pH of bulk tailings in the unplanted control after 60 d. A separate one-way ANOVA was performed to compare the pH of bulk, rhizosphere-influenced, and the unplanted control 60 d samples for each plant species. Treatment means with different letters are significantly different at $p < 0.05$ (Tukey's HSD test; $n = 3$).

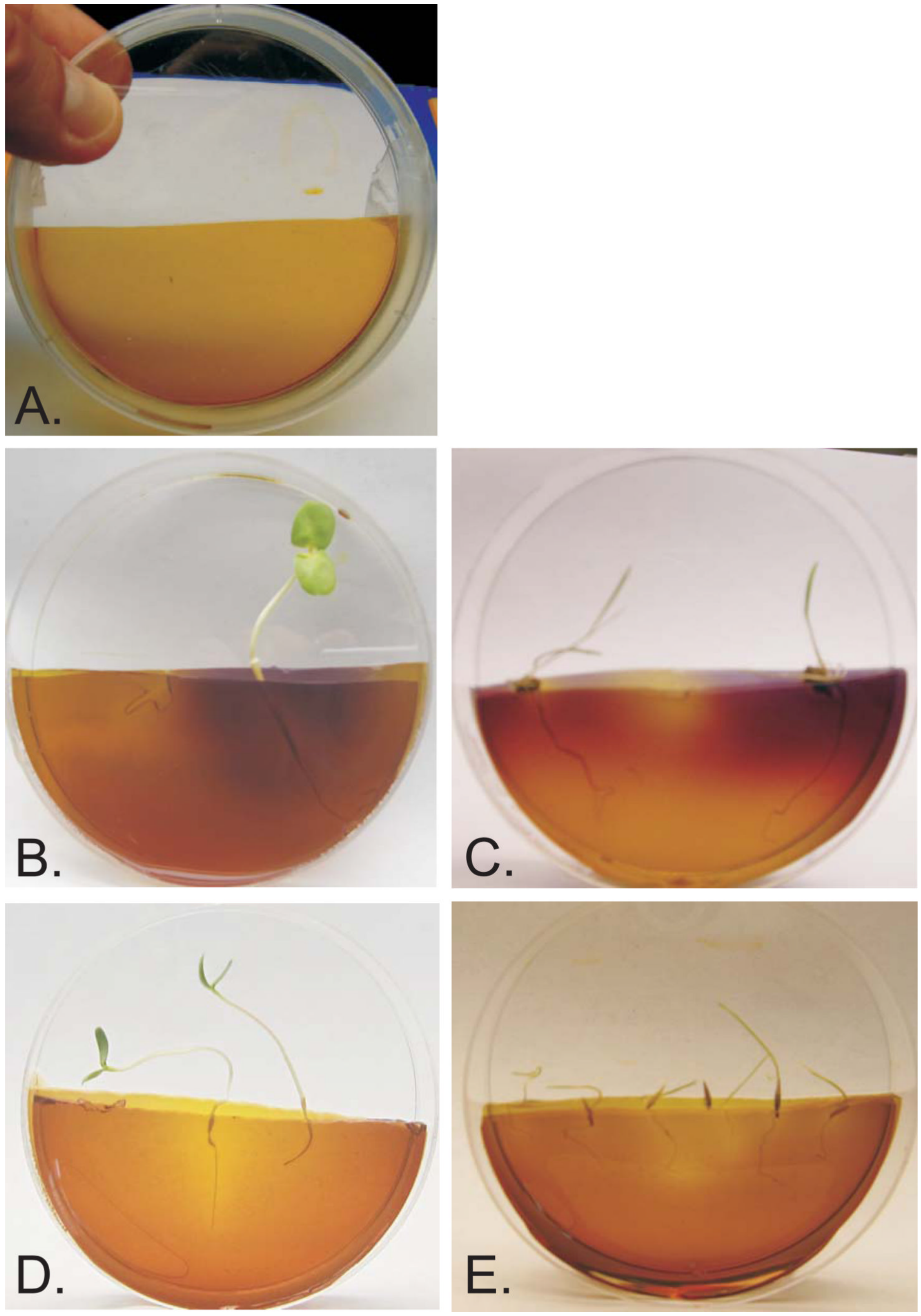

Fig. 4.

Effect of seedling roots on pH in agarose medium. Seedlings roots were tested sandwiched between agarose slabs amended with the pH indicator bromocresol purple. The slabs appear light yellow when the pH is ≤ 5.2, orange at pH 5.5, and purple at pH ≥ 6.8. (A) control plate at pH 5.5, (B) mesquite and (C) buffalo grass showing alkalization around the root, (D) mountain mahogany and (E) arizona fescue showing a lighter yellow color around the root indicating a more acidic pH.

Table 1

Characteristics of IKMHSS tailings immediately following compost amendment.

Tailings Treatment	pH	EC (ms cm^{-1})	NHC[1] CFU g^{-1}	Iron Oxidizers MPN g^{-1}
Unamended Tailings	2.5 ± 0.1	13.5 ± 0.04	$6.7 \pm 2.3 \times 10^2$	1.6×10^4
Compost alone	9.7 ± 0.1	28.7 ± 1.07	$3.0 \pm 0.5 \times 10^7$	n.d.[2]
Tailings + 10% compost	3.6 ± 0.1	17.6 ± 1.78	$2.1 \pm 0.70 \times 10^3$	9.0×10^3
Tailings + 15% compost	4.2 ± 0.1	19.5 ± 1.24	$3.4 \pm 0.40 \times 10^4$	2.7×10^3
Tailings + 20% compost	5.8 ± 0.1	21.4 ± 2.58	$1.6 \pm 0.64 \times 10^6$	5.3×10^2

[1]NHC = Neutrophilic heterotrophic bacteria

[2]n.d.= not determined

Table 2

Accumulation of metal(loid)s in plants shoot tissues after 60 d of growth in IKMHSS mine tailings.

Element	Total[1] mg kg^{-1}	DATL[2] mg kg^{-1}	Plant species	Accumulation of metal(loid)s in the shoot[3] (mg kg^{-1})		
				10% compost	15% compost	10 vs 15% compost (t-test)
As	2593	≤ 30	Buffalo grass	26.7 ± 6.51 a	26.7 ± 5.63 a	NS
			Mesquite	10.0 ± 7.98 b	3.97 ± 3.22 b	NS
			Quailbush	14.2 ± 5.62 ab	16.9 ± 5.77 b	NS
			Catclaw acacia	9.90 ± 3.84 b	7.37 ± 2.97 b	NS
Pb	2197	≤ 100	Buffalo grass	9.79 ± 6.13 a	8.96 ± 4.19 a	NS
			Mesquite	6.11 ± 5.85 a	1.00 ± 0.49 b	NS
			Quailbush	2.42 ± 1.96 a	3.89 ± 3.59 b	NS
			Catclaw acacia	3.84 ± 1.69 a	1.11 ± 0.15 b	NS
Zn	2003	≤ 500	Buffalo grass	369 ± 86.6 a	241 ± 22.5 b	NS
			Mesquite	476 ± 92.8 ab	326 ± 171 b	NS
			Quailbush	1033 ± 416 b	1092 ± 289 a	NS
			Catclaw acacia	642 ± 247 ab	269 ± 30.4 b	*
			Buffalo grass	1.33 ± 0.48 ab	0.69 ± 0.18 a b	NS
Cd	7.1	≤ 10	Mesquite	0.42 ± 0.08 b	0.32 ± 0.19 b	NS
			Quailbush	2.10 ± 0.21 a	2.96 ± 1.94 a	NS
			Catclaw acacia	1.21 ± 0.67 ab	0.35 ± 0.05 b	NS
			Buffalo grass	18.5 ± 10.0 a	14.2 ± 4.42 a	NS
Cu	127	≤ 40	Mesquite	12.4 ± 2.98 a	12.8 ± 4.38 a	NS
			Quailbush	14.5 ± 3.28 a	11.9 ± 5.17 a	NS
			Catclaw acacia	9.88 ± 1.97 a	7.14 ± 1.17 a	NS
			Buffalo grass	1.85 ± 1.23 a	0.73 ± 0.08 a	NS
Ni	9.67	≤ 100	Mesquite	1.22 ± 0.39 a	0.69 ± 0.32 a	NS
			Quailbush	2.36 ± 0.48 a	1.55 ± 0.38 a	NS

Element	Total[1] mg kg^{-1}	DATL[2] mg kg^{-1}	Plant species	Accumulation of metal(loid)s in the shoot[3] (mg kg^{-1})		
				10% compost	15% compost	10 vs 15% compost (t-test)
			Catclaw acacia	2.44 ± 1.48 a	2.01 ± 1.62 a	NS

[1] Total metal(loid) concentration in the Iron King tailings before planting. Values are average ± standard deviation (n = 3).

[2] DATL= domestic animal toxicity limit. Values listed are maximum tolerable levels for cattle (National Research Council, 2005).

[3] Values with different letters are significantly different at $p < 0.05$ (one-way ANOVA, Tukey's test) for each column corresponding to each element. t-test for each row (NS = no significant difference;

* = significant difference).

Table 3

Effect of compost rate and plant species on aqueous extractable metal(loid)s extracted from Iron King mine tailings after 60 d.

Plant species	----------Aqueous extractable concentration[1] ---------- (mg kg^{-1})			Analysis of variance[2]
	Unamended	10% Compost	15% Compost	
Unplanted control		15.4 ± 7.5	1.74 ± 1.3	- Compost rate: *
Buffalo grass	As	14.9 ± 2.0	1.52 ± 0.32	- Plant species: NS
Mesquite	139 ± 10.0	8.0 ± 5.6	1.39 ± 0.48	- Compost rate - plant
Quailbush		13.6 ± 2.5	0.775 ± 0.21	species combination: *
Catclaw acacia		18.4 ± 3.9	1.02 ± 0.32	
Unplanted control		0.005 ± 0.003	0.002 ± 0.0005	- Compost rate: *
Buffalo grass	Pb	0.004 ± 0.0006	0.022 ± 0.011	- Plant species: NS
Mesquite	0.060 ± 0.004	0.002 ± 0.0008	0.024 ± 0.017	- Compost rate - plant
Quailbush		0.003 ± 0.0008	0.006 ± 0.005	species combination: *
Catclaw acacia		0.001 ± 0.00009	0.009 ± 0.011	
Unplanted control		241± 92	43.0 ± 9.7	- Compost rate: *
Buffalo grass	Zn	244 ± 26	26.8 ± 7.2	- Plant species: *
Mesquite	1496 ± 71	357 ± 83	34.9 ± 21.8	- Compost rate - plant
Quailbush		189 ± 88	246 ± 34	species combination: *
Catclaw acacia		373 ± 89	128 ± 73	
Unplanted control		0.594± 0.23	0.075 ± 0.044	- Compost rate: *
Buffalo grass	Cd	0.987 ± 0.66	0.009 ± 0.015	- Plant species: NS
Mesquite	5.14 ± 0.22	1.18 ± 0.24	0.022 ± 0.033	- Compost rate - plant
Quailbush		0.668 ± 0.19	0.163 ± 0.047	species combination: *
Catclaw acacia		0.962 ± 0.21	0.066 ± 0.070	
Unplanted control		6.40 ± 1.69	0.995 ± 0.762	- Compost rate: *
Buffalo grass	Cu	5.04 ± 3.86	0.178 ± 0.102	- Plant species: NS
Mesquite	56.7 ± 1.57	4.67 ± 2.84	0.154 ± 0.077	- Compost rate - plant
Quailbush		5.05 ± 1.06	0.193 ± 0.103	species combination: *
Catclaw acacia		4.57 ± 3.15	0.219 ± 0.069	
Unplanted control		0.428 ± 0.215	0.085 ± 0.041	- Compost rate: *
Buffalo grass	Ni	0.649 ± 0.431	0.057 ± 0.008	- Plant species: NS
Mesquite	0.94 ± 0.052	0.728 ± 0.178	0.054 ± 0.014	- Compost rate - plant
Quailbush		0.442 ± 0.096	0.113 ± 0.004	species combination: *
Catclaw acacia		0.612 ± 0.125	0.068 ± 0.024	
Unplanted control		848 ± 398	116 ± 63	- Compost rate: *
Buffalo grass	Al	978 ± 521	1.1 ± 0.3	- Plant species: NS
Mesquite	2100 ± 167	1270 ± 254	1.2 ± 0.7	- Compost rate - plant

Plant species	----------Aqueous extractable concentration[1] ---------- (mg kg^{-1})			Analysis of variance[2]
	Unamended	**10% Compost**	**15% Compost**	
Quailbush		777 ± 192	21.6 ± 5.8	species combination: *
Catclaw acacia		1060 ± 149	6.1 ± 4.8	

[1]Values are means ± SD ($n = 3$).

[2]Statistical analysis was carried out using two-way ANOVA, Tukey's test, $\alpha = 0.05$.