Biochars Reduce Mine Land Soil Bioavailable Metals

J. A. Ippolito,* C. M. Berry, D. G. Strawn, J. M. Novak, J. Levine, and A. Harley

Abstract

Biochar has been proposed as an amendment to remediate mine land soils; however, it could be advantageous and novel if feedstocks local to mine land sites were used for biochar production. Two different feedstocks (pine beetle–killed lodgepole pine [*Pinus contorta*] and tamarisk [*Tamarix* spp.]), within close proximity to mine land–affected soils, were used to create biochars to determine if they have the potential to reduce metal bioavailability. Four different mine land soils, contaminated with various amounts of Cd, Cu, Pb, and Zn, received increasing amounts of biochar (0, 5, 10, and 15% by wt). Soil pH and metal bioavailability were determined, and the European Community Bureau of Reference (BCR) sequential extraction procedure was used to identify pools responsible for potential shifts in bioavailability. Increasing biochar application rates caused increases in soil pH (initial, 3.97; final, 7.49) and 55 to 100% (i.e., no longer detectable) decreases in metal bioavailability. The BCR procedure supported the association of Cd with carbonates, Cu and Zn with oxyhydroxides and carbonates, and Pb with oxyhydroxides; these phases were likely responsible for the reduction in heavy metal bioavailability. This study proved that both of these feedstocks local to abandoned mining operations could be used to create biochars and reduce heavy metal bioavailability in mine land soils.

Core Ideas

• Local feedstocks were used to create biochars used for local mine land reclamation.

• Novel creation and use of biochar were used to solve a localized issue.

• Biochar application caused bioavailable Cd, Cu, Zn, Pb reduction.

• Metal carbonate and oxyhydroxide precipitates formed after biochar application.

HEAVY METAL movement into soils and water bodies by former mining operations worldwide pose serious threats of public health, safety, and the environment. An estimated 500,000 abandoned mines exist in the United States alone (BLM, 2016). Within the western United States lies approximately 160,000 abandoned mining operations contaminating more than 40% of the area's headwater stream reaches (USEPA, 2000); approximately 33,000 of these mines cause contamination via acid generation (Mittal, 2011). This condition can increase heavy metal bioavailability in mine-affected soils and waters. Reducing heavy metal bioavailability is paramount for site reclamation.

Reducing mine-affected soil heavy metal bioavailability typically begins with lime addition. Lime increases soil pH, reducing metal bioavailability via sorption or precipitation reactions. Biochars may have a similar impact because they often are alkaline. Thus, in metal-containing acidic mine soils, the suitability of biochar for reducing metal bioavailability may hold some promise.

Previous research has shown that biochars can remove various metals from solution (Mohan et al., 2014). For example, Uchimiya et al. (2010) showed that broiler litter (*Gallus gallus domesticus*) biochar immobilized Cd, Cu, Ni, and Pb from solution via cation exchange, reaction with phosphate-based biochar ligands, some precipitation, and interaction with C=C π electrons. Similarly, Harvey et al. (2011) and Sánchez-Polo and Rivera-Utrilla (2002) suggested that biochars can sorb solution Cd via π bonding mechanisms. Moreover, several pig (*Sus domesticus*) and cow (*Bos taurus*) manure–based biochars were used by Kolodyńska et al. (2012) to successfully sorb Cd, Cu, Zn, and Pb. In that study, the maximum metal sorption occurred between pH 5 and 6. The authors suggested that maximum binding sites are present in this pH range, whereas at greater pH values sorption capacity decreases with concomitant precipitation as metal hydroxides, carbonates, or phosphates. Xu et al. (2013) used dairy manure biochar to sorb Cd, Cu, and Zn. Their results showed that metal sorption was mainly due to precipitation with

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carbonate or phosphate species, with less sorption due to surface complexation or π electron bonding. Kim et al. (2015) used soybean [*Glycine max* (L.) Merr.] stover and orange (*Citrus x sinensis*) peel biochars to remove aqueous Cd and Pb. The biochars sorbed 70 to near 100% (i.e., near limit of detection [LOD]) of the metals, with Cd found mainly in exchangeable and carbonate fractions, whereas Pb was associated with Fe-Mn oxides and residual fractions (i.e., strongly associated with mineral crystalline structures). Lu et al. (2012) used sewage sludge–derived biochar to sorb Pb over a pH range of 2 to 5. Across the pH range, the authors observed 38 to 42% Pb sorption associated with organic hydroxyl and carboxyl functional groups and 58 to 62% Pb sorption as coprecipitated or complexed on mineral surfaces. Tong et al. (2011) used peanut straw (*Arachis hypogaea* L.), soybean straw, and canola straw (*Brassica napus* L.) biochars to sorb Cu from solution. The authors found that greater negative surface charges on biochar led to greater Cu sorption via surface complexation. Ippolito et al. (2012) used X-ray absorption fine structure spectroscopy to study the effect of pecan [*Carya illinoinensis* (Wangenh.) K. Koch] shell biochar to sorb solution Cu. Results showed that under acidic conditions, Cu was complexed with organic ligands, whereas under neutral to alkaline pH conditions, Cu formed associations with organic ligands and precipitated as carbonate, oxide, and hydroxide mineral phases. The above results suggest that biochar has numerous binding mechanisms for metals and thus may be suitable for reducing soil metal bioavailability.

Several studies have applied biochars to metal-contaminated soils to study metal bioavailability. Beesley et al. (2010) mixed hardwood biochar into a soil (1:2 v/v) containing elevated Cu, Cd, and Zn concentrations. The authors showed that, as compared with controls, pore water Cu concentrations increased due to associations with dissolved organic carbon; pore water Cd and Zn decreased likely due to reduced metal solubility associated with a biochar-induced soil pH increase. Novak et al. (2009) observed a similar reduction in leachate Zn when a pecan shell–based biochar (0, 0.5, 1, 2% w/w) was added to a loamy sand. In a follow-up study to Beesley et al. (2010), Beesley and Marmiroli (2011) used scanning electron microscopy, noting that the mixed hardwood biochar sorbed soil leachate Cd and Zn as surface complexes. Park et al. (2011) added either chicken manure– or green waste–derived biochar to two different soils (5% w/w) contaminated with Cd and Pb or with Cu and observed decreases in Cd and Pb bioavailability with both biochars. Ahmad et al. (2016) mixed six different biochars into two different soils contaminated with Pb and Cu or with Pb and Zn (10% w/w). Biochars increased soil pH, and most biochars immobilized heavy metals via functional group complexation and metal-hydroxide precipitation. Zhu et al. (2015) noted an increase in soil pH and decreases in exchangeable Cd, Cr, Cu, Ni, Pb, and Zn when wine lees–based (material from a wine processing factory) biochar was added to a heavy metal–contaminated soil (0.5 and 1% w/w). The authors then used a European Community Bureau of Reference (BCR) (Nemati et al., 2009) sequential extraction procedure to identify metal speciation pools. Their results showed that exchangeable and Fe/Mn oxyhydroxide–associated metals decreased, whereas metals in the residual phase increased. This latter fraction represented metals associated with crystalline mineral structures, a relatively resistant and stable fraction.

Other studies have applied biochar to heavy metal–affected mine land soils, observing responses similar to those described above. Fellet et al. (2011) added increasing amounts of orchard prune residue biochar to mine tailings (0, 1, 5, and 10% w/w) contaminated with Cd, Pb, and Zn and observed metal bioavailability decreases with biochar application. Fellet et al. (2014) used the same mine tailings but amended it with either orchard prune residue, fir tree (*Abies* sp.) pellet, or manure pellet biochars (0, 1.5, and 3% w/w) and noted results similar to Fellet et al. (2011). Kelly et al. (2014) added increasing amounts of beetle-killed lodgepole pine biochar to two heavy metal–contaminated mine soils (0, 10, 20, and 30% w/w). Biochar reduced leachate Cd, Cu, Pb, and Zn from one mine soil but increased leachate Cd and Zn in the second mine soil. The authors attributed the increase in leachate Cd and Zn to a relative lack in pH change and greater Cd and Zn concentrations in the second mine soil as compared with the first. Trakal et al. (2011) added increasing amounts of willow (*Salix* sp.) biochar to a smelter-affected soil and noted enhanced Cu and Pb sorption but no change in Cd or Zn sorption. Houben and Sonnet (2015) added miscanthus straw (*Miscanthus sinensis* Andersson) biochar to a Cd-, Pb-, and Zn-bearing soil adjacent to a smelting facility. As with previous studies, the authors noted an increase in soil pH likely associated with the dissolution of biochar-borne oxides, hydroxides, and carbonate phases. Houben and Sonnet (2015) also fractionated the soil metal pools, showing that biochar reduced Cd and Zn in the exchangeable pool (similar to Zhu et al. [2015]) while increasing these metals in the carbonate pool.

The above studies illustrate biochar's utility for sorbing and thus reducing heavy metal bioavailability from waters and soils. However, with the exception of Kelly et al. (2014) and Trakal et al. (2011), who used beetle-killed lodgepole pine or willows as feedstocks, many of the above studies used feedstocks not inherent or within close proximity to mine land sites. It would be advantageous if a local feedstock were used for biochar production, thus providing an economic advantage (e.g., transportation cost reduction) because (large) tonnages of biochar are likely required for site application. Here, we propose the use of beetle-killed lodgepole pine as well as tamarisk as ideal biochar feedstocks for reclaiming the approximately 33,000 acid-generating mines in the western United States. In the western United States, the mountain pine beetle has killed sufficient lodgepole pine covering 7.1×10^6 ha (Hart et al., 2015), and tamarisk, an invasive woody species with characteristics similar to weedy plants (e.g., high seed productivity), has been found growing on approximately 4.0×10^5 to 6.5×10^5 ha (BLM, 2007). This makes these two feedstocks ideal biochar choices for potential western US mine land reclamation. We used four different western US mine land soils with the hypothesis that lodgepole pine and tamarisk biochar would reduce heavy metal bioavailability and shift metal pools to more resistant phases.

Materials and Methods
Biochar

Beetle-killed lodgepole pine and tamarisk were obtained from a site near Kremmling, CO, and in the Willow Creek floodplain below Creede, CO, respectively. Both materials were chipped before thermal conversion via pyrolysis. Biochar Solutions Inc.

converted raw wood product into biochar using a two-stage process. In the first stage, the feedstock was initially held between 500 and 700°C for <1 min under an O_2–limited environment. This process took place in a primary reactor with all heat for the process produced via an exothermic partial oxidation reaction. Temperatures were controlled by managing the ratio of available air to biomass and ensuring that it was below the combustion ratio. This management of air to biomass allowed for the preservation of solid carbon (i.e., biochar) while driving off nitrogen, oxygen, hydrogen, and other biomass constituent components. In the second stage, the material was pyrolyzed between 300 and 550°C for approximately 15 min in an anaerobic environment. The pyrolysis gas produced in the first stage of the process was used as sweep gas for the second stage and was primarily composed of N_2, H_2, CO, CH_4, volatile organic carbon phases, and trace gases; no oxygen was available during the second stage. After the approximately 15-min hold time, the biochar was removed from the pyrolyzer, cooled, and passed through a 0.25-mm sieve.

Both biochars were analyzed for pH and electrical conductivity using a saturated paste extract (Rhoades, 1996; Thomas, 1996) for total C and N via dry combustion (Nelson and Sommers, 1996), NO_3–N and NH_4–N using a 2 M KCl extract (Mulvaney, 1996), and organic N content (i.e., the difference between total and inorganic N). Biochar total metal concentrations were determined by using an $HClO_4$–HNO_3–HF-HCl digestion (Soltanpour et al., 1996) followed by elemental analysis using inductively coupled plasma–optical emission spectrometry (ICP–OES). Biochar characteristics are presented in Table 1.

Mine Land Soils

Four different soils were collected near historic abandoned mining sites in the western United States. Surface soil (0–15 cm) was collected in the Willow Creek floodplain below Creede, CO, an area contaminated with Cd, Pb, and Zn. One alluvial mine tailing soil was collected at two different depths (0–5 and 5–10 cm) near Leadville, CO, an area contaminated with Cd,

Cu, and Zn. Surface soil (0–15 cm) was collected in an area of the Coeur d'Alene River Basin in northern Idaho (Black Rock Slough), an area affected by mine and milling waste deposition contaminated with Cd, Cu, Pb, and Zn. All soils were air-dried and ground to pass a 2-mm sieve. Relative extractable metal concentrations were determined by weighing 3.00 g soil into a 50-mL centrifuge tube. Next, 30 mL of 0.01 M $CaCl_2$ (i.e., a measure of trace metal bioavailability [Pueyo et al., 2004]) was added to each tube. The tubes were shaken at 120 rpm for 2 h and centrifuged at ~500 × g, and then the samples were filtered through a 0.45-μm membrane filter. A drop of concentrated HNO_3 was added, and the solution was analyzed for Cd, Cu, Pb, and Zn via ICP–OES. Total soil metals were also determined using a 4 M HNO_3 digestion (Bradford et al., 1975). Data are presented in Table 2.

Experimental Design: Biochar Effects on Metal Bioavailability

Lodgepole pine or tamarisk biochar was weighed (0.00, 0.15, 0.30, or 0.45 g) into 50-mL centrifuge tubes. The different soils were then placed into the centrifuge tubes (3.00, 2.85, 2.70, and 2.55 g) to equal 0, 5, 10, or 15% biochar by weight. Next, 30 mL of 0.01 M $CaCl_2$ was added to each tube. The tubes were shaken at 120 rpm for 2 h and centrifuged (~500 × g), and solution pH was measured. The supernatant was then analyzed for bioavailable metals as described above. Final metal concentrations were adjusted based on the amount of soil per treatment. All treatments were run in triplicate.

Experimental Design: Biochar Effects on Soil Metal Fractionation

After the metal bioavailability measurement, the material in the centrifuge tubes was air-dried and homogenized. A 1.00-g sample of dried soil was placed in a 50-mL centrifuge tube and extracted using the four-step European Community Bureau of Reference (BCR) sequential extraction procedure according to Ure et al. (1993). Briefly, the four-step sequential extraction procedure operationally defines metal pools as (i) soluble, carbonates, exchange sites (40 mL 0.11 M acetic acid; 16 h shake time at 20°C); (ii) iron and manganese oxyhydroxides (40 mL 0.1 M hydroxylamine hydrochloride adjusted to pH 2 with concentrated nitric acid, made fresh the day of extraction; 16 h shake time at 20°C); (iii) organically bound and sulfides (10 mL of 8.8 M hydrogen peroxide and digested at 20°C for 1 h with lids on loosely and occasional manual shaking; digestion was then continued at 85°C in a water bath, with lids on loosely for 1 h; heated digestion continued with lids completely removed, and the tubes were gently shaken periodically until the volume was reduced to 3 mL; another 10-mL aliquot of 8.8 M hydrogen peroxide was added, and the tubes heated again at 85°C in a water bath, with lids on loosely for 1 h; heated digestion continued with lids completely removed, and the tubes were gently shaken periodically until the volume was reduced to 1 mL; 40 mL of 1 M ammonium acetate was added to the cooled residue, the tubes were recapped and shaken for 16 h at 20°C); and (iv) residual, or metals associated with crystalline mineral structures, a relatively resistant and stable fraction (soil/biochar mixtures were allowed to air dry 20°C in the centrifuge tubes; air-dried material was removed from the centrifuge tube and placed in digestion

Table 1. Lodgepole pine and tamarisk biochar characteristics.

Property	Lodgepole pine biochar	Tamarisk biochar
pH	9.1	10.4
EC,† dS m^{-1}	0.70	12.4
Total C, %	87.8	76.9
Total N, %	0.43	1.37
C/N ratio	204	56
Organic N, %	0.43	1.37
NH_4–N, mg kg^{-1}	2.8	6.5
NO_3–N, mg kg^{-1}	2.7	2.9
Total Ca, %	0.45	0.89
Total Mg, %	0.25	0.73
Total K, %	0.22	1.63
Total Na, %	<0.01	1.00
Total P, %	0.03	0.23
Total Al, %	0.04	0.02
Total Fe, %	0.06	0.03
Total Cd, mg kg^{-1}	0.07	0.10
Total Cu, mg kg^{-1}	6.0	27.2
Total Pb, mg kg^{-1}	<0.005	<0.005
Total Zn, mg kg^{-1}	35.9	58.3

† Electrical conductivity.

Table 2. Bioavailable and total Cd, Cu, Pb, and Zn concentrations in four mine land soils as determined by 0.01 M CaCl$_2$ extraction (Pueyo et al., 2004) and 4 M HNO$_3$ digestion (Bradford et al., 1975).

Soil	Bioavailable metals			
	Cd	Cu	Pb	Zn
	———————————————— mg kg^{-1} ————————————————			
Creede, CO	6.38 (0.29)†	0.12 (0.00)	8.47 (0.32)	541 (24.0)
Leadville, CO (0–5 cm)	27.4 (3.98)	3.21 (0.45)	0.00 (0.00)	2,410 (303)
Leadville, CO (5–10 cm)	9.72 (0.26)	2.73 (0.19)	0.09 (0.15)	887 (36.5)
Black Rock Slough soil	2.29 (0.13)	0.78 (0.06)	32.0 (1.01)	146 (3.51)
	Total metals			
Creede, CO	32.9 (3.29)	102 (11.9)	4,370 (196)	5,080 (975)
Leadville, CO (0–5 cm)	103 (1.56)	1,230 (24.9)	4,840 (25.3)	18,700 (449)
Leadville, CO (5–10 cm)	40.8 (9.28)	1,430 (279)	4,490 (764)	7,900 (1,850)
Black Rock Slough soil	12.8 (0.06)	96.2 (0.37)	5,530 (19.7)	1,120 (16.9)

† Values are average ± SD (*n* = 3).

tubes, with weights recorded; 1 mL of deionized water was added to create a slurry; 7 mL of concentrated hydrochloric acid and 2 mL of concentrated nitric acid (i.e., aqua regia) were added, and the mixtures were allowed to predigest overnight; the next day the digestion tubes were placed in a block digester at 105°C for 2 h; the tubes were removed, cooled, and brought to a 50-mL final volume and filtered through a 0.45-μm membrane). After each sequential extraction shaking period, tubes were centrifuged, and the liquid was decanted and filtered through a 0.45-μm membrane and analyzed for Cd, Cu, and Pb concentration using ICP–OES. All treatments were run in triplicate, and metal concentrations were adjusted based on the amount of soil per treatment.

All data were analyzed separately for each soil using PROC GLM in SAS version 9.4 (SAS Institute, 2013) at α = 0.05. When significance was observed, Fisher's protected least significant difference (Steel and Torrie, 1980) was used to indicate differences between treatment means.

Results and Discussion
Soil pH

Increasing lodgepole pine biochar application rate caused a significant pH increase in all four soils; tamarisk biochar acted similarly (Table 3). Both biochars were alkaline (lodgepole biochar, pH 9.1; tamarisk biochar, pH 10.4) and likely contained carbonates as well as –COO$^-$ and –O$^-$ functional groups (Yuan et al., 2011) that contributed to acidity reduction. Other researchers have observed similar results (Kelly et al., 2014; Zhu et al., 2015).

Metal Bioavailability

Increasing lodgepole pine and tamarisk biochar application rates caused significant reductions in CaCl$_2$–extractable Cd, Cu, Pb, and Zn in all four mine land soils studied (Fig. 1 and 2). This suggests decreased bioavailability for uptake by plants, as observed by Zhu et al. (2015). Cadmium, Cu, Pb, and Zn were reduced by 55 to 100% (LOD), 91 to 100% (LOD), 84 to 100% (LOD), and 60 to 100% (LOD), respectively. Others have shown similar results. Novak et al. (2009), Laird et al. (2010), and Beesley and Marmiroli (2011) noticed decreased Zn, Cu and Zn, and Cd and Zn, respectively, in leachate (i.e., potentially bioavailable) after biochar application to soil. Park et al. (2011) and Fellet et al. (2011, 2014) observed reductions in extractable and bioavailable Cd, Cu, Pb, and Zn when biochar was added to metal-laden soils. Yuan et al. (2011) suggested that reduction in metal bioavailability was due to the formation of metal-carbonate species and reactions with surface functional groups, which may be a sequestration mechanism. Formation of metal-oxyhydroxide species has also been shown to be a mechanism responsible for decreased metal bioavailability (Ippolito et al., 2012). To gain insight into potential phase(s) responsible for the reductions in heavy metal bioavailability, the BCR heavy metal fractionation procedure was used.

Metal Fractionation

Sequential extraction procedures, such as BCR, have been used to operationally define soil metal pools in various settings (Guerra et al., 2007; Ippolito et al., 2009; Nemati et al., 2009),

Table 3. The effect of increasing lodgepole pine (pH 9.1) or tamarisk (pH 10.4) biochar rate on pH of four mine land soils.

Biochar and application rate	Creede, CO, soil (0–15 cm)	Leadville, CO, soil (0–5 cm)	Leadville, CO, soil (5–10 cm)	Black Rock Slough, ID, soil (0–15 cm)
Lodgepole pine biochar				
0%	5.44 (0.04)c†	5.33 (0.06)c	5.12 (0.04)c	3.97 (0.01)c
5%	7.09 (0.03)a	5.86 (0.03)b	5.91 (0.02)b	5.20 (0.06)b
10%	6.36 (0.00)b	6.10 (0.01)a	6.21 (0.01)a	6.40 (0.03)a
15%	6.39 (0.00)b	6.22 (0.05)a	6.26 (0.01)a	6.33 (0.12)a
Tamarisk biochar				
0%	5.44 (0.04)d	5.33 (0.06)d	5.12 (0.04)d	3.97 (0.01)d
5%	6.27 (0.00)c	6.01 (0.04)c	6.03 (0.02)c	5.82 (0.06)c
10%	7.05 (0.03)b	6.54 (0.01)b	6.71 (0.04)b	7.13 (0.01)b
15%	7.17 (0.03)a	6.72 (0.04)a	6.90 (0.01)a	7.49 (0.01)a

† Values are means ± SD (*n* = 3). For an individual biochar and soil, different letters following means denote significant differences at α = 0.05.
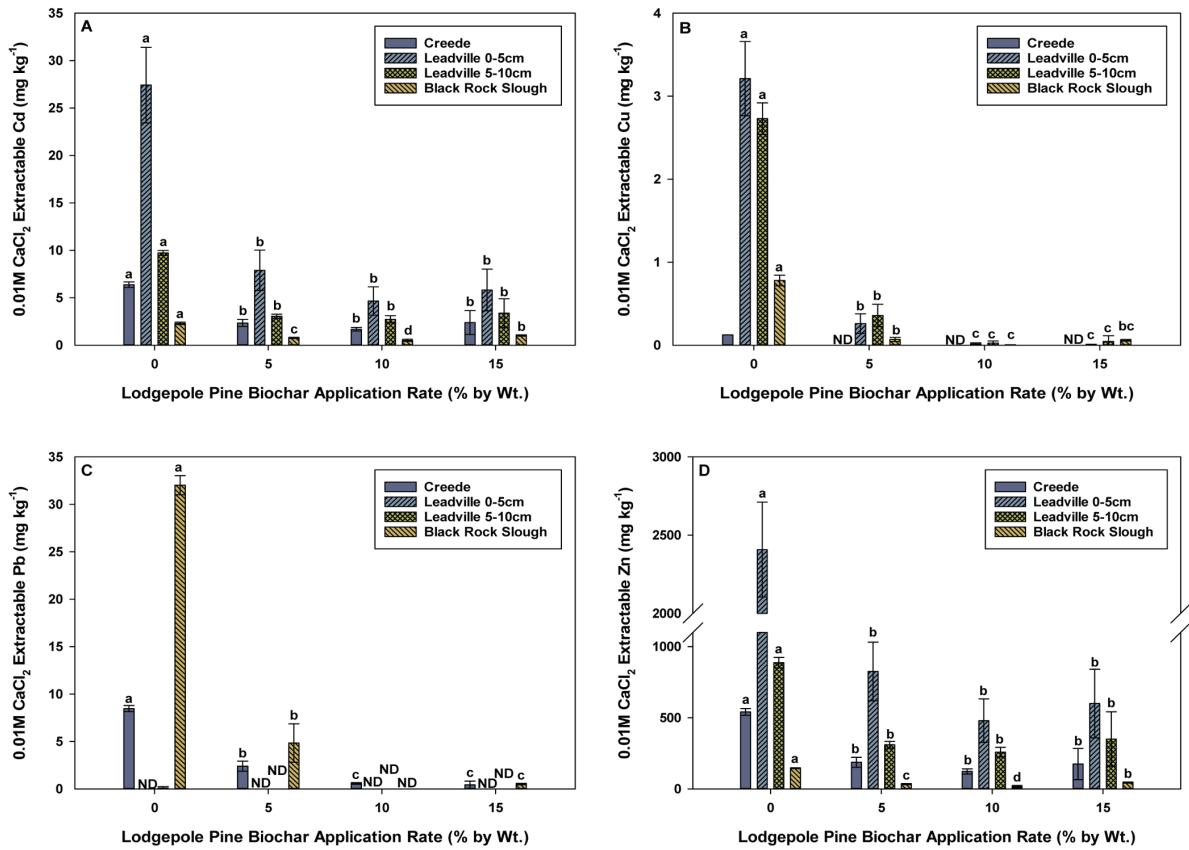
Fig. 1. The effect of increasing lodgepole pine biochar rate on bioavailable Cd (A), Cu (B), Pb (C), and Zn (D) in four mine land soils. Different letters above bars, for individual soils, denote significant differences at $\alpha = 0.05$. Values are means ($n = 3$) $\pm$ SD. ND, nondetectable.
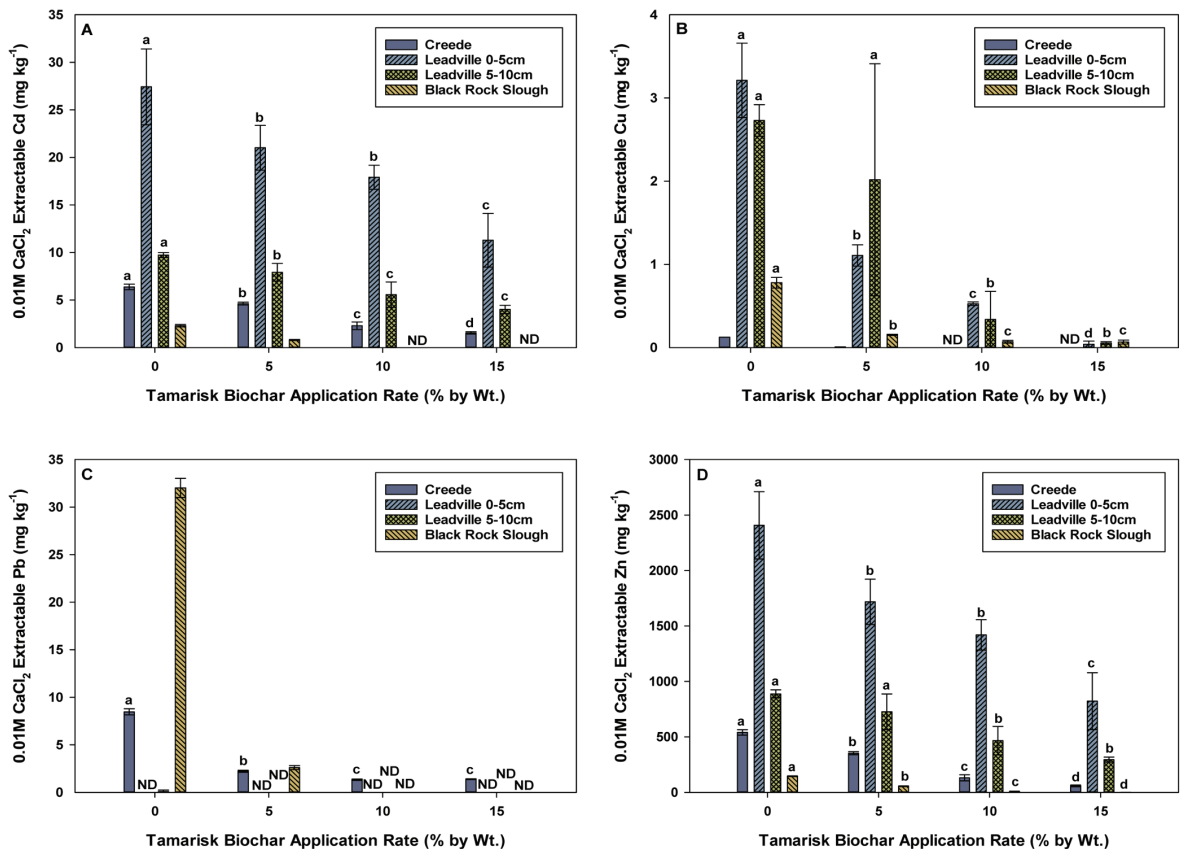

Fig. 2. The effect of increasing tamarisk biochar rate on bioavailable Cd (A), Cu (B), Pb (C), and Zn (D) in four mine land soils. Different letters above bars, for individual soils, denote significant differences at $\alpha = 0.05$. Values are means ($n = 3$) $\pm$ SD. ND, nondetectable.

including under biochar application (Houben and Sonnet, 2015; Kim et al., 2015; Zhu et al., 2015). As stated by Houben and Sonnet (2015), sequential extraction procedures suffer from a lack of selectivity and element redistribution during extraction, yet their use is justified when the goal is to compare differences in a soil as affected by different treatments, as in the current study.

The chemical fractionation results of mine land soils as affected by increasing lodgepole pine or tamarisk biochar are shown in Fig. 3 and 4, respectively. The main fraction of Cd in all soils was the soluble/exchangeable/carbonate phase (Fig. 3A and 4A); the biochar application rate tended to increase Cd in this phase. A similar result was found by Zhu et al. (2015). It was unlikely that Cd increased in the soluble portion of the first sequential extraction step because an increase in the bioavailable Cd would have been evident (Fig. 1A and 2A). Supporting this notion, Beesley et al. (2010) showed that biochar reduced the water-soluble Cd concentration in a metal-contaminated soil. It is impossible to separate the exchangeable and carbonate pools within the first step of the BCR sequential extraction procedure. However, Houben and Sonnet (2015) used a five-step sequential extraction procedure (i.e., the Tessier scheme [Tessier et al., 1979]) after miscanthus straw biochar was added to a metal-bearing soil. The authors showed that biochar reduced Cd in the exchangeable pool and increased Cd in the carbonate pool. Park et al. (2011) showed that biochar increased soil pH, suggesting that this promoted the precipitation of $Cd(CO_3)$. Yuan et al. (2011) found that carbonates were the major alkaline component in biochars generated at temperatures similar to those used in the current study. Thus, in the current study, if Cd was associated with the carbonate pool, this would likely have been the result of biochar increasing soil pH and promoting $CdCO_3$ precipitation, which could explain the decrease in Cd bioavailability. Maintaining system pH may help prevent dissolution and release of Cd back into the soil solution.

The main fraction of Cu found in all soils was the residual phase (Fig. 3B and 4B), similar to the findings of Zhu et al. (2015). Increasing biochar amendment rates did not show any trends in the residual extractable Cu concentrations. Increasing the biochar application rate significantly decreased Cu bound in the oxidizable fraction in several soils, opposite of the findings by Park et al. (2011). However, increasing the biochar application rate increased Cu in the Fe/Mn oxyhydroxide phase and in several instances in the soluble/exchangeable/carbonate pool when significance was present. The Fe/Mn oxyhydroxide phase represents metals that could be released under reduced conditions, and thus it would be important to avoid such conditions during and after site reclamation. Copper in the soluble/exchangeable/carbonate and Fe/Mn oxyhydroxide pools are likely associated with carbonates, oxides, and hydroxides, as shown by others (Ippolito et al., 2012; Park et al., 2011; Uchimiya et al., 2011). The oxyhydroxide and carbonate phases could explain the decrease in Cu bioavailability with increasing biochar application rate.

The Creede and Black Rock Slough soils contained elevated bioavailable Pb concentrations as compared with the Leadville soils (Fig. 1C and 2C) and thus are the focus of the BCR sequential extraction for Pb. Within the Creede soil, the main Pb fraction was the Fe/Mn oxyhydroxide, whereas in the Black Rock Slough soil, both the Fe/Mn oxyhydroxide and residual fractions dominated (Fig. 3C and 4C). Lead

bioavailability decreased in the Creede soil, yet no clear trends in the sequential extraction procedure could explain the decrease. In the Black Rock Slough soil, biochar applied at 5% (by wt) decreased Pb extractability in the residual phase. In contrast, Zhu et al. (2015) and Park et al. (2011) showed that Pb in the residual phase increased with increasing biochar application rate, similar to that observed when tamarisk biochar was applied at 15% by wt. Also in the Black Rock Slough soil, increasing lodgepole pine biochar application rate caused Pb to increase in the Fe/Mn oxyhydroxide phase, which may explain the decrease in Pb bioavailability in this soil. In support of this contention, Cao et al. (2009) showed that a dairy manure biochar caused Pb to precipitate as a mixed carbonate-hydroxide phase. Lead has also been shown to have an affinity for certain oxides found in wood charcoal (Machida et al., 2005), and Ahmad et al. (2016) showed that biochar caused Pb to form a metal hydroxide phase due to a biochar induced pH increase.

Most of the extractable Zn occurred in the soluble/exchangeable/carbonate and residual fractions (Fig. 3D and 4D). Increasing biochar application rate tended to decrease Zn in the residual fraction and increase Zn in the soluble/exchangeable/carbonate fraction. In the two Leadville soils, concentrations of Zn in the Fe/Mn oxyhydroxide fraction increased. The oxyhydroxide and soluble/exchangeable/carbonate phases were likely responsible for the decrease in bioavailable Zn. Ahmad et al. (2016) showed that biochar promoted Zn to form metal-hydroxide precipitates due to induced pH increases. As with Cd, if the Zn increases in the soluble/exchangeable/carbonate fraction had been due to soluble Zn, an increase in the bioavailable Zn would have been evident (Fig. 1D and 2D). Beesley et al. (2010) showed that water-soluble Zn decreased when biochar was applied to a metal-contaminated soil. Houben and Sonnet (2015) noted Zn decreases in the exchangeable phase and increases in the carbonate phase, similar to their Cd findings. The shift between exchangeable and carbonate phases was attributed to the increase in soil pH promoting metal precipitation (as shown by Ahmad et al. [2016]), which could have occurred in the mine soils used in the current study. Houben and Sonnet (2015) observed a significant relationship between Zn in the Fe/Mn oxide phase and an increase in soil pH, which is similar to the findings observed in this study. Houben and Sonnet (2015) emphasized that metals associated with oxides can be released under acidic conditions, and thus the need in mine land reclamation to properly account for acid generation and maintain a desired soil pH.

Comparison between Biochars and Rates

Although both biochars acted similarly throughout the study, tamarisk biochar increased soil pH to a greater extent as compared with lodgepole pine biochar across the biochar application range studied (Table 3). Yet, when bioavailable metal concentration differences were present, lodgepole pine biochar reduced metal bioavailability to a greater extent than tamarisk biochar (data not shown). Thus, lodgepole pine biochar would be the suggested biochar for these four mine land soils. Furthermore, based on the data presented in Fig. 2, an application rate between
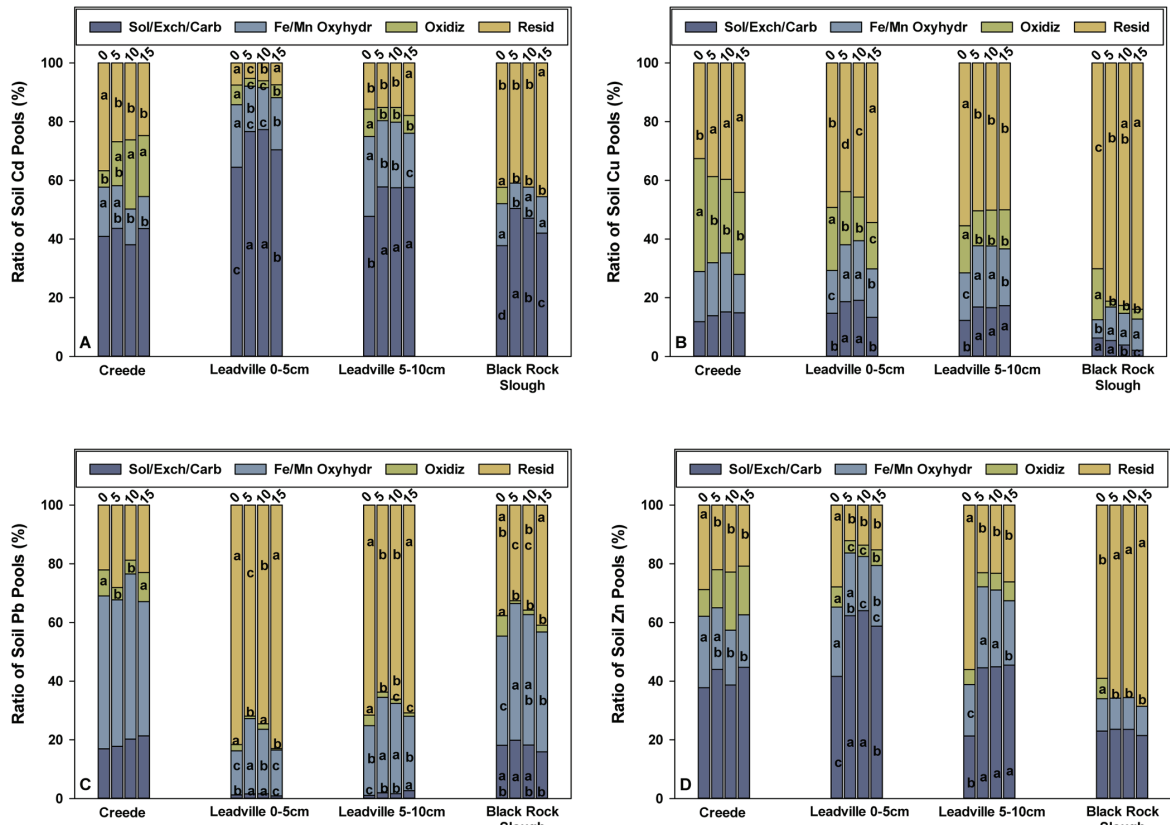
Fig. 3. The effect of increasing lodgepole pine biochar rate (0, 5, 10, or 15% by wt.) on the ratio of soluble/exchangeable/carbonate, Fe/Mn oxyhydroxide, oxidizable, and residual fractions of Cd (A), Cu (B), Pb (C), and Zn (D) in four mine land soils. Different letters within a particular fraction for an individual soil denote significant differences at $\alpha = 0.05$. No letters present denotes nonsignificance.
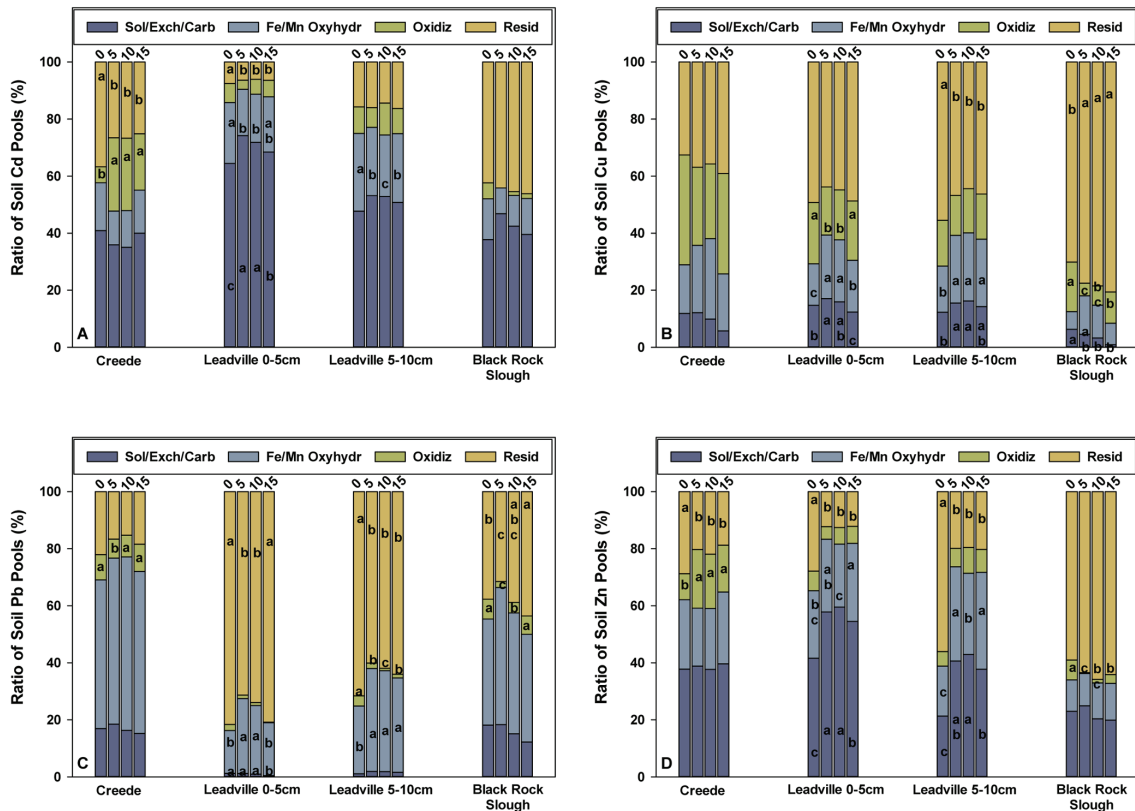

Fig. 4. The effect of increasing tamarisk biochar rate (0, 5, 10, or 15% by wt.) on the ratio of soluble/exchangeable/carbonate, Fe/Mn oxyhydroxide, oxidizable, and residual fractions of Cd (A), Cu (B), Pb (C), and Zn (D) in four mine land soils. Different letters within a particular fraction for an individual soil denote significant differences at $\alpha = 0.05$. No letters present denotes nonsignificance.

5 and 10% would be suggested as the target application rate to reduce metal bioavailability with the least amount of biochar.

Conclusions

Mining operations worldwide have the potential to cause environmental contamination by generating acidity. This acidity can subsequently increase heavy metal bioavailability in mine-affected soils. Reducing heavy metal bioavailability via increasing soil pH and sequestering heavy metals is necessary for site reclamation success. A laboratory study was conducted to prove that feedstocks (pine beetle–killed lodgepole pine or tamarisk) within close proximity to four mine-affected soils could be successfully used to create biochars that reduce heavy metal bioavailability. Both biochars were able to increase soil pH and subsequently reduce Cd, Cu, Pb, and Zn bioavailability. A BCR sequential extraction procedure was then used to operationally define soil phase(s) responsible for the reduction in heavy metal bioavailability. The BCR procedure showed that Cd was associated with carbonates, Cu and Zn with oxyhydroxides and carbonates, and Pb with oxyhydroxides. The stability of these "remediated" phases in the environment for reduced bioavailability requires maintaining elevated pH and accounting for possible future acid generation under reclamation conditions. These findings support the utility of local feedstocks for biochar creation and use for reducing heavy metal bioavailability in nearby metal-contaminated mine land soils.

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