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Effects of biochar and *Arbuscular mycorrhizae* on bioavailability of potentially toxic elements in an aged contaminated soil

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Biochar pyrolyzed from corn stalks at 300 °C/500 °C and arbuscular mycorrhizae (AMF) were examined independently and in combination as possible treatments for soil remediation contaminated with Cd, Cr, Ni, Cu, Pb, Zn after 35 years following land application of sewage sludge in the 1970s. The results showed that biochar significantly decreased the heavy metal concentrations and their bioavailability for plants, and both biochars had similar such effects. AMF inoculation of corn plants had little effect on heavy metal bioavailability in either control or biochar amended soil, and no interaction between biochar and AMF was observed. Changes in DTPA extractable metals following biochar addition to soil were correlated with metal uptake by plants, whereas pore water metal concentrations were not predictive indicators. This research demonstrates positive benefits from biochar application for contaminated soil remediation, but remain ambiguous with regard to the benefits of simultaneous AMF inoculation on reduction of heavy metal bioavailability.

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1. Introduction

Contamination of agricultural soils by potentially toxic elements (PTEs) is a common problem on soils that have received applications of bio-solids, that are co-mixed with waste materials containing heavy metals. While regulations for land disposal of composted sewage sludge now limit the total amounts of PTEs to levels that have been deemed safe, the application of these materials to land prior to the implementation of safer practices in past decades has left a legacy problem of soil contamination (Epstein, 2003; Koo et al., 2013; Torri et al., 2014). Various methods for remediation of contaminated soils have been developed, including land excavation, soil washing, phytoremediation, and stabilization to reduce PTE bioavailability (Kiikkila et al., 2001; Perez De Mora et al., 2005; Fellet et al., 2011; Beesley et al., 2014). Nonetheless, effective low cost practices are very much needed to solve this problem. One such method that is receiving interest is the use of biochars that can adsorb PTEs and decrease their bioavailability to plants and prevent uptake and food chain transfer (Puga et al., 2015). Interest in the potential of biochars for remediation of contaminated soils is also gaining momentum with the advocacy for incorporation of biochars into soils as a means to improve soil quality and fertility.

Biochar is produced by thermally degrading (charring or pyrolyzing) a biomass-derived feedstock under oxygen-limited conditions. When incorporated into agriculture soil, biochar can improve soil fertility and may also be an option for enhancing soil C stocks and mitigating greenhouse gas emissions from world cropland (Lehmann et al., 2006; Lehmann, 2007; Roberts et al., 2010). Biochar has a large surface area with various functional groups that may bind cations, and is usually alkaline, thus decreasing metal solubility (Lehmann et al., 2006; Hossain et al., 2010), which would reduce metal bioavailability in soils.

Experiments using biochar as a soil amendment in contaminated soils have so far been encouraging, but as with much research on heavy metals have mainly investigated changes in bioavailability in short term studies with metal spiked soils. Beesley and Marmiroli (2011) observed biochar can rapidly reduce the mobility of selected contaminants in this polluted soil system, with especially encouraging results for Cd. Likewise, Namgay et al. (2010) found significantly decreased availability of Cd and Pb with biochar application in a pot experiment. Beesley et al. (2014) studied the influence of organic wastes on trace element mobility and toxicity in amended contaminated soils and showed that

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biochar reduced free metal concentrations furthest, but dissolved organic carbon primarily controlled metal mobility after amendment with compost. Bian et al. (2013) used a cross-site field experiment to study biochar soil amendment as a solution to prevent Cd-tainted rice from China which showed that Biochar treatment at 20–40 t ha^{-1} reduced rice Cd by 20%–90% from metal polluted rice fields and Biochar amendment caused Cd immobilization primarily due to the liming effect by biochar. Altogether, the positive results associated with addition of biochar to agricultural land suggest that biochar amendments may be used to reduce heavy metal availability for restoration of contaminated soils.

Arbuscular mycorrhizal fungi (AMF) are thought to be one of the most important soil microbial groups that affect metal uptake by plants and metal immobilization in soils (Piotrowski and Rillig, 2008) and are commonly introduced into soil for land reclamation (Renker et al., 2004). AMF form symbioses with approximately 2/3 of plant species and provide their hosts with benefits including increased access to immobile nutrients, especially phosphorus, improved water relations, and greater pathogen resistance (Newsham et al., 1995; Smith and Read, 2008). There is also evidence that AMF are able to increase plant tolerance to PTEs. This occurs through binding of metals to the fungal hyphae (Gonzalez-Chavez et al., 2002) and complexation of metals by glomalin (Gonzalez-Chavez et al., 2004), which is a glycoprotein produced by all AMF that have been tested to date (Wright et al., 1996, Wright and Upadhyaya, 1998; Nichols, 2003). Glomalin-bound copper in a soil contaminated by copper smelting operations has been measured at concentrations from 3.7 to 89 mg Cu per gram of soil (Cornejo et al., 2008). AMF hyphal and glomalin production should therefore be taken into account when phytostabilization technologies are used in polluted soils. This ability of AMF to sequester and accumulate PTEs in a non-toxic form may help to increase plant fitness and soil quality in polluted areas.

Soil amendments that increase AMF abundance could be beneficial to plant hosts and result in improved soil quality via influences on soil structure and heavy metal immobilization (Rillig et al., 2006). Specifically, the effect of AMF inoculation on Cd speciation was a decrease in the inorganic bound Cd fraction with concomitant increases in the residual Cd fraction (Aghababaei et al., 2014). Recent studies indicate that soil biochar amendments can increase AMF percent root colonization on plants in acidic soils (Ezawa et al., 2002; Matsubara et al., 2002; Yamato et al., 2006). It can thus be hypothesized that biochar may indirectly increase glomalin production by increasing AMF colonization. Metal glomalin complexes could further be stabilized by sequestration in the biochar matrix thereby reducing bioavailability and uptake of PTEs into plants. The objectives of this research were to examine the individual and interactive effects of biochar and AMF on bioavailability and uptake of PTEs from an aged heavy-metal contaminated soil with a prior history of annual sewage sludge applications and use as an agricultural soil. Experiments were conducted to examine specific mechanisms of metal retention by biochar, glomalin production by AMF and potential synergisms for contaminant immobilization over time.

2. Materials and methods

2.1. Soil characteristics

The soil used in this experiment came from a long-term bio-solids land application experiment site located at the Moreno Field Station of the University of California, Riverside, California. At this field site, windrow-composted bio-solids from the Metropolitan Water Reclamation District of Greater Chicago (designated as MWRD here after) was applied at a dry weight equivalent rate of 45 ton ha^{-1} yr^{-1} to a sandy loam soil for five years from 1978 to 1982. During and after the termination of the bio-solids applications, the experimental plots were continuously cropped annually with a variety of field crops until 1990, after which the field was fallowed. A composite soil sample was taken by collected of soil from random locations within field site that were taken from the top 20 cm of the soil profile using a steel shovel. The soil was then transported to the laboratory where it was air-dried and passed through a 2-mm sieve to homogenize and uniformly mix the soil prior to its use for the experiment.

Corn stalks used as the feedstock for biochar were obtained from a farm at Live Oak Canyon Rd. Redlands, California, The corn stalks were washed with water, dried in open air under shade for 1 week, and then oven dried at 80 °C for 2 d. The feedstock (120 g) was placed in a stainless steel cylinder fitted with an internal steel pipe connected to a manifold that delivered N_2 gas during pyrolysis to maintain anaerobic conditions while allowing venting of biogas. The feedstock was pyrolyzed for 2 h in a muffle furnace at 300 °C or at 500 °C, respectively, to produce two biochar products. Approximately, 30–40% of the dried corn stalk biomass was converted to BC after pyrolysis. The charred residues were ground to pass through a 2 mm sieve, and stored in plastic bags under room temperature for three months until use. Chemical characteristics of the soil and biochar used in the experiment are listed in Table 1.

2.2. Plant growth and metal uptake

A growth chamber experiment was conducted to study the combined effect of biochar and AMF on heavy metal uptake by corn plants grown on the bio-solids treated sandy soil. Using a fully factorial complete design, six treatments were compared (2 AMF × 3 biochar): M0B0 (no AMF/no biochar control); M1B0 (AMF inoculated seedlings in soil without biochar); M0B3 (uninoculated seedlings with biochar 300 °C); M1B3 (AMF inoculated seedlings with biochar 300 °C amended soil), M0B5 (uninoculated seedlings with biochar 500 °C); M1B5 (AMF inoculated seedlings with biochar 500 °C amended soil).

All of the soils were autoclaved before addition of biochar and inoculation with AMF. Plants were grown in plastic cones (3.8 cm × 21 cm, SC-10 Super, Cone-tainer), and were arranged randomly with 6 replicates. Each 164 mL cone contained approximately 200 g dry soil at a bulk density of 1.4 g cm^{-3}, using biochar application rates equivalent to 1.5% biochar or approximately 22.5 t ha^{-1} in the surface 20 cm depth. Plants were fertilized with

Table 1
PTE concentrations in biochar and soil used in the experiment.

Material/PTE (mg/kg)	Cd	Cr	Cu	Ni	Pb	Zn
Biochar 300 °C[a]	0.19 ± 0.10	2.63 ± 1.40	13.1 ± 2.26	1.38 ± 0.66	2.44 ± 0.91	199.1 ± 66.14
Biochar 500 °C[a]	0.18 ± 0.05	1.93 ± 0.63	8.7 ± 0.91	1.75 ± 0.68	6.04 ± 0.88	191.7 ± 51.51
Biosolid amended soil[b]	170 ± 6.84	2478 ± 138	1200 ± 53	482 ± 15.58	775 ± 29.17	3493 ± 158
Max contaminant level[c]	85	3000	4300	420	840	7500

[a] Values are means of 3 replicates.
[b] Values are means of 5 replicates.
[c] US EPA Part 503 rules for land application.

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