

Article

Use of Biochar and Industrial Hemp for Remediation of Heavy-Metal-Contaminated Soil: Root Uptake and Translocations for Cd, Pb, and Zn

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Abstract: Phytoremediation has been reported as a more energy-efficient, and therefore cost-effective, method of environmental restoration compared to traditional remediation methods for heavy-metal-contaminated soils. Biochar has been shown to have variable effects on remediation potential in heavy-metal-contaminated soils. The objective of this study was to evaluate the effects of soil contamination level (i.e., low, medium, and high), industrial hemp (*Cannabis sativa* L.) cultivar (i.e., ‘Carmagnola’ and ‘Jinma’), biochar rate (i.e., 0, 2, 5, and 10% by volume), and their interactions on root tissue Cd, Pb, and Zn concentrations and uptakes; whole-plant Cd, Pb, and Zn uptakes; and translocation factors after 90 days of hemp growth in contaminated soil from the Tar Creek Superfund Site near Picher, Oklahoma. Hemp removal of Cd, Pb, and Zn differed between soil contamination levels ($p < 0.01$), but was unaffected ($p > 0.05$) by the hemp cultivar or biochar rate, except for total Zn uptake. Total Zn uptake was affected ($p = 0.02$) by the biochar rate in the medium- and high-contaminated soils, where total plant Zn uptake in the high-contaminated soil was numerically the largest with 10% biochar (0.28 mg cm^{-2}) and in the medium-contaminated soil was numerically the largest with 2% biochar (0.07 mg cm^{-2}), but was unaffected ($p > 0.05$) by the biochar rate in the low-contaminated soil. The translocation factor for Zn uptake in the low and medium soils was >1 , indicating industrial hemp as a potential Zn hyper-accumulator up to a threshold soil contamination level. Results demonstrate that biochar amendment has the potential to enhance hemp’s remediation capability of heavy-metal-contaminated soils.

Keywords: soil contamination; heavy metals; phytoremediation; biochar; industrial hemp



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1. Introduction

Soil contamination, particularly from heavy metals, poses a major environmental hazard, resulting in potential toxicities and bioaccumulation. Mining sites are a major contributor to anthropogenic environmental degradation and pollution via heavy metals, not only leading to soil contamination, but also water contamination. In particular, lead (Pb) contamination in water bodies has significant negative implications for community health, increasing the risk of developmental delays and nervous system and brain damage in children [1].

Lead, cadmium (Cd), and zinc (Zn) exposure at the Tar Creek Superfund site, near Picher in northeast Oklahoma, is an example of extensive environmental heavy metal contamination [2]. Tar Creek’s environmental contamination was so extensive that, in 1980, the Governor of Oklahoma created a Tar Creek task force to investigate the effects of the acid-mine drainage on surface water in the area. In 1983, Tar Creek was added

to the Environmental Protection Agency's (EPA) National Priorities List because of the estimated 23,701,200 m³ of mine tailings, or chat, which contaminated surrounding soils as well as local surface waters and groundwater [3,4]. However, remediation of heavy-metal-contaminated soils/sediments and/or water resources is expensive, as well as time- and resource-consuming.

Plants, and their associated rhizosphere microbiota, have been used to assist with environmental clean-ups and/or to render pollutants less harmful (i.e., phytoremediation). Phytoremediation has grown in popularity and acceptance as a cheaper and less invasive alternative or complement to conventional remediation strategies, such as excavation and removal, incineration, chemical oxidation, soil washing, and electrical coagulation [5,6].

Previous studies have shown that certain plant species have a greater affinity than others for the uptake of pollutants, such as various heavy metals, organic contaminants, radionuclides, and/or pesticides from the soil [5]. Plants have several mechanisms that can be used to benefit environmental clean-up efforts, such as pollutant volatilization, stabilization, extraction, and degradation. Different plant processes are suitable for different pollutants depending on whether the pollutant is organic or inorganic [5].

Mining waste, or tailings, such as the material causing contamination at the Tar Creek Superfund site, is an inorganic substance often containing substantial amounts of heavy metals, which can be absorbed and stabilized in plant tissues [7]. Heavy metals can have deleterious impacts on the immediate and surrounding environments and wildlife inhabitants, such as soil and water contamination and growth abnormalities [1,3,4], as well as human health, such as with elevated frequencies of certain cancers [1]. Traditional engineering-based remediation methods are often both invasive and expensive, altering soil structure and aggregate stability and affecting biological function of the area impacted by mining activities [8]. Using plants offers a more natural means to help remediate contaminated areas.

In 1998, at the Institute of Bast in Ukraine, hemp (*Cannabis sativa* L.) was planted near the Chernobyl nuclear power plant to remove radioactive contaminants. The experimental planting led to the discovery of hemp's potential for soil remediation [9]. Hemp is a fast-growing, deep-rooted, large-biomass-producing, hardy crop, which are all traits that are desirable for phytoremediation. Studies report that hemp can accumulate heavy metals in significant amounts, specifically with a 50- to 100-fold bioconcentration factor (BCF), which is the ratio of pollutant concentration in plant parts to that in the soil [2,5,10]. Further studies reported that hemp is a hyper-accumulating plant for certain heavy metals, meaning it is a plant that absorbs toxins to a greater tissue concentration than the concentration in the soil in which the plant is growing [9].

Phytoremediation in general has several limitations. Primarily, there is a threshold of toxicity and/or pollution beyond which a plant cannot grow and survive [5]. Secondly, the magnitude of pollutant uptake into plant tissues is ultimately relatively small and requires further management after stabilization and removal [5]. To compensate for some of the plant-growth limitations, soil amendments, such as biochar, can be added to the soil to potentially enhance phytoremediation efforts.

Biochar is a highly variable substance produced by the pyrolysis of organic materials, such as plant biomass. Biochar has increased in production and popularity from various organic feedstocks due to biochar's potential to be used as a soil amendment to relieve some of the limitations on plant growth in heavy-metal-contaminated soil [2,11,12]. Consequently, biochar has many applications in industry and environmental management. Biochar can enhance plant growth by improving soil fertility and other physical, chemical, and/or hydraulic properties of the soil [13]. Biochar can be added to raise soil pH and improve adsorption and cation exchange due to carboxyl-group formation following oxidation.

Adsorption of heavy metal cations, via biochar's generally large cation exchange capacity, can render the heavy metal cations less soluble or insoluble in soil solution and reduces heavy metal bioavailability in the environment [2].

Several studies have been conducted evaluating the effects of biochar on heavy metal availability in contaminated soils [2,14,15]. Liu et al. [14] concluded that the addition of modified coconut (*Cocos nucifera*) shell biochar to contaminated soils greatly reduced heavy metal availability and determined that the biochar could be a suitable amendment for in situ soil remediation. Jiang et al. [15] added rice (*Oryza sativa*)-straw-derived biochar to a simulated contaminated soil and reported significantly reduced soil concentrations of acid-soluble copper (Cu) and Pb.

Thurston et al. [2] recently evaluated the effects of a hemp cultivar and biochar rate in various levels of combined Pb-, Zn-, and Cd-contaminated soil from the Tar Creek Superfund Site near Picher, OK. Averaged across biochar rates, the 'Carmagnola' cultivar accumulated 331 mg Pb kg⁻¹ from a high-contaminated soil and did not differ from the 'Jinma' cultivar for aboveground-tissue-Pb concentration after 90 days of growth in heavy-metal-contaminated soils [2]. Thurston et al. [2] also reported that, across all treatment combinations (i.e., two hemp cultivars, four biochar rates, and three soil contamination levels), biochar heavy metal concentrations were the largest in the 2% (v/v) and smallest in the 10% (v/v) biochar rates.

Traditional remediation procedures are expensive and invasive, where the projected cost for the remediation of the Tar Creek Superfund site specifically exceeds \$167 million [16]. Phytoremediation is proposed as a more cost-effective technique to traditional remediation methods, as well as potentially being much less disruptive to the local ecosystem and biota. Coupled with biochar, phytoremediation with industrial hemp may provide a viable, alternative strategy to remediate heavy-metal-contaminated soil.

There has been extensive research on phytoremediation and its viability as an in situ remediation technique [5–9], as well as research on biochar and industrial hemp and related fiber crops as phytoremediators [10–12,14,15]. However, there has been little research on the interaction between industrial hemp and biochar to help remove heavy metals from contaminated soil. The objective of this study was to evaluate the effects of soil contamination level (i.e., low, medium, and high), hemp cultivar (i.e., 'Carmagnola' and 'Jinma'), biochar rate (i.e., 0, 2, 5, and 10% by volume), and their interactions on root tissue Cd, Pb, and Zn concentrations and uptakes; whole-plant Cd, Pb, and Zn uptakes; and translocation factors after 90 days of hemp growth in heavy-metal-contaminated soil in a greenhouse. Based on recent greenhouse work by Thurston et al. [2], it was hypothesized that (i) the 'Carmagnola' and 'Jinma' cultivars will not differ in their Cd, Pb, and Zn removal from the soil; (ii) the biochar rate will not impact heavy metal uptake and accumulation; and (iii) hemp root tissues will have a greater concentration and uptake of heavy metals when grown in more severely contaminated soil.

2. Materials and Methods

This study was conducted as an extension of an initial greenhouse study performed at the University of Arkansas, Division of Agriculture Milo J. Shult Agricultural Research and Extension Center in Fayetteville, AR, during Summer and Fall 2021 that evaluated biochar rate and hemp cultivar for phytoremediation of heavy-metal-contaminated soils [2,17]. Specifically, after hemp was grown in contaminated soil for 90 days, Thurston [17] evaluated the effects of soil contamination level, hemp cultivar, biochar rate, and their interactions on root, aboveground, and whole-plant dry matter; aboveground tissue Cd, Pb, and Zn concentrations; uptakes; and bioconcentration factors. However, the current study evaluated the effects of soil contamination level, hemp cultivar, biochar rate, and their interactions on

root tissue Cd, Pb, and Zn concentrations and uptakes; whole-plant Cd, Pb, and Zn uptakes; and heavy metal translocation factors. Translocation factors are calculated as the ratio of above- to belowground elemental uptake and is often used to determine if a plant can translocate more into the above- than belowground tissue (i.e., translocation factor > 1) [5].

2.1. Soil Collection, Processing, and Analyses

Seven 18.9 L (5 gallon) buckets of heavy-metal-contaminated soil from the top 10–15 cm were collected in June 2021 from three different locations within an approximate 22 ha area surrounding a former chat-processing area at the Tar Creek Superfund Site near Picher, OK [17].

Field estimates of the heavy metal concentrations were established using a field-portable, hand-held X-ray fluorescence spectrometer (model S1-Titan, Bruker, Berlin, Germany) several days before soil collection. The three soils were then semi-quantitatively categorized as being of a low (~500–600 mg Pb kg⁻¹, <1000 mg Zn kg⁻¹, and <20 mg Cd kg⁻¹), medium (~1500–1800 mg Pb kg⁻¹, 2000 mg Zn kg⁻¹, and 60 mg Cd kg⁻¹), and high (~5500 mg Pb kg⁻¹, 13,000 mg Zn kg⁻¹, and 123 mg Cd kg⁻¹) level of contamination [17].

As described by Thurston [17], after collection, soils were sieved, air-dried in a greenhouse, and manually homogenized. After homogenization, three ~200 g sub-samples were collected from each soil group for physical and chemical property analyses. Sub-samples were oven-dried in a forced-draft oven at 70 °C for 48 h, then crushed with a mortar and pestle. Sand, silt, and clay fractions were measured using a modified 12 h hydrometer procedure [18]. Soil pH and electrical conductivity were measured potentiometrically in a 1:2 soil/water suspension [19,20]. Soil organic matter concentration was measured by loss on ignition [21]. Total carbon and total nitrogen were measured by high-temperature combustion using a Variomax C/N analyzer (Elementar Americas, Inc., Mt. Laurel, NJ, USA). The soil C/N ratio was calculated based on measured total carbon and total nitrogen concentrations. Total recoverable (TR) Pb, Zn, and Cd concentrations were also measured by acid digestion [22]. Table 1 summarizes the initial soil property differences between the three soils used in the greenhouse study. Briefly, TR Pb concentration in the high-contaminated soil was 10.5 times greater than in the medium-contaminated soil, which was 3.2 times greater than in the low-contaminated soil (Table 1). Total recoverable Zn concentration in the high-contaminated soil was 4.0 times greater than in the medium, which was 7.0 times greater than in the low soil (Table 1). Total recoverable Cd concentration in the high-contaminated soil was 3.1 times greater than in the medium, which was 7.5 times greater than in the low soil (Table 1).

Table 1. Summary of initial soil property differences between three soils from Tar Creek Superfund site used in greenhouse study [17].

Soil Property	Soil Contamination Level			<i>p</i>
	Low	Medium	High	
	Total recoverable elements (mg kg ⁻¹)			
Pb	308 c	978 b	10,251 a	<0.01
Zn	763 c	5353 b	21,179 a	<0.01
Cd	4.6 c	34.4 b	107 a	<0.01
Sand (g g ⁻¹)	0.23 a, [†]	0.44 a	0.35 b	<0.01
Silt (g g ⁻¹)	0.48 b	0.45 b	0.56 a	<0.01
Clay (g g ⁻¹)	0.28 a	0.11 b	0.09 b	<0.01
pH	6.27 b	6.23 b	6.53 a	0.01
Electrical conductivity (dS m ⁻¹)	1.95 a	1.35 b	1.32 b	0.01
Soil organic matter (%)	3.1 b	2.1 c	4.6 a	<0.01
Total C (%)	0.88 c	1.12 b	3.69 a	<0.01
Total N (%)	0.10 b	0.07 c	0.17 a	<0.01
C:N ratio	9.1 c	15.9 b	22.3 a	<0.01

[†] Means in a row with different letters are different at *p* < 0.05.

2.2. Treatments Evaluated and Experimental Design

This study evaluated the effects of three treatments: (i) four biochar rates (i.e., 0, 2, 5, and 10% by volume), (ii) two hemp cultivars (i.e., ‘Carmagnola’ and ‘JinMa’), and three levels of soil contamination (i.e., low, medium, and high). A Douglas fir (*Pseudotsuga menziesii*) feedstock was used in a slow-pyrolysis kiln to create the biochar used in this experiment [2]. Though the exact pyrolysis temperature was unknown due to being proprietary, the material created and used in this study was medium-sized, 3–5 mm flakes, with a pH of 8.93, surface area of $308 \text{ m}^2 \text{ g}^{-1}$, and ash content of 2.1% [2]. The final biochar material also had initial mean Cd, Pb, and Zn concentrations of 0.15, 2.5, and 15.8 mg kg^{-1} [2]. The biochar rates used in this study were based on personal communication recommendations from BiocharNow (Berthoud, CO, USA), the company that supplied the biochar used in this project, where the 2 and 5% by volume rates represented typical biochar field application rates used in the industry.

Four replications (i.e., blocks) of each soil/biochar/cultivar treatment combination were prepared, for a total of 96 individual experimental units (i.e., pots). Each block had 24 experimental units that were organized in a randomized complete block design on two adjacent greenhouse benches [17].

2.3. Pot Preparation, Hemp Establishment, and Water Management

Soil and biochar, 2000 g total, were added to plastic pots with a base diameter of 12 cm, top inside diameter of 17.5 cm, and height of 18 cm. Biochar amendment rates were 0, 2, 5, and 10% by volume to match prior studies’ amendment rates [11,12]. Biochar masses (i.e., 10, 25, and 50 g) were then added to plastic bags with soil (i.e., 1990, 1975, and 1950 g, respectively) and manually shaken for 2 min in a circular motion to homogenize the soil–biochar mixture in a manner similar to incorporation by tillage. To overcome initial soil nutrient deficiencies that would have negatively affected plant establishment and growth [17], nitrogen (249 kg N ha^{-1}) and phosphorus (P; $58.7 \text{ kg P ha}^{-1}$) in the form of urea (46-0-0) and triple superphosphate (TSP; 0-46-0), respectively, were added to all pots and 0, 66.9, and $100 \text{ kg of potassium (K) ha}^{-1}$ in the form of potash (0-0-60) were added to the low-, medium-, and high-contaminated soils, respectively. Fertilizer was added to the soil at the same time biochar was added to the soil [2,17]. Due to sub-optimal growing conditions in the greenhouse (i.e., excessive heat), initial attempts to germinate hemp seeds directly into the contaminated soil were unsuccessful; thus, hemp seeds needed to be germinated and establish viable growth in potting soil [2,17]. Once plants grew past the seed/leaf stage and entered the vegetative stage of growth, seedlings were transplanted into pots containing the contaminated soils [2,17]. Pots were placed on greenhouse benches ~ 2 m below growth lights, which were adjusted as plant height increased.

A watering scheme was developed for each soil group based on their respective individual soil characteristics [17]. Based on mean sand, clay, and SOM concentrations, gravimetric moisture content at field capacity was estimated using the Soil, Plant, Water, Atmosphere (SPAW) model [23]. Bulk density was also estimated for each soil/biochar combination. A Theta Probe (SM150T, Delta-T Devices, Inc., Houston, TX, USA) was used to measure volumetric soil water content in the top 6 cm of soil and was calibrated to determine the target water volume application required to result in the estimated moisture field capacity. The Theta Probe was then used prior to each watering, and a look-up chart was developed to specify water volume to apply to the nearest milliliter. Watering occurred on an every-other-day basis and watering procedures were applied separately to each soil/biochar treatment combination [2].

The greenhouse was set to 12 h intervals between light and dark periods. The climate-controlled greenhouse used forced-air evaporative cooling with exhaust fans to buffer

the air temperature during the warm season (i.e., April through September) [2]. During October through March, a ceiling-mounted, electronically controlled heater was used to control the greenhouse air temperature [2]. Hemp plants were grown in the contaminated soil for a total of 90 days. Additional details regarding pot preparation, plant establishment, and watering are described in the studies of Thurston et al. [2] and Thurston [17].

2.4. Plant Sample Collection, Processing, and Analyses

After 90 days of growth in the contaminated soil, aboveground plant tissue was collected by cutting the plant's stem at the soil surface. Root samples were collected from the pots and hand-washed to remove any excess soil present. Belowground tissues were oven-dried for 48 h at 65 °C, then finely ground to pass a 1 mm mesh screen.

Using a modified EPA 3050B procedure [22], 0.5 g of finely ground root material was digested using 5 mL of nitric acid for 24 h and 3 mL of 30% hydrogen peroxide, 1 mL every 24 h over a period of 72 h, then heated and refluxed at 120 °C for 3 h following the procedure for acid digestion of plant tissue by inductively coupled plasma optical emissions spectrometry (ICP-OES; model FHS16, Spectro Arcos, Wilmington, MA, USA) [24,25]. Tissue extracts were diluted to 25 mL and filtered through Whatman 42 filter paper and analyzed for total Pb, Zn, and Cd concentrations by atomic absorption spectrometry [17].

Internal validity could have suffered from small variations in filter paper, mesh sample sorting to remove adsorbed biochar, and sample weighing. To combat the potential internal validity issues, a test batch of samples was created to determine if there was a significant difference in results based on whether biochar was carefully manually removed from root samples or not. After digesting the test samples, results showed that sorting did not have a significant effect on heavy metal concentrations.

Root tissue concentrations plus root dry matter data from Thurston et al. [2], on a replicate-by-replicate basis, were used to calculate Cd, Pb, and Zn uptakes in the root tissue. Root tissue Cd, Pb, and Zn uptakes were added to aboveground tissue Cd, Pb, and Zn uptakes from Thurston et al. [2] to determine and report whole-plant Cd, Pb, and Zn uptakes. The ratio of above- to belowground heavy metal uptake was calculated for Cd, Pb, and Zn and analyzed as the translocation factor (TF) [5] to determine if hemp is a hyperaccumulator plant.

2.5. Data Analyses

Similar to Thurston et al.'s study [2], a three-factor analysis of variance (ANOVA) was conducted using the PROC GLIMMIX procedure in SAS (version 9.4, SAS Institute, Inc., Cary, NC, USA), based on a completely random design, to evaluate effects of soil contamination level, hemp cultivar, biochar rate, and their interactions on root Cd, Pb, and Zn tissue concentrations and uptakes; whole-plant Cd, Pb, and Zn uptakes; and Cd, Pb, and Zn translocation factors. All data analyses were conducted using a gamma distribution to achieve data normality [2] and homogeneity of variance was assumed. The threshold $p \leq 0.05$ was used to judge significance. Treatment means were separated by least significant difference when appropriate.

3. Results and Discussion

Thurston et al. [2] recently measured and reported below- and aboveground hemp tissue dry matter and aboveground tissue heavy metal concentrations and calculated aboveground heavy metal uptakes. In the current study, belowground heavy metal concentrations were measured and, coupled with data regarding belowground dry matter reported by Thurston et al. [2], belowground heavy metal uptakes were calculated and reported. In addition, belowground heavy metal uptakes from the current study were

added to the aboveground heavy metal uptakes reported by Thurston et al. [2] to calculate total plant heavy metal uptakes. Furthermore, aboveground heavy metal uptakes recorded by Thurston et al. [2] were divided by belowground heavy metal uptakes generated in the current study to calculate the TF.

3.1. Belowground Concentrations

After 90 days of hemp growth in contaminated soil, belowground Cd, Pb, and Zn concentrations all differed ($p < 0.01$) between soils (Table 2). However, neither the hemp cultivar nor biochar rate affected ($p > 0.05$) belowground Cd, Pb, or Zn concentrations (Table 2). The belowground Cd concentration was numerically largest in the medium-contaminated soil, which did not differ from the high-contaminated soil, where both were at least 14.6 times greater than from the low-contaminated soil, which had the lowest belowground Cd concentration (Table 3). The belowground Pb concentration was largest in the high-contaminated soil, which was 13.1 times greater than in the medium-contaminated soil, which was 3.0 times greater than in the low-contaminated soil, which had the lowest belowground Pb concentration (Table 3). Similar to Pb, the belowground Zn concentration was largest in the high-contaminated soil, which was 4.3 times greater than in the medium-contaminated soil, which was 5.0 times greater than in the low-contaminated soil, which had the lowest belowground Zn concentration (Table 3). With a well-established, initial heavy metal concentration gradient between the low-, medium-, and high-contaminated soils used in this study (Table 1), it stands to reason that the belowground heavy metal tissue concentrations would follow a similar pattern. Many similar aboveground tissue responses in the three soils were reported by Thurston et al. [2], with the exception that both the Pb and Zn concentrations differed between cultivars among soils, Pb concentration also differed between biochar rates within soils, and Cd concentrations differed between cultivar/biochar rate/soil combinations.

Table 2. A summary for the analysis of variance for the effect of soil contamination level, hemp cultivar (Cult), biochar (BC) application rate, and their interactions on belowground (BG) heavy metal [i.e., cadmium (Cd), lead (Pb), and zinc (Zn)] tissue concentrations and uptakes, total plant tissue uptake, and translocation factors (TF) for hemp grown in the greenhouse in contaminated soil from the Tar Creek Superfund site.

Plant Property	Source of Variation						
	Soil	Cult	Soil × Cult	BC	Soil × BC	Cult × BC	Soil × Cult × BC
	p						
BG Cd concentration	<0.01	0.61	0.33	0.54	0.82	0.31	0.92
BG Pb concentration	<0.01	0.19	0.29	0.26	0.58	0.41	0.24
BG Zn concentration	<0.01	0.24	0.36	0.79	0.19	0.97	0.63
BG Cd uptake	<0.01	0.64	0.88	0.66	0.70	0.66	0.98
BG Pb uptake	<0.01	0.26	0.85	0.24	0.29	0.70	0.73
BG Zn uptake	<0.01	0.41	0.75	0.70	0.16	1.00	0.96
Total Cd uptake	<0.01	0.60	0.80	0.63	0.65	0.65	0.99
Total Pb uptake	<0.01	0.08	0.85	0.35	0.10	0.74	0.75
Total Zn uptake	<0.01	0.10	0.94	0.58	0.02	0.98	0.96
TF Cd	0.04	0.91	0.82	0.38	0.09	0.67	0.66
TF Pb	<0.01	0.83	0.22	0.51	0.29	0.69	0.20
TF Zn	<0.01	0.95	0.60	0.68	0.52	0.99	0.96

Table 3. Soil contamination level effects on belowground (BG) heavy metal [i.e., cadmium (Cd), lead (Pb), and zinc (Zn)] tissue concentrations and uptakes, total plant tissue uptake, and translocation factors (TF) for hemp grown in the greenhouse in contaminated soil from the Tar Creek Superfund site.

Plant Property	Soil Contamination Level		
	Low	Medium	High
BG Cd concentration (mg kg ⁻¹)	23.8 b, [†]	439.7 a	348.3 a
BG Pb concentration (mg kg ⁻¹)	145.0 c	437.4 b	5751 a
BG Zn concentration (mg kg ⁻¹)	430.1 c	2150 b	9223 a
BG Cd uptake (mg cm ⁻²)	0.0003 b	0.0061 a	0.0049 a
BG Pb uptake (mg cm ⁻²)	0.0022 c	0.0061 b	0.083 a
BG Zn uptake (mg cm ⁻²)	0.0064 c	0.03 b	0.14 a
Total Cd uptake (mg cm ⁻²)	0.0004 b	0.0068 a	0.0058 a
Total Pb uptake (mg cm ⁻²)	0.0031 c	0.0084 b	0.09 a
TF Cd	0.32 a	0.18 b	0.25 ab
TF Pb	0.79 a	0.63 a	0.10 b
TF Zn	1.5 a	1.1 a	0.47 b

[†] Means in a row with different letter are significantly different at $p < 0.05$.

Linger et al. [26] reported that hemp roots were tolerant of Cd present in the soil, accumulating over 800 mg Cd kg⁻¹ in the root tissue without a decline in plant growth or development. Aboveground hemp tissue accumulated less than 100 mg Cd kg⁻¹, and the more contaminated the soil, the more Cd the plant accumulated [26]. The study used two different Cd concentrations available to the plant in the soil: 17.3 ± 2.0 mg Cd kg⁻¹ and 71.7 ± 8.2 mg Cd kg⁻¹ [26], which were similar to the Cd concentrations in the low- and medium-contamination soils used in the current study. Linger et al. [26] concluded that hemp roots showed hyperaccumulator-like potential depending on their growth stage, where juvenile roots accumulate more Cd than older roots. The hemp plants may have accumulated more Cd in the belowground than in the aboveground tissue because large Cd levels can impair photosynthetic activity and growth [26].

Stonehouse et al. [10] studied selenium (Se) accumulation in hemp tissues, and reported that increasing selenate levels in the soil resulted in increased tissue-Se accumulation up to a threshold of ~ 40 μ M Se. In contrast to the current study, Stonehouse et al. [10] reported greater Se concentrations in the aboveground tissue and seeds and significantly lower Se concentrations in the root tissue.

Xu et al. [27] investigated Pb accumulation and distribution in various organs of industrial hemp. Their results showed that an increasing soil/Pb concentration corresponded to an increase in Pb concentration in the plant [27]. The tissue/Pb concentration was 2 to 7 times greater in the roots than in the aboveground tissue and 6 to 25 times greater than in the seeds [27]. Xu et al. [27] attributed the large root-tissue Pb concentration to the plants' response to heavy metal stress, where hemp plants will trap heavy metals in the belowground tissue to reduce damage to aboveground photosynthetic and respiratory tissues.

3.2. Belowground Uptakes

After 90 days of hemp growth in contaminated soil, similar to belowground concentrations, belowground Cd, Pb, and Zn uptakes all differed ($p < 0.01$) between soils, but were unaffected ($p > 0.05$) by the hemp cultivar or biochar rate (Table 2). The belowground Cd uptake was numerically largest in the medium-contaminated soil, which did not differ from the high-contaminated soil, where both were at least 16.3 times greater than in the low-contaminated soil, which had the lowest belowground Cd uptake (Table 3). The belowground Pb uptake was largest in the high-contaminated soil, which was 13.6 times greater than in the medium-contaminated soil, which was 2.8 times greater than in the

low-contaminated soil, which had the lowest belowground Pb uptake (Table 3). The belowground Zn uptake was largest in the high-contaminated soil, which was 4.7 times greater than in the medium-contaminated soil, which was 4.7 times greater than in the low-contaminated soil, which had the lowest belowground Zn uptake (Table 3).

Linger et al. [26] reported that industrial hemp accumulated, on average, 832 $\mu\text{g Cd}$ in the aerial parts of the plant when grown in soil with a Cd concentration of 17 mg kg^{-1} . However, no belowground heavy metal concentration or uptake results were reported [26].

Candito et al. [28] reported heavy metal uptakes for Cd, Pb, and thallium (Tl) in the root and aboveground tissues of industrial hemp as well as whole-plant uptake. Plants were grown in soils of varying contamination (Cd = 7.8 and 8.4 mg kg^{-1} , Pb = 20.3 and 35.2 mg kg^{-1} , and Tl = 3.1 and 7.9 mg kg^{-1}) [28]. Roots accumulated 2.63 and 1.69 $\text{g ha}^{-1} \text{ year}^{-1}$ of Cd, 1.25 and 1.60 $\text{g ha}^{-1} \text{ year}^{-1}$ of Zn, and 2.68 and 3.29 $\text{g ha}^{-1} \text{ year}^{-1}$ of Tl from the respective contaminated soils [28]. Similar to the current study, Candito et al. [28] was one of the few studies that reported metal uptakes in addition to tissue concentrations.

3.3. Total Plant Uptakes

After 90 days of hemp growth in contaminated soil, similar to belowground concentrations and uptakes, total plant Cd and Pb uptakes differed ($p < 0.01$) between soils, but were unaffected ($p > 0.05$) by the hemp cultivar or biochar rate, while total plant Zn uptake differed ($p = 0.02$) between soil/biochar rate combinations and was also unaffected ($p > 0.05$) by the hemp cultivar (Table 2). The total plant Cd uptake was numerically largest in the medium-contaminated soil, which did not differ from the high-contaminated soil, where both were at least 14.5 times greater than in the low-contaminated soil, which had the lowest total plant Cd uptake (Table 3). The total plant Pb uptake was largest in the high-contaminated soil, which was 10.7 times greater than in the medium-contaminated soil, which was 2.7 times greater than in the low-contaminated soil, which had the lowest total Pb uptake (Table 3).

The total plant Zn uptake was numerically largest in the high-contaminated soil with 10% biochar (0.28 mg cm^{-2}), which did not differ from the high-contaminated soil with 5% biochar, and was smallest in the low-contaminated soil with 0, 2, 5, and 10% biochar ($< 0.02 \text{ mg cm}^{-2}$), which did not differ (Figure 1). Consequently, within the low-contaminated soil, the total plant Zn uptake did not differ ($p > 0.05$) between biochar rates (Figure 1). Within the medium-contaminated soil, the total plant Zn uptake was numerically the largest with a 2% biochar rate and numerically the smallest with a 10% biochar rate, while the 0 and 5% biochar rates had intermediate uptake (Figure 1). Within the high-contaminated soil, the total plant Zn uptake did not differ ($p > 0.05$) between the 5 and 10% biochar rates, but the total plant Zn uptakes with the 0 and 2% biochar rates, which did not differ between themselves, were less than the total plant Zn uptake with the 10% biochar rate, but also similar to that of the 5% biochar (Figure 1). It is unclear why biochar improved Zn uptake; however, the biochar's affinity for Zn adsorption may have been decreased, considering that the biochar's initial Zn concentration was much greater than the initial Cd or Pb concentration, leaving more soluble Zn in solution to be available for plant uptake.

Within each individual biochar rate, the total plant Zn uptakes in all three soils differed between one another, where the high-contaminated soil always had significantly the largest uptake, the medium-contaminated soil always had intermediate uptake, and the low-contaminated soil always significantly the smallest uptake (Figure 1). The total plant Zn uptake in the unamended control for the low- and medium-contaminated soils did not differ ($p > 0.05$) from any of the three respective biochar-amended treatments (Figure 1). However, the total plant Zn uptake in the unamended control for the high-contaminated

soil was similar to that with the 2 and 5% biochar rates but was lower than that with the 10% biochar rate (Figure 1). No relevant studies exist that quantify total plant heavy metal uptakes from directly measured below- and aboveground plant dry matter and heavy metal concentrations, which is a unique and novel attribute of the current study.

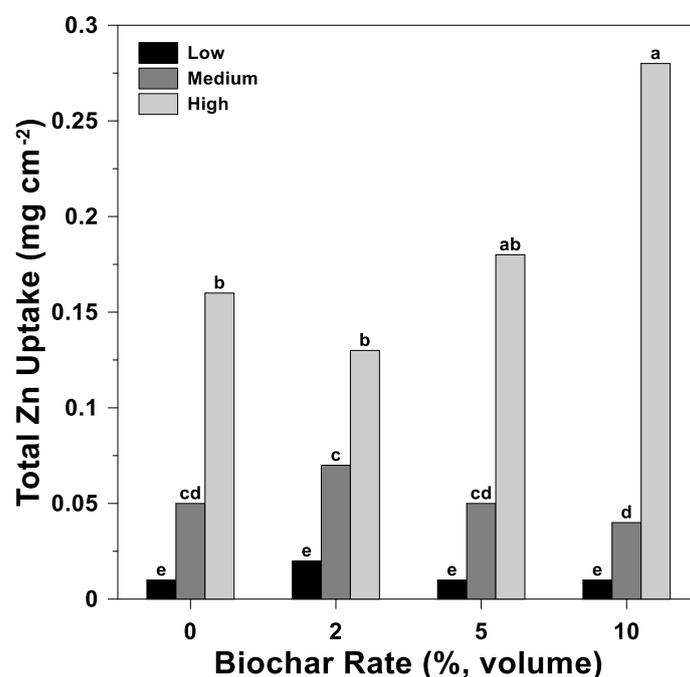


Figure 1. Total zinc (Zn) uptake by industrial hemp with different biochar rates (i.e., 0, 2, 5, and 10% by volume) and across three levels of soil contamination using soil from Tar Creek Superfund site. Different letters atop bars indicate significant difference at $p < 0.05$.

3.4. Translocation Factors

After 90 days of hemp growth in contaminated soil, similar to the belowground concentrations and uptakes, the Cd, Pb, and Zn TFs differed ($p < 0.04$) between soils, but were unaffected ($p > 0.05$) by the hemp cultivar and biochar rate (Table 2). As the ratio of above- to belowground heavy metal uptake changed, the TF for Cd was numerically largest in the low-contaminated soil, which did not differ from the high-contaminated soil, and was numerically lowest in the medium-contaminated soil, which also did not differ from the high-contaminated soil. Thus, the TF for Cd was 1.8 times greater in the low- than in the medium-contaminated soil (Table 3). Similar to Cd, the TF for Pb was also numerically largest in the low- but did not differ from the medium-contaminated soil, where both were at least 6.3 times greater than in the high-contaminated soil, which had the lowest TF for Pb (Table 3). Similar to Pb and Cd, the TF for Zn was also largest in the low- and did not differ from the medium-contaminated soil, where both were at least 2.3 times greater than in the high-contaminated soil, which had the lowest TF for Zn (Table 3).

A possible explanation for all three heavy metals having the numerically largest TF in the low-contaminated soil and numerically smallest TF in the medium- or high-contaminated soils is that only so much of each heavy metal is available to the plant for uptake and translocation. The more severely contaminated soils may have had a greater concentration of bioavailable heavy metals for the hemp to take up, such as with the total Zn uptake, but the resulting proportion in the above- relative to the belowground tissue was less prominent in the high- than in the low-contamination soil. Another possible explanation is that, with an increasing concentration of heavy metals in the soil, the microorganisms present in the rhizosphere, which can enhance heavy metal solubility

through the increased release of carbon dioxide (CO₂) and organic matter decomposition, may have been killed off, thus hindering translocation [29].

A plant is classified as a hyperaccumulator if it has a TF > 1 [30]. Among the three heavy metals tested in this study, only the TF for Zn was greater than 1 from the low- and medium-contamination soils. Consequently, hemp may be a potential hyperaccumulator for Zn, but the results do not support the conclusion that hemp is a potential hyperaccumulator for all three heavy metals or that hemp is a hyperaccumulator in severely contaminated soils.

3.5. Implications

Though this study was conducted with ~2000 g of biochar-amended contaminated soil under controlled greenhouse conditions, the results of this study support that industrial hemp has the potential to successfully remove heavy metals from soil and translocate them into plant tissue. Phytoremediation is a rapidly growing field with great potential for mild-to-moderate environmental remediation, but more research is needed, including greenhouse and field studies, on phytoremediation's viability in severe cases of environmental contamination where in situ remediation is utilized. The results from this study also suggest that the incorporation of biochar into contaminated soils may enhance industrial hemp's uptake of Zn, but may not significantly improve Pb or Cd uptake.

4. Conclusions

The objective of this study was to evaluate the combination of industrial hemp and biochar for remediating heavy-metal-contaminated soils. This study evaluated three different levels of soil contamination, two industrial hemp cultivars, four biochar amendment rates, and their interactions on root tissue Cd, Pb, and Zn concentrations and uptakes; whole-plant Cd, Pb, and Zn uptakes; and translocation factors after 90 days of growth in contaminated soil from the Tar Creek Superfund Site near Picher, Oklahoma.

For Cd, Pb, and Zn, belowground concentrations and uptakes were greater in the medium- and high-contaminated soils than in the low-contaminated soils, which supported the hypothesis that hemp root tissues would have a greater concentration and uptake of heavy metals when grown in more severely contaminated soils. The results from this study also supported the hypothesis that the two hemp cultivars, 'Carmagnola' and 'Jinma', did not differ in their ability to absorb Cd, Pb, and Zn from the soil. Belowground Cd, Pb, and Zn concentrations and uptakes all differed significantly between soils and were unaffected by the biochar rate or hemp cultivar. The total plant Cd, Pb, and Zn uptakes also differed between soils and were unaffected by the hemp cultivar. However, the total plant Zn uptake differed with each biochar rate at different soil contamination levels, where the high-contaminated soil uptake was always significantly the largest, that of the medium-contaminated soil was always intermediate, and that of the low-contaminated soil was always significantly the smallest. The total plant Zn uptake in the unamended control in the low-contamination soil did not differ with different biochar rates. The total plant Zn uptake for the high-contamination soil was lower in the 0 and 2% treatments than the 10% treatment. The results from this study suggest that biochar positively impacts Zn uptake in more severely contaminated soils, but not Cd or Pb or in less contaminated soils. The results were similar to the aboveground tissue responses recently reported by Thurston et al. [2] as part of the same study, with the exception that the Pb and Zn concentrations differed between hemp cultivars in different soils, Pb concentrations differed between biochar rates in different soils, and Cd concentrations differed between cultivar/biochar/soil combinations.

This research contributed to the greater field of environmental restoration and phytoremediation in that the results quantified total plant heavy metal uptakes from directly

measured above- and belowground plant dry matter and heavy metal concentrations. Further research could be conducted to identify other soil amendments that may be beneficial in phytoremediation, industrial hemp's capacity to remediate other heavy metals, and/or other plant species' ability to remove and translocate heavy metals.

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