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Assessing Biochar and Industrial Hemp to Remediate Heavy-metal-contaminated Soil

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Abstract

Environmental degradation is becoming increasingly prevalent as global industrialization runs rampant. Anthropogenic activity, such as mining, deposits inorganic pollutants into the environment, leading to potential soil and water contamination. Traditional, engineering-based remediation and containment procedures alter soil structure and aggregate stability and affect the biological function of the area impacted by mining activities. Phytoremediation is a more energy-efficient, and therefore cost-effective, method of environmental restoration. Phytoremediation, on its own, works in soils that are less contaminated so that the selected plant can actually grow. Soil amendments, such as biochar, can be added to improve remediation potential in more 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'), Douglas fir (*Pseudotsuga menziesii*)-derived 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 among soil-contamination levels ($P < 0.01$), but was unaffected ($P > 0.05$) by hemp cultivar or biochar rate, except for total Zn uptake. Total Cd uptake was greatest from the high- (0.0058 mg cm^{-2}), which did not differ from the medium-, and was significantly greater than from the low-contaminated soil (0.0004 mg cm^{-2}). Total Pb uptake was greatest from the high- (0.09 mg cm^{-2}) than the other two soils, while the medium- (0.0084 mg cm^{-2}) was also greater than from the low-contaminated soil (0.0031 mg cm^{-2}). Total Zn uptake was affected ($P = 0.02$) by biochar rate in the medium- and high-contaminated soils, where total plant Zn uptake in the high- was numerically largest with 10% biochar (0.28 mg cm^{-}

^{2}) and, in the medium- was numerically largest with 2% biochar (0.07 mg cm^{-2}), but was unaffected ($P > 0.05$) by 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 hyperaccumulator up to a threshold soil-contamination level. Results demonstrate that biochar amendment has the potential to enhance hemp's ability to remediate heavy-metal-contaminated soils.

Introduction

Background and Need

Mining began in Picher, OK in 1904 with the discovery of sphalerite and galena, which contain lead (Pb) and zinc (Zn). The Picher Minefield, part of the Tri-state Mining District, produced 118,306 Mg (130,410 tons) of Pb and 679,712 Mg (749,254 tons) of Zn between 1904 and 1970 (Beattie et al., 2017). Most of the ore smelting occurred on-site or nearby, generating massive amounts of waste, called chat. All mining operations came to a halt in the 1970s after greater-than-normal blood-lead levels were discovered in children in the area (Beattie et al., 2017).

Soil contamination poses a potential major environmental hazard, resulting in potential toxicity and bioaccumulation. Mining sites are a major contributor to anthropogenic environmental degradation and pollution, and remediation is expensive as well as being time- and resource-consuming. Mining waste can lead to not only soil contamination, but water contamination as well. As seen in places like Flint, MI in recent years, lead contamination in water bodies has significant negative implications for community health, increasing the risk of developmental delay and nervous system and brain damage in children (Briffa et al., 2020).

Industrial hemp *(Cannabis sativa* L.*)* is a promising biological agent in environmental restoration because of its deep roots, fast growth, large biomass, and hardiness. Other studies in the area of phytoremediation report that hemp grows well on contaminated soils, specifically with radionuclides, and can accumulate large amounts of heavy metals, such as cobalt, copper, manganese, nickel, Pb, and Zn, into hemp's aboveground tissue (Meer et al., 2005; Stonehouse et al., 2020).

Biochar is another promising option to mitigate the growing issue of environmental contamination and pollution (Qiu et al., 2022). Biochar is a highly variable, carbon-rich substance, produced by pyrolysis under oxygen-limited/hypoxic conditions. Biochar has applications in carbon sequestration and improving general soil fertility and other soil properties, such as pH, cation exchange capacity, and water-holding capacity. Biochar’s high chemical reactivity stems from the fact that it is highly porous and possesses a very large specific surface area. Consequently, biochar is capable of immobilizing heavy metals present in soils, reducing their toxicity and bioavailability by raising soil pH (Antonangelo and Zhang, 2019).

Problem Statement

The problem being explored in this project was finding more cost-effective and less intrusive methods for environmental restoration. Environmental degradation is becoming increasingly prevalent as industrialization runs rampant globally. The projected cost for remediation of the Tar Creek Superfund site exceeds $167 million (USEPA, 2008). Studies have shown plants are effective at removing heavy metals, organic contaminants, radionuclides, and pesticides (Kafle et al., 2022). Thus, phytoremediation may be more cost-effective than industrial methods of environmental remediation, such as dredging, excavation, and incineration, as well is potentially 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, thus warranting investigation.

Purpose Statement

The specific purpose of this project was to assess the effects of the combination of biochar rate and industrial hemp cultivar on remediating a gradient of heavy-metal-contaminated soils.

Literature Review

In this review of relevant literature, the following themes were identified: phytoremediation, biochar, industrial hemp (*Cannabis sativa L.)*, and the Tar Creek Superfund Site. This literature review addresses these key themes in greater detail below.

Phytoremediation

The use of plants and their associated microbiota to perform environmental cleanup or to render pollutants harmless is called phytoremediation (Kafle et al., 2022). Phytoremediation is by no means a new technology, but, within the past decade, phytoremediation has gained greater acceptance as a cheaper and less-invasive alternative or complement to conventional remediation strategies, such as incineration, chemical oxidation, soil washing, and electrical coagulation (Wang and Delavar, 2023).

Studies have shown that some plants are more effective than other plants at absorbing various heavy metals, organic contaminants, radionuclides, and pesticides from the soil (Kafle et al., 2022). Plants have several different 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.

Mining waste, or tailings, is an inorganic pollutant, which cannot be degraded, but can be sequestered or stabilized in plant tissues (Chibuike and Obiora, 2014). Heavy metals are the primary constituent of mine tailings, and heavy metals can have disastrous impacts on the immediate and surrounding environment and wildlife inhabitants, as well as on human health. Traditional engineering-based remediation methods are often both invasive and expensive.

Remediation and containment procedures, such as the construction of tailing dams, landfilling, encapsulation, and surface capping, alter soil structure and aggregate stability and affect the biological function of the area impacted by mining activities (Sánchez-Castro et al., 2023). Using plants offers a more natural means to help remediate contaminated areas. However, one of several limitations to using only plants for remediation is that there is a threshold of toxicity and pollution beyond which a plant cannot grow. Secondly, the magnitude of heavy metal, or other pollutant, absorption into plant tissues is ultimately relatively small. Furthermore, once stabilized in plant tissue, the pollutant-containing plant material still needs to be managed in some way. To compensate for some of these limitations on plant growth, amendments such as biochar can be added to the soil. In addition to its own ability to adsorb pollutants, biochar may also work synergistically with certain plants to enhance remediation capabilities.

A study was conducted by Bian et al. (2017) that identified moso bamboo (*Phyllostachys praecox*) as a potential phytoremediator of heavy-metal-contaminated soils. The study grew moso bamboo independently, as well as in combination with a cadmium and zinc hyperaccumulator succulent species (*Sedum plumbizincicola*), in copper (Cu)-, Zn-, and cadmium (Cd)-contaminated soil in southwest Hangzhou, China (Bian et al., 2017). The site was a former paddy field that had been irrigated with contaminated water from a galvanization factory, resulting in heavy-metal accumulation in the soil (Bian et al., 2017). Bian et al. (2017) reported that moso bamboo and *S. plumbizincicola* grew well on the polluted soil and accumulated more heavy metals when intercropped than bamboo grown alone.

Petelka et al. (2019) conducted a study on the phytoremediation potential of 25 native plant species grown in soil from a former mine-tailing site associated with the Damang Gold Mine in southwest Ghana, which was contaminated with arsenic (As), Pb, mercury (Hg), and Cu.

Native species were selected based on large productivity and vitality, large abundance, and local and literature knowledge on the species (Petelka et al., 2019). Species of trees, palms, shrubs, herbs, ferns, and grasses were grown (Petelka et al., 2019). Plant shoots and soil samples were analyzed for total concentrations of As, Pb, Hg, and Cu, and it was reported that 12 plant species had relatively large Cu bioaccumulation, indicating that the species could be useful in phytoextraction of Cu from contaminated soil.

Biochar

Biochar is a highly variable substance produced by pyrolysis of organic materials, such as plant biomass. Biochar has many applications in industry and agriculture, as well as gaining popularity as a potential reducer of greenhouse gas emissions. In agriculture, biochar's predominant use is as a soil amendment to improve soil fertility and other soil physical, chemical, and/or hydraulic properties (Weber and Quicker, 2018). Biochar can be added to raise the soil pH and improve adsorption and cation exchange due to carboxyl group formation following oxidization (Weil and Brady, 2017). Adsorption of heavy-metal cations by biochar renders the heavy-metal cations less soluble to insoluble in soil solution and reduces heavy-metal bioavailability in the environment (Thurston, 2023). The large cation exchange capacities for biochar come from production at lower temperatures relative to pyrolysis, in which surface area is significantly increased and functional groups are present to provide negative charges (Weber and Quicker, 2018).

Liu et al. (2018) studied the effect of modified coconut (*Cocos nucifera*) shell biochar on the availability of Cd-, nickel-, and Zn-contaminated soil. Contaminated soil was collected from paddy-field topsoil in Mianzhu County, Sichuan Province, China (Liu et al., 2018). Liu et al.

(2018) reported that the addition of modified coconut shell biochar greatly reduced availability of heavy metals and determined that the biochar could be a suitable amendment for in-situ soil remediation.

Jiang et al. (2012) studied the immobilization of Cu (II), Pb (II), and Cd (II) by rice (*Oryza sativa*)-straw-derived biochar added to a simulated, polluted Ultisol. Soil was collected from a pristine area in Liuzhou, China and then had heavy metals added in the forms of $NaNO_3$, $Cu(NO_3)_2$, $Pb(NO_3)_2$, and $Cd(NO_3)_2$ (Jiang et al., 2012). The addition of biochar increased pH and made the Ultisol surface charge negative, creating an advantageous environment for heavy-metal immobilization (Jiang et al., 2012). The study concluded that acid-soluble Cu and Pb concentrations decreased significantly after biochar amendment and that, while acid-soluble Cd decreased numerically, the Cd decrease was not enough to be significant.

Industrial Hemp

In 1998, at the Institute of Bast in Ukraine, hemp was planted near the Chernobyl nuclear power plant to remove radioactive contaminants. The hemp planting led to the discovery of hemp's potential in soil remediation (Ahmad et al., 2016).

Hemp is a fast-growing, deep-rooted, large-biomass-producing, hardy crop, where all these traits are desirable for phytoremediation. Other studies report that hemp compares to sunflower *(Helianthus annuus)* when grown on soils contaminated with heavy metals, like radionuclides (Stonehouse et al., 2020; Meers et al., 2005). Studies report that hemp can accumulate these elements in significant amounts [i.e., 50- to 100-fold bioconcentration factor (BCF), where BCF is defined as the ratio of pollutant concentration in plant parts to that in the soil (Kafle et al., 2022; Stonehouse et al., 2020)]. Further studies reported that hemp is a hyperaccumulating plant, which is a plant that absorbs toxins to a greater tissue concentration than the soil in which plant is growing (Ahmad et al., 2016).

Stonehouse et al. (2020) performed a field study in selenium (Se)-contaminated agricultural areas in Colorado and reported 15-25 µg of Se g^{-1} in hemp seeds and 5-10 µg Se g^{-1} in the leaves and flowers. In the same study, industrial hemp (variety 'Workhorse', Colorado Cultivars Co., Eaton, CO) was grown in Turface, a gravel growth medium, in a controlled greenhouse amended with 40-320 µ*M* selenate at intervals expected to induce plant toxicity (Stonehouse et al., 2020). The industrial hemp plants showed complete tolerance up to 160 µ*M* selenate and accumulated up to 1300 mg of Se kg^{-1} shoot dry weight, which categorizes industrial hemp as a hyperaccumulator (Stonehouse et al., 2020). Selenium is an essential nutrient at low levels for many organisms, but becomes toxic at large concentrations, thus industrial hemp's phytoremediation and biofortification capacity has positive implications for both Se deficiency and toxicity in soil.

Ahmad et al. (2016) performed a study at a site contaminated with Zn, Cu, cobalt (Co), nickel (Ni), chromium (Cr), and Cd near the Kohi Noor textile mills in Rawalpindi, Pakistan. Results showed that industrial hemp leaf tissue accumulated 1350 mg Cu kg^{-1}, 151 mg Cd kg^{-1}, and 123 mg Ni kg^{-1}, suggesting that industrial hemp is viable for remediation of soils contaminated with Cu, Cd, and Ni (Ahmad et al., 2016).

Tar Creek

Mining began in Picher, OK in 1904 with the discovery of sphalerite and galena, which contain Pb, Zn, and Cd. The Picher Minefield, part of the Tri-state Mining District, hereafter referred to as the Tar Creek site after Tar Creek that flows through part of the former minefield near Picher, produced 118,306 Mg (130,410 tons) of Pb and 679,712 Mg (749,254 tons) of Zn during its 70 years of operation (Beattie et al., 2017). Most of the ore smelting occurred on-site or nearby, generating massive amounts of waste, called chat. Chat from Tar Creek contains Pb, Zn, and Cd concentrations deleterious to human health and the environment. All mining operations came to a halt in the 1970s after greater-than-normal blood-Pb levels were identified in area children, which was attributed to environmental contamination from the mining and chat-processing activities at the Tar Creek site (Beattie et al., 2017). After mining operations were ceased, abandoned underground mine shafts and cavities filled with water, flowing to the surface and into Tar Creek, killing all downstream biota (USEPA, 2024).

Heavy metals in the soil affect organic pollutants' degradability, effectively doubling the pollution effect. In water systems, biomagnification of heavy metals is a problem because humans are typically the last of the food chain, where all the metals have accumulated. Heavy metals persist in the environment for a long time and can react with other elements present in sediment or soil, becoming more toxic (Briffa et al., 2020).

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 National Priorities List by the Environmental Protection Agency (EPA) because of its estimated 23,701,200 m^3 (31 million yd^3) of chat, which contaminated soils, as well as surface and groundwater (USEPA, 2024; ODEQ, 2020). Clean-up for the Tar Creek area has spanned from 1984 to present day. Remediation strategies for Tar Creek include plugging abandoned wells, excavating contaminated soil at residences and public areas, injecting chat into underground mine caverns, containing mine waste in trenches, constructing an on-site repository for consolidation and management of contaminated soil and mine waste, and supporting voluntary relocation for residents (USEPA, 2024). The EPA has conducted five-year reviews at Tar Creek, the most recent one being in 2020, and concluded that current remediation efforts are protective of the environment and human health in the short term. The repairs have cost in the hundreds of millions of dollars (USEPA, 2019).

Recent Research

Thurston et al. (2024) evaluated the effects of 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. Thurston et al. (2024) reported that the ‘Carmagnola’ industrial hemp cultivar accumulated 47.1 mg Cd kg^{-1} and the ‘Jinma’ cultivar accumulated 40.6 mg Cd kg^{-1} in aboveground tissue when grown in a high-contaminated soil. 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 (Thurston et al., 2024). Zinc concentration was largest in ‘Carmagnola’ grown in a high-contaminated soils, accumulating 1715 mg Zn kg^{-1} in aboveground tissue and ‘Jinma’ responded similarly (Thurston et al., 2024). In general, heavy-metal concentrations and uptakes in aboveground plant tissue were greatest in a high- and lowest in a low-contaminated soil, likely due to the high-contaminated soil possessing the largest heavy-metal concentration. Industrial hemp’s capacity to sequester heavy metals in its aboveground plant tissues has important implications for belowground hemp response to heavy-metal-contaminated soil, because before being translocated to aboveground tissues, heavy metals had to be taken up by roots first.

Thurston et al. (2024) 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 largest in the 2% (v/v) and smallest in the 10% (v/v) biochar rates (Thurston et al., 2024). Thurston et al. (2024) attributed the results to the particle size of the biochar pieces and the biochar's subsequent exposed surface area, as well as the strength of the heavy-metal-concentration gradient that results from adding biochar to contaminated soil. Biochar is a highly variable substance, where particle size and source material can greatly impact biochar's behavior in the soil and biochar's ability to adsorb cations.

Thurston et al. (2024) also converted biochar-heavy-metal concentrations to uptakes in order to assess the removal of plant-available heavy metals. Results from heavy-metal uptakes showed that Cd and Zn uptakes differed only among biochar rates, resulting in an equivalent biochar uptake of 21.6 kg Cd ha^{-1} and 1440 kg Zn ha^{-1} from 90 days of exposure to contaminated soil (Thurston et al., 2024). Biochar-Pb uptake differed among biochar (BC) rate-cultivar combinations, with 10% BC resulting in ~ 990 kg Pb ha^{-1} with 'Carmagnola' and ~ 1300 kg Pb ha^{-1} with 'Jinma' (Thurston et al., 2024). Results from Thurston et al. (2024) showed that biochar may be a beneficial soil amendment to increase remediation of heavy-metal-contaminated soil.

Justification

There has been extensive research on phytoremediation and phytoremediation's viability as an *in-situ* remediation strategy, as well as research on biochar and on industrial hemp and other fiber crops as phytoremediators. However, there has been little research on the interactions between biochar and phytoremediation, more specifically the interactions between industrial hemp and biochar to help remove heavy metals from heavy-metal-contaminated soil.

Objective and Testable Hypotheses

The specific 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.

It was hypothesized that i) ‘Carmagnola’ and ‘Jinma’ cultivars will not differ in their Cd, Pb, and Zn removal from the soil, ii) 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.

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 (Thurston, 2023). Specifically, after hemp was grown in contaminated soil for 90 days, Thurston (2023) 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 and whole-plant Cd, Pb, and Zn uptakes.

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 (Thurston, 2023).

Field estimates of the heavy metal concentrations were established using a field-portable x-ray fluorescence spectrometer 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}, 13000 mg Zn kg^{-1}, and 123 mg Cd kg^{-1}) level of contamination (Thurston, 2023).

As described by Thurston (2023), 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 hours, then crushed with a mortar and pestle. Sand, silt, and clay fractions were measured using a modified 12-hour hydrometer procedure (Gee and Or, 2002). Soil pH and electrical conductivity were measured potentiometrically in a 1:2 soil-water suspension (Sikora and Kissel, 2014; Wang et al., 2014). Soil organic matter concentration was measured by loss-on-ignition (Zhang and Wang, 2014). Total carbon and total nitrogen were measured by high-temperature combustion using a Variomax C/N analyzer

(Elementar Americas, Inc.). 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 (USEPA, 1996). Table 1 summarizes the initial soil property differences among the three soils used in the greenhouse study. Briefly, TR-Pb concentration in the high was 10.5 times greater than in the medium, which was 3.2 times greater than in the low soil (Table 1). Total-recoverable-Zn concentration in the high 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 was 3.1 times greater than in the medium, which was 7.5 times greater than in the low soil (Table 1).

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). 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 (Thurston, 2023).

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 (Kim et al., 2015; Antonangelo et al., 2019). 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 minutes in a circular motion to homogenize the soil-biochar mixture in a manner similar to incorporation by tillage. Nitrogen (249 kg N ha^{-1}) and phosphorus (P; 58.7 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 kg potassium (K) ha^{-1} in the form of potash (0-0-60) were added to the low, medium, and high-contamination soils, respectively. Fertilizer was added to the soil at the same time biochar was added to the soil (Thurston, 2023). Hemp seeds were initially germinated in potting soil (Thurston, 2023). Once plants grew past the seed-leaf stage and entered the vegetative stage of growth, seedlings were transplanted into pots containing the contaminated soils. 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 (Thurston, 2023). Based on mean sand, clay, and SOM concentrations, gravimetric moisture content at field capacity was estimated using the Soil, Plant, Water, Atmosphere (SPAW) model (Saxton and Rawls, 2006). Bulk density was also estimated for each soil-biochar combination. A Theta Probe (SM150T, Delta-T Devices, Inc., Houston, TX) was used to measure volumetric soil water content in the top 6 cm of soil and was calibrated to determine the target water volume required to apply 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.

The greenhouse was set to 12-hour intervals between light and dark periods. Hemp plants were grown in the contaminated soil for a total of 90 days. Additional details regarding pot preparation, plant establishment, and watering were described in Thurston (2023).

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 hours at 65°C, then finely ground to pass a 1-mm mesh screen.

Using a modified EPA 3050B procedure (USEPA, 1996), 0.5 g of finely ground root material were digested using 5 mL of nitric acid for 24 hours and 3 mL of 30% hydrogen peroxide, 1 mL every 24 hours over a period of 72 hours, then heated and refluxed at 120ºC for 3 hours following the procedure for acid digestion of plant tissue by inductively coupled plasma-optical emissions spectrometry (ICP-OES; Huang and Schulte, 1985; Zarcinas et al., 1987). 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 (Thurston, 2023).

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 (2023), 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. (2024) 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; Kafle et al., 2022) to determine if hemp is a hyperaccumulator plant.

Data Analyses

Similar to Thurston et al. (2024), a three-factor analysis of variance (ANOVA) was conducted using the PROC GLIMMIX procedure in SAS (version 9.4, SAS Institute, Inc., Cary, NC), 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 (Thurston et al., 2024). The threshold $P \leq 0.05$ was used to judge significance. Treatment means were separated by least significant difference when appropriate.

Results and Discussion

Thurston et al. (2024) 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 belowground dry matter from Thurston et al. (2024), belowground heavy-metal uptakes were calculated and reported. In addition, belowground heavy-metal uptakes from the current study were added to aboveground heavy-metal uptakes reported by Thurston et al. (2024) to calculate total plant heavy-metal uptakes. Furthermore, aboveground heavy-metal uptakes from Thurston et al. (2024) were divided by belowground heavy-metal uptakes generated in the current study to calculate the TF.

Belowground Concentrations

After 90 days of hemp growth in contaminated soil, belowground Cd, Pb, and Zn concentrations all differed ($P < 0.01$) among soils (Table 2). However, neither hemp cultivar nor biochar rate affected ($P > 0.05$) belowground Cd, Pb, or Zn concentrations (Table 2). Belowground Cd concentration was numerically largest from the medium, which did not differ from the high, where both were at least 14.6 times greater than from the low soil, which had the lowest belowground Cd concentration (Table 3). Belowground Pb concentration was largest from the high, which was 13.1 times greater than from the medium, which was 3.0 times greater than from the low soil, which had the lowest belowground Pb concentration (Table 3). Similar to Pb, belowground Zn concentration was largest from the high, which was 4.3 times greater than from the medium, which was 5.0 times greater than from the low soil, which had the lowest belowground Zn concentration (Table 3). With a well-established, initial, heavy-metal-concentration gradient among the low, medium, and high 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 among the three soils were reported by Thurston et al. (2024), with the exception that both Pb and Zn concentrations differed between cultivars among soils, Pb concentration also differed among biochar rates within soils, and Cd concentrations differed among cultivar-biochar rate-soil combinations.

Linger et al. (2005) reported that hemp roots were tolerant of Cd present in the soil, accumulating to 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 (Linger et al., 2005). 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} (Linger et al., 2005), which were similar to the Cd concentrations in the low and medium soils used in the current study. Linger et al. (2005) concluded that hemp roots have shown 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 (Linger et al., 2005).

Stonehouse et al. (2020) 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 µM Se. In contrast to the current study, Stonehouse et al. (2020) reported greater Se concentrations in the aboveground tissue and seeds and significantly lower Se concentrations in the root tissue (Stonehouse et al., 2020).

Xu et al. (2021) investigated Pb accumulation and distribution in various organs of industrial hemp. Results showed that increasing soil-Pb concentration corresponded to an increase in Pb concentration in the plant (Xu et al., 2021). Tissue-Pb concentration was 2 to 7 times greater in roots than in aboveground tissue and 6 to 25 times greater than in the seeds (Xu et al., 2021). Xu et al. (2021) 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.

Belowground Uptakes

After 90 days of hemp growth in contaminated soil, similar to belowground concentrations, belowground Cd, Pb, and Zn uptakes all differed ($P < 0.01$) among soils, but were unaffected ($P > 0.05$) by hemp cultivar or biochar rate (Table 2). Belowground Cd uptakes were numerically largest from the medium, which did not differ from the high, where both were at least 16.3 times greater than from the low soil, which had the lowest belowground Cd uptake (Table 3). Belowground Pb uptakes were largest from the high, which was 13.6 times greater than from the medium, which was 2.8 times greater than from the low soil, which had the lowest belowground Pb uptake (Table 3). Belowground Zn uptake was largest from the high, which was 4.7 times greater than from the medium, which was 4.7 times greater than from the low soil, which had the lowest belowground Zn uptake (Table 3).

Linger et al. (2005) reported that industrial hemp accumulated, on average, 832 μg 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 (Linger et al., 2005).

Candito et al. (2004) reported heavy-metal uptakes for Cd, Pb, and thallium (Tl) in 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}). Roots accumulated 2.63 and 1.69 g ha^{-1} year^{-1} of Cd, 1.25and 1.60 g ha^{-1} year^{-1} of Zn, and 2.68 and 3.29 g ha^{-1} year^{-1} of Tl from the respective contaminated soils. Similar to the current study, Candito et al. (2004) was one of the few studies that reported metal uptakes in addition to tissue concentrations.

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 ($P < 0.01$) among soils, but were unaffected ($P > 0.05$) by hemp cultivar or biochar rate, while total plant Zn uptake differed ($P = 0.02$) among soil-biochar rate combinations and was also unaffected ($P > 0.05$) by hemp cultivar (Table 2). Total plant Cd uptake was numerically largest from the medium, which did not differ from the high, where both were at least 14.5 times greater than from the low soil, which had the lowest total plant Cd uptake (Table 3). Total plant Pb uptake was largest from the high, which was 10.7 times greater than from the medium, which was 2.7 times greater than from the low soil, which had the lowest total Pb uptake (Table 3).

Total plant Zn uptake was numerically largest from the high soil with 10% biochar (0.28 mg cm^{-2}), which did not differ from the high soil with 5% biochar, and was smallest from the low soil with 0, 2, 5, and 10% biochar (< 0.02 mg cm^{-2}), which did not differ (Figure 2). Consequently, within the low soil, total plant Zn uptake did not differ ($P > 0.05$) among biochar rates (Figure 2). Within the medium soil, total plant Zn uptake was numerically largest with 2% biochar rate and numerically smallest with 10% biochar, while the 0 and 5% biochar rates were intermediate (Figure 2). Within the high soil, total plant Zn uptake did not differ ($P > 0.05$) between the 5 and 10% biochar rates, but total plant Zn uptake in the 0 and 2% biochar rates, which did not differ, were less than the total plant Zn uptake in the 10% biochar rate, but also similar to that in the 5% biochar (Figure 2).

Within each individual biochar rate, total plant Zn uptake in all three soils differed among one another, where the high was always significantly largest, the medium was always intermediate, and the low soil was always significantly smallest (Figure 2). Total plant Zn uptake in the unamended control for the low and medium soils did not differ ($P > 0.05$) from any of the three respective biochar-amended treatments (Figure 2). However, total plant Zn uptake in the unamended control for the high soil was similar to that with 2 and 5% biochar rates but was lower than with 10% biochar rate (Figure 2). No relevant studies exist that quantified 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.

Translocation Factor

After 90 days of hemp growth in contaminated soil, similar to belowground concentrations and uptakes, the Cd, Pb, and Zn TFs differed ($P < 0.04$) among soils, but were unaffected ($P > 0.05$) by hemp cultivar and biochar rate (Table 2). As the ratio of above- to belowground heavy-metal uptake, the TF for Cd was numerically largest for the low, which did not differ from high, and was numerically lowest from the medium, which also did not differ from the high soil, thus the TF for Cd was 1.8 times greater for the low than for the medium soil (Table 3). Similar to Cd, the TF for Pb was also numerically largest from the low, but did not differ from the medium, where both were at least 6.3 times greater than from the high soil, which had the lowest TF for Pb (Table 3). Similar to Pb and Cd, the TF for Zn was also largest from the low, did not differ from the medium, and where both were at least 2.3 times greater than from the high 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 soil and numerically smallest TF in the medium or high soil is that only so much of the heavy metals are available to the plant for translocation and uptake, where the more severely contaminated soils may have had a lower concentration of bioavailable heavy metals for the hemp to absorb and translocate. 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 (CO_2) and organic matter decomposition, may have been killed off, thus hindering translocation (Wang and Hu, 2023).

A plant is classified as a hyperaccumulator if it has a TF > 1 (Usman et al., 2019), Among the three heavy metals tested in this study, only the TF for Zn was greater than 1 from the low and medium soils. Consequently, hemp may be a potential hyperaccumulator for Zn, but 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.

Implications

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 on phytoremediation’s viability in severe cases of environmental contamination where in-situ remediation is utilized. Results from this study also suggest that the incorporation of biochar into contaminated soils may enhance industrial hemp’s uptake of Zn, but did not significantly improve Pb or Cd uptake.

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 from the medium and high soils than from the low 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. 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 among soils and were unaffected by biochar rate or hemp cultivar. Total plant Cd, Pb, and Zn uptakes also differed among soils and were unaffected by hemp cultivar. However, total plant Zn uptake differed within each biochar rate among soil-contamination levels, where the high soil was always significantly largest, the medium soil was always intermediate, and the low soil was always significantly smallest. Total plant Zn uptake in the unamended control in the low soil did not differ among biochar rates. Total plant Zn uptake for the high soil was lower in the 0 and 2% treatments than the 10% treatment. Results from this study suggest biochar positively impacts Zn uptake in more severely contaminated soils, but not Cd or Pb or in less contaminated soils. Results were similar to the aboveground tissue responses recently reported by Thurston et al.

(2024) as part of the same study, with the exception that Pb and Zn concentrations differed between hemp cultivar among soils, Pb concentrations differed between biochar rates within soils, and Cd concentrations differed among cultivar-biochar-soil combinations.

This research contributed to the greater field of environmental restoration and phytoremediation in that 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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TABLE 1 Summary of initial soil properties differences among the three soils from the Tar Creek Superfund site used in the greenhouse study [adapted from Thurston (2023)].

	Soil-contamination level			
Soil property	**Low**	**Medium**	**High**	***P***
Total recoverable elements (mg kg^{-1})				
Pb	308 c	978 b	10251 a	< 0.01
Zn	763 c	5353 b	21179 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 $P < 0.05$

TABLE 2 Analysis of variance summary of 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 factor (TF) for hemp grown in the greenhouse in contaminated soil from the Tar Creek Superfund site.

	Source of variation						
Plant property	**Soil**	**Cult**	**Soil x Cult**	**BC**	**Soil x BC**	**Cult x BC**	**Soil x Cult x 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 factor (TF) for hemp grown in the greenhouse in contaminated soil from the Tar Creek Superfund site.

	Soil-contamination level		
Plant property	**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 $P < 0.05$

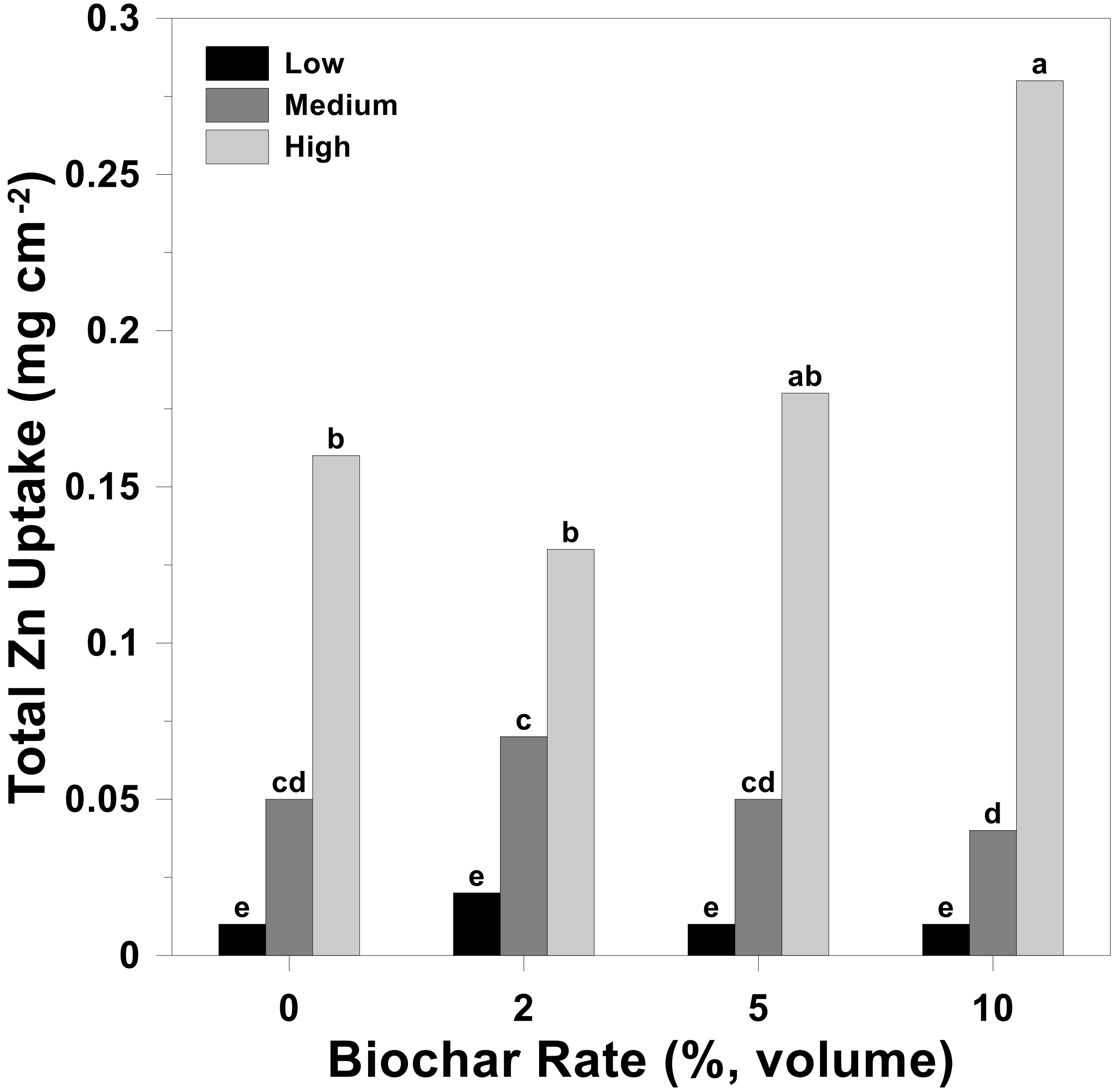

FIGURE 1 Total zinc (Zn) uptake by industrial hemp among biochar rates (i.e., 0, 2, 5, and 10% by volume) and across three levels of contaminated soil from the Tar Creek Superfund site. Different letters atop bars indicate a significant difference at $P < 0.05$.