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Bioaccumulation of heavy metals in hot pepper grown in biochar and animal manure-amended soil

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ABSTRACT

Heavy metals are non-biodegradable and persist in the environment longer, contaminating the food chain. Phytoremediation has emerged as a reliable, eco-friendly, cost-effective and sustainable approach that utilizes plants to remediate soil or water contaminants. The study assesses the ability of hot pepper (*Capsicum annuum* cv. Georgia Flame) grown under seven soil amendments- vermicompost, inorganic fertilizer, sewage sludge, organic fertilizer, chicken manure, horse manure and biochar on heavy metals remediation. The research was conducted in an RCBD split-plot design and heavy metal concentrations were analyzed using an inductively coupled plasma-optical emission Spectrometry (ICP-OES). The results revealed that the average concentration of K (105.69 $mgKg^{-1}$) and Mg (12.8 $mgKg^{-1}$) were the highest in hot pepper fruits. However, Zn (0.16 $mgKg^{-1}$) was found in the maximum amount among all heavy metals, whereas Cr (0.002 $mgKg^{-1}$) was found in the minimum amount. Biochar with amendments enhanced the bioaccumulation of heavy metals, suggesting it as an essential amendment for heavy metal remediation. The average bioaccumulation factor (BAF) of Pb (0.72), Mn (0.07) and Cr (0.16) were less than 1, indicating no effect on soil metal remediation. Conversely, Cd (5.69), Mg (22.76), Zn (10.07), Ni (117.2) and Cu (46.37) exhibited a BAF value exceeding 1, suggesting that hot peppers have the potential to remediate these particular soil metals. Though Cd, Ni, Mg, Cu and Zn showed a BAF value greater than 1, their accumulation in the fruits remained within the permissible limit. Hence, hot pepper is considered a potential crop for remediating heavy metals.

1. Introduction

In the present world, industrial and agricultural activities have increased with the rapid population growth. This leads to heavy metal contamination in soil, water and plants, posing a significant risk to plant vitality, soil health and human well-being [1]. Heavy metals persist in the environment for an extended period as they are non-biodegradable. This results in the accumulation of heavy metals in living organisms through biomagnification [2]. Heavy metals are elements with higher atomic density, more than 4 g/cm^3 [3]. They are classified as essential and non-essential elements. Essentials include boron (B), copper (Cu), iron (Fe), molybdenum (Mo), nickel (Ni), and zinc (Zn), while non-essential includes Cadmium (Cd), Silver (Hg) and Lead (Pb) [3]. Essential elements in trace amounts help in the proper growth and development of plants. Non-essential elements are phytotoxic and have significant health and environmental risks. Heavy metals like Cd, Pb, Cr, As, Hg, Ni, Cu, and Zn contaminate agricultural soil if present at low contamination levels. This induces toxicity in plants, which affects their growth and development [4,5]. This contamination impairs soil fertility through the disruption of microbial activity, which plays a vital role in the maintenance of healthy soil [1].

The impacts of heavy metals are seen in both their deficiency and excess in plants. This impact can be physiological disruption or can impair critical processes such as nitrogen uptake and photosynthesis [6]. Heavy metals like Pb, Cd, Ni, and Cu accumulate in the food chain, transferring from one trophic level to another. This leads to serious health problems like cancer and cardiovascular diseases in human health [7]. Additionally, they pollute water resources through seepage [8]. Over time, these metals accumulate in plants and contaminate the food chain. Hence, it is very crucial to regularly check heavy metals in foods for public safety, especially for globally consumed crops like hot pepper [7].

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Among the traditional methods, physical and chemical approaches for remediating heavy metals are expensive and inefficient. These methods generate secondary waste, which is also challenging to manage and control [9]. Hence, phytoremediation offers an environmentally friendly, cost-effective, and sustainable alternative that uses plants to remediate heavy metal contaminants [10]. The process involves the absorption of contaminants by plant roots and transforming them into less toxic forms. Some plants immobilize heavy metals after absorption and control the level of contamination [11]. The plants help to keep soil health intact and maintain biodiversity. This makes phytoremediation applicable in urban areas and agricultural land [12–14].

Around 400 species of plant from 45 families like Fabaceae, Euphorbiaceae, Asteraceae, Lamiaceae and Brassicaceae are identified as having hyperaccumulation ability, acting as potential for effective remediation in varied ecological settings [15]. Many plants possess the capability to remediate pollutants influenced by environmental factors such as light intensity, temperature, precipitation and soil properties [16]. Both woody and herbaceous plants vary in heavy metal accumulation and transgenic plants further enhance metal uptake and detoxification, increasing the scope of phytoremediation [10,17]. While crops from different families have been studied for their phytoremediation ability, few studies have been undertaken on the possible application of hot pepper in phytoremediation [18]. Hot pepper (*Capsicum spp*.) is known for its culinary and medicinal benefits and belongs to the Solanaceae family. It is best used for its pungent flavour and nutritional value. Studies have shown that Hot pepper accumulates heavy metals like Cd, Co, Cr, Cu, Pb, Fe, Mn, and Zn, making it potential for phytoremediation [19]. A study by Antonious and Kochhar [20] suggested that hot pepper, grown as a potential phytoremediation crop, performed better by incorporating soil amendments like biochar, vermicompost, horse manure and chicken manure in the remediation process. The phytoremediation potential of plants is significantly enhanced by soil amendments that improve soil properties like pH, organic matter content and cation exchange capacity, which play an important role in metal solubility and its uptake [21].

Very little research has been conducted to evaluate hot pepper phytoremediation ability with contradictory results. A study by Antonious (2016) [22] on *Capsicum annum* showed the phyto accumulation properties of heavy metals such as Cu, Zn and Mo. Contrary to this finding, Lidikova et al. (2021) [23] found that no accumulation of heavy metals like Mn, Zn, Cr, Cu, Ni, Cd, Pb and Hg occurred in *capsicum annum* when grown in heavy metals contaminated soil. Hence, these contradictory findings do not provide the correct information about the phytoremediation potential of hot peppers. As it is considered an important part of the global diet, it is crucial to assess its phytoremediation ability. Also, it is necessary to understand the effects of different soil amendments on heavy metal accumulation and their impact on human health. Hence, the primary objective of this study is to conceptualize phytoremediation by investigating the potential use of hot pepper in the remediation of heavy metals under different soil management practices. The concentration of heavy metals in soil, amendments and hot pepper fruits can be quantified using ICP-OES and compared with the permissible limit set by FAO/WHO/US-EPA to ensure the safety level of hot pepper consumption. According to current research into the subject, hot pepper consumption seems harmless as far as exposure to heavy metals is concerned. Most studies reflect that heavy metals in the hot pepper were below their permissible limit [24,25]. However, monitoring hot peppers should be done when grown with organic and inorganic soil amendments to quantify heavy metal accumulations in the edible parts, as these amendments also include heavy metals and show a significant effect on metal uptake [22]. Testing and monitoring for heavy metals should be done stringently in crops before consumption to ensure food safety [26].

2. Materials and methods

2.1. Experimental design

A field experiment was conducted using an RCBD split-plot design with three replications at the University of Kentucky farm. Six soil amendments (vermicompost, inorganic fertilizer, sewage sludge, organic fertilizer, chicken manure and horse manure) and native soil were applied as soil treatments. Each treatment was mixed with biochar to analyze if biochar had any effect on heavy metal uptake. Vermicompost was sourced from Wiggle Worm Soil Builder, Avenue Union Grove, WI, USA. Sewage sludge was from the Metropolitan sewer district, Louisville, KY, USA, chicken manure from Alltech Chicken Facility, Lexington, KY, USA and horse manure from HighPointe farm and Training Center La Grange, KY, USA. Inorganic fertilizer, organic fertilizer and biochar were acquired from Lowe's of Frankfort, KY, USA The native soil (No mulch, NM) was used as a control treatment characterized as Bluegrass Maury Silty loam consisting of 6 % and 56 % silt and 38 % clay. The experimental plot had no cropping history and was left fallow for many years. (Table 2) shows the properties and the elements composition of each soil amendment.

2.2. Cultivation practices

The guideline for the cultivation of the hot pepper was adopted from the Vegetable Production Guide for Commercial Growers [27]. Soil amendments were applied and tilled up to the depth of 15 cm on the topsoil. Each soil amendment was measured and applied to specific plot in order to supply 5 % of N and avoid variability in N content in each treatment (Table 1). Hot pepper seedlings (*Capsicum annuum* cv. Georgia Flame) were transplanted in the prepared plots at 3–5 cm depth at a spacing of 2 ft. between plants and rows. Uniform irrigation was supplied using a drip irrigation system. Three applications of insecticide Brigade 2 E of FMC Corporation were applied at the rate of 0.46 Lha-1 at 15-day intervals to control insect infestation.

2.3. Soil sampling and data collection

Soil samples from each experimental plot were taken from 15 cm topsoil using a soil core sampler equipped with a plastic liner before plantation and after the harvest. Three plants were selected randomly for the samples and their fruits were collected from each plot. The fresh fruits were washed with deionized water to eliminate external contaminants. Fruit samples underwent oven drying at 65 °C for 48 h. Both soil and fruit-dried samples were ground using a mortar and pestle and passed through a nonmetal sieve to achieve a particle size of 2 mm. Ground samples were re-dried to achieve constant weight and packed in plastic bags for analysis. The metal analysis was performed using ICP-OES at the Chemistry and Environmental Toxicology lab at Kentucky State University. A study by Da Cruz et al. (2022) [28] validated that ICP-OES allows the accurate quantification of elements and evaluates

Table 1
Application rate of soil amendments in the research plots (University of Kentucky South Farm, Lexington, KY).

Soil Amendments	Application Rate (gm^{-2})
Sewage Sludge (SS)	224.54
Vermicompost (Vermi)	1120.52
Chicken manure (CM)	487.23
Horse manure (HM)	350.27
Inorganic Fertilizer (Inorganic- Fert.)	24.35
Organic fertilizer (Organic-Fert.)	2240.00
Biochar	500.00

the effectiveness of the bioremediation process in complex matrices, including plant tissues and soil. A study accomplished by Senila et al. (2014) [29] demonstrated the accuracy and reliability of ICP-OES for determining essential and non-essential elements in medicinal plants and their extracts.

2.4. Metal analysis

For the digestion of the plant and soil samples, 1 g of each dry sample was kept in a glass flask, 10 ml of concentrated nitric acid (HNO_3) was added and the mixture was left overnight. The mixture was then heated on hot plates at 125 °C for 4 h. The digested mixture was diluted with deionized water to a volume of 50 ml and filtered using Whatman filter paper No.1. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) in standard mode following SW-846. EPA 6010 B method was used for quantifying the metal concentration [30]. The calibration of the equipment was prepared with multielement standards (Tru Q 500 ml, PerkinElmer) and working dilution was made with concentrations of 0,1, 10 and 100 ppm. Calibration curves were generated referencing working dilution maintained below the 10 % margin. Three replicates of each sample were tested to reduce the error % and arithmetic means were calculated with a mean difference below 5 %. As per the PerkinElmer company, Instrumental Detection Limits (IDL) for the ICP-OES used were 0.0027 mg kg^{-1} for Cd, 0.0054 mg kg^{-1} for Cu, 0.0002 mg kg^{-1} for Cr, 0.00004 mg kg^{-1} for Mg, 0.048 mg kg^{-1} for Ni, 0.0001 mg kg^{-1} for Mn, 0.042 mg kg^{-1} for Pb, 0.001 mg kg^{-1} for K and 0.0018 mg kg^{-1} for Zn.

2.5. Bioaccumulation Factor (BAF)

To determine the bioaccumulation factor of heavy metals, the following formula by Ekere et al. (2020) [31] and Nepal et al. (2024) [32] was applied.

$$BAF = \frac{Total\ heavy\ metal\ concentration\ in\ fruit}{Total\ heavy\ metal\ concentration\ in\ soil}$$

Where BAF > 1 indicates high accumulation, 0.1 < BAF ≤ 1 indicates moderate accumulation and BAF ≤ 0.1 indicates low accumulation.

2.6. Statistical analysis

The statistical analysis was accomplished using R software version 4.3.2. The Shapiro-Wilk test was performed to observe if the data were normally distributed, followed by an analysis of variance (ANOVA) to observe any significant difference in metal accumulation due to different soil treatments.

3. Results

3.1. Properties of native soil and the amendments used

Soil characteristics varied significantly across different soil types (Table 2). Soil pH ranged from 5.7 in the SS to 7.6 in the biochar. Mg was highest in NM and lowest in organic manure. The concentrations of Cd, Cr, Ni, Pb, and Cu showed substantial variation within all treatments. Vermicompost showed the maximum Cu (3.69 ppm) concentration, whereas SS had the highest concentration of Zn (1.58 ppm) and Cr (0.355 ppm). For the biochar, the carbon concentration is mentioned as 50,623 ppm which is equivalent to 5.06 % C. And it had the highest electrical conductivity (410.33μS cm^{-1}), indicating a higher concentration of soluble salts. These results indicated a sufficiently distinct network. Also, soil amendments show varied concentrations of heavy metals, with inorganic fertilizer and biochar influencing different soil parameters.

Table 2
Properties of the soil amendments and native soil (no mulch) used in the research field.

Soil Characteristics	Native Soil (NM)	CM	HM	Inorganic Fertilizer	Organic Fertilizer	SS	Vermicompost	Biochar
KCl Soil pH	6.2 ± 0.05 e	7.3 ± 0.06 b	7.3 ± 0.01 b	6.9 ± 0.005c	5.8 ± 0.059 f	5.7 ± 0.01 f	6.5 ± 0.001 d	7.6 ± 0.02 a
Soil-Water pH	5.63 ± 0.02 b	6.057 ± 0.26 a	5.64 ± 0.09 b	6.15 ± 0.1 a	5.78 ± 0.13 b	5.67 ± 0.1 b	5.71 ± 0.13 b	6.35 ± 0.002c
P, ppm	95.83 ± 10.2 a	89.33 ± 6.64 a	116 ± 50 a	121.3 ± 47.9 a	94.83 ± 10.32 a	100.33 ± 10.69 a	87.67 ± 9.46 a	42.79 ± 0.28 b
K, ppm	336.17 ± 12.06 d	100.115 ± 1.39 d	56.782 ± 0.368 e	1024.03 ± 0.387 ab	383.386 ± 0.386 b	12.771 ± 0.89 g	141.308 ± 0.25c	22.387 ± 4.079 f
C, ppm	1091.7 ± 44.9 cd	1160.8 ± 51 b	1067.2 ± 12.2 d	1155.3 ± 28.1 bc	1112.8 ± 43.9 bcd	1050 ± 28.2 d	1230.2 ± 27.9 a	50623 ± 5.53 a
Mg, ppm	130.33 ± 2.84c	24.278 ± 0.200c	104.34 ± 0.430 a	13.356 ± 0.126 e	7.532 ± 0.333 f	18.094 ± 0.228 d	36.308 ± 0.347 b	13.396 ± 1.238 e
Zn, ppm	6.65 ± 0.82 bc	0.499 ± 0.013 cd	0.543 ± 0.052c	0.107 ± 0.003 d	0.366 ± 0.029 cd	1.581 ± 0.085 a	1.166 ± 0.331 b	0.292 ± 0.011 cd
Cd, ppm	0.07 ± 0.00 ab	0.0491 ± 0.002 a	0.047 ± 0.010 a	0.0123 ± 0.0005 b	0.046 ± 0.005 a	0.041 ± 0.002 a	0.063 ± 0.020 a	0.042 ± 0.003 a
Cr, ppm	0.04 ± 0 a	0.002 ± 0.000 d	0.039 ± 0.008c	0.284 ± 0.003 b	0.029 ± 0.003c	0.355 ± 0.006 a	0.0332 ± 0.006c	0.032 ± 0.010c
Ni, ppm	0.46 ± 0.09 a	0.045 ± 0.003c	0.069 ± 0.011 b	0.007 ± 0.000 d	0.028 ± 0.003 cd	0.100 ± 0.009 a	0.012 ± 0.001 d	0.022 ± 0.008 d
Pb, ppm	9.183 ± 1.17 ab	0.019 ± 0.000c	0.012 ± 0.002c	0.034 ± 0.010 b	0.019 ± 0.002c	0.163 ± 0.007 a	0.006 ± 0.000c	0.012 ± 0.001c
Cu, ppm	3.22 ± 0.11c	0.027 ± 0.020 d	0.156 ± 0.027c	0.042 ± 0.001 d	0.004 ± 0.000 d	0.754 ± 0.0195 b	3.693 ± 0.076 a	0.255 ± 0.026c
EC, μS cm^{-1}	101.53 ± 6.05c	5.18 ± 0.233 e	2.66 ± 0.171 e	134.76 ± 0.145 b	27.9 ± 0.115 d	3.78 ± 0.008 e	21.03 ± 0.066 d	410.33 ± 5.92 a
N-NO_3, ppm	26.67 ± 4.51c	18.33 ± 9.24c	25 ± 6 b	79.33 ± 8.39 a	32.67 ± 8.5 bc	20 ± 3.46c	37.33 ± 11.85 b	92.62 ± 0.02 d
N-NH_4, ppm	5.67 ± 3.79 b	66.7 ± 61.4 a	3.3 ± 0.58 b	50.7 ± 56.6 ab	47 ± 23.5 ab	29.7 ± 17.9 ab	3.667 ± 0.58 b	35.82 ± 0.215 d

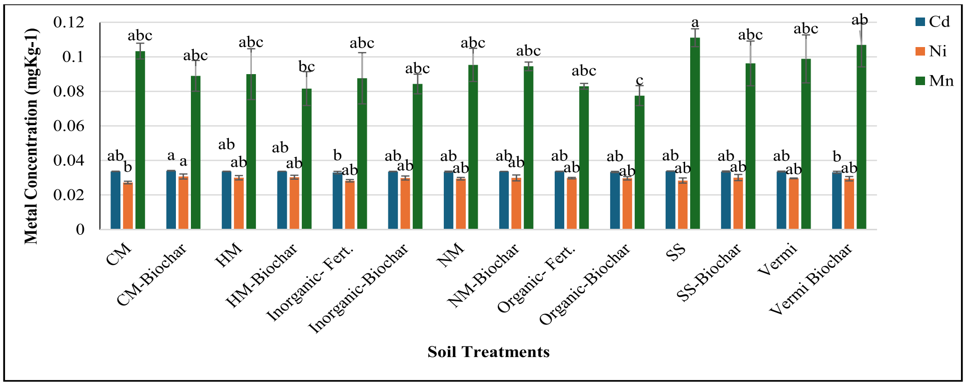

Fig. 1. Heavy metals concentration (Cd, Ni and Mn) on hot pepper fruits grown under different soil amendments. Bars in the figure with a different letter(s) within each bar show the significant difference at ($p \leq 0.05$) determined using Tukey's HSD test.

3.2. Metal concentration in fruits

The results showed that the concentration of Cd, Ni, Mn, Zn and Mg in fruits differed significantly ($P < 0.05$) among all the soil treatments, demonstrating the influence of soil amendment on the absorption of metals. In contrast, Pb, Cr, Cu and K did not differ significantly, suggesting that metals were either uniformly distributed across treatments or metals uptake was not affected by soil amendments (Figs. 1, 2, 3). The average concentration of metals detected in the fruits was found in increasing order as Cr < Pb < Ni < Cd < Mn < Cu < Zn < Mg < K among all the amended soils. The average concentration of K (105.689 ppm) was maximum, whereas Cr (0.002 ppm) had minimum concentration in the fruit part. The concentration of Pb and Zn was highest in soil amended by inorganic fertilizer. Similarly, Cd and Ni concentration was observed to be the maximum in CM-Biochar amended soil. Mn was found to be maximum in SS. Concentration of Cr was maximum in HM-Biochar. SS- Biochar amended soil showed the maximum concentration of Mg. Cu was highest in organic fertilizer-amended soil. K concentration was found to be maximum in the fruits grown in CM-Biochar, and Zn was maximum in Inorganic-Biochar amended soil.

3.3. Metal concentration in the soil after plantation

The results showed that Pb, Cd, Mn, Mg, Ni, Cu, Cr, and Zn concentrations in soils with different amendments differed significantly ($P < 0.05$). However, there was no significant difference in K concentrations (Figs. 4, 5, 6). The mean metal contents in the modified soils increased in order as: Ni < Cd < Cu < Cr < Zn < Pb < K < Mg < Mn. Mn had the highest mean concentration (1.469 ppm), while Ni had the lowest (0.001 ppm) among all amendments. SS-Biochar amended soil showed the highest concentration of Pb and Zn, whereas inorganic fertilizer resulted the maximum concentrations of Cd and Mg. The highest Ni concentration was recorded in Vermi-Biochar amended soil. Organic Biochar exhibited the highest Mn, Cr, and K levels, while HM-Biochar contained the maximum Cu concentration.

3.4. BAF values in hot pepper

The bioaccumulation factor (BAF) values of Cd, Mg and Mn significantly differed among all the amended soil. In contrast, the Pb, Cr, Zn, Ni, Cu, and K did not differ significantly (Figs. 7,8,9). BAF values of Cd, Mg, Zn, Ni, Cu, and K were greater than 1 (BAF > 1) indicating metal accmulation, whereas Pb, Mn and Cr had the lower BAF values (BAF < 1) showing no accumulation (only absorption).

Soil treated with HM-Biochar had the highest BAF for Cr. Cu had the highest BAF in SS-Biochar-added soil, while Zn had the highest in Inorganic biochar-amended soil. The highest BAF for Pb was observed in native soil. Cd, Mg, and K had the greatest BAF values in CM-Biochar-treated soil, while Ni had the highest in soil amended with Inorganic Biochar. Similarly, Vermi-Biochar-treated soil had the highest BAF for Mn.

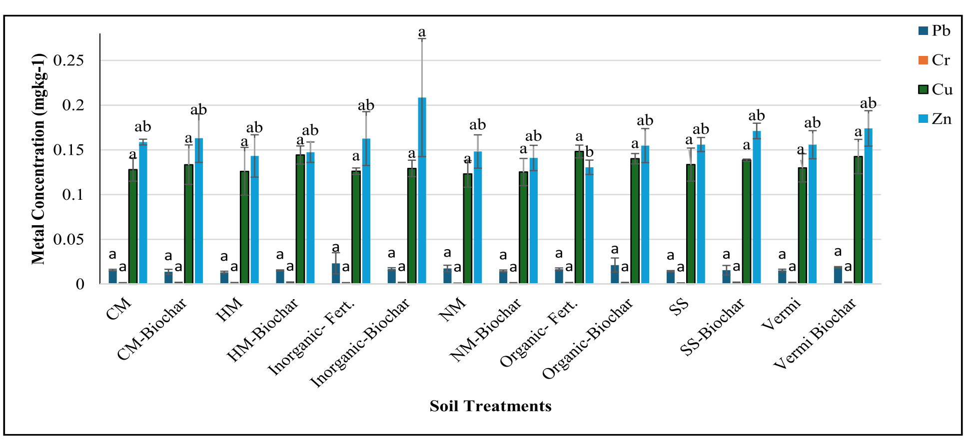

Fig. 2. Heavy metals concentration (Pb, Cr, Cu and Zn) in hot pepper fruits grown under different soil amendments. Bars in the figure with a different letter(s) within each bar show the significant difference at ($p \leq 0.05$) determined using Tukey's HSD test.

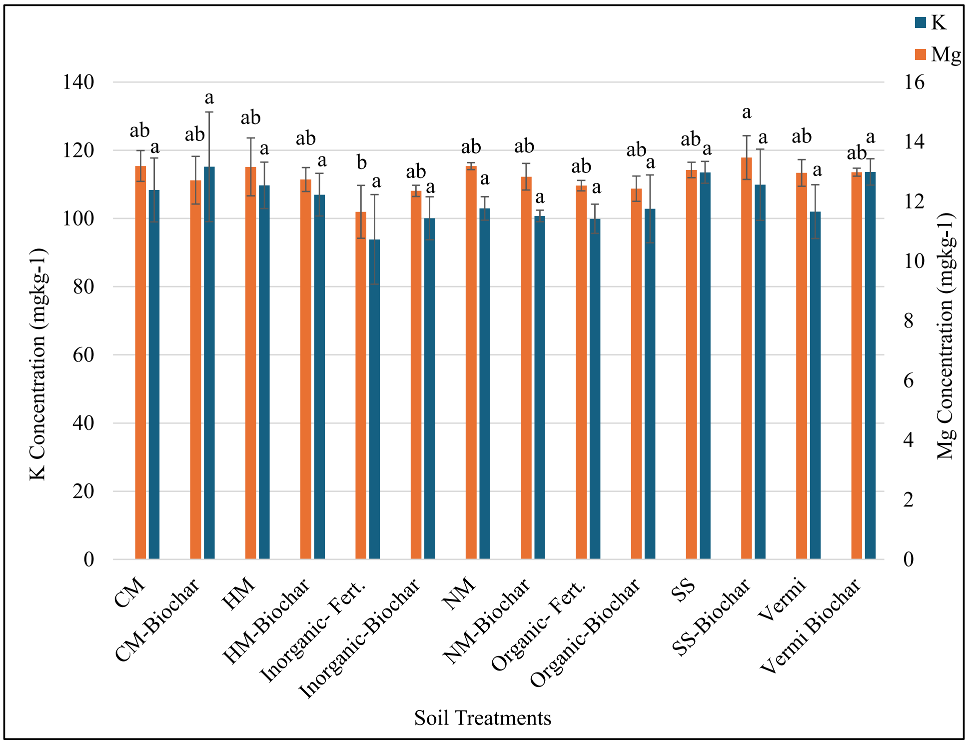

Fig. 3. Magnesium (Mg) and Potassium (K) concentration in hot pepper fruits grown under different soil amendments. Bars in the figure with a different letter(s) within each bar show the significant difference at ($p \leq 0.05$) determined using Tukey's HSD test.

4. Discussion

4.1. Metal concentration in soil and fruit

To evaluate the bioaccumulation capacity of hot pepper for heavy metals, it is essential to know the heavy metal concentration in different soil amendments and their effect. These inputs serve as indirect sources of contamination. Despite detectable concentrations of heavy metals in certain amendments, a consistent pattern between fruit accumulation and metal content in the amendments was not observed. This suggests that soil chemistry, pH, bioavailability and plant-specific uptake mechanisms might play the decisive role over metal concentration alone [36,37]. Few studies have suggested that total metal concentrations in amendments or soil are not always predictive of uptake in plants due to the complex microbial interaction, chelation process and organic matter content, which impact the mobility of metals [38,39].

Toxic metals like Pb and Cr absorption in hot pepper fruits was minimal, possibly due to the higher accumulation of such toxic metals in the roots compared to the shoot parts. A similar result was observed in the study conducted by Amin et al. (2019) [40] where the phytoaccumulation of Cr was observed to accumulate higher in the root zone than in the shoot zone. The root exudates of the plants tend to show selective uptake mechanisms where they filter the toxic metals into the membranes of the exudates and avoid translocation to the other aerial parts [41].

Pb absorption and accmulation of Zn were highest in fruits grown in soil amended with inorganic fertilizer. The increased availability of Pb in soil treated with inorganic fertilizer is associated with soil pH because inorganic fertilizers contain nitrogen in the form of ammonium, which acidifies the soil during nitrification. In comparison to other soil amendments, acidic soil improves Pb mobility, allowing plants to absorb more of it [42].

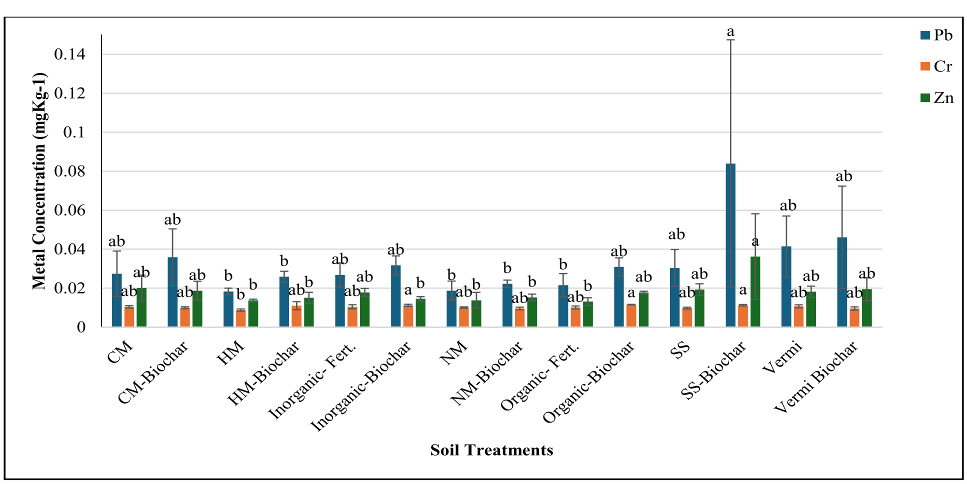

Fig. 4. Heavy metals concentration (Pb, Cr and Zn) in soil amended with different soil amendments. Bars in the figure with a different letter(s) within each bar show the significant difference at ($p \leq 0.05$) determined using Tukey's HSD test.

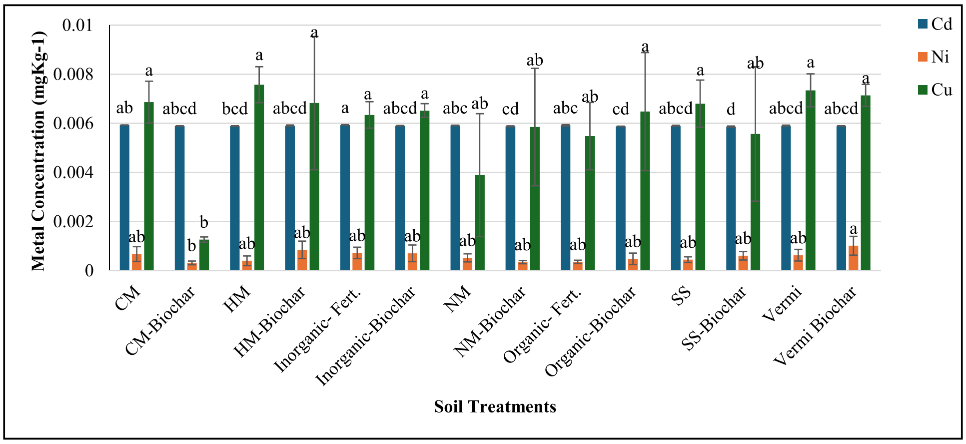

Fig. 5. Heavy metals concentration (Cd, Ni and Cu) in soil amended with different soil amendments. Bars in the figure with a different letter(s) within each bar show the significant difference at (p ≤ 0.05) determined using Tukey's HSD test.

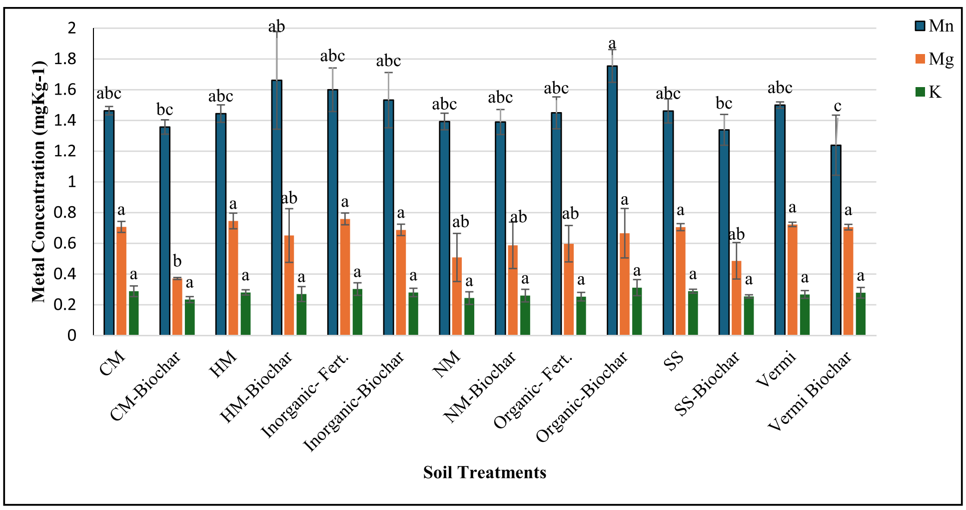

Fig. 6. Metals concentration (Mn, Mg and K) in soil amended with different soil amendments. Bars in the figure with a different letter(s) within each bar show the significant difference at (p ≤ 0.05) determined using Tukey's HSD test.

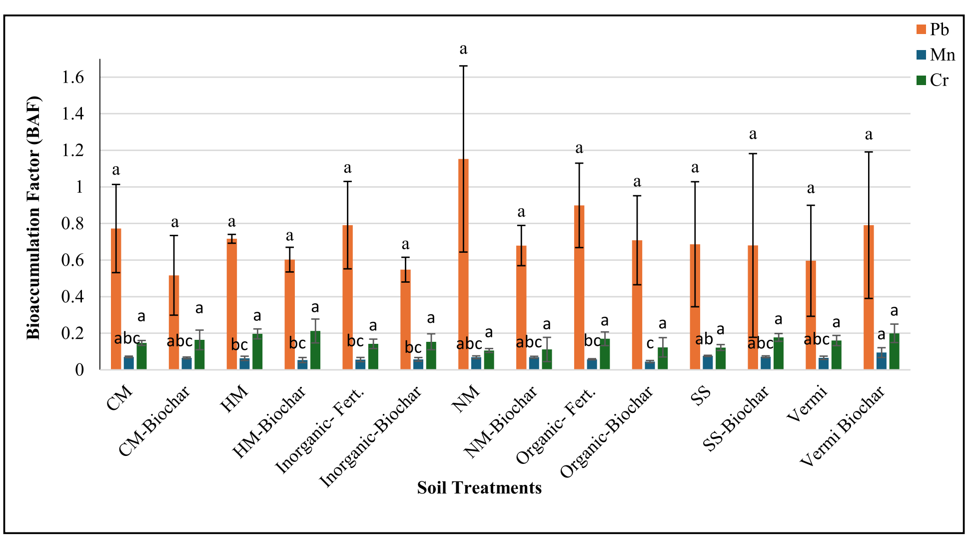

Fig. 7. BAF values of metals (Pb, Mn and Cr) in hot pepper fruits grown under different soil amendments. Bars in the figure with a different letter(s) within each bar show the significant difference at (p ≤ 0.05) determined using Tukey's HSD test.

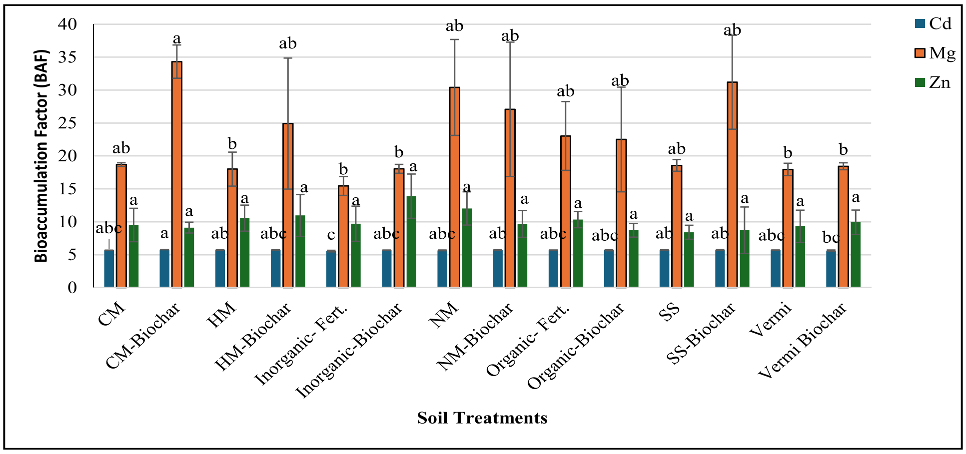

Fig. 8. BAF values of metals (Cd, Mg and Zn) in hot pepper fruits grown under different soil amendments. Bars in the figure with a different letter(s) within each bar show the significant difference at ($p \leq 0.05$) determined using Tukey's HSD test.

Nitrogen supports the uptake of Zn in inorganic amended soil [43]. Nie et al.(2017) [44] and Xue et al.(2012) [45] found a significant positive correlation between N and Zn concentration. Inorganic fertilizers show higher solubility and availability of heavy metals, enhancing more uptake by plants. Hence, the concentration of Pb and Zn was found to be maximum in fruits of hot peppers grown in inorganic fertilizer-amended soil [46].

The maximum accumulation of Cu was observed in soil amended with organic fertilizer. Organic matter boosts the activity of endophytic bacteria, which release enzymes such as phosphatase and dehydrogenase. These enzymes help to break down organic complexes, releasing Cu and increasing its bioavailability to plants [47].

In the study, biochar was amended with different Amendments like chicken manure, horse manure, sewage sludge, and inorganic fertilizers. It has been observed that most of the heavy metals showed their higher accumulation in biochar-amended soil. Though the mean accumulation of toxic metals like Cr, Cd and Ni was found to be low in hot pepper fruits, it has been observed that the accumulation of these heavy metals is higher in HM-biochar and CM-biochar, compared to other soil treatments.

Biochar, a porous material from biomass pyrolysis, is an effective sorbent for heavy metals, immobilizing them in soil through surface adsorption and chemical reactions. Its porous structure and high surface area reduce bioavailability to plants [48]. But when Biochar is amended with inorganic fertilizers, the acidification in the soil can release the complexes of the heavy metals, increasing their uptake by plants [49]. A similar case was observed with the organic matter, where the microbial activities enhanced by the organic matter, increased the solubility of the heavy metals and made them available for plants to uptake [50].

Mn absorption was found maximum in sewage sludge. Sewage sludge contains high amounts of organic carbon, nitrogen, phosphorous, and trace elements, including Mn [35]. During decomposition, the organic acid formed causes chelation of the Mn, resulting in its increased mobility and solubility in soil [51]. The enzymatic activity influenced by sewage sludge's long-term application enhanced the rhizosphere's phosphatase activity [50]. It also increased the urease activity (16–43 %) in the rhizosphere of Ransom soyabean. Hence, Park et al. (2011) [50] concludes that these activities directly influenced the remediation of heavy metals in soils.

Potassium is considered an essential macronutrient for the growth and development of the plant and influences various physiological activities like osmoregulation, enzyme activation, photosynthesis, etc. [52]. Hence, its maximum accumulation can be attributed to its significant role in plant metabolism and translocation within plant tissue. Maximum absorption of K might be due to different factors like soil moisture and soil temperature, as high soil moisture usually increases K availability to the plant roots and high temperature leads to high physiological and root activities, leading to maximum K uptake [53]. N, K, Mg and Ca greatly influence the pungency of the hot pepper due to the presence of Capsaicin. This might be another major reason for the high uptake of K and Mg in hot pepper fruits [54].

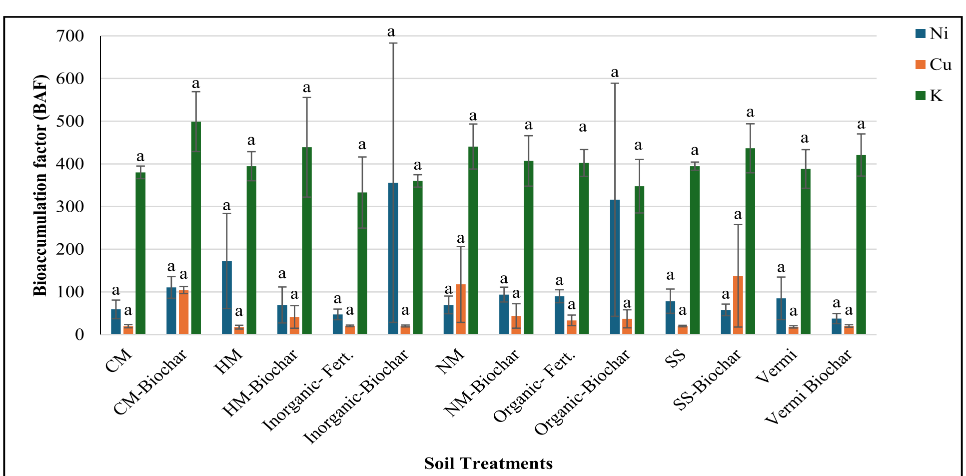

Fig. 9. BAF values of metals (Ni, Cu and K) in hot pepper fruits grown under different soil amendments. Bars in the figure with a different letter(s) within each bar show the significant difference at ($p \leq 0.05$) determined using Tukey's HSD test.

4.2. BAF values in hot pepper

High BAF values indicate the effectiveness of the phytoextraction process, which is when plants uptake the heavy metals and accumulate them in plants parts. Similarly, lower BAF values were associated with the phyto-stabilization process, where the absorption and mobility of heavy metals are reduced, lowering their bioavailability. This indicates the stabilization of metals rather than the accumulation [11].

Based on this, it can be observed that metals like Cd, Mg, Zn, Ni, Cu, and K with BAF values greater than 1 have higher accumulation in hot peppers, signifying the effective phyto extraction of metals for bioremediation. Accumulation of heavy metals in plant parts is one of the important factors that defines the efficiency of plants for phytoremediation [55]. Metals like Pb, Mn and Cr with lower BAF values indicate the phyto-stabilization process of hot pepper in stabilizing these metals, thus reducing their bioavailability.

A study by Antonious et al.(2010) [56] and Nepal et al. (2024) [57] suggests that BAF values below 1 neither show the accumulation ability nor pose any harm to human health. In contrast, BAF values greater than 1 indicate higher metal accumulation and pose potential consumer hazards. Contrary to this finding, our study presents data on the accumulation of heavy metals both in plants and soil to be within the permissible limits by FAO, WHO and US-EPA (Table 3). This suggests hot pepper poses no threat to human health despite the accumulation of heavy metals as they are below the permissible limits. Also, Ugulu et al. (2021) [19] demonstrated that the accumulation of heavy metals in hot peppers were within the safe limits for human consumption.

The improved soil structure, increased water retention and enhanced cation exchange capacity as a result of the application of biochar increased the availability of Mg and K to plants [58]. Mg and K buildup in the fruits of plants such as hot peppers growing in modified soils is primarily due to increased nutrient availability from the amendments, better soil microbial activity, pH modification and chelation processes. These systems work together to improve plants' uptake of magnesium and potassium, resulting in their maximum accumulation in the fruit part.

Soil amendments with biochar enhance the bioavailability of heavy metals, thus increasing the BAF values. The combination improves the physical properties of soil, soil pH, cation exchange capacity (CEC) and increases the bioavailability of heavy metals like Cd, Mn and Ni [59]. The innovative combination of soil amendments, such as Ethylene Diamine Tetra Acetic acid (EDTA) with biochar and charcoal, has significantly enhanced the phytoremediation capacity of plants like *Brassica junceae* and *Typha latifolia*, enhancing both biomass and metal accumulation [60]. Similar physiology of biochar can be associated with heavy metals like Cr, Cu, Zn, Pb, Cd, Mg, K, Ni and Mn, whose BAF values were observed high in different soil amendments with biochar.

Table 3
Permissible limit of metals in plants and soil by FAO, WHO and US-EPA [33–35].

Metals	Permissible limit in soil (mg kg^{-1})	Permissible limit in crops (mg kg^{-1})
Pb	90–400	0.3
Cd	0.5	0.2
Ni	5	1.5
Mn	500	200
Cr	0.2	1.5
Mg	NA	NA
Cu	12	10
K	NA	NA
Zn	60	50

NA: Not available

5. Limitations and future research direction

This study is limited to single growing season data. As phytoremediation is long-term process which is influenced by climatic condition, cumulative soil plant interaction and seasonal variation. It is very crucial to consider many repeated trials to validate the results. Hence, further multiyear research studies are recommended to assess the sustainability and stability of the phytoremediation findings across different climatic conditions and varying seasons.

Furthermore, it is also recommended to incorporate detailed plant growth and physiological parameters along with heavy metal uptake information. Variables such as fruit yield, plant canopy, root length, biomass accumulation, root to shoot ratio and chlorophyll content provide valuable context to understand the relationship between plant performance and remediation efficiency.

6. Conclusion

This study showed that hot pepper (*Capsicum annuum* cv. Georgia Flame) can act as a phytoremediation plant while being safe for human consumption. Hot pepper showed higher accumulation of Cd, Mg, Zn, Ni, Cu and K with BAF value exceeding one while low accumulation of heavy metals like Pb, Mn and Cr with BAF values below 1. The accumulation of all the studied heavy metals was within the permissible limit set by FAO, WHO and US-EPA, confirming the safety for human consumption.

BAF values exceeding one indicated higher accumulation of heavy metal by plants, making it an effective phytoextractor, while the BAF value below 1 indicated stabilization of the heavy metals within the rhizosphere.

Biochar mixed with organic and inorganic amendments has been shown to considerably increase the concentrations of toxic heavy metals like Pb, Cr, Ni, etc. due to the chelating and soil chemical properties. The highest accumulation of Cd and Ni was recorded in soil amended with CM-biochar. In HM-biochar, Cr absorption was highest at the highest BAF value.

This study provides insight into many phytoremediation elements, such as physiological responses to phytoremediation and different types of soil amendments. Connecting the gaps between all these factors could resurrect cost-efficient, ecofriendly and sustainable approaches such as phytoremediation as a long-term and successful solution to environmental contamination.

This study suggests that hot pepper can remediate heavy metals like Pb, Cd, Ni, Mn, Cu, Cr and Zn from the contaminated site. The concentrations of the heavy metals in fruit tissues were also below the permissible limits, confirming the safety for human consumption.

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CRediT authorship contribution statement

Basanta Neupane: Writing – original draft, Methodology, Formal analysis, Conceptualization. **Mohammad H. Dawood:** Writing – review & editing, Supervision. **George F. Antonious:** Writing – review & editing, Methodology, Conceptualization. **Anjan Nepal:** Writing – review & editing, Methodology.

Data availability

The study's original contribution is mentioned in the article. Contact the corresponding author at basanta.neupane@kysu.edu for further inquiries.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: George F. Antonious reports financial support was provided by US Department of Agriculture. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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