

Effect of Poultry Litter and Biochar Application Methods on Nutrient Loss

By

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A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 3, 2024

Keywords: Preferential flow, soil leachate, phosphorus, metals, poultry litter, biochar, water quality, flow interruption

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Abstract

High levels of phosphorus (P) and trace metals, such as copper (Cu) and zinc (Zn), can cause significant water quality issues when delivered to water bodies. This study investigated the impact of different poultry litter application methods on P and metals leaching in pastures using rainfall simulation on soils containing macropores. The major objectives of this study were to examine the effectiveness of different application methods (surface vs subsurface) of poultry litter and pine wood pristine biochar on the leaching of dissolved reactive phosphorus (DRP) and trace metals via preferential flows. Intact undisturbed soil cores were used for column-based successive rainfall simulation experiments. Treatments were surface-applied poultry litter, subsurface-banded poultry litter, surface-applied poultry litter + biochar, subsurface-banded poultry litter + biochar, and control (no biochar and nor poultry litter). Results showed that treated columns leached significantly greater amounts of dissolved reactive phosphorus (DRP) than control columns. However, DRP leaching decreased with successive rainfall events. Subsurface application proved to be more effective than surface broadcasting in controlling DRP losses. Additionally, the pristine biochar used in this study did not help reduce DRP leaching.

Further, treated columns leached significantly higher amounts of copper and zinc than control columns, likely due to the presence of metals in the soluble fraction in poultry litter and changes in soil conditions such as pH and dissolved organic matter. There was no significant difference in the leaching of aluminum and iron between treated and control columns, as most aluminum and iron were not present in the mobile fraction in both poultry litter and soil. With successive simulations, the leaching of copper, zinc, and aluminum decreased, while the subsurface leaching of iron increased in columns treated with poultry litter, likely due to reducing conditions and the flow interruption technique. Incorporating pristine biochar with poultry litter

reduced the leaching of iron and aluminum more effectively than copper and zinc. This study provides an overview of the effectiveness of manure and biochar application on the leaching and deposition of P and metals in soil columns and prompts further in-depth research on nutrient and metal management in agricultural ecosystems.

Acknowledgments

With immense pleasure I would be privileged to express my deep sense of gratitude and indebtedness to those whose assistance was indispensable for the completion of my master's thesis and project work. Special thanks to my advisor Dr. Jasmeet Lamba, for giving me such a good project, valuable guidance, constant supervision, ceaseless encouragement, constructive ideas and painstaking assistance during my project work and preparation of my project.

No words can suffice my feelings of gratitude to my parents S. Sher Singh & Smt. Kulwant Kaur, my sister Prabhjyot Kaur whose blessings, love, immense patience, understanding and never-ending support were the constant source of inspiration during the tenure of my degree.

I would like to acknowledge my lab mates, Vishawjot Sandhu, Ranveer Singh, Atiqur Rahman, Tanvir, Rakesh, Preetika Kaur, and Kritika for their support and fruitful discussions. I highly appreciate the collaborative effort and willingness of Ranveer and Atiqur to assist with experiments whenever needed. Also, I would like to thank my other friends Rajveer, Jagdeep, Annu, Ramandeep, Amandeep and Dildeep for their continued readiness to help me whenever I reached out.

Finally, I extend my sincere gratitude to Destinne Ray, Jack Morgan, James Johnson, Bobby Bradford, and Caroline Whiting for their valuable assistance in the lab whenever needed.

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Chapter 1

Introduction

1.1 Background

In the United States, the poultry industry is a massive and vertically integrated sector that plays a crucial role in the nation's economy and global food supply. According to NASS. (2021), the Southeastern United States contributes around 66% to the nation's annual output in terms of broiler production. Among these southeastern states, Georgia, Alabama, Mississippi, and Arkansas emerge as the key players in this industry (Bryant et al., 2022). Consequently, the Southeastern region finds itself abundant in poultry litter (PL), with Alabama alone generating approximately 1.8 million Mg of PL annually (Kpombrekou et al., 2016).

Poultry litter is a popular soil supplement and offers a rich blend of organic carbon, nitrogen, phosphorus, potassium, and other essential macronutrients (Watts et al., 2010). However, it also contains the metals such as magnesium (Mg), sodium (Na), aluminum (Al), zinc (Zn), arsenic (As), and copper (Cu) (Lamba et al., 2019). To manage this waste efficiently and sustainably, PL is commonly spread across soil surfaces of agricultural croplands, providing an economical and environmentally friendly solution (Lin et al., 2018).

The benefits of PL application are manifold. It enhances soil fertility, promotes plant growth, and boosts biomass yield (Jaja et al., 2022; Netthisinghe et al., 2016), making it an attractive alternative to expensive inorganic fertilizers (Schronce et al., 2022). However, according to Hoover et al. (2019), prolonged use of PL can lead to environmental degradation, chiefly through non-point source pollution. Eutrophication from phosphorus and ecotoxicity due to elevated levels of micronutrients like copper and zinc negatively impacts soil-water systems (Crippen et al., 2016).

In agricultural fields, the movement of nutrients into surface waters primarily occurs through surface runoff and subsurface flow (King et al., 2015). Extensive research has focused on managing surface runoff to mitigate soil erosion (Pote et al., 2011; Torbert et al., 2002; Watts et al., 2011). The subsurface movement of nutrients is equally crucial for addressing water eutrophication (Li et al., 2013). Subsurface movement is particularly prevalent in soils with low sorption capacity and high preferential flow through soil macropores (King et al., 2015; Sims et al., 1998).

To maintain water quality and mitigate environmental risks, agricultural practices must evolve. Implementing strategies such as nutrient management planning, soil testing, and precise application rate determination can optimize PL utilization while minimizing its environmental impacts. Adopting various application techniques and management practices is essential for ensuring the sustainable use of PL nutrients while protecting water resources (Bolan et al., 2010; Feng et al., 2019).

The development of new methods, such as subsurface band application of PL, has emerged as an alternative to conventional surface broadcast methods for addressing concerns related to PL application (Way et al., 2013). This approach involves placing manure in specific locations within the soil, minimizing nutrient loss from volatilization or runoff (Kulesza et al., 2014; Pote et al., 2011; Tewolde et al., 2009, 2015, 2018). Researchers have found this method to be more effective in delivering nutrients to crops and increasing yields compared to traditional surface broadcasting (Pote et al., 2011; Tewolde et al., 2009, 2015, 2018). Furthermore, it is believed that subsurface application of PL can reduce leaching, making it an attractive option for sustainable agriculture (Way et al., 2013).

This study seeks to determine the efficacy of the subsurface application method in minimizing contaminant loss in leaching. Additionally, another promising technique for mitigating contaminant loss in leachate is the use of biochar as a soil amendment. Biochar, a solid material derived from biomass carbonization, can enhance soil functionality, and reduce greenhouse gas emissions from biomass decomposition (Shakya et al., 2017). The use of biochar in soil has gained attention from scientists due to its potential to reduce contaminant leaching (Y. Li et al., 2020). Therefore, this study aims to assess the effectiveness of biochar in combination with PL to quantify its impact on reducing leaching and subsurface contaminant movement. Through this investigation, valuable insights into the efficacy of these strategies in minimizing contaminant loss and enhancing agricultural sustainability will be provided.

1.2 Research Objective

The primary goal of this study was to understand the impact of soil's physical and chemical properties on the nutrient and metal leaching in a no-till pasture field located in the Sand Mountain region of north Alabama by adopting different application types of poultry litter such as surface application and subsurface application, and to check the impact of biochar when combined with poultry litter for same application types. To quantify the leaching behavior, a flow interruption technique was used which analyze the concentration of nutrients such as phosphorus, copper, zinc, iron, and aluminum in leachate over time rather than a single rain event. The study mainly focused on laboratory-based rainfall simulation experiments on soil columns to analyze leachate samples and soil samples at different depths for dissolved phosphorus, and heavy metals. The major objectives of this study were:

Objective 1: Investigating the impact of poultry litter application method and combined application method of poultry litter and biochar on phosphorus leaching in agricultural soils using a flow interruption technique.

Objective 2: Understanding metal leaching from poultry litter and biochar application methods in agricultural soil by using a flow interruption technique.

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Chapter 2

Investigating the Impact of Poultry Litter Application Method and Biochar on Phosphorus Leaching

2.1 Introduction

The United States is the largest producer and second-largest exporter of poultry meat (Bishop et al., 2015). According to USDA-NASS (2024) in 2023, in the United States, the majority of broiler chicken feeding operations were concentrated in the southeastern US, with Georgia and Alabama having the greatest production. Alabama produces over 1 billion birds per year generating about 1.8 million Mg of poultry litter (PL). Poultry litter comprises a mixture of manure, waste bedding, feathers, and other components (Pote et al., 2011). Poultry litter is a valuable source of nutrients for agricultural use as it contains essential plant nutrients such as nitrogen, phosphorus, potassium, and micronutrients, making it a nutritionally rich fertilizer (Maroccia, 2022). Studies have shown that the application of PL can lead to increased biomass yield and improved nutrient concentration in forages (Jaja et al., 2022). On the contrary, the long-term and repeated application of PL in agriculture lead to increased levels of phosphorus (P) in soils (Chakraborty et al., 2021), raising the risk of eutrophication in surface water bodies (Malhotra et al., 2024).

The major cause of eutrophication is a high concentration of P in PL, and repeated application of PL may make soil's labile P forms more abundant (Jeon et al., 2015). These labile P result in greater total P and dissolved reactive phosphorus (DRP) loss in runoff (Roswall et al., 2022). The application of PL is typically based on the crop nitrogen (N) requirement since PL typically consists of approximately 3% N and 1.5% P so, application based on an N rate often leads to excessive application of P (Ashworth et al., 2021). Phosphorus transport in agricultural systems

can occur either through surface or subsurface pathways. The majority of research on P transport from agricultural fields primarily concentrated on surface runoff and the development of management techniques aimed at minimizing soil erosion (King et al., 2015a; Pote et al., 2009, 2011; Watts et al., 2011). However, the vertical movement of P, i.e. subsurface flow, is also a major concern for eutrophication of water bodies (Beauchemin et al., 1998; Li et al., 2013). The subsurface movement of P can be notable in watersheds characterized by elevated soil P levels (such as those in areas with animal and specialty crop farming), low P sorption capacity (found in watersheds with sandy soil or soils rich in organic matter), and artificial drainage systems (such as tiles and ditches) (King et al., 2015; Sims et al., 1998). Most of the increase in leachate P concentration after manure application originates from the water-soluble P present in the manure (Kang et al., 2011; Katuwal et al., 2022; Lamba et al., 2013; Toor et al., 2016). Therefore, it is crucial for agricultural practices to consider the potential impact of PL application and to implement proper management strategies to minimize nutrient runoff and leaching to protect water quality.

The application of PL is typically done using surface broadcasting, a method where the litter is spread evenly over the soil surface. To address the drawbacks associated with traditional surface broadcasting methods a new method subsurface band application of solid manure has emerged as an innovative technique (Way et al., 2013). Many researchers concluded that the subsurface application approach appears to be more effective in delivering nutrients to the crop compared to conventional surface broadcasting methods, as it involves PL in the soil at a specific location where the nutrients are shielded from volatilization or runoff (Kulesza et al., 2014; Tewolde et al., 2015, 2018). Additionally, studies have shown that utilizing subsurface banding for PL can effectively mitigate nutrient losses in runoff from pastures and hay fields, leading to a remarkable reduction

of up to 95% in P losses when compared to surface broadcast application (Lamba et al., 2013; Watts et al., 2015). According to Tewolde et al. (2022), subsurface banding is a more financially advantageous strategy compared to conventional surface broadcasting. However, research on quantifying the loss of P in leachate as a function of surface and subsurface application of P is limited. This is especially important in regions where the loss of P via soil macropores is important (Budhathoki et al., 2022; Malhotra et al., 2024). Another approach used to mitigate P loss involves the utilization of biochar (BC). Biomass, such as manure, agricultural wastes, and sewage sludge, is pyrolyzed at relatively low temperatures ($<700^{\circ}\text{C}$) to form BC, a substance rich in carbon (L. Yang et al., 2021). In general, some advantages of using BC include reduction in greenhouse gas emissions and erosion, while increasing nutrient availability, soil organic matter, water retention, microbial activity, and crop production (Mohan et al., 2014). Several studies reported that BC applications are effective in reducing the nutrient leaching in the soil matrix (Li et al., 2019; Lv et al., 2021; Pratiwi et al., 2016; Ukwattage et al., 2020). The reduction in nutrient leaching is due to the presence of various functional groups on the BC's surface (carboxyl, hydroxyl, and methyl groups), which can chemically bind and retain nutrients (Alkharabsheh et al., 2021). A few studies also reported that BC application was an ineffective strategy to reduce leaching of nutrients, such as P (Lu et al., 2022; Nguyen et al., 2020; Xie et al., 2021). According to Toluwase et al. (2020), the combined application of PL and BC could be an effective strategy to retain nutrients in the soil and prevent leaching. Thus, adding biochar to PL may be another management practice to reduce nutrient leaching.

Studying P transport through preferential flow pathways is crucial for quantifying P losses from agricultural soils, assessing water pollution risks, and developing sustainable soil and nutrient management practices to mitigate these issues (Abou et al., 2004; Koch et al., 2023; Makowski et

al., 2020). Thus, the objective of this study was to examine the impact of different methods (surface vs. subsurface band) of applying manure on the leaching of nutrients (DRP), using intact undisturbed soil columns. The objective includes quantifying preferential transportation with successive rainfall events. Moreover, the effect of pristine BC, which is unmodified biochar, was also assessed to show how it would affect DRP leaching when combined with PL. Based on prior research, it was hypothesized that subsurface band application of PL will reduce the DRP concentration in leachate. Also, when PL is combined with pristine BC, it works better than PL alone in reducing DRP leaching (Su et al., 2019). To achieve these objectives, the flow interruption technique was employed to conduct successive rainfall events with intervals between simulations. This unique approach allows us to quantify how the pattern of DRP leaching changes with different rainfall events following poultry litter application.

2.2 Materials and Methods

2.2.1 Study Area

The soil columns used in this research were obtained from a hillslope pasture field situated at the Sand Mountain Research and Extension Center (SMREC) in north Alabama (34°17'02.6" N, 85°57'51.8" W) (Fig. 1), and an elevation of approximately 350 m above mean sea level. The study site is an approximately 0.40 ha field with a slope length of 80 m. Cattle had been grazing on this field for over 15 years before sampling, with approximately 50 cattle grazing the entire area at any given time. For optimal growth and long-term persistence of the tall fescue (*Festuca arundinacea* Schreb.) plants, grazing was halted when the grass height reached 100 mm or less. The slope gradient of the site was 3.4%.

The soils present at the study site were Hartsells fine sandy loam (classified as fine-loamy, siliceous, subactive, thermic Typic Hapludults) and Wynnville fine sandy loam (classified as fine-loamy, siliceous, subactive, thermic Glossic Fragiudults). The Hartsells soil exhibits good drainage with moderate permeability and is typically found on broad, smooth plateaus, mountaintops, or hilltops. The parent material of these soils consists of loamy residuum resulting from the weathering of sandstone and shale (Soil Survey Staff, 2020). The Wynnville series is comprised of moderately well-drained soils with slow permeability, also derived from sandstone and shale. Notably, a restrictive hard pan layer near the soil surface was observed at the study site, which could contribute to the formation of a perched water table during intense rainfall events (Budhathoki et al., 2022). Based on a 30-year analysis of normal weather patterns, the mean annual precipitation in this region was 1340 mm, while the mean annual temperature was 14.8 °C. Tall fescue, a cool-season grass, had grown on the study site for over 40 years.

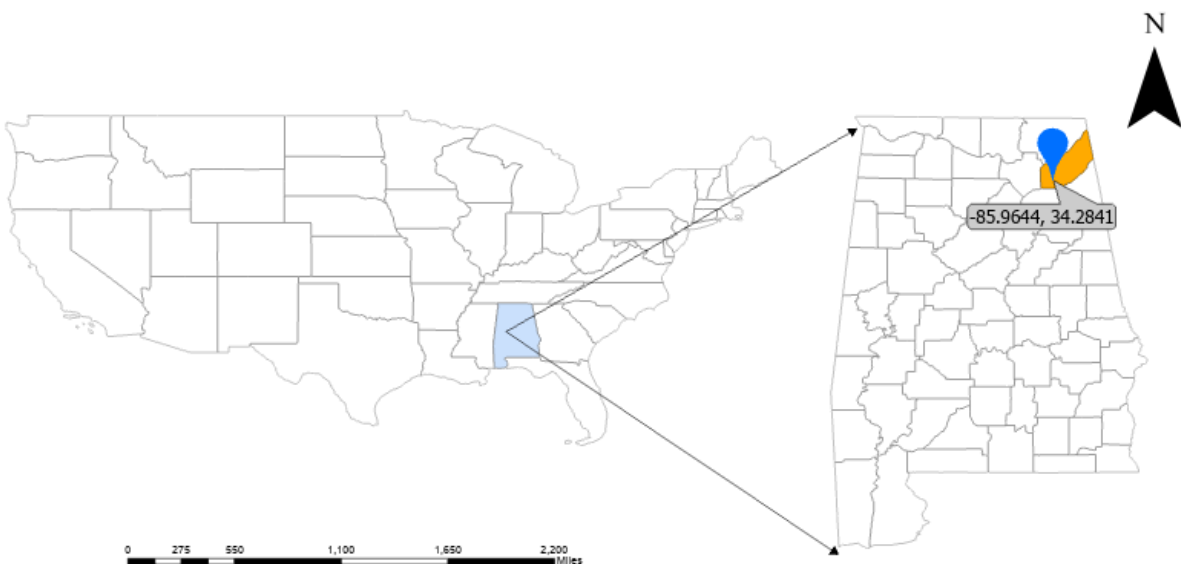


Figure 1. Location of the study site at the Sand Mountain Research and Extension Center (SMREC) in north Alabama.

2.2.2 Sample Collection

A total of 15 undisturbed intact soil cores, 150 mm diameter x 500 mm depth, were collected from the field by using polyvinyl chloride (PVC) pipes. The cores were collected by using a hydraulic system mounted on a tractor (Prior et al., 2004) (Fig. 2). Additionally, disturbed soil samples ($n = 42$) from different depth intervals adjacent to the location of the undisturbed soil cores were collected and analyzed for various physiochemical properties of soil at depth intervals of 0-5 cm, 5-10 cm, and 10-25 cm.



Figure 2. Tractor mounted hydraulic device is used to collect the soil cores.

2.2.3 Soil Column Preparation and Rainfall Simulation

The soil column preparation and rainfall simulation protocols were adapted from a recent study (Malhotra et al., 2024). Soil column preparation was conducted at Auburn University's Soil and Water Laboratory and Biological Engineering Research Lab (BERL), Auburn, AL. The soil cores were placed in a vertical position and saturated with warm water. This saturation process was

carried out from the base upwards and lasted for 48 hours to either deactivate or remove earthworms. After 48 hours, the soil cores were subjected to a draining process that lasted approximately 3 days to ensure uniform soil moisture levels among all the soil columns (Budhathoki et al., 2022; Zopp et al., 2016). The columns were stored at a temperature of 4°C until the commencement of the rainfall simulation experiments. One day before the experiment, the lower half of each soil column was removed by cutting, so each soil core used in the experiment was the top 25 cm of the original core. This depth was chosen because most nutrient accumulation and preferential flow occurs in the top 25 cm of soil (Budhathoki et al., 2022; Malhotra et al., 2024). Next, liquid petroleum jelly was inserted into the space between the soil and the inner surface of the PVC pipe to reduce selective flow along the wall of the column, especially in saturated conditions (Yu et al., 2007). To occupy the vertical gap (3 cm) between the soil and a PVC cap mounted to the bottom of the pipe, acid-washed sand was employed. The soil columns were then subjected to rainfall simulation experiments.

The rainfall simulation system included various components such as the soil column, carboys, a peristaltic pump, a securely sealed PVC base cap, a system for collecting leachate, and a cap designed to simulate rainfall. (Fig. 3). A Masterflex L/S multichannel peristaltic pump (Cole Parmer, Vernon Hills, IL) pumped rainfall solution (0.01 M potassium chloride) and tracer solution (0.01 M potassium bromide) to the cap of the rainfall simulator. Five treatments were utilized in the rainfall Simulation experiment: 1) surface broadcasting of PL, 2) subsurface band application of PL, 3) surface broadcasting of PL and biochar, 4) subsurface band application of PL and biochar, and 5) a control (no litter or biochar). For each subsurface band, the band centerline was along a diameter line of the soil core, the width of the band was around 60 mm, the vertical dimension of the band was around 50-100 mm, and a 50-100 mm layer of soil was above the top of the band.

The application rate of the PL, applied on a wet basis was 10 Mg ha⁻¹. The application rate of the biochar was 10 Mg dry biochar ha⁻¹ and the biochar moisture content was 51% wet basis, so the biochar application rate, applied on a wet basis, was 20.4 Mg ha⁻¹. When both PL and biochar were applied together, the moist basis application rates were 10 Mg ha⁻¹ for the PL and 20.4 Mg ha⁻¹ for the biochar. For a given application rate, the mass of material applied per unit length of band is inversely proportional to the band spacing. For the subsurface band applications, the band in each column was representative of a 117.3 mm band spacing used in the field. Each soil column was subjected to three simulations, with a gap of five days between consecutive simulations. The first step of the rainfall simulation experiment was to saturate the soil core through capillary action from the bottom with 0.01 M KCl solution. The solution was applied until ponding to a depth of 3-5 cm was achieved on top of the soil column. After this, the column was irrigated from the top with a constant rate of 0.01 M KCl solution with an application rate sufficient to maintain a head of 2-3 cm on the surface of the soil, to maintain a saturated flow condition. When the saturated flow condition was attained, 0.01 M sodium bromide, as a conservative tracer, was applied to get breakthrough curves for preferential flow (Zopp et al., 2016). Afterward, the KCl solution was once again utilized to rinse out the bromide (Br⁻) from the column. Plastic acid-washed bottles were used to collect the effluent from the bottom of the soil core for subsequent chemical analysis. The accumulated pore volumes were determined using the following method:

$$\text{Pore volume} = V / (V_c [1 - \rho_b / \rho_s])$$

Where V represents the volume of effluent collected, V_c denotes the bulk volume of the soil column, ρ_b , and ρ_s denotes the bulk density and particle density (2.65 Mg m⁻³) of the soil, respectively. The relative concentration was determined by:

$$\text{Relative concentration} = C / C_0$$

Where C is the concentration of Br in leachate samples and C_0 is the concentration of Br in rainwater. Breakthrough curves (BTCs) were generated for each column, depicting the relative concentration of Br in the effluent against pore volume. Acid-washed bottles (500 mL) were used to collect leachate, corresponding to three to four pore volumes. This range depended on the point at which the relative concentration of Br (C/C_0) reached a negligible level. The quantification of Br ion concentration in the leachate was performed using a bromide combination ion-selective electrode (model HI4102, Hannah Instruments Inc., Smithfield, RI).

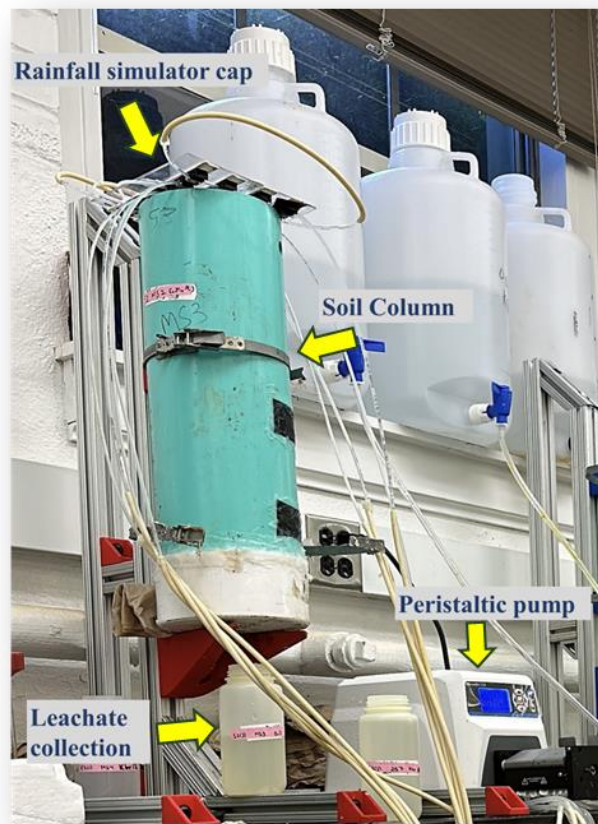


Figure 3. The lab setup shows a rainfall simulation experiment, including soil columns, carboys, a peristaltic pump, rainfall simulation caps, and leachate collection bottles.

2.2.4 Physical and Chemical Analysis of PL, Soil, Biochar, and Leachate Samples

2.2.4.1 PL, Soil Sample, and Leachate Sample Analysis

For this experiment, the PL was from broiler producer in north Alabama. The litter was stored at 4°C and analyzed for properties including pH, moisture content, metal content and DRP. The soil bulk density was calculated, dividing the weight of the oven-dried soil sample by V, the volume of the collected sample. The hydrometer method was used to determine the soil texture (Bouyoucos, 1962). The loss on ignition method was used to determine soil organic matter content (Huisman et al., 2013). For chemical analysis, soil samples were dried at 60°C for 48 hours, followed by sieving through a 2-mm mesh sieve. The soil pH and EC were assessed using VWR Symphony electrodes (VWR Scientific Inc., Radnor, PA) with a soil-to-water ratio of 1:10 for soil, and a manure-to-water ratio of 1:3 for PL, as specified by Carter et al. (2008). The DRP levels in the soil samples were analyzed with an extraction process using a 1:10 ratio of soil to deionized water (Kovar et al., 2009). The soil water suspension was shaken for one hour using a mechanical shaker, and aliquots obtained through centrifuging were passed through a 0.45 µm filter. Then DRP was analyzed using the molybdenum blue colorimetric method developed by Murphy et al. (1962), using a Seal Analytical AQ2 automated discrete analyzer (Seal Analytical Inc., Mequon, WI). Leachate samples filtered through a 0.45 µm filter were used to determine DRP.

2.2.4.2 Characterization and Physicochemical Analysis of Biochar

The pristine pine wood biochar used in this study was purchased from Waste to Energy Inc. (Slocomb, AL). Morphological and physio-chemical properties of biochar were analyzed and included carbon, hydrogen, nitrogen, and sulfur (CHNS) composition, ash content, point of zero charge (pH_{ZPC}), electrical conductivity, pH, and cation exchange capacity (CEC) (Wang et al., 2022). Moreover, the P content of biochar was analyzed by using inductively coupled plasma-

optical emission spectrometry (ICP-OES) following EPA Method 200.2 (Martin et al., 1994). Water extractable phosphorus of biochar was analyzed by using double digestion method with the help of ICP-OES by following EPA Method 200.8.

2.2.5 Statistical Analysis

For statistical analysis, SAS version 9.4 (SAS Institute Inc, Cary, NC) was used. PROC GLIMMIX was used to determine the effect of Simulation and treatment on the leachate nutrient concentration. Simulation and treatment, and their interaction, were considered fixed effects, while replication and replication*time were considered random effects. Simulation was considered as a repeated measure in the model. Data were transformed by using a log-normal transformation to maximize data fitness, and data were back-transformed to show the original means in the graphs. When significant main effects or interactions were found, mean separations were performed using the least significant difference (LSD) test, to compare means of treatment and time at a significance level of $P < 0.05$.

2.3 Results and Discussions

2.3.1 Physical and Chemical Analysis of PL, Soil, and Biochar Properties

The concentration of total phosphorus (TP) in PL was $14.1 \pm 0.03 \text{ g kg}^{-1}$ (Table 1). The TP concentration was consistent with the range ($12\text{--}30 \text{ g kg}^{-1}$) typically found in poultry litter from Alabama producers (He et al., 2008). The pH of the poultry litter was approximately 7, which is typical for most manures (Chastain et al., n.d.). The bedding material used for broiler production was pine shavings, which is often used in the southeastern US (Sharma et al., 2015).

Table 1. Properties of PL used for soil column rainfall simulation (mean $\pm$ standard error).

Bedding Material	Pine shavings
pH	7.37 $\pm$ 0.33
Moisture content (wet basis), %	12.17 $\pm$ 0.03
Total N, g kg ⁻¹	54.3 $\pm$ 0.08
Total P, g kg ⁻¹	14.1 $\pm$ 0.03
Total K, g kg ⁻¹	36 $\pm$ 0.01
Dissolved reactive phosphorus (DRP), g kg ⁻¹	1.6 $\pm$ 0.007

The physicochemical properties (pH, EC, organic matter, dry bulk density, and the composition percentage of sand, silt, and clay) of soil exhibit variations across different depths within the soil profile (Table. 2). In this study, we investigated three different depths: 0-5 cm, 5-10 cm, and 10-25 cm, with organic matter contents of 4.90%, 3.26%, and 2.60%, respectively. The average dry bulk density for the 0-5 cm and 5-10 cm depth range was consistent at 1.19 g cm⁻³. However, for the 10-25 cm depth range, the dry bulk density increased to 1.34 g cm⁻³. Sand content was 60%, 63%, and 66% for the soil at depths of 0-5, 5-10, and 10-25 cm, respectively.

Table 2. Average pH, EC ($\mu\text{S cm}^{-1}$), organic matter (%), bulk density (g cm^{-3}), sand (%), silt (%), and clay (%) of soil at three depth ranges. Values representing the mean $\pm$ standard error.

Depth (cm)	pH	EC ($\mu\text{S cm}^{-1}$)	Organic matter (%)	Dry bulk density (g cm^{-3})	Sand (%)	Silt (%)	Clay (%)
0-5	6.14 $\pm$ 0.74	239 $\pm$ 78.93	4.90 $\pm$ 0.60	1.19 $\pm$ 0.06	60 $\pm$ 4.43	31 $\pm$ 4.68	8 $\pm$ 0.49
5-10	5.93 $\pm$ 0.80	98.83 $\pm$ 35.30	3.26 $\pm$ 0.02	1.19 $\pm$ 0.02	63 $\pm$ 3.03	30 $\pm$ 2.83	7 $\pm$ 0.57
10-25	5.68 $\pm$ 0.37	123.56 $\pm$ 57.73	2.60 $\pm$ 0.15	1.34 $\pm$ 0.08	66 $\pm$ 3.89	26 $\pm$ 3.39	8 $\pm$ 0.50

The average electrical conductivity of the soil was $153.8 \mu\text{S cm}^{-1}$ in the 0-25 cm depth range, and this level has been found to be detrimental to many crops as it is lower than the minimum threshold value of $4000 \mu\text{S cm}^{-1}$ as mentioned by Kingery et al. (1994). The soil profile (0 – 25 cm) was somewhat acidic, with a mean pH of 5.92. This is mainly due to the properties of the sandstone parent material.

Physiochemical properties of pine wood biochar, encompassing its carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) contents, ash content, point of zero charges, pH, and electrical conductivity, are summarized in Table 3. The carbon content of biochar was more than 80%, which represents stable nature of biochar (Wijitkosum et al., 2019). The H, S, and N content values were 1.1%, 0.30%, and 0.40%, respectively. The ash content and pH_{zpc} of biochar were 7% by weight and 9.4, respectively. The pH_{zpc} indicates the domination of basic groups on the surface of biochar (Fiol et al., 2009). This basic surface charge was negative because the pH of the biochar sample

was 9.9, which was greater than pH_{zpc} . The negative charge was possibly due to ionization or dissociation of acidic oxygen surface groups (Tran et al., 2016).

Table 3. Physicochemical properties of biochar: characteristics and composition analysis.

Values representing the mean $\pm$ standard error.

C, wt. %	83 ± 1
H, wt. %	1.1 ± 0.1
S, wt. %	0.30 ± 0.1
N, wt. %	0.40 ± 0.01
Ash, wt. %	7.0 ± 0.2
pH_{zpc}	9.4
Electrical conductivity, $\mu\text{S cm}^{-1}$	1547
pH of biochar solution	9.9
P, mg Kg^{-1}	456.7 ± 16.6
Water extractable phosphorus (WEP), mg Kg^{-1}	16.61 ± 0.26

2.3.2 Bromide Breakthrough Curve Analysis

Breakthrough curves (BTCs) were used to identify the preferential flow through soil columns. All the soil columns used in this study showed the relative concentration of bromide (Br^-) is observed to be more than 0.5 before one pore volume, which indicates that there is an early breakthrough of Br^- (Fig. 4). The solute concentration ratio (C/C_0) of 0.5 observed before 1 pore volume indicates preferential flow (Malhotra et al., 2024; Mon et al., 2006; Cheng et al., 2014). Moreover, the long tail and asymmetrical shape of the Br^- BTC, as observed in our study, also indicate preferential

flow (Zhang et al., 2015). Similarly, according to Bouma et al., (1979), rapid transportation of tracer solution and early breakthrough curves represent the presence of large and continuous pores in unpacked soil columns. A study by Budhathoki et al. (2022) also reported a dense network of macropores in this region. So, in the Budhathoki et al. (2022) study, the dense macropore network system, rapid transportation of Br^- , and the shape of BTCs from all soil columns could be linked with preferential flow through soil macropores (Fig. 4) as reported by Luo et al. (2010) and Zhang et al. (2015).

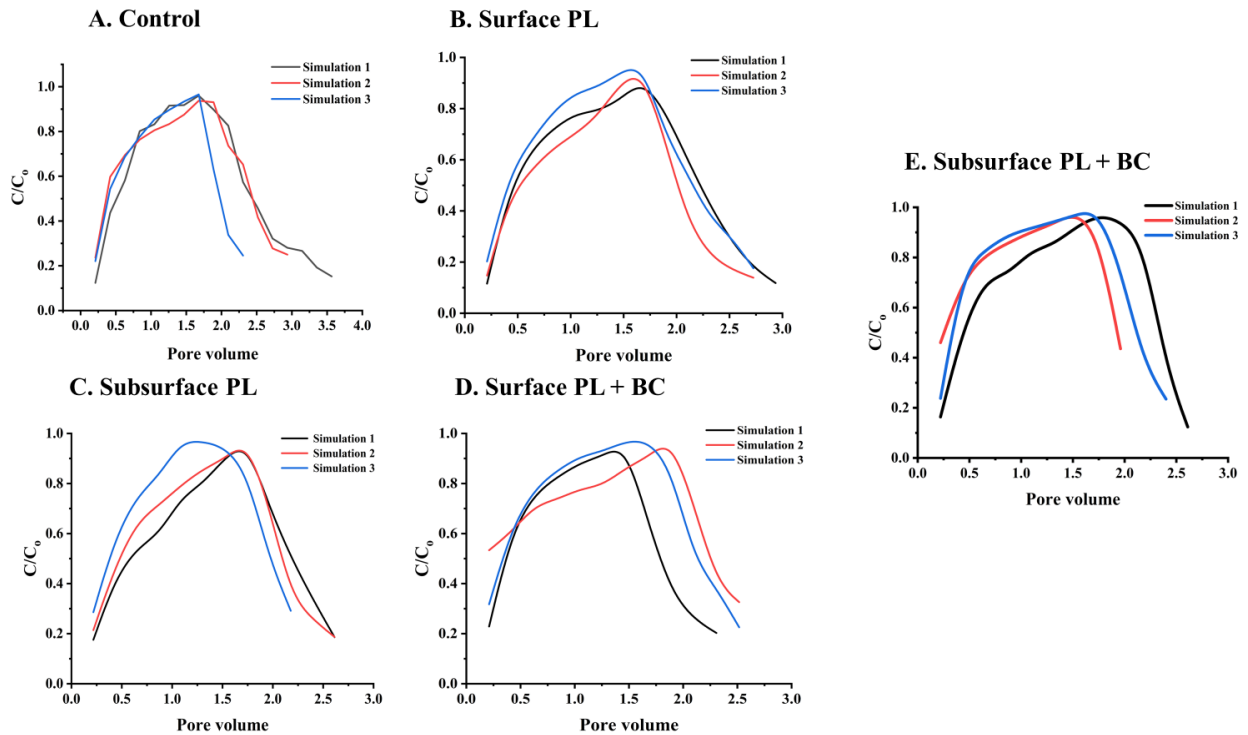


Figure 4. Breakthrough curves for relative concentration of bromide with respect to pore volume. Where PL: Poultry litter; BC: Biochar.

2.3.3 Nutrient Breakthrough Curves

Leachate samples were collected from three simulation trials that were subjected to analysis for dissolved reactive phosphorus (DRP) concentration as a function of different treatments. Treated

columns with PL or with combined application of PL + BC leached a higher concentration of DRP than untreated columns. Fig. 5 represents the breakthrough curves of DRP with respect to pore volume. For columns treated with surface PL, DRP leaching was higher in simulation 1 than simulations 2 and 3. The highest DRP concentration in leachate samples from simulation 1 was 3.37 mg L^{-1} , occurring before one pore volume (i.e., 0.43 pore volume), indicating the existence of macropores and preferential flow (Mon et al., 2006). After attaining its peak value, the leaching of DRP showed a declining trend with respect to pore volume. For simulations 2 and 3, breakthrough curves were similar to one another, and these simulations leached significantly lower amounts of DRP over time than simulation 1. Regarding subsurface band application of PL, in simulation 1, the concentration of DRP in leachate attained its peak (1.30 mg L^{-1}) at about 0.60 pore volume. The concentration then followed the same declining trend as in surface applied PL treatment. In simulation 2, the trend differed, as the concentration of leached DRP did not show a consistent declining trend, and it ranged from 0.56 mg L^{-1} to 0.98 mg L^{-1} . The concentration of leached DRP in simulation 3 was lower than in simulation 1 and simulation 2. In the surface application of PL + BC treatment, the concentration of leached DRP reached a maximum (14.8 mg L^{-1}) in simulation 1 before 0.5 pore volume, and then declined. For simulations 2 and 3, the concentration of DRP in the leachate was significantly lower than for simulation 1. The highest amount of DRP leached was 4 mg L^{-1} and 2 mg L^{-1} in simulations 2 and 3, respectively. For the subsurface band treatment of PL and BC, in simulation 1, the maximum concentration of DRP (4.58 mg L^{-1}) was leached before 0.5 pore volume. After that, the leached amounts of DRP in simulations 2 and 3 was very close to one another, and they followed the same pattern over time. From these breakthrough curves, we found that all treated columns leached a higher concentration of DRP than the control. The applied PL and BC used in this study contributed to increasing the

DRP concentration in leachate. Primarily the results of our study showed the first flush phenomenon in simulation 1, i.e., immediate transport of P to subsurface soil after application of manure (Brock et al., 2007). For simulations 2 and 3, the DRP concentration in the leachate was reduced, relative to simulation 1, and this is in agreement with Katuwal et al. (2022). Moreover, the rapid response of DRP to leachate samples after manure application indicates the presence of preferential flow pathways that connect the surface soil to subsurface ground (Kleinman et al., 2009).

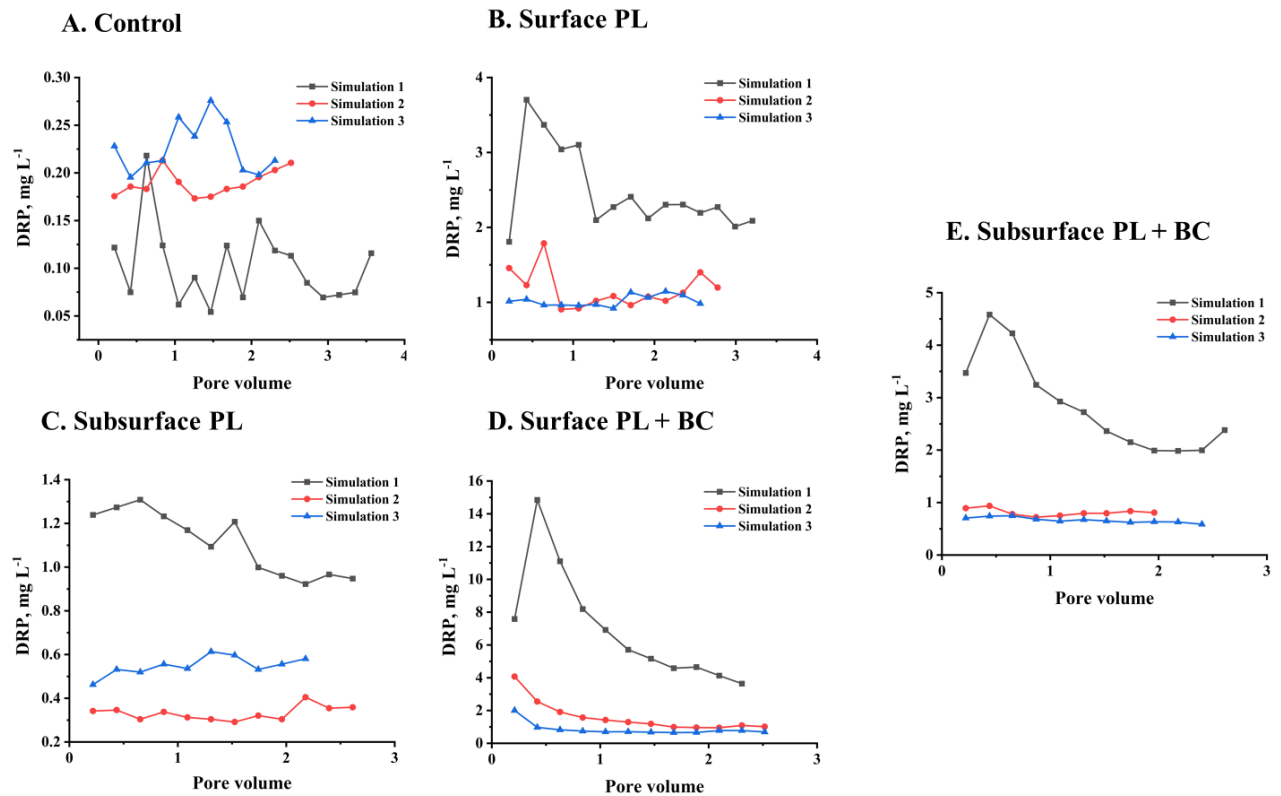


Figure 5. Breakthrough curves for dissolved reactive phosphorus (DRP) with respect to pore volume. Where PL: Poultry litter; BC: Biochar.

2.3.4 DRP Leaching from Different PL Methods

In simulation 1, we found that surface broadcast PL had leached significantly greater DRP (4.0 mg L^{-1}) than the subsurface banded PL treatment (2.48 mg L^{-1}) and the control treatment (0.20 mg L^{-1}) (Fig. 6). The results from the simulation 2 demonstrated that surface broadcast PL leached 1.22 mg L^{-1} DRP, which was significantly greater than the subsurface banded PL (1.47 mg L^{-1}) and control (0.42 mg L^{-1}) treatments. (Fig. 6). In Simulation 3, we observed that treatments included surface broadcast PL leached greater DRP (0.99 mg L^{-1}) as compared to the control treatment (0.31 mg L^{-1}).

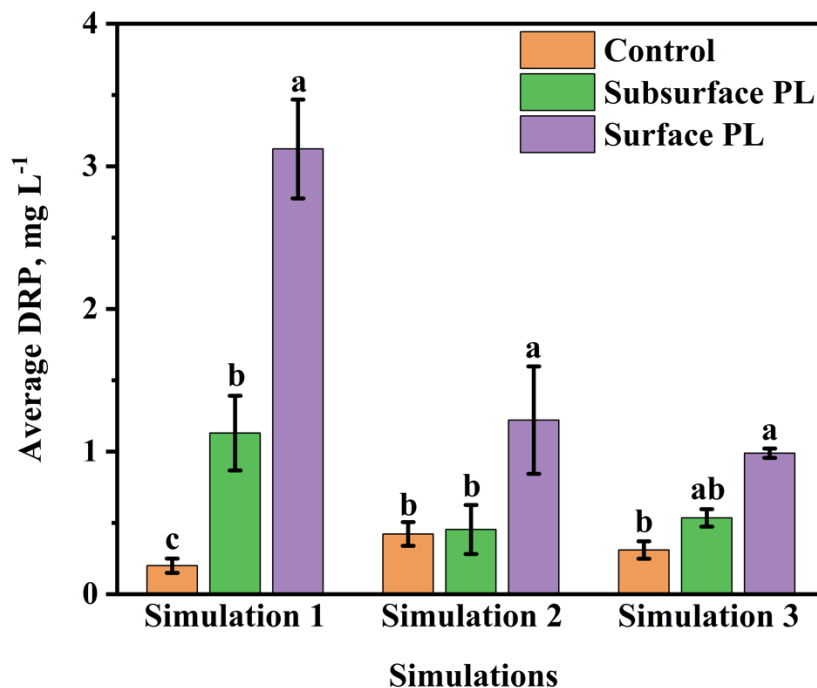


Figure 6. Dissolved reactive phosphorus, through control and PL treated columns in each simulation. Each bar indicates standard error. Means followed by different letters showed significant difference at significant level of 0.05. Where PL: Poultry litter.

The results indicated that treated columns leached a higher concentration of DRP than the control columns. A previous study conducted by Hoover et al. (2019) found that PL leached a greater amount of DRP than control treatments as PL acts as a source of P. For each of the three simulations, the amount of leached DRP was greatest for surface applied litter treatment, intermediate for the subsurface banded treatment, and least for the control. According to Kleinman et al. (2015), surface application of PL causes immediate transport of DRP to the soil profile after manure application. This immediate transport of DRP after litter application could be due to preferential flow pathways (Kleinman et al., 2009). As shown by Budhathoki et al. (2022), soil macropores are prevalent at the study site, which likely resulted in a high loss of DRP in the leachate. Further, in the surface broadcasting method, ponded water was in direct contact with surface-applied PL, further increasing the loss of DRP via preferential pathways (Williams et al., 2018). Whereas, when PL was applied in subsurface bands, we found that the concentration of DRP was less as compared to the surface-applied method. The subsurface band application of PL reduces the direct contact between water moving through the soil profile and litter (Watts et al., 2015). In addition, creating a trench for subsurface band application of PL can reduce macropore continuity and cause soil compaction, which could have reduced DRP leaching in this treatment (Lamba et al., 2013). The findings are in agreement with the previous studies showing that subsurface application could help reduce P leaching (Glæsner et al., 2011; Grant et al., 2019; Williams et al., (2018).

2.3.5 Use of Biochar with Poultry Litter

In simulation 1, both surface and subsurface applications of PL + BC led to a significant increase in leached DRP, ranging from 2.48 to 4.0 mg L⁻¹, in contrast to the control treatment, which had a minimal DRP level of 0.20 mg L⁻¹ (Fig. 7). Similarly, simulations 2 and 3 followed the same trend,

with PL + BC applications resulting in significantly higher DRP levels compared to the control. In the second simulation, DRP levels ranged from 1.22 to 1.47 mg L⁻¹ for PL + BC treatments, while the control group had a lower DRP level of 0.42 mg L⁻¹ (Fig. 7). Likewise, in the third simulation, DRP levels surged to 0.81 to 1.20 mg L⁻¹ for surface and subsurface PL + BC applications, respectively, significantly surpassing the control group's DRP level of 0.31 mg L⁻¹.

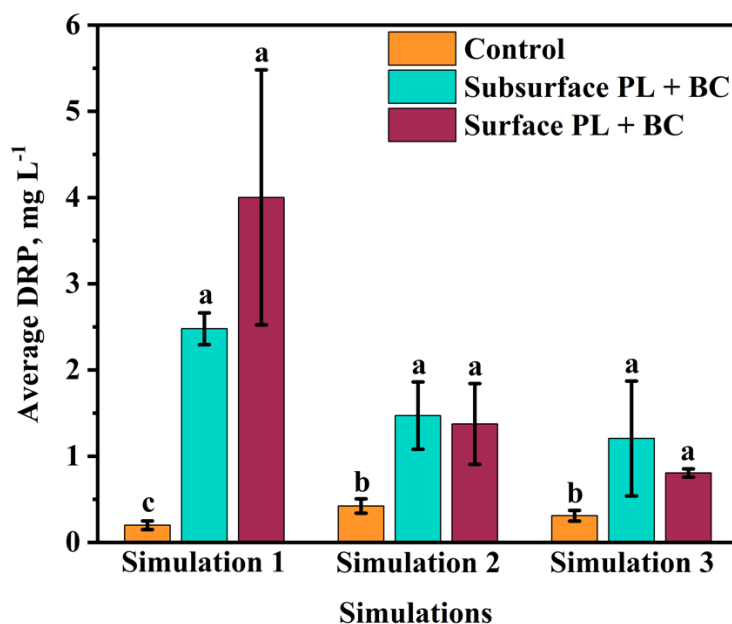


Figure 7. Dissolved reactive phosphorus, through control and PL + BC treated columns in each simulation. Each bar indicates standard error. Means followed by different letters showed significant difference at significant level of 0.05. Where PL: Poultry litter; BC: Biochar.

Considering the effect of PL + BC, leachate concentration of DRP was numerically higher in surface application in simulation 1 than the subsurface method, however it was not statistically significant (Fig. 7). Then in simulation 2 and 3, no significant difference was observed in DRP leaching from the surface and subsurface methods. Overall, the introduction of BC with PL leads

to higher DRP loss compared to PL alone, but the difference was not statistically significant. Thus, use of pristine BC in addition to the PL did not reduce the DRP in the leachate. To quantify this further, we collected leachate from the application of PL and PL + BC, and found that pristine BC does not help to mitigate DRP leaching from PL (Fig. 8). From Fig. 8, the DRP leaching curves from PL alone and combined PL + BC applications are very similar, nearly overlying one another, demonstrating that biochar offered no mitigation of DRP leaching. This effect might be attributed to BC's contribution to increased P leaching, as reported by Angst et al. (2013) and Li et al. (2019). They reported that the presence of BC significantly raised the cumulative amount of $\text{PO}_4^{3-}\text{-P}$ in soil leachate due to the water-soluble P content in BC ash. Moreover, the BC used in this study had a phosphorus concentration of 456.7 mg kg^{-1} , and WEP was 16.6 mg kg^{-1} , which was also contributing towards leaching. Schnell et al. (2012) also reported that BC application contributed to increased concentration of DRP in runoff studies, as BC itself releases P into the soil matrix. Another reason for the increase in the loss of DRP might be the slightly acidic nature of the soil, as BC releases more orthophosphate as soil acidity increases (Wei et al., 2013). Also, Madiba et al. (2016) reported that co-application of biochar with phosphate fertilizer significantly increases the P leaching, compared to an unfertilized control. Similarly, Bradley et al. (2015) also reported that application of BC to manure was not an effective method to control P leaching. Moreover, it has been observed that pristine BC was not effective in adsorbing contaminants, i.e., less removal for metals from soil and water, as untreated BC exhibits a lower binding capacity than modified BC (Phiri et al., 2024).

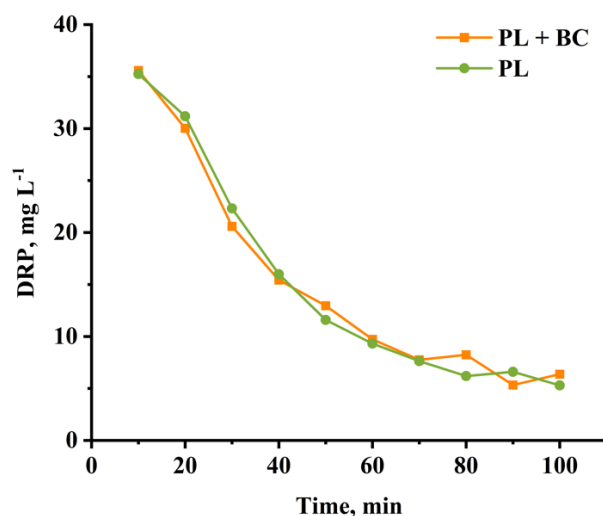


Figure 8. DRP leaching from BC and PL + BC only with respect to time. Where PL: Poultry litter; BC: Biochar.

2.3.6 DRP Leaching Respective to Simulation

In our study, we observed that columns treated with PL and PL + BC, using both surface and subsurface methods, leached significantly higher amounts of DRP in the first simulation compared to subsequent simulations. In contrast, the control treatment exhibited a different trend, with DRP concentrations significantly higher in simulation 2 compared to simulation 1 (Fig. 9). The higher DRP concentrations observed in the first simulation for all treated columns can be attributed to the immediate transport of DRP into the soil profile following manure application. This aligns with findings by Sharpley et al. (2001), which indicate that the highest risk of P loss occurs during the initial rainfall events post-fertilizer application. Similar findings were reported by Gentry et al. (2007) and Smith et al. (2007), such that frequent P losses occur during these early events. Additionally, according to Smith et al. (2007), nutrient export can be notably high if the interval is short between application and the first rainfall event.

The amount of leached DRP was lower for simulations 2 and 3 than for simulation 1 (Fig. 9). Similar patterns were noted across various research studies conducted on soil columns and tile drainage, where the level of P leaching decreased with each subsequent rainfall event following the initial application of PL (Feyereisen et al., 2010; Williams et al., 2016). Also, research by Beven et al. (2013) and Geohring et al. (2001) indicated that in agricultural soils, the walls of macropores are often already saturated with P, which limits the chance for P to absorb and leach through these pathways.

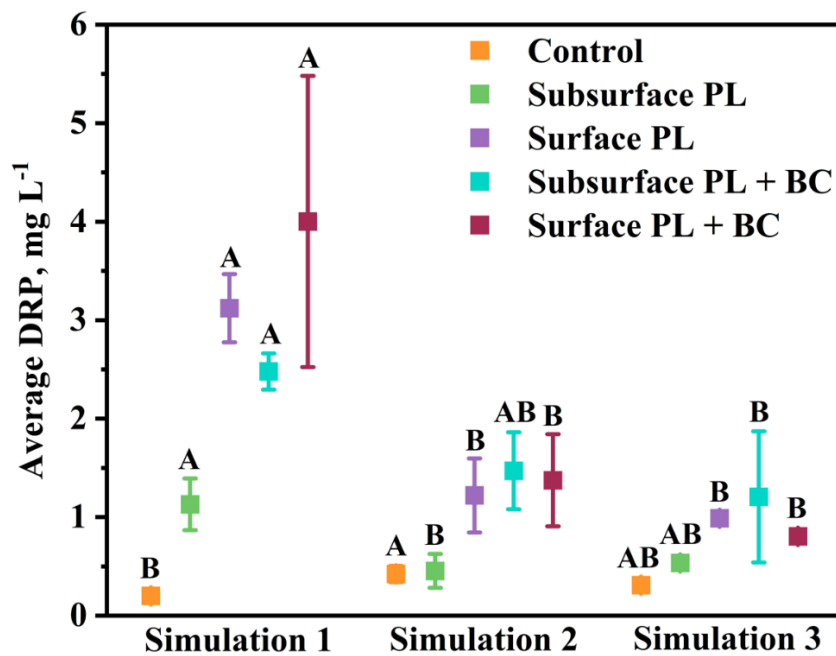


Figure 9. Significant differences for dissolved reactive phosphorus leaching were observed with respect to three simulations from five treatments. Each bar indicates standard error. Means followed by different letters showed significant differences at a significant level of 0.05. Where PL: Poultry litter; BC: Biochar.

2.3.7 DRP Loading

Table 4 presents the DRP loading in the leachate from the control and the treatment columns treated with PL and PL + BC in each simulation. In our study, we found that in simulation 1, the soil columns treated with the surface application method with PL alone or PL + BC combined, the DRP loading in the leachate was significantly greater than for both subsurface banded PL treatment and the control treatment. Also, when PL + BC was applied using subsurface banding, the DRP loading in the leachate was significantly higher compared to the control treatment. Then, in simulations 2, DRP loading in leachate samples were significantly higher from subsurface banded PL + BC, surface PL, and surface PL + BC treatments than subsurface banded PL and control treatments. In simulation 3, there were no significant differences observed between treated and control columns because most of the leachable DRP was released during the initial rainfall events (Williams et al., 2016).

There was higher loss of nutrients when we applied PL by using the surface broadcast method. In our study loading of DRP in leachate was higher for the surface-applied treatments than for the subsurface banded and control treatments. Similar results were reported by Glæsner et al. (2011). When treatments were applied using subsurface banding, DRP loading in leachate was lower than surface-applied treatments. This is because the subsurface band application limited the contact between the PL and the water moving down the soil profile (Lamba et al., 2013). Also, during the application, subsurface banding causes compaction of soil, which also leads to reduced nutrient loading in the soil profile.

Table 4. Loading of dissolved reactive phosphorus (DRP) in the leachate for all columns, for the various treatments and for the three simulations. Values shown in parentheses are standard errors. Means followed by different letters showed a significant difference at alpha 0.05 between treatment*Simulations.

Average loading (g ha ⁻¹) with standard error			
Treatment	Simulation		
	Simulation 1	Simulation 2	Simulation 3
Control	1038±(380) F	1484±(404) FE	954±(253) F
Subsurface PL	4531±(1238) BC	1401±(411) FE	1660±(104) DFE
Subsurface PL + BC	8344±(392) BA	4697±(1379) BC	3317±(1575) DCE
Surface PL	13465±(1504) A	4492±(1385) BC	3542±(113) DC
Surface PL + BC	13414±(4129) A	4669±(1590) BC	2737±(159) DCE

2.3.8 Implications of Study

This study offers critical insights into soil management practices and their environmental impacts. The findings of this study support the hypothesis that the method of PL application significantly influences P leaching, with subsurface band application proving more effective in reducing DRP leaching compared to surface broadcasting. This has important implications for agricultural practices, indicating that farmers should consider subsurface application techniques to mitigate nutrient runoff and enhance nutrient retention in the soil (Dell et al., 2011; Watts et al., 2015b).

Practically, this study highlights the need for appropriate application methods to minimize environmental impacts. Promoting subsurface band application as a best practice for PL management can lead to improved air and water quality while also improving crop nutrient uptake and potentially reducing fertilizer needs (Ashworth et al., 2021; Watts et al., 2019). These findings can inform the development of agricultural guidelines and policies that encourage subsurface application of organic amendments like PL. Updating environmental regulations to incorporate these recommendations can promote sustainable farming practices and protect water resources from phosphorus pollution.

While the study found that pristine BC was not effective in reducing DRP leaching, it opens avenues for future research. Further studies should explore different types of BC, application rates, and combinations with other soil amendments. Moreover, investigation of the impact of modified BC on nutrient leaching could provide valuable insights into improving nutrient management practices and mitigating environmental impacts.

Reducing P leaching through improved application methods can have significant environmental benefits, including the prevention of harmful algal blooms and the preservation of aquatic ecosystems. By adopting these practices, the agricultural sector can contribute to more sustainable and environmentally friendly food production systems. Implementing subsurface application methods may have initial costs, but the long-term benefits of improved soil health and reduced environmental impact can outweigh these expenses. Educating farmers on the economic and environmental advantages of these practices can facilitate their adoption. By integrating these findings into agricultural practices and policies, we can achieve better environmental stewardship and sustainability in farming and in the environment.

2.4 Conclusions

A study was conducted to investigate the effect of the placement of PL and combined application of PL and biochar on the leaching of DRP in successive rainfall events. Soil columns were collected from a pasture field having a dense macropore system. Five treatments were applied: unamended control (0 Mg ha^{-1}), surface broadcasting of PL (10 Mg ha^{-1}), subsurface band application of PL (10 Mg ha^{-1}), surface application of PL (10 Mg ha^{-1}) and biochar (10 Mg ha^{-1}), subsurface application of PL (10 Mg ha^{-1}) and biochar (10 Mg ha^{-1}). For each treatment, three simulations were performed (simulation 1 (0th day), simulation 2 (5th day), and simulation 3 (10th day)). The bromide breakthrough curves showed the existence of preferential flow. After performing rainfall simulation experiments, we found that in simulation 1, treated columns leached a significantly greater amount of DRP in the leachate sample than in control columns. In simulations 2 and 3, DRP leaching was less than in simulation 1. In terms of application methods, subsurface application is better than surface broadcasting. Also, the pristine biochar used in this study was not an effective way to hold or reduce DRP leaching.

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Chapter 3

Elucidating Impact of Poultry Litter and Biochar Application Method on Metal Leaching

3.1 Introduction

The broiler industry in the USA has a rich history and has evolved into a highly industrialized and vertically integrated sector (Bukari, 2014). The USA is the world's largest producer and the second-largest exporter of poultry meat, as well as a major egg producer (Benavides et al., 2020; Davis et al., 2013). Among the states in the USA, Alabama alone produces around 1.09 billion broilers annually, constituting approximately 12.3% of the total US broiler production (Lamba et al., 2019). As the poultry industry grows, it increases the challenges of properly managing the manure produced, due to its environmental concerns on soil, air, and water quality (Rahman et al., 2022). Worldwide, more than 90% of poultry manure is commonly dispersed on land near poultry farms (Babuna et al., 2005). Similarly, in Alabama, around 1.8 Mg of poultry litter is produced annually by the broiler industry. Most of the poultry litter is applied to land and farms close to poultry farms (Chakraborty et al., 2021).

Poultry litter (PL) is a combination of manure, feathers, and various bedding materials such as wood shavings, peanut hulls, rice hulls, or other similar materials (Mitchell et al., 2005). Poultry litter is a rich source of nutrients, such as nitrogen (N), phosphorus (P), and potassium (K) for crops (Scholarlycommons et al., 2022). Apart from these macronutrients, PL also contains trace metals (e.g., magnesium [Mg], sodium [Na], and aluminum [Al]) and heavy metals (e.g., zinc [Zn], arsenic [As], and copper [Cu]) (Lamba et al., 2019). These elements are required for plant growth, but an excess of these elements leads to toxicity in plant species, which further impacts organisms that consume these plants (Gerber et al., 2007). Moreover, the accumulation of these metals in soil

from repeated application of PL (Aljohani, 2023) negatively impacts soil fertility and chemistry and degrades surface and groundwater quality (Xiao et al., 2022). Higher concentrations of heavy metals in soils also threaten the environment and significantly impact human health (Jalali et al., 2008). The United States Environmental Protection Agency (USEPA) has grouped metals like Zn, Al, chromium (Cr), Cu, and nickel (Ni) as potentially toxic elements (PTEs) because of their non-biodegradable and toxic nature as they impact the food chain (Goren et al., 2022). Moreover, Königer et al. (2021) stated that these heavy metals, even at low concentrations, can seriously impact soil diversity and microbiota. Therefore, the concentration of these metals should be monitored regularly, as government agencies set various criteria for these metals. According to the USEPA, the concentration of Zn should not exceed 0.12 mg L^{-1} in freshwater. If Zn concentration in drinking water exceeds the defined range, it can cause nausea and vomiting in children, especially under age 2 (Noulas et al., 2018). Furthermore, higher concentrations of Al in acidic soil, i.e., Al toxicity, hinder plant growth in soils with a pH lower than 5.5 (Rout et al., 2001). Aluminum also inhibits microbial processes by restricting root growth and affecting plants' water and nutrient absorption capacity (Singh et al., 2017). Other metals, such as Ni, are also phytotoxic at higher concentrations. Ni toxicity impacts plant growth, causing chlorosis, necrosis, and wilting and negatively affecting photosynthesis and respiration (Bhalerao et al., 2015). Additionally, Singh et al. (2017) reported that Ni is harmful for the human body as it may lead to asthma, respiratory problems, and heart disorders. Similarly, Fe negatively affects plants' growth and development, carbon metabolism, enzymatic activities, respiration processes, and the efficiency of photosynthesis (Zahra et al., 2021). However, metals such as Cr are required by the body to perform catabolic processes and control blood pressure and glucose levels, but in excess, they can cause serious health repercussions like allergies, skin rashes, irritations, nosebleeds, stomach

ulcers, breathing problems, weakened immune systems, and even lung cancer (Singh et al., 2017). Metal, such as Cu, plays an important role in the physiological functions of the human body, but its higher concentration can cause effects related to the intestine and liver (Ahmad et al., 2021).

Heavy metals exhibit significant persistence within the soil and can accumulate within plants over time (Lu et al., 2022; Naila et al., 2019). The sorption and desorption behaviors of heavy metals in soil primarily depend on factors such as soil pH, redox potential, and the composition of soil minerals, including metal hydroxides, phosphates, clays, metal oxides, and organic and inorganic matter (Lu et al., 2022). However, Camobreco et al. (1996) demonstrated that preferential flow significantly enhances the mobility and speed of metal transport to groundwater. Therefore, in recent years, there has been considerable focus on the movement of metals within soil profiles (Liu et al., 2019; Suszek Gonçalves et al., 2020). Moreover, research has indicated that the concentration of metals can escalate to toxic levels with the prolonged application of manure (Xiu-Zhen et al., 2008). Dissolved organic matter (DOM) from poultry litter application can effectively contribute organic ligands to the formation of complexes with heavy metals in the soil (Bolton et al., 1991). These complexes have the potential to migrate downward and degrade groundwater quality (Li et al., 1996). The leachability and mobility of heavy metals are determined not solely by their total concentrations in the soil but also by the soil's physicochemical properties and various environmental factors (Jalali et al., 2007).

No-till practices in the field lead to greater water infiltration (Ajayi et al., 2021). Moreover, the formation of large pores in the soil as a result of organisms and the proliferation of plant roots throughout the soil matrix (Cavaliere et al., 2009) increases preferential flow (Kim et al., 2008). According to McMullen et al. (2014), metal leaching through preferential flow after the application of poultry manure needs further research.

Many studies have reported metal accumulation in soil at varying depths after the application of poultry litter using the surface broadcasting method (Adeli et al., 2007; Daigh et al., 2009). To reduce the fate and transportation of nutrients, new method was developed to apply poultry litter, which is subsurface method. Most researchers focused on the fate and transport of nutrients, particularly phosphorus and nitrogen, in agricultural fields treated with poultry litter using subsurface application methods (Lamba et al., 2013; Liu et al., 2016; Watts et al., 2015b) . However, limited research has quantified the impact of subsurface poultry litter application on metal fate and transport processes (Lamba et al., 2019). Moreover, there are only a few studies that use undisturbed soil columns to quantify metal leaching due to poultry litter application methods (Adeli et al., 2007; McLaren et al., 2003).

Meanwhile, use of biochar (BC) has been increasing in agricultural sector and is considered an effective adsorbent that can immobilize heavy metals in soil and the environment, as well as increase soil productivity (Igalavithana et al., 2017). Biochar is derived from either plant or animal-based materials and is considered an environmentally friendly sorbent (Jha et al., 2023). The physicochemical properties of BC, such as its porous structure, active functional groups, extended specific surface area, cation exchange capacity (CEC), and high organic carbon content, contribute to its ability to adsorb and retain organic and inorganic compounds (Fahmi et al., 2018). Different studies have reported that the application of BC to contaminated soil results in a reduction of metal leaching and the mobility of heavy metals (Fahmi et al., 2018; Yang et al., 2021). However, limited work has been done to evaluate the effectiveness of BC to investigate metal leaching when it is applied along with PL via surface and subsurface applications.

Therefore, this research aims to investigate metal leaching as a function of biochar and poultry litter application methods. Specific objectives of this study were (a) quantifying the impact of the

poultry litter application method on metal leaching and (b) investigating the effect of biochar on the loss of metals in leachate. Our research builds on previous work conducted by Budhathoki et al. (2022), which has highlighted the presence of substantial macropores at the study site, which can enhance the loss of contaminants via preferential flow pathways.

3.2 Materials and Methods

3.2.1 Study Site

The study site was a pasture field at the Sand Mountain Research and Extension Center (SMREC) in the Sand Mountain region in northeast Alabama (34°17'02.6"N, 85°57'51.8"W), an area known for broiler production within the state (Malhotra et al., 2024). The pasture field covered approximately 0.40 hectares, with a slope length of 80 meters. The Kentucky 31 Tall Fescue (*Festuca arundinacea* Schreb), a cool-season grass, has been established at the study site for over 40 years. This field had not received any broiler litter application for at least 13 years prior to the start of the study. The soil type at the study site includes Hartsells (fine-loamy, siliceous, thermic, Typic Hapludults) and Wynnville (fine-loamy, siliceous, thermic, Glossic Fragiudults) types, which have different drainage qualities and come from weathered sandstone and shale (Budhathoki et al., 2022). The area receives about 1340 mm of rain each year, with an average temperature of 14.8 °C. Previous research has shown that soil macropores can be responsible for the enhanced leaching of contaminants via preferential flow pathways in this region (Budhathoki et al., 2022; Malhotra et al., 2024).

3.2.2 Soil Sampling and Preparation for Rainfall Simulation

For this study, 15 undisturbed soil cores were collected from the field, using a hydraulic system attached to a tractor (Prior et al., 2004). The cores used for the experiment were 150 mm in

diameter and 500 mm deep. The soil cores were carefully extracted to keep them intact. Following sampling, the airspace within each soil core (inside the PVC pipe) was filled with bubble wrap packing material, and then a PVC end cap was securely fitted over each end of the pipe before transporting them. To transport these cores without disturbing them, they were cushioned with wood shavings. These cores were maintained at 4°C before further analyses. Additionally, along with the undisturbed cores, 15 disturbed soil samples were also collected from nearby locations at various depths for chemical analysis, including pH, metal concentration, electrical conductivity, bulk density, organic matter content, and texture.

Before the rainfall simulation experiments, the 2 cm vertical gap between the soil and the lower end of the PVC pipe was filled with acid-washed sand. Each column was covered with a stainless-steel mesh (75 µm) and supported by a PVC drain cover with a central drain hole connected to tubing for leachate collection. Additionally, the horizontal space between the soil and the pipe walls was filled with liquid petroleum jelly to minimize bypass flow (McLeod et al., 2014).

3.2.3 Rainfall Simulation Experiment

The rainfall Simulation protocols were based on a recent study conducted by Malhotra et al. (2024). Initially, soil columns were cut to a depth of 25 cm because preferential flow predominantly occur in the upper 25 cm of soil due to dense macropore network system (Budhathoki et al., 2022). Then, the soil columns were subjected for saturation for a 24-hour period with a 0.01 M KCl solution through capillary action to maintain a constant head of 2-3 cm on the soil surface. Further, five different treatments were applied, which included 1) surface application of PL and 2) subsurface band application of poultry litter 3) surface application of combined poultry litter and biochar, 4) subsurface band application of combined poultry litter and biochar, and 5) a control treatment. The application rate of the PL, applied on a wet basis was 10 Mg ha⁻¹. The application

rate of the biochar was 10 Mg dry biochar ha⁻¹ and the biochar moisture content was 51% wet basis, so the biochar application rate, applied on a wet basis, was 20.4 Mg ha⁻¹. When both PL and biochar were applied together, the moist basis application rates were 10 Mg ha⁻¹ for the PL and 20.4 Mg ha⁻¹ for the biochar. The subsurface bands were positioned with their centerlines aligned along the diameter of each soil core. Each band measured approximately 60 mm in width and varied between 50-100 mm in vertical dimension. A layer of soil, ranging from 50 to 100 mm in thickness, covered the top of each band. For each treatment, three rainfall simulations were carried out at a gap of 5 days to investigate temporal dynamics in the loss of metals. Each treatment had three replications. For rainfall simulations, a peristaltic pump (Masterflex L/S; Cole-Parmer, Vernon Hills, IL) was used to add a leaching solution to the soil columns. Initially, a saturated flow condition was achieved by adding the 0.01 M KCl solution, and throughout the simulation, the head (2-3 cm) was maintained to ensure saturated flow conditions. Then, a bromide (Br⁻) solution (0.01 M KBr) was applied. Bromide is commonly used as a conservative tracer due to its non-toxic nature and minimal sorption onto soils with low anion exchange capacity (Sobotkova et al., 2014). Once the Br⁻ concentration in the leachate matched the input concentration, the columns were washed with a 0.01 M KCl solution until the Br⁻ concentration consistently diminished in the leachate.

3.2.4 Physical and Chemical Analysis of Leachate, Soil, Manure, and Biochar

The leachate collected from soil columns was analyzed for the concentration of Br⁻ by using a Br⁻ combination ion-selective electrode (ISE) (model HI4102, Hannah Instruments Inc., Smithfield, RI). To check the preferential flow through soil columns, the Br⁻ breakthrough curve (BTC) was used (Ersahin et al., 2002). For Br⁻ BTCs, graphs were plotted against the ratio of the Br⁻ concentration in the effluent to the number of pore volumes collected. The relative concentrations

were obtained by dividing the measured Br^- concentrations (C_i) by the initial concentration (C_0) (Shahmohammadi et al., 2022). The number of pore volumes collected (PV) for column effluents was calculated by:

$$\text{PV} = V_c/V_0$$

V_c is the cumulative volume of column effluent (mL), and V_0 is the amount of water within the soil column (mL), which is calculated by multiplying the column's total volume by the soil's saturated water content. The leachate samples were also analyzed for pH and EC using respective pH and EC meters (VWR symphony electrodes, VWR International, Inc., Radnor, PA). Further, the leachate samples were analyzed for metals (Fe, Al, Zn, Cu, Cr, and Ni) using ICP-OES (EPA 200.8), before performing the analyses on ICP-OES, the samples were acid-digested using the block digester by following EPA Method 200.2 (Martin et al., 1994).

For soil analysis, the samples were air-dried, grounded, and sieved through a 2 mm mesh before analysis. A hydrometer was used to measure sand, silt, and clay particle percentages (Bouyoucos, 1962). Organic matter was measured using the weight loss on ignition method described by Malhotra et al. (2020). Bulk density was measured by using the ratio of the mass of the oven-dried sample (105°C) to the volume of the sample collected. Soil samples (1:10 soil:water) underwent pH and EC analysis via a pH and EC meter (VWR Scientific Inc., Radnor, PA) (Carter et al., 2007). Soil total metals were quantified using inductively coupled plasma-optical emission spectrometry (ICP-OES) following EPA Method 200.8. Before analysis, the samples were digested using a microwave digester, performing total digestion with concentrated nitric acid in accordance with EPA Method 3052 (USEPA, 1995). To analyze the different metal fractions in the soil, such as soluble, exchangeable, carbonate, amorphously bound oxide, crystalline hydrous oxide, organic-

bound, and residual fractions, a sequential extraction procedure was used on soil samples from a depth of 0-25 cm as described by Gleyzes et al. (2002).

The poultry litter used in this study was collected from northern Alabama. For this study, PL was analyzed for various properties such as pH, moisture content, and total metals. The pH of poultry manure samples (1:3 manure-to-water ratio) was measured using a pH meter (VWR Scientific Inc., Radnor, PA), following the method described by Carter et al. (2007). The metal content of the PL was analyzed by using dry ashing method as described by Wolf et al. (2003) and Isaac (1998). The moisture content of PL was determined as described by Hoskins et al. (2003). For metal fractionation of PL, a modified BCR method was used, as reported by Rauret et al., (1999).

Biochar used in this study was prepared from pine wood. This pine wood biochar was purchased from Waste to Energy Inc. (Slocomb, Alabama, USA). Moreover, this biochar was earlier used by Wang et al. (2022). Various morphological and physicochemical properties of the biochar were thoroughly analyzed, including CHNS composition, ash content, point of zero charge (pH_{ZPC}), and pH (Wang et al., 2022). Metal content of biochar was analyzed by using ICP-OES through EPA Method 200.2 (Martin et al., 1994).

3.2.5 Statistical Analysis

Statistical analysis was conducted using SAS version 9.4 (SAS Institute Inc, Cary, NC). The PROC GLIMMIX method was employed to assess the impact of time and treatment on leachate nutrient concentration. Time and treatment, along with their interaction, were considered fixed effects, while “rep” and “rep*time” were included as random effects, treating time as a repeated measure in the model. Data were transformed using a log-normal distribution to improve data fit, and back transformation was applied to present the original means in the graphs. Multiple comparisons of

treatment and time means were performed using the least significant difference (LSD) test, with a significance level of 0.05.

3.3 Results and Discussion

3.3.1 Soil Properties

The physical and chemical properties of the soil are presented in Table 1. The electrical conductivity (EC) of the soil was 0.163 dS m^{-1} , which indicates a lower soluble salt content (Malhotra et al., 2024). The average pH of the soil for each depth increment was 5.9, which is within the range as reported by HE et al. (2019) in the study conducted in the Sand Mountain region. Organic matter was showing a declining trend, 4.9%, 3.26%, and 2.60%, with respect to depth 0-5 cm, 5-10 cm, and 10-25 cm, respectively. Besides, the texture of the soil was sandy loam and the proportion of sand, silt, and clay distribution in soil samples at different depths is mentioned in the Table. 5.

Table 5. Chemical and physical properties of the soil samples at varying depths (0-5 cm, 5-10 cm, and 10-25 cm) along with standard errors.

Depth (cm)	Organic matter (%)	pH	EC ($\mu\text{S cm}^{-1}$)	Dry bulk density (g cm^{-3})	Sand (%)	Silt (%)	Clay (%)
0-5	4.90 $\pm$ 0.60	6.14 $\pm$ 0.74	239 $\pm$ 78.93	1.19 $\pm$ 0.06	60 $\pm$ 4.43	31 $\pm$ 4.68	8 $\pm$ 0.49
5-10	3.26 $\pm$ 0.02	5.93 $\pm$ 0.46	98.8 $\pm$ 35.30	1.19 $\pm$ 0.02	63 $\pm$ 3.03	30 $\pm$ 2.83	7 $\pm$ 0.57
10-25	2.60 $\pm$ 0.15	5.68 $\pm$ 0.36	123.59 $\pm$ 57.73	1.34 $\pm$ 0.05	66 $\pm$ 3.89	26 $\pm$ 3.39	8 $\pm$ 0.50

The results from the sequential extraction procedure showed that a large percentage of Al, Zn, and Fe were bound to the residual fraction (Fig. 10) compared to the sum of other fractions. According to Borgese et al. (2013), the proportion of metals linked to the residual fraction indicates that a significant amount of metals in the soil are strongly bound to clay minerals. Metals bound to minerals in the residual fraction are only soluble in strong acid solutions (Sungur et al., 2015). Among all the metals, Cu showed the highest proportion associated with organic matter, approximately 20%. Metals such as Al and Zn were mostly attached to the residual fraction in the 0–25 cm soil pool. Many other studies have also reported that most of the Zn in soil was associated with the residual fraction, indicating that it is tightly bound within the soil profile (Liu et al., 2018; Makuch et al., 2021; Zahedifar, 2017). The major proportion of Fe was strongly bound to the residual and crystalline fractions. Similar findings have been reported by Shahbazi et al. (2019).

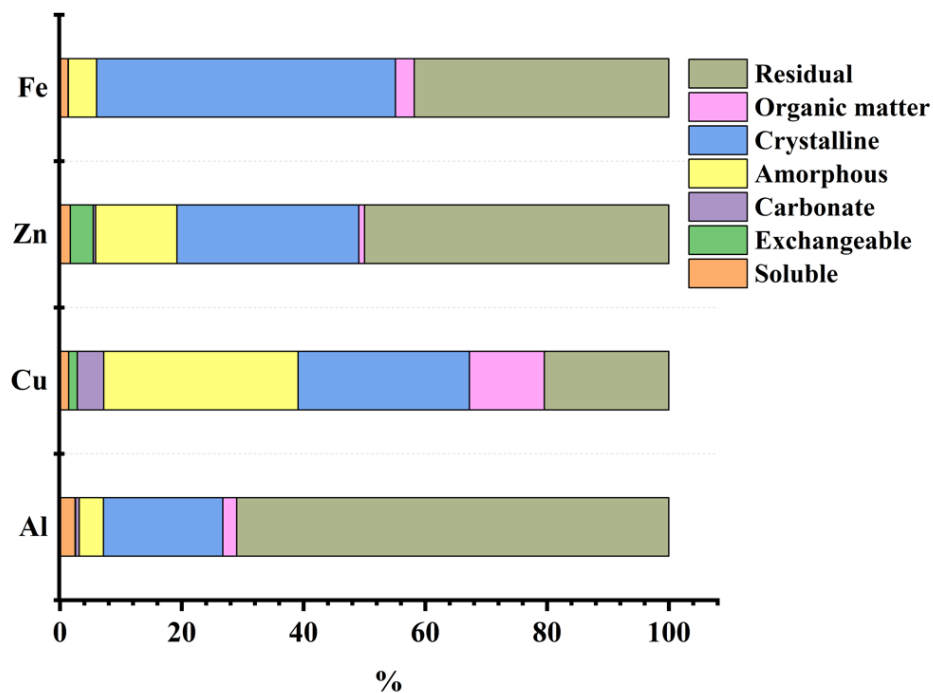


Figure 10. Overall percentage distribution of metals in the different fractions obtained from the sequential extraction procedure for soil at a depth of 0-25 cm.

Figure 11. depicts the initial concentration of metals in soil samples collected in proximity to the intact-undisturbed soil cores (0-5 cm, 5-10 cm, and 10-25 cm). In all soil columns, we found that Al and Fe were the most dominant metals than Cu and Zn. Their concentration is increasing with respect to the depth of soil. Whereas concentration of Zn was found to be higher in surface soil, decreasing with depth of soil. Moreover, the concentration of Cu was higher in 5-10 cm soil (Malhotra et al., 2023)

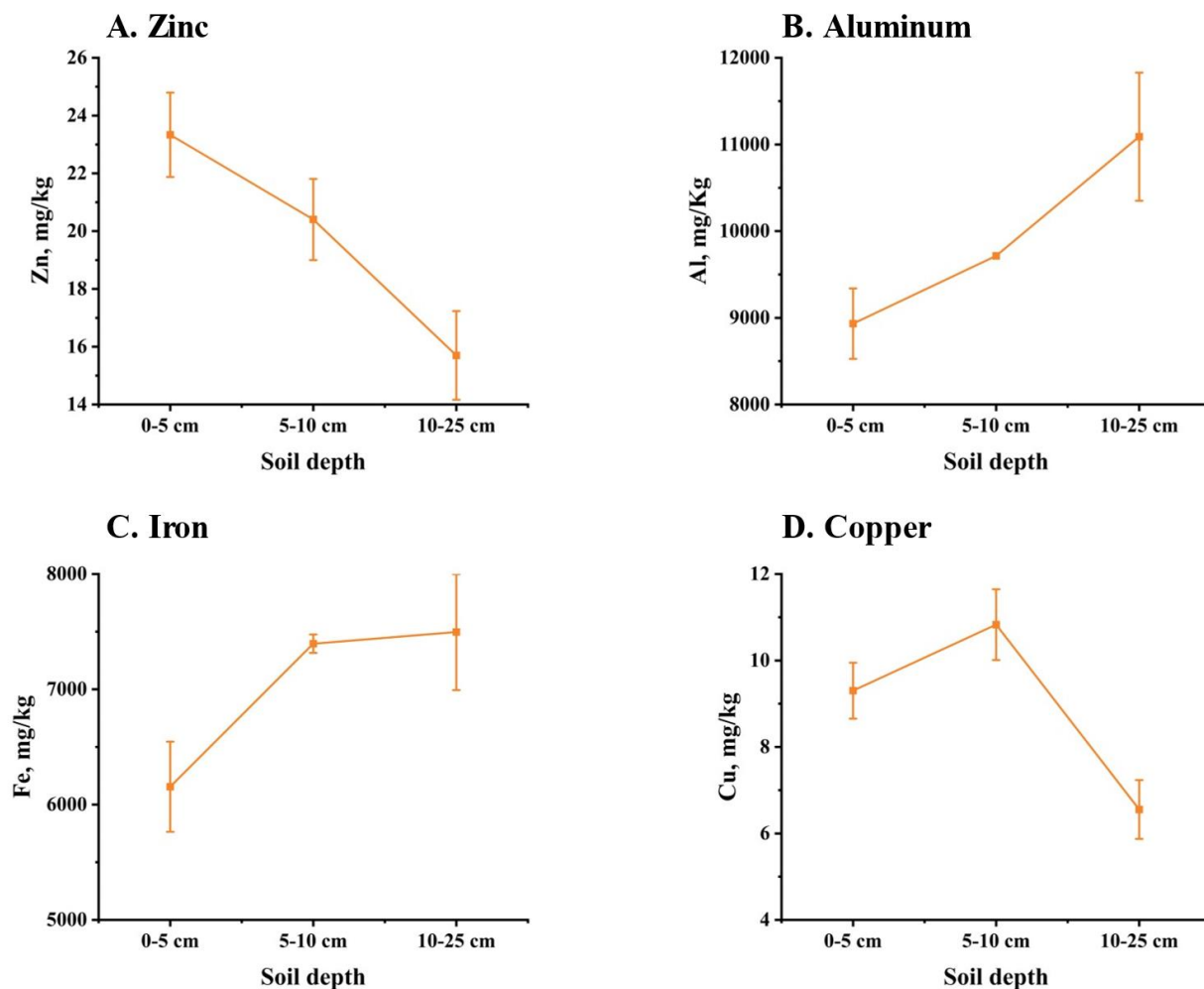


Figure 11. Distribution of total metals within soil profile from 0 to 25 cm depth. Each bar represents a standard error.

3.3.2 Poultry Litter Properties

The PL used in this study was quantified for different metal concentrations (Al, Cu, Fe, and Zn) (Table 6). The average pH of PL was 7.37, close to the typical pH of poultry litter (Lin et al., 2018b). Moisture content of the PL was 12.17 %, which was slightly lower than the average moisture content in litters (A. Sharpley, n.d.).

Table 6. Physical and chemical properties of the poultry litter, along with standard errors.

pH	Moisture content (wet basis), %	Al, mg kg ⁻¹	Cu, mg kg ⁻¹	Fe, mg kg ⁻¹	Zn, mg kg ⁻¹
7.37±0.33	12.17±0.03	1890±20.67	184±2.00	2291±56	356.33±9.67

Fig. 12 represents the different proportions of metal in PL. The sequential extraction procedure was performed to quantify the different fractions of acid-soluble (water soluble, exchangeable, and acid soluble), reducible (Mn-oxides, amorphous Fe oxide, and crystalline Fe-oxides), oxidizable, and residual (Rauret et al., 1999). The proportion of Zn was near about 50% which was present as acid soluble in PL followed by Cu, Fe, and Al. The acid soluble part was easily destroyed in acidic environment and easily influenced by pH values (Wang et al., 2022). Large proportion (48%) of Cu was attached to organic matter such as oxidizable fraction in PL than another metals. According to Wang et al. (2018), the organic-bound fraction was simulated in oxidizing conditions. Major portion of Fe and Al was present in reducible and residual portion of PL. Reducible portion was released under reducing conditions (Wang et al., 2022), while residual proportion was less bioavailable (Malhotra et al, 2023).

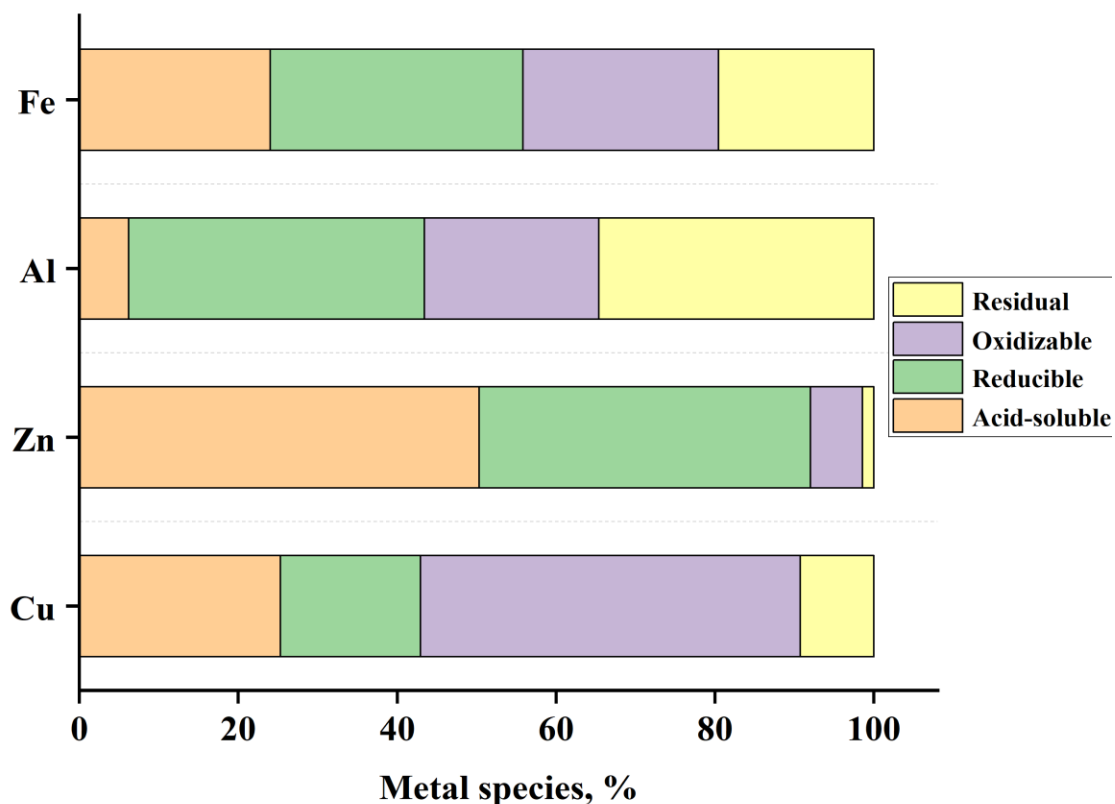


Figure 12. Distribution of metal percentage within PL in different fractions (residual, oxidizable, reducible, and acid-soluble).

3.3.3 Biochar Properties

The characterization of pine wood biochar was performed in a previous study (Wang et al. (2022), which included physicochemical properties, detailing its carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) contents, ash content, point of zero charge, pH, and metals present in Table 7. Physicochemical properties of biochar indicate that the carbon content of biochar was more than 80%, which is considered stable according to Wijitkosum et al. (2019). Low ash content has been observed for pine wood biochar due to lower inorganic constituents. Importantly, the pH of biochar solution is 9.9, whereas pH_{pzc} has been found to be 9.4, which suggests that biochar is stable in

solutions and behaves alkaline nature. Besides, trace elements like Cu, Zn, Fe, and Al concentrations were 13.1, 7.0, 846.7, and 7.21 mg Kg⁻¹, respectively.

Table 7. Physicochemical properties of biochar: characteristics and composition analysis.

C, wt. %	83 ± 1
H, wt. %	1.1 ± 0.1
S, wt. %	0.30 ± 0.1
N, wt. %	0.40 ± 0.01
Ash, wt. %	7.0 ± 0.2
pH_{zpc}	9.4
pH of biochar solution	9.9
Cu, mg Kg⁻¹	13.1 ± 1.6
Zn, mg Kg⁻¹	7.0 ± 2.0
Fe, mg Kg⁻¹	846.7 ± 56.4
Al, mg Kg⁻¹	7.21 ± 4.2

3.3.4 Bromide Breakthrough Curves

The shape of BTCs is useful for defining preferential flow (Marinas et al., 2013). In this study, the shape of BTCs was asymmetrical and had a long tail, indicating preferential flow through soil columns from each treatment (Fig. 13), as found by Luo et al. (2010). Moreover, in this study, the Br⁻ concentration was noted to exceed 0.5 prior to reaching one pore volume, indicating an early breakthrough of Br⁻ (Cheng et al., 2014). Thus, this early breakthrough and rapid transportation represent large and continuous macropores in undisturbed soil columns, as reported by Budhathoki

et al. (2022) based on X-ray CT scanning analysis. Soil macropores result from biological activities such as old root channels or earthworm burrowing (Kang et al., 2011). These macropores are responsible for preferential flow, which can lead to the transportation of applied fertilizers and other chemicals over considerable distances within a watershed (Wang et al., 2011).

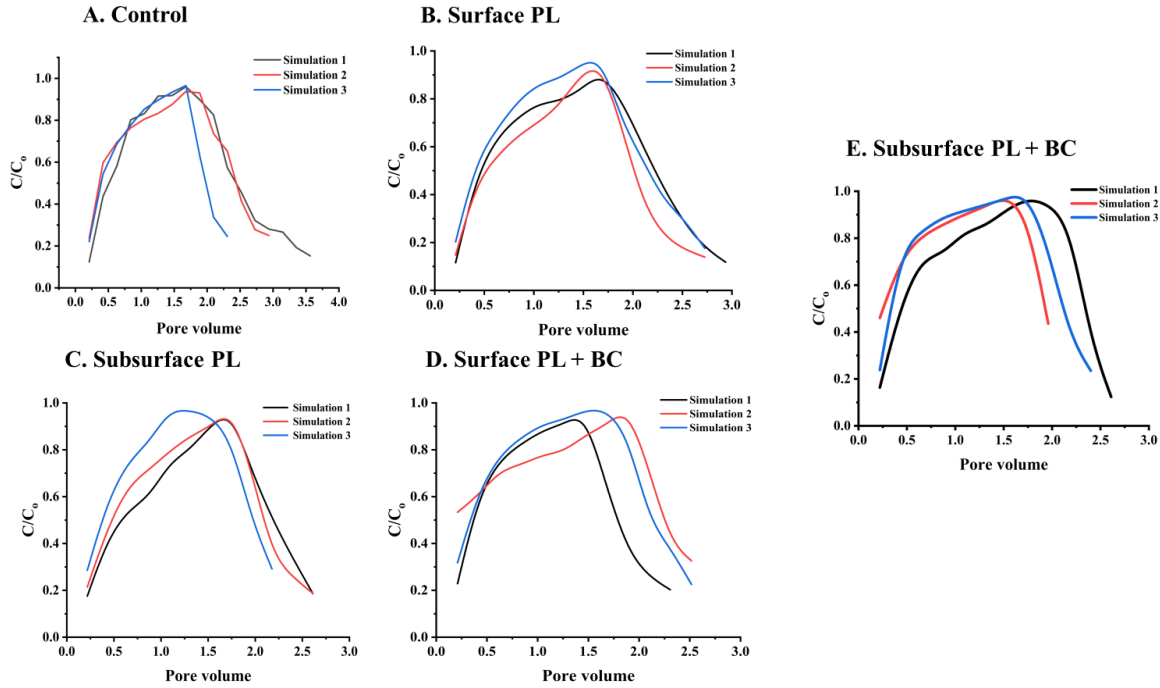


Figure 13. Breakthrough curves for relative concentration of bromide with respect to pore volume. Where PL: Poultry litter; BC: Biochar.

3.3.5 Metal Leaching

In general, surface and subsurface applications of PL and combined application of BC and PL enhanced the leaching of metals via preferential flows present in the soil columns. The concentrations of metals such as Ni and Cr in the leachate samples were below the detection limit from both the control and treated columns. In contrast, Cu, Zn, Al, and Fe were detected in the leachate samples collected from the soil columns before and after the treatments. In this study, the

metals Cu and Zn behaved similarly in leaching patterns. The movement of these metals depends upon dissolved organic matter (DOM), pH, and the ligands (Draszawka et al., 2017; Suszek et al., 2020). The average concentrations of leached metals (Cu, Zn, Al, and Fe) from the five treatments are represented in Figs. 14, 15, and 16. For Cu, we found that concentration was significantly ($P<0.05$) greater in treatment surface PL + BC (0.14 mg L^{-1}) than in control treatment (0.005 mg L^{-1}) as well as subsurface PL (0.08 mg L^{-1}) in simulation 1 (Fig. 14 A). In addition, the treatments of subsurface PL, surface PL, and subsurface PL + BC (0.06 mg L^{-1} to 0.10 mg L^{-1}) resulted in a significantly higher release of Cu in the leachate samples compared to the control treatment ($P<0.05$). Then, in simulation 2, all treated columns leached a significantly higher amount of Cu in leachate samples (0.02 mg L^{-1} to 0.03 mg L^{-1}) than in control. Moreover, in simulation 3, the concentration of Cu in leachate from subsurface PL + BC (0.01 mg L^{-1}) was significantly greater than in the control treatment (0.005 mg L^{-1}) at a significance level of 0.05.

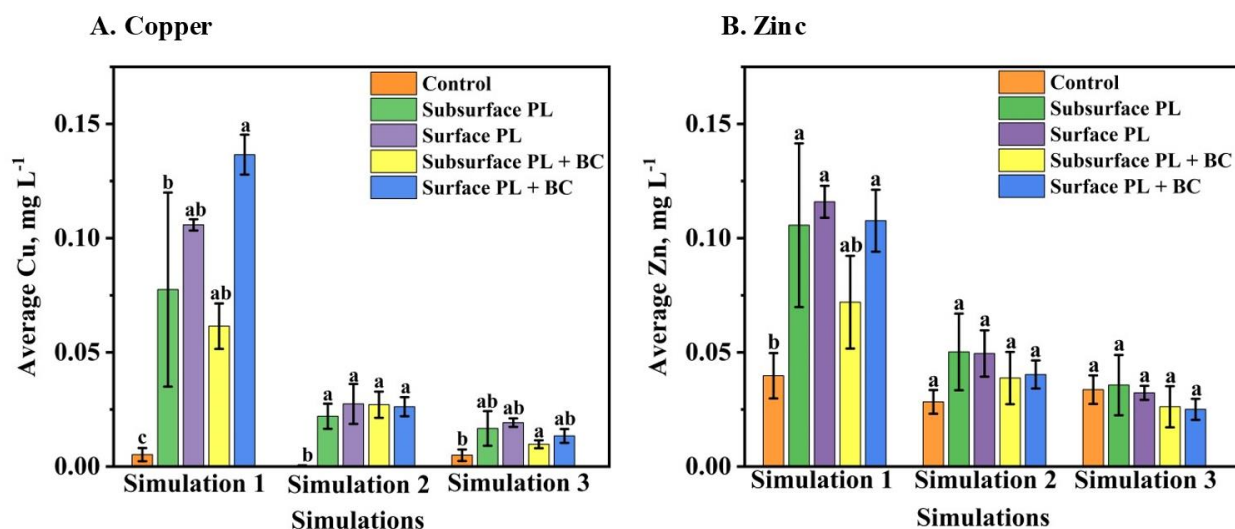


Figure 14. Leaching of metals (A. Copper, B. Zinc) through all five treatments in each simulation. Each bar indicates standard error. Within each simulation means followed by

different letters were significantly different at the significant level of 0.05 between each treatment within the simulation. Where PL: Poultry litter; BC: Biochar.

Zinc is considered a secondary contaminant in drinking water, with a secondary maximum contaminant level (SMCL) of 5 mg L⁻¹. The average concentration of Zn collected from both the treated as well as control columns was less than the suggested secondary limit throughout the simulations (Fig. 14 B). In simulation 1, the concentration of Zn leaching from treated columns such as subsurface PL, surface PL, and surface PL + BC ranged from 0.11 mg L⁻¹ to 0.12 mg L⁻¹, significantly ($P<0.05$) greater than the control treatment (0.04 mg L⁻¹). Then, in simulation 2 and simulation 3, the average concentration of Zn in the leachate sample from the control and treated columns did not show any significant difference ($P<0.05$).

We found that a large proportion of Cu was associated with the fractions of Fe/Mn oxides or organic matter in a soil (Fig. 10). According to Sun et al. (2019), this fraction of Cu tends to be less mobile, and less available for leaching. Thus, in control columns, Cu concentration was lower in all simulations than in treated columns. While Zn was present in soluble form in soil (Fig. 10). Thus, the leachate concentration of Zn was higher in control columns than Cu. In all treated columns, average Cu and Zn concentrations were significantly ($P<0.05$) higher in leachate samples in simulation 1 than control columns. The treated columns leached greater Cu and Zn in leachate in simulation 1 because these metals in poultry litter were associated with exchangeable, soluble, and carbonate fractions (Fig. 12) and therefore were more mobile (Li et al., 2021). The interaction between metals and dissolved organic matter (DOM) in soil, resulting in the formation of complexes, can significantly influence the adsorption processes and mobility of these metals (Suszek et al., 2020). Moreover, Zn forms complexes with DOM but is not highly stable (Amrheln et al., 1992). When PL or combined application of PL + BC was applied on the surface of soil

columns, it increased the pH of the soil solution than subsurface treatments in simulation 1 (Table. 8). According to Draszawka et al., (2017), with the increase in pH, a higher proportion of Cu and Zn can be complexed with organic matter, resulting in less of it being adsorbed by clay particles. Thus, Cu mobilizes more due to stable complex formation than Zn. A similar finding was reported by Suszek et al. (2020) that metals like Cu readily form stable complexes with DOM, as compared to Zn, tend to exhibit greater mobility within the soil. However, when PL or combined application of PL + BC was applied using the subsurface method, the pH of the soil solution was reduced in first simulation. At lower pH, metal ions contribute to leaching. A similar finding was reported by Zheng et al. (2012). We found that most of the leachable metals got leached during initial rainfall event (Katuwal et al., 2022) such that in simulation 2 and 3 concentration of Cu and Zn in leachate sample were lower as compared to simulation 1. Moreover, when BC was incorporated with PL, it was unable to hold or absorb the Cu and Zn. It was likely due to pristine BC used in this study because pristine BC has limited effectiveness in removing heavy metals from soil and water as reported by Phiri et al. (2024). Thus, results indicated that BC incorporation along with PL did not help reduce concentration of Cu and Zn in leachate samples

Table 8. Mean $\pm$ SE values of pH of leachate samples from all five treatments and through all three Simulations

	Simulation 1	Simulation 2	Simulation3
Control	5.81 $\pm$ 0.12	5.76 $\pm$ 0.15	5.67 $\pm$ 0.16
Subsurface PL	5.48 $\pm$ 0.23	6.05 $\pm$ 0.21	5.76 $\pm$ 0.43
Surface PL	5.90 $\pm$ 0.08	5.84 $\pm$ 0.18	5.70 $\pm$ 0.29
Subsurface PL + BC	5.61 $\pm$ 0.19	6.46 $\pm$ 0.22	6.05 $\pm$ 0.45
Surface PL + BC	6.03 $\pm$ 0.26	6.03 $\pm$ 0.24	5.87 $\pm$ 0.22

For Fe, we found that in simulation 1 (Fig. 15), the average concentration of Fe was 0.19 mg L⁻¹ in the surface PL + BC treatment, significantly ($P<0.05$) greater than the control treatment (0.06 mg L⁻¹). Then, in simulation 2, the concentration of Fe in leachate from surface PL treatment (0.43 mg L⁻¹) was significantly ($P<0.05$) higher than in control (0.02 mg L⁻¹) and surface PL + BC treatment (0.10 mg L⁻¹). The subsurface PL and subsurface PL + BC treatments leached significantly greater amounts of Fe than control ($P<0.05$). Further, in simulation 3, the highest amount of Fe was leached by surface application of PL, which was 1.41 mg L⁻¹, significantly greater than other treatments ($P<0.05$). Similarly, the subsurface application of PL leached a significantly ($P<0.05$) greater amount of Fe (0.34 mg L⁻¹) in leachate samples than the surface as well as subsurface application of PL + BC and control. The values of Fe in leachate samples from the surface as well as subsurface PL + BC (0.14 and 0.09 mg L⁻¹) leached significantly greater Fe

than the control ($P < 0.05$). The leachate concentration of Fe in simulation 1 was less in control columns. The reason behind this could be a notable proportion of iron in soils was linked to the crystalline form, especially within the residual fraction (Fig. 10), where it was tightly bound within the soil mineral structure (Hori et al., 2015). While, in simulation 1, Fe leaching from surface PL + BC treatment was higher than control because of presence of iron in PL and BC. After simulation 1, we found that in simulation 2 and 3 the concentration of Fe leached from surface applied method of PL increasing significantly. A similar trend was observed in the case of subsurface application of PL in simulation 3. This could be possible because of reducing conditions and flow interruption techniques. According to Schelde et al. (2002), the flow interruption technique results in the replenishment of colloidal particle reserves in the soil. The generation of dissolved ferrous Fe through the dissolution of Fe oxides increased with the duration of flow interruption under reducing conditions (Carstens et al., 2017). This leads to increased formation of iron oxide colloids when flow is re-established, and the solution is transported into oxidizing regions. Ferrous Fe is a more soluble form than ferric Fe (Zinati et al., 2005). Moreover, Zhu et al. (2014) also reported an increase in colloidal Fe in the effluent of natural soil under reducing conditions following flow stagnation. The incorporation of BC along with PL reduced the Fe leaching in all three Simulations and in both surface and subsurface methods. The potential reasoning behind this is the increase in soil solution pH with the addition of BC (Table. 8), as BC is considered a promising material that can improve pH (Zhang et al., 2019). With increase in pH ferrous Fe is oxidized to its ferric form (Payne et al., n.d.). According to USEPA (2003), at higher soil pH, ferric Fe readily precipitates as insoluble Fe oxides and oxyhydroxides, such as goethite and hematite. The solubility of iron in soil was likely controlled by these insoluble ferric oxides (Lindsay, 1988).

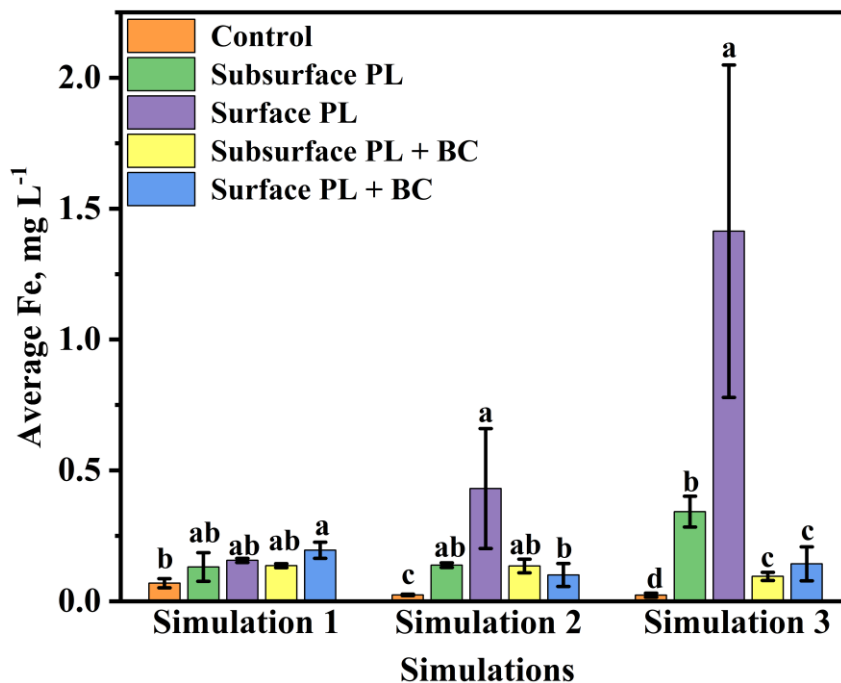


Figure 15. Leaching of iron through all five treatments in each simulation. Each bar indicates standard error. Within each simulation means followed by different letters were significantly different at significant level of 0.05 between each treatment within simulation. Where PL: Poultry litter; BC: Biochar.

Furthermore, the mean flow weighted concentration of Al in leachate samples from control treatment in simulation 1 was 0.53 mg L⁻¹, having no significant difference with treated columns with PL and PL + BC (Fig. 16). from the Al concentration in columns treated with surface application of PL (0.88 mg L⁻¹) was significantly ($P < 0.05$) greater than surface PL + BC (0.40 mg L⁻¹) in Simulation 1. In simulation 2, a similar pattern was observed, Al metal concentration was significantly ($P < 0.05$) greater in surface PL treatment (0.49 mg L⁻¹) than surface PL + BC (0.21 mg L⁻¹). Further, in simulation 3, it was found that the concentration of Al was 0.55 mg L⁻¹ in

subsurface PL treatment, significantly ($P<0.05$) greater than surface PL + BC (0.17 mg L^{-1}) and control treatment (0.18 mg L^{-1}). The control columns did not leach a significant amount of Al in the leachate sample because most of the Al in soils was mainly present in the residual fraction (Fig. 10). Zheng et al. (2012), also reported that Al present in the residual fraction was characterized by lower mobility and reduced susceptibility to leaching when compared to the exchangeable, oxide-bound, and organic matter-bound fractions. Moreover, the solubility and leachability of Al depend on the pH (Rahman et al., 2018). In acidic conditions, characterized by low pH, aluminum becomes more soluble and mobile, thereby increasing the potential for leaching. According to Draszawka-Bolzan. (2017), at a pH below 6, the oxides of Fe and Mn dissolved, liberating absorbed metal ions into the solution. Moreover, when BC was incorporated along with PL, it reduced the leaching of Al in leachate samples than surface applied PL alone. This became possible due to the increase in leachate pH caused by the addition of BC (Table. 8). Similar finding was reported by Shetty et al. (2020), the application of BC increases soil pH, thereby reducing the solubility of Al. Moreover, Shi et al. (2018), reported that BC enhances the transformation of Al into stable states, such as organically bound or sorbed hydroxyl Al, thus reducing the Al leaching. A similar trend was observed in simulation 2. In simulation 3, the leaching of Al was higher from subsurface PL treatment, among other treatments, but not significantly.

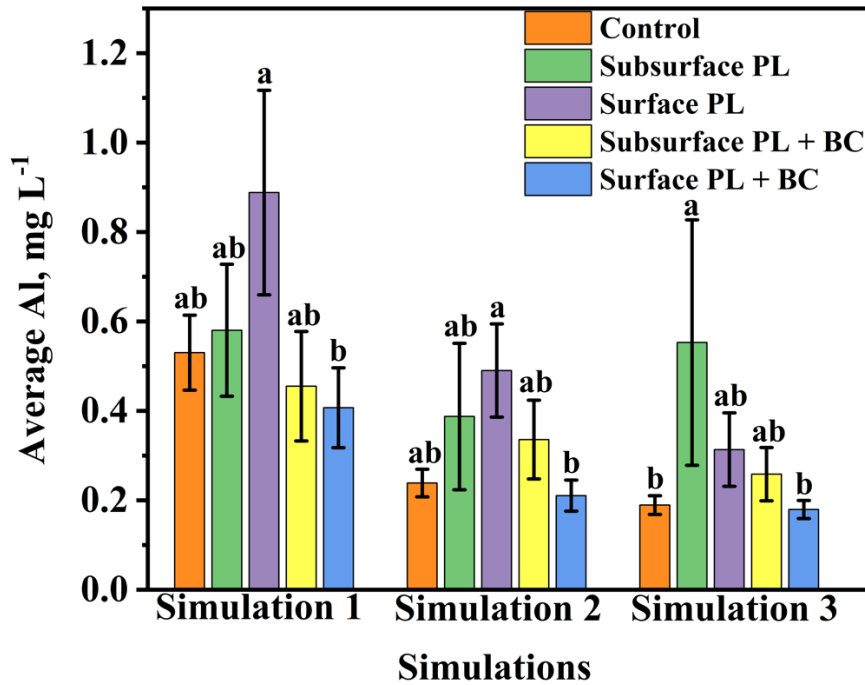


Figure 16. Leaching of Al through all five treatments in each simulation. Each bar indicates standard error. Within each simulation means followed by different letters were significantly different at a significant level of 0.05 between each treatment within a simulation. Where PL: Poultry litter; BC: Biochar.

3.3.6 Metal Leaching with Time

Fig. 17 represents the leaching of metals from each treatment with respect to simulations. We found that most metals, such as Zn, Al, and Cu, leached from all treatments during the first rainfall simulation. Then, in the next simulations (simulations 2 and 3), concentrations of metals in leachate samples were significantly lower than in simulation 1. A study conducted by Sharpley et al. (2001) reported that the greatest risk of metal leaching, such as phosphorus, occurs during the immediate aftermath of fertilizer or manure application, particularly during initial rainfall events.

A similar finding was reported by Page et al. (2014) and Sun et al. (2020), that following manure application, initial rainfall events typically lead to greater leaching of heavy metals from agricultural soils compared to subsequent rainfall events. A similar pattern was observed for Fe in control, surface PL+BC, and subsurface PL+BC treatments. Whereas when PL was applied alone, the trend was the opposite for surface and subsurface methods. The leaching of Fe increased with successive simulations. The concentration of Fe in leachate samples was significantly greater in simulation 3 than in simulations 1 and 2. According to Carstens et al. (2017) the duration of flow interruption under reducing conditions increased dissolved ferrous Fe by dissolution of Fe oxide. Ferrous Fe is more soluble than ferric Fe (Zinati et al., 2005). Similarly, Zhu et al. (2014) also noted an increase in colloidal iron in the effluent of natural soil under reducing conditions after periods of flow stagnation. Moreover, a study conducted by Malhotra et al. (2023) reported the loss of colloidal particles in this region.

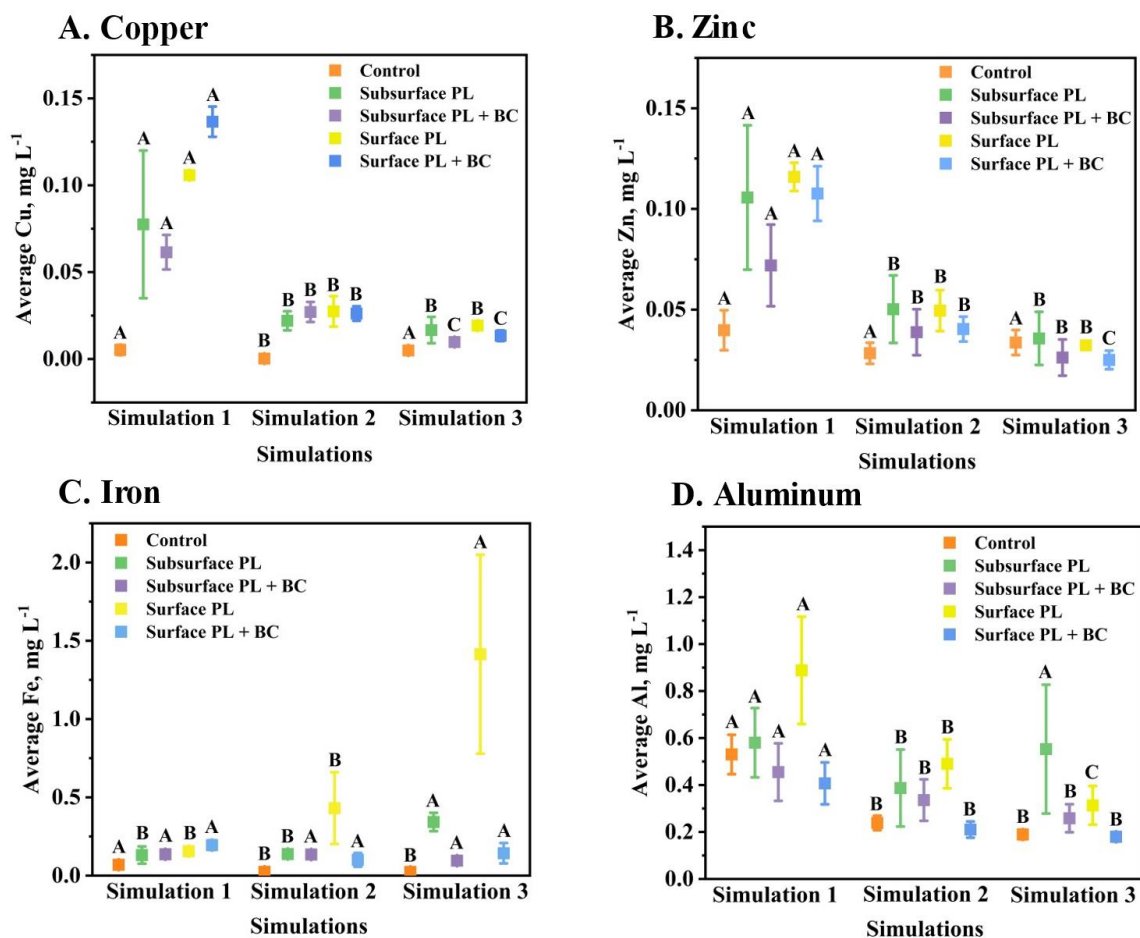


Figure 17. Leaching of metals (A. Copper, B. Zinc, C. Iron, D. Aluminum) through all five treatments in each simulation. Each bar indicates standard error. Within each treatment, means followed by different letters were significantly different at a significant level of 0.05 between each simulation within each treatment. Where PL: Poultry litter; BC: Biochar.

3.3.7 Metal Loading

Table 9 presents the loading of metals (Cu, Zn, Fe, and Al) in leachate samples from all five treatments across each Simulation. For Cu, in Simulation 1, the loading was significantly ($P < 0.05$) higher in surface-applied treatment (surface PL + BC) compared to other treatments (subsurface PL, subsurface PL + BC, surface PL, and control). In Simulations 2 and 3, treated columns leached

significantly ($P<0.05$) greater loading of Cu in leachate samples than control columns. For Zn, in Simulation 1, loading of Zn in leachate samples were significantly ($P<0.05$) greater from surface PL and surface PL + BC treatments than subsurface PL + BC and control treatments. However, in Simulations 2 and 3, the Zn loading did not differ significantly among the treatments. In the case of Al, the four treatments (surface PL, subsurface PL, subsurface PL + BC, and control) exhibited significantly ($P<0.05$) higher loading compared to the surface PL + BC treatment in Simulation 1. This trend did not continue in Simulations 2 and 3, where no significant differences in Al loading were observed within treatments. Overall, for metals (Cu, Zn, and Al), a significantly higher amount of metal loss was observed in the initial Simulation compared to the successive Simulations. The trend followed by loading of metals was consistent with concentration loss of metals. Thus, loading of these metals decreases with respect to successive rainfall events. Similar finding was reported by Katuwal et al. (2022), such that nutrient concentrations reduced over successive rainfall events.

In the case of Fe, there was no significant difference in loading among all treatments in Simulation 1. In Simulation 2, loading of Fe in leachate samples were significantly greater from treated columns than control columns. Surface PL treatment showed significantly ($P<0.05$) greater loading of Fe than surface PL + BC and control treatments. However, in Simulation 3, surface-applied PL treatment showed significantly ($P<0.05$) greater Fe loading compared to other treatments. Specifically, for surface PL and subsurface-applied PL, the Fe loading was higher in Simulation 3. This trend, opposite to that observed for other metals, was due to the reducing conditions caused by the flow interruption technique. As previously discussed, these reducing conditions led to the dissolution of ferric Fe into ferrous Fe. Ferrous Fe is more soluble (Zinati et al., 2005), resulting in greater Fe loss in subsequent Simulations.

Table 9. Loading of metals (Cu, Zn, Al, and Fe) in the leachate for all columns for various treatments for the three simulations. Values shown in parentheses are standard errors. Means followed by different letters showed a significant difference at alpha 0.05 between treatment*Simulations.

Average Loading for Cu (g ha⁻¹) with standard error			
Treatment	Simulation		
	Simulation 1	Simulation 2	Simulation 3
Control	20.76±(10.88) A	0.94±(0.94) D	14.15±(7.49) D
Subsurface PL	291.58±(175.45) A	73.60±(21.99) C	55.67±(26.42) C
Subsurface PL + BC	223.64±(47.11) A	83.04±(20.44) CB	29.25±(4.72) CD
Surface PL	439.73±(19.50) A	100.97±(32.25) CB	67.94±(1.63) C
Surface PL + BC	477.47±(37.42) B	87.76±(9.94) CB	47.18±(11.60) C

Average Loading for Zn (g ha⁻¹) with standard error			
Treatment	Simulation		
	Simulation 1	Simulation 2	Simulation 3
Control	192.50±(59.67) EDC	101.91±(14.25) EF	99.08±(11.79) EF
Subsurface PL	378.39±(132.18) AC	166.08±(58.50) ED	109.46±(33.19) EF

Subsurface PL + BC	253.83±(64.35) BDC	111.35±(23.70) EF	79.26±(28.36) F
Surface PL	480.30±(22.19) A	182.12±(37.35) EDC	114.18±(2.50) EF
Surface PL + BC	377.45±(54.21) A	134.94±(14.09) EDF	87.76±(17.06) EF

Average Loading for Fe (g ha⁻¹) with standard error

Treatment	Simulation		
	Simulation 1	Simulation 2	Simulation 3
Control	329 ±(89) D	89±(7) E	68±(18) E
Subsurface PL	483±(235) CD	460±(64) CBD	1119±(241) B
Subsurface PL + BC	488±(37) CBD	406±(84) CBD	285±(38) D
Surface PL	647±(20) CBD	1585±(843) B	5163±(2493) A
Surface PL + BC	687±(129) CBD	328±(128) D	494±(217) CBD

Average Loading for Al (g ha⁻¹) with standard error

Treatment	Simulation		
	Simulation 1	Simulation 2	Simulation 3
Control	2465.68±(273.38) A	867.19±(97.30) B	564.28±(44.62) B

Subsurface PL	2073.13±(587.24) A	1322.95±(573.35) B	1239.92±(300.32) B
Subsurface PL + BC	1611.70±(391.95) A	970.04±(170.92) B	785.09±(194.87) B
Surface PL	3502.71±(886.63) A	1781.55±(399.36) B	1139.89±(377.46) B
Surface PL + BC	1412.60±(289.79) B	719.98±(131.37) B	630.34±(89.32) B

3.3.8 Implications of Study

The implication of this study extends to a broader perspective such as sustainable agriculture, soil health, water quality, climate change mitigation, and policy development. The research underscores the importance of optimizing poultry litter application methods and incorporating biochar to promote sustainable agricultural practices. By understanding how these factors influence metal leaching, farmers can enhance soil fertility while minimizing environmental contamination, aligning with global efforts to develop sustainable agricultural systems. Incorporating biochar is also an important because the previous findings highlight the role of biochar in improving soil structure, increasing nutrient retention, and enhancing microbial activity, contributing to long-term soil health essential for sustainable crop production and ecosystem resilience (Singh et al., 2023).

Protecting water quality is another significant implication, as metal leaching from agricultural soils can contaminate surface and groundwater resources. Insights from this study can inform practices that safeguard water bodies, which is crucial for communities relying on these sources for drinking water, agriculture, and recreation. Additionally, biochar's carbon sequestration potential supports

climate change mitigation by storing carbon in soils for extended periods, making it a valuable tool for reducing greenhouse gas emissions and enhancing carbon sinks (Gupta et al., 2020).

The study's findings have important policy and regulatory implications, providing evidence to guide the development of standards for using poultry litter and biochar in agriculture. Policymakers can create guidelines that ensure the safe and effective use of these amendments, protecting both agricultural productivity and environmental health. Regulations might include recommendations for soil testing, appropriate application methods, and biochar use to mitigate leaching risks.

Finally, this research opens avenues for further studies on the interactions between soil amendments and metal leaching, encouraging innovation in developing new amendments, such as modified biochar, that optimize agricultural sustainability and environmental protection. Moreover, by conducting field studies, we can address the complex dynamics of metal leaching, poultry litter application, and biochar use; the study can contribute to a broader understanding of achieving sustainable and resilient agricultural systems in the face of global challenges.

3.4 Conclusions

To conclude, BTCs showed preferential flow occurring through soil columns. Metals such as Ni and Cr were below the range of detection limits in leachate samples. While metals such as Cu, Zn, Al, and Fe are present in leachate samples in significant amounts. The leaching of metals mostly depends upon the initial amount of metals available in the soil. Treated columns leached significantly greater amounts of Cu and Zn in leachate samples than in control. It may be due to soluble metals present in poultry litter and due to changes in soil conditions such as soil solution pH and dissolved organic matter. The formation of complexes between metals and DOM in soil

greatly impacts the adsorption processes and mobility of these metals. In the case of Al and Fe, treated columns did not differ significantly from the control columns because most of the Al and Fe in soil and litter were not present in the mobile fraction. Moreover, with successive simulations, the leaching of Cu, Zn, and Al decreases, whereas from columns treated with PL through the surface, the subsurface leaching of Fe increases with respect to simulation. The possible reason behind this could be reducing conditions and using the flow interruption technique because flow interruption under reducing conditions replenishes colloidal particles and increases dissolved ferrous iron from Fe oxide dissolution, enhancing its solubility compared to ferric iron. Incorporation of pristine BC along with PL reduces the leaching of metals such as Fe and Al than Cu and Zn.

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Chapter 4

General Conclusions and Recommendations

4.1 Conclusions

The study evaluated different PL application methods, including surface and subsurface techniques. Additionally, it assessed the impact of the combined use of BC and PL on P and metal leaching through preferential flow pathways in no-till agricultural soil using undisturbed soil columns. The study provided valuable insights into nutrient and metal leaching dynamics in soils with an extensive network of macropores. From these findings, the following conclusions were drawn:

1. Non-adsorbing tracer Br⁻ BTCs revealed preferential flow in no-till soils, highlighting the critical need to account for these pathways in developing effective best management practices.
2. In terms of DRP leaching, the subsurface application of poultry litter reduces P leaching relative to surface broadcast of litter primarily by decreasing direct water-litter contact. Additionally, reduction in nutrient leaching loss through subsurface bands due to soil compaction caused during trench creation and disruption of macropore continuity.
3. Incorporating pristine biochar did not reduce or retain DRP from leachate; instead, it increased cumulative ortho-P in soil leachate due to its high water-soluble P content. Therefore, combining pristine biochar with poultry litter was found to be not an effective way to mitigate DRP leaching.
4. In terms of rainfall simulation timing, treated columns with PL and PL + BC, using both surface and subsurface methods, initially leached significantly higher amounts of DRP

compared to subsequent simulations, attributed to the immediate transport of DRP into the soil profile following manure application.

5. Cu and Zn leaching patterns vary with treatment method. Incorporating biochar with poultry litter did not reduce Cu and Zn concentrations. Leaching was highest in simulation 1 compared to simulations 2 and 3.
6. The solubility and leachability of Al depend on pH levels. Applying PL increased Al leaching because pH of soil solution after application of PL through any method was lower as compared to combined application of PL + BC. Thus, incorporating BC with PL reduced Al leaching by raising the leachate pH. Aluminum leaching also decreased with simulations.
7. Iron leaching exhibited an opposite trend compared to Cu, Zn, and Al. Regardless of the application method (surface or subsurface), Fe leaching increased with each simulation due to flow interruption under reducing conditions. Surface application of PL alone resulted in higher Fe leaching than subsurface method of PL alone. However, incorporating biochar (BC) with PL reduced Fe leaching across all simulations and application methods.

4.2 Recommendation for Future Studies

1. Long-term application of animal manure can alter the physical and chemical properties of soil. Future experiments should be conducted on field sites with varying histories of litter applications, such as short-term (less than 6 years), medium-term (10-15 years), and long-term (over 25 years), to understand the impact of litter application duration on nutrient and metal transport dynamics.
2. For further studies on the interactions between soil amendments and nutrient leaching, it is important to encourage innovation in developing new amendments, particularly focusing on

modified biochar. Modified biochar can enhance its ability to retain nutrients and reduce leaching. By incorporating functional groups or minerals into biochar, its nutrient adsorption capacity can be significantly improved. This will not only optimize nutrient use efficiency in agricultural systems but also minimize the risk of nutrient runoff into water bodies.