

2021

Evaluating the Effects of Residual Broiler Litter and Copper in Soil on Hemp (*Cannabis sativa* L.) Fertility and Cannabinoid Production

Sarah E. Forden
Murray State University

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Evaluating the Effects of Residual Broiler Litter and Copper
in Soil on Hemp (*Cannabis sativa* L.) Fertility and Cannabinoid Production

A Thesis

Presented to

the Faculty of the Hutson School of Agriculture

Murray State University

Murray, Kentucky

In Partial Fulfillment

of the Requirements of the Degree

of Master of Science

by Sarah E. Forden

July 2021

Acknowledgments

I would like to thank Dr. David Ferguson for all the time, work, and guidance he offered up while accomplishing this research. He put countless hours into the completion of this study and I am forever grateful. Dr. David Ferguson has been a great mentor to me and has encouraged me to be the best I can be. I have been blessed by him since our first meeting before I came to Murray State, and I am so honored to have been able to work alongside and learn from him. Also, a huge thank you to Dr. Megan Taylor and Dr. Iin Handayani for their advice and guidance in completing this thesis.

I would like to thank Mallorie Snider, Mounica Talasila, and Bhavesh Mudupu for their hard work and help in measuring out plots and plant spacing as well as hemp transplanting and harvest. I would also like to thank Austin Hogancamp, Julie Boyd, and the MSU Arboretum Staff for their help in planting and/or harvesting the hemp. I would also like to acknowledge and thank Huichang Cha and the Breathitt Veterinary Lab for the CBD, THC, and metal analysis. Thank you to the Murray State Center for Agricultural Hemp for their resources, help, and guidance and Certis USA, LLC. for supplying the Gemstar LC insecticide.

Thank you to my parents who have supported me in more ways than one throughout this educational accomplishment. They have encouraged me every single step of the way. I would also to thank God for His guidance, discernment, and constant comfort. I am so thankful to Him for all the wonderful people and hard work that made this study possible.

Abstract

This study encases two separate experiments observing hemp (*Cannabis sativa* L.) and soil fertility with the use of broiler litter and copper. The long-term broiler litter research plots were established in 1997 and the copper fertility research plots were not established until 2009. Both experiments use a randomized complete block design with the broiler litter experiment composed of four replications and eight treatments at the following rates: 0, 1, 2, 3, 4, 5, 6, and 7 tons BL * ac⁻¹. The copper experiment was designed with four replications and three treatments at the following rates: 0, 10, 20 lbs Cu * ac⁻¹. This is the first growing season with hemp, previous crops include corn and soybeans. A protected urea nitrogen fertilizer was hand spread before planting to give 100 lbs N * ac⁻¹ as a pre-plant application. The hemp was transplanted by hand in the established no-till field on June 13th, 2020 in 40-inch wide rows with intra-row spacing at 36 inches. The transplants were produced using feminized seed and the Queen Dream cultivar. The data collected throughout this study includes: plant height, plant tissue analysis, hemp flower bud yields, and soil analysis. In addition, bud samples were analyzed for CBD, THC, and heavy metal content. There was no statistical difference between the broiler litter treatments and hemp flower bud yields. Yield for the eight broiler litter treatments were as follows: 2443, 1969, 2413, 2605, 2281, 2569, 2737, 2725 lbs * ac⁻¹ for the 0, 1, 2, 3, 5, 6, and 7 tons * ac⁻¹ treatments, respectively. However, yields tended to increase with additional rates of broiler litter. The soil fertility differences between treatments remained significant for many factors, including soil phosphorus and potassium levels. The lack of statistical difference in the yields may be

due to the amount of variability in the yield data. In the copper experiment, there was no statistical difference between the copper treatments and the hemp flower bud yields.

Yield for the three copper treatments were as follows: 2197, 2557, 2179 lbs * ac⁻¹ for the 0, 10, and 20 lbs Cu * ac⁻¹ treatments, respectively. However, the higher yield, CBD, THC values of the 10 lbs Cu * ac⁻¹ treatment deserves further study. There was still a statistical difference in soil copper levels between treatments. In both experiments, the data supports the need for repeating experiments to provide a better picture of soil fertility effects on hemp cultivation in the future.

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Chapter 1

Introduction

Using broiler litter as a substitute for commercial fertilizers has continued to grow in popularity as it is both an inexpensive option and readily available to farmers. About 10.2 million tons of broiler litter is produced in the United States on an annual basis, with most of that broiler litter coming from southern states (Dunkley et al., 2011). The production of broiler chickens is concentrated in the southeastern and south-central states, producing 85% of the total broiler meat found in the United States (Paudel & McIntosh, 2005). Broiler litter is one of the highest value manures due to its unique nutrient content (Rasnake, 1996). Broiler litter is composed of essential nutrients such as nitrogen, phosphorus, and potassium but also secondary and micronutrients that are necessary for plant growth. In addition, broiler litter has the ability to build soil organic matter and mineralizable soil nitrogen, which enables crops to be less dependent on nitrogen additions, creating more sustainable agroecosystems (Hoover et al., 2019). Broiler litter is typically best utilized with row crops such as corn, cotton, and soybeans, as well as grass pastures or hayfields (Rasnake, 1996). It has been determined that the continuous use of broiler litter year after year is likely to cause high phosphorus levels in the soil if phosphorus is not efficiently utilized by the crop. This residual phosphorus can run off into bodies of water, creating dead zones, which can be harmful to aquatic life (United States Environmental Protection Agency, n.d.). However, legume crops can be used in a rotation to help utilize excess phosphorus and potassium.

In 1997, a broiler litter experiment was developed by Dr. John Mikulcik on the Murray State University Pullen Farm. This study was created in order to determine if the nutrients in broiler litter were as readily available to plants as they are from commercial fertilizers and to observe the impact of broiler litter on soil, corn, and soybean fertility. While this research initially started with broiler litter applications every year, these historic plots have not been treated with broiler litter since 2018 due to the increasing phosphorus levels. This study will examine the residual effects of the previous broiler litter applications on crop fertility. Preceding research on these historic plots also identified that of the micronutrients that broiler litter contains, copper was found to be correlated with soybean yield (Upchurch, 2008). However, with the increase in hemp (*Cannabis sativa* L.) markets and production, the fertility requirements for successful cannabinoid production and high yields in hemp is of interest. Therefore, with this in mind, the need to assess the residual effects of broiler litter and copper on hemp fertility parameters and cannabinoid production is necessary in order for farmers to make informed decisions about optimizing their hemp production.

Statement of the Problem

What are the effects of residual broiler litter on hemp fertility parameters in addition to CBD and THC? Additionally, what are the effects of residual soil copper levels on hemp fertility parameters as well as CBD and THC?

Purpose of the Study

The purpose of these studies was to evaluate the long-term, residual effects of broiler litter and copper in soil on hemp yield, fertility, and cannabinoid content. The broiler litter experiment began in 1997 and has been used to study fertility in a number of

crops. Findings from this research experiment suggested a correlation between copper uptake and soybean yield. A separate copper experiment was started in 2009 to follow up on previous results related to the broiler litter experiment. The broiler litter plots have not been treated with broiler litter since 2018 and the copper plots have not been treated with copper since 2009. Corn and soybeans have been studied on these particular fertility plots in the past. However, in the fall of 2018, industrial hemp became a legal commodity to grow in the United States. Due to the increasing interest in hemp production, knowledge of soil fertility requirements for hemp is needed.

Research Questions/Hypotheses

Hypothesis 1:

H₀1: There will not be statistically significant differences in soil nutrients, plant tissue nutrients, THC, CBD, and hemp crop yields in the plots treated with higher rates of broiler litter.

H₁1: There will be statistically significant differences in soil nutrients, plant tissue nutrients, THC, CBD, and hemp crop yields in the plots treated with higher rates of broiler litter.

Hypothesis 2:

H₀2: There will not be statistically significant differences in soil nutrients, plant tissue nutrients, THC, CBD, and hemp crop yields in the plots treated with higher rates of copper in the soil.

H₁2: There will be statistically significant differences in soil test nutrients, plant tissue nutrients, THC, CBD, and hemp crop yields in plots treated with higher rates of copper in the soil.

Theoretical/Conceptual Framework

The framework of this study was based on the methods of the original investigator, Dr. John Mikulcik. He organized the broiler litter study into eight different treatments, zero to seven tons of broiler litter per acre. Broiler litter has not been applied to these plots since 2018, as the residual effects of these treatments are of interest.

The copper study began after a correlation between soybean yield and the amount of copper in soybean plant tissues was observed during the long-term broiler litter experiment (Upchurch, 2008). In 2009, Joshua Scott began the copper experiment by applying a pre-plant application of copper sulfate solutions at rates of zero, ten, and twenty pounds of copper per acre (Scott, 2010). Copper has not been applied to these plots since the initial application in 2009, as the residual effects of these treatments are of interest.

Assumptions

1. All applications of treatments were made uniformly.
2. All pesticide treatments were applied uniformly.
3. All plots have the same no-till tillage history.
4. All data was collected accurately.

Delimitations

1. This study is not designed to investigate the residual effects of broiler litter and copper applications on hemp pests, weeds, or diseases.
2. This study is designed to investigate the residual effects of broiler litter and copper applications on soil fertility, hemp fertility, THC, CBD, and crop yield.

Limitations

1. Limited by the rainy weather conditions of the 2020 growing season.
2. Study conducted on exclusively no-till plots.
3. Limited to the plots soil type of Grenada silt loam with fragipan.
4. Only one variety of hemp per growing season will be used in this study.

Definition Terms

Broiler Litter – A mixture of poultry manure and bedding materials that has been removed from poultry houses.

Hemp Bud- The fully dried flower that is produced by the female hemp plant.

CBD- Also known as cannabidiol, an abundant and non-intoxicating cannabinoid found in *Cannabis sativa* L.

THC- Also known as delta-(9)-tetrahydrocannabinol, the primary psychoactive cannabinoid in *Cannabis sativa* L.

Significance of the Study

A study of the residual effects of broiler litter and copper on soil fertility, hemp fertility, THC, CBD, and yield will be beneficial to both hemp farmers and agronomists looking to engage these particular treatments in their fertility programs. It may also give insight into specific nutrients that may affect cannabinoid production in hemp.

Chapter 2

Review of Literature

This chapter will review literature that is related to this research study. This review will highlight the current fertilization trends being utilized and the effects of broiler litter use on soil fertility and crop yield. In addition, it will highlight the known impact of soil applications of copper on plant uptake and crop yield.

Current Fertilization Trends

In general, fertilizers and soil amendments are derived from virgin raw material, wastes, and composts and are sold in solid, liquid, and gaseous forms. Most commercial fertilizers contain the three basic elements essential for plant growth: nitrogen, phosphorus, and potassium. However, some commercial fertilizers also contain micronutrients such as calcium, sulfur, zinc, and other metals that are necessary for plant growth (United States Environmental Protection Agency, n.d.). Anhydrous ammonia, ammonium nitrate, ammonium sulfate, and urea are the most commonly used nitrogen materials utilized for nitrogen fertilization in the United States. Diammonium phosphate and monoammonium phosphate are the most frequently used phosphorus fertilizers in the United States, while potassium chloride is the most commonly used potassium fertilizer (United States Department of Agriculture, 2019). Only 5% of U.S. croplands are fertilized with livestock manure. The pattern of manure use is dependent on the type of livestock manure available from a reasonable distance and the total distance that the manure needs to be moved as transport costs can be expensive (MacDonald et al., 2009).

Yet, the use of manure for fertilizer is growing in popularity due to its ability to provide a plethora of nutrients outside of nitrogen, phosphorus, and potassium while also increasing the activity of microbes in the soil. However, environmental concerns also become an issue with the use of broiler litter due to the rapid increase of soil phosphorus levels associated with its application. Rasnake (1996) concluded that alternating the use of broiler litter with nitrogen fertilizer year to year may negate this issue as broiler litter will still provide enough residual phosphorus and potassium even with decreased application rates.

Effects of Broiler Litter Use on Soil Fertility

Broiler litter is comprised of chicken feces and urine along with the bedding materials used in broiler houses, such as sawdust, pine shavings, and peanut hulls. Broiler litter provides the primary plant nutrients of nitrogen (N), phosphorus (P_2O_5), and potassium (K_2O), the secondary plant nutrients of calcium (Ca), magnesium (Mg), and sulfur (S), and essential minuscule amounts of micronutrients such as copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), and boron (B) (Prasad and Stanford, 2019). Specifically, broiler litter is able to provide up to 57 to 60 pounds of N per ton, 69 to 72 pounds of P_2O_5 per ton, and around 46 pounds of K_2O per ton. In addition, broiler litter also provides 42 to 44 pounds of Ca per ton, 8.1 to 8.7 pounds of Mg per ton, and 12.1 to 14 pounds of S per ton (Chastain et al., n.d.). However, the nutrient make-up of broiler litter may vary depending on the age of the litter, length of storage, the number of flocks between broiler house cleanouts, amount and type of bedding material, moisture content, litter pH, feed rations, and the type of broiler housing system used (Prasad and Stanford, 2019).

While the importance of nitrogen, phosphorus, and potassium is well known, the secondary nutrients of calcium, magnesium, and sulfur are just as vital. Calcium provides structural support to the cell walls of plants and serves as a "secondary messenger" when plants are stressed. Magnesium is the central atom in the midst of four nitrogen atoms in the chlorophyll molecule and is extensively involved in photosynthesis. Sulfur is also essential to the photosynthetic process and a necessary element in plant protein synthesis (Oldham, 2019).

Adeli et al. (2010) conducted a three-year study to determine the effects of broiler litter compared to commercial fertilizers on soil nutrient content and quality at equivalent nitrogen rates. The treatments included annual applications of broiler litter at 0, 1.0, 2.0, 2.5, 3.0, 4.5, and 6.0 tons * ac⁻¹ and yearly applications of commercial fertilizer at 30, 60, 80, 100, 120, and 150 lbs N * ac⁻¹. It was determined that broiler litter applications increased soil total carbon, microbial biomass, potassium, extractable soil phosphorus, soil cation exchange capacity, and the stability of soil aggregate with increasing application rates. Furthermore, it was determined that broiler litter is more effective in improving soil's chemical, biological, and physical components than traditional commercial fertilizer.

A separate study conducted by Adeli et al. (2007) determined that soil surface (0-6 in) carbon, copper, zinc, and arsenic significantly increased with increasing broiler litter applications but these increases did not exceed the normal range and therefore did not pose a potential threat to the surrounding ecosystems. Soil phosphorus, however, does pose a threat as levels can build up in the soil over time with repeated broiler litter

applications if the crop is not actively taking up nutrients, leading to environmental concerns such as dead zones (Carpenter, 2008).

Effects of Broiler Litter Use on Crop Yield

Being informed about the effects of broiler litter on soil properties is essential, but relevant information on broiler litter's impact on yield is of great value to producers. As previously mentioned, broiler litter contains not only primary and secondary nutrients but also essential micronutrients such as copper, manganese, and zinc that most commercial fertilizers do not contain. These micronutrients are of great importance when looking to increase yields.

Adeli et al. (2005) conducted a study in order to compare the effects of broiler litter and commercial fertilizer on soybean yield. Broiler litter was applied at rates to obtain 0, 35.5, 71.0, and 142.0 lbs N * ac⁻¹ and commercial fertilizers were applied at rates equivalent to the broiler litter's nitrogen and phosphorus content. Soybean yield was greater with broiler litter than commercial fertilizer as the application of broiler litter increased soybean yield by 9%. It is possible that broiler litter outperformed commercial fertilizer because of the micronutrients found within the broiler litter that are not typically found in commercial fertilizers. However, a ten-year broiler litter experiment conducted by Netthisinghe et al. (2016) determined that broiler litter provided only similar corn yields compared to corn yields on plots treated with inorganic fertilizer.

A four-year field study was conducted by Gascho et al. (2001), where broiler litter at rates of 0, 2.0, 4.0, and 6.0 tons * ac⁻¹ were broadcast one to three weeks before summer and winter crops. The summer crops were cotton, pearl millet, and peanut, while the winter crops were wheat and oilseed canola. The broiler litter applications positively

affected the production of cotton, canola, pearl millet, and wheat but decreased peanut yield. In addition, *Rhizoctonia* limb rot damage in peanuts was increased, and canola experienced severe lodging due to *Sclerotinia* damage during the final year of the four-year study.

Effects of Copper Applications on Plant Uptake and Crop Yield

Heavy metals such as copper, zinc, nickel, cobalt, and manganese are essential trace elements in plants, yet these metals can become very toxic to the plant at high concentrations (Arru et al., 2004). However, in a study done by Gupta and Kalra (2006), it was determined that copper applications of up 44.61 lbs Cu * ac⁻¹ had no detrimental effects or yield reductions in barley and wheat. Copper plays an essential role in photosynthetic and respiratory transport chains, oxidative stress protection, and cell wall metabolism (Yruela, 2009). An experiment conducted by Syuhada et al. (2014) that included foliar applications of copper on corn plants at the following rates, 0, 0.4, 3.0, and 6.0 lbs Cu * ac⁻¹, concluded that the foliar applications of copper on corn leaves significantly increased corn yields with increasing application rates.

Khan et al. (2008) conducted an experiment with *Cannabis sativa* L. plants in five different locations in Pakistan. Each of these locations contained varying amounts of copper within the soil, and results showed that the plant uptake of copper was significantly different between sites, which was surmised to be due to the initial varying concentrations of copper found in the soils. This brought into question hemp's (*Cannabis sativa* L.) role as a phytoremediator and if the accumulation of heavy metals from different soil types in the consumable tissues of this plant is cause for concern.

In contrast, according to a report done by Murdock and Ritchey (2012), neither yield responses nor symptomatic indicators to copper have been found in the state of Kentucky. However, in 2008, Upchurch found a positive correlation between the plant uptake of copper and soybean yield in Murray, Kentucky, during a long-term broiler litter experiment. These findings lead Scott (2010) to begin a separate copper experiment with pre-plant soil applied applications of copper at the rates of 0, 10, and 20 lbs Cu * ac⁻¹. Scott found no statistical differences in soybean yield between the three treatments, as well as no significant differences the in plant uptake copper. Yet, from 2013 to 2019 on the same copper plots, there was a statistically significant yield increase in corn at the 10 lbs Cu * ac⁻¹ rate when all six years were added together (Unpublished data).

Chapter Summary

Chapter two discussed and reviewed the current fertilization trends being utilized within the United States and its comparison to current broiler litter use. The effects of broiler litter on soil fertility and crop yield were also reviewed. It was determined that broiler litter often outperforms commercial fertilizers due to the micronutrients found within the litter as well as improving chemical and biological activity within the soil. However, some studies did suggest that the use of broiler litter on crop yield was only comparable to inorganic fertilizers and may also cause higher disease ratings in certain crops. Previous research also suggests that certain levels of copper have the potential to increase yields without symptomatic indicators.

Chapter 3

Methodology

Introduction

This chapter will describe the research methods used to successfully complete these experiments. The following sections are located within this chapter: context of the study, research design, source of data, data collection procedures, and data analysis.

Context of Study

This study was conducted on Murray State University's Pullen Farm, where the research plots have been located since 1997. This project was a continuation of a long-term broiler litter and copper study in order to observe the residual effects of these soil amendments on hemp fertility and cannabinoid production. No broiler litter or copper treatments were applied in the 2020 growing season. Broiler litter has not been applied since 2018, and copper has not been applied since 2009.

Research Design

This experiment utilized a randomized complete block design for both the broiler litter and copper studies. The dimensions for each plot within the broiler litter and copper experiments were 17.4 feet long by 15 feet wide. The broiler litter studies included 32 plots, with 8 of those plots in each replication equaling a total of 4 replications (Fig. 1). The copper study included 12 plots, with 3 of those plots in each replication equaling a total of 4 replications (Fig. 2).


Target#- Treatment		Doran Road		Blue#-Plot#
Rep. 4	Rep. 3	Rep. 2	Rep. 1	
5 ²⁵	3 ¹⁷	7 ⁹	7 ¹	
6 ²⁶	1 ¹⁸	4 ¹⁰	2 ²	
0 ²⁷	6 ¹⁹	2 ¹¹	3 ³	
2 ²⁸	2 ²⁰	5 ¹²	4 ⁴	
3 ²⁹	0 ²¹	1 ¹³	1 ⁵	
1 ³⁰	4 ²²	6 ¹⁴	5 ⁶	
4 ³¹	5 ²³	3 ¹⁵	6 ⁷	
7 ³²	7 ²⁴	0 ¹⁶	0 ⁸	
Low-Lying Swag				
Copper Plots				

Figure 1. Pullen Farm Experimental Design for Broiler Litter Plots. The large number in the center represents the treatments of broiler litter (tons broiler litter * ac^{-1}) applied in 2018. The number in the upper right corner represents the plot number.

Doran Road 			
Broiler Litter Plots			
Low-Lying Swag			
Large# = Treatment		Red# = Plot#	
20 ^{C3}	10 ^{C2}	0 ^{C1}	Rep. 1
10 ^{C6}	20 ^{C5}	0 ^{C4}	Rep. 2
0 ^{C9}	10 ^{C8}	20 ^{C7}	Rep. 3
0 ^{C12}	20 ^{C11}	10 ^{C10}	Rep. 4

Figure 2. Pullen Farm Experimental Design for Copper Plots. The large number in the center represents the copper treatments (lbs Cu * ac⁻¹) applied in 2009. The number in the upper right corner represents the plot number.

Source of Data

On May 21st, 2020 prior to transplanting, all plots were fertilized with urea-treated with NutriSphere to provide 100 pounds of nitrogen per acre. Two-week-old feminized hemp seedlings of the 'Queen Dream' variety were hand transplanted the second week of June 2020 in 40-inch wide rows with intra-row spacing at 36-inches allowing for 4,356 hemp plants per acre. Herbicides were not applied to the hemp crop, so alleys between

plots were mowed once a week, and a mini-rototiller was used between hemp rows for weed control. The mini-rototiller was cleaned between use in each plot in order to prevent cross-contamination of soils. The weeds surrounding the hemp plants were regularly removed with a garden hoe.

On August 12th through August 19th of 2020, plant tissue samples were taken, air dried, and sent to Water's Lab for nutrient analysis. Only in the copper experiment, on August 21, potassium magnesium sulfate was hand spread to all plots to provide 30 lbs $K_2O * ac^{-1}$, 14.7 lbs $Mg * ac^{-1}$, and 30 lbs $S * ac^{-1}$ because low levels of potassium and magnesium were found the plant tissue analysis results.

A *Heliothis* trap was set up on August 25th in order to monitor possible corn earworm populations. On August 29th, the pesticide GemStar was applied to the hemp plants. In order to provide 10 fl. oz. of GemStar $* ac^{-1}$ with 0.25% (v/v) NIS, the solution was spread using a CO_2 backpack sprayer with high-boom clearance. Unfortunately, it rained about 0.2 inches after the initial application and was only minimally effective.

Upon observation, corn earworm counts were still high, and worms were much larger than a quarter of an inch which is the maximum length suggested for effective GemStar use. Dipel, another biological pesticide, was used to combat the prevalence of corn earworms. On September 7th, DiPel was applied to the hemp plants to provide 1.0 lb product $* ac^{-1}$ with 0.25% (v/v) non-ionic surfactant.

Plant height measurements from the broiler litter plots were taken September 1st through September 4th, and plant height measurements from the copper plots were taken September 8th through September 10th. On October 3rd, 2020 replication one and replication two from the copper plots were harvested. Replication three of the copper

plots were harvested on October 5th, and replication four of the copper plots were harvested on October 7th, along with the first replication in the broiler litter plots. On October 8th, replication two of the broiler litter plots were harvested, and the following day, replication three and half of replication four (includes control) were harvested. Rain from hurricane Delta shut down harvest early, and the other half of replication four was not completed until October 13th, 2020. Plants were then stripped of bud and bud leaves over a tarp-covered in nylon netting. The nylon netting containing the bud was then fashioned into a sack and placed on shelves within the greenhouse with steady temperatures around 65° F for drying. The flower bud harvest was taken to the lab for analysis the last week of October. Soil samples were taken post-harvest on November 17th through November 18th of 2020.

Data Collection Procedures

Multiple data collections were made in the same fashion for both the broiler litter and copper experiments. Plant height was taken from each plot by measuring each hemp plant located in the innermost rows for a total of 12 plants per plot. In order to collect data for the plant tissue analysis, the third fully developed leaf from ten flowering plants in the innermost rows from each plot were collected and placed in brown paper bags, air dried, and sent to Water's Lab for analysis.

Harvest of the broiler litter and copper experiment included four plants located centrally in rows two and three of each plot that were cut down as close to the ground as possible using limb-loppers. In order to collect yield data, the dried hemp bud from each plot was removed from the nylon netting and placed in a bin, and weighed. In order to prepare hemp bud samples for THC, CBD, and heavy metal analysis, sub-samples of the

dried bud from each plot were ground using a blender and placed in 50 ml tubes, labeled, and mailed to the Breathitt Veterinary Center for analysis.

Composite soil samples were pulled from every single plot post-harvest. Six cores were obtained from each plot at a depth of 4 inches, mixed together, and sent to Water's Lab for analysis. Basic soil test #4 was used to analyze these samples.

Data Analysis

The data was statistically analyzed using the SAS analytics software (SAS Institute, 2013). Analysis of variance (ANOVA) was used to interpret the data gathered from the plant tissue analysis, soil analysis, THC analysis, CBD analysis, yield, and heavy metal analysis of the hemp bud. A general linear model (GLM) was used to interpret the data from the plant height collection. These statistical tests were used to identify any significant difference between treatments within both studies. When a statistical difference did occur, the least significant difference (LSD) was determined and used to separate the means.

Chapter Summary

Chapter three was a review of the research design, what data was collected, how the data was collected, and how the data was analyzed. The experimental design of this study is a randomized complete block design. The data collected consisted of plant tissue samples, plant height, yield, soil samples, and bud analysis for THC, CBD, and heavy metal content. Analysis of variance was used to determine the statistical differences in the plant tissue analysis, the soil analysis, the THC and CBD analysis, the heavy metal

content of the hemp bud, and the yield between treatments. A general linear model was used to determine significant differences in height between treatments.

Chapter 4

Results

Introduction

This chapter will assess the data collected during the 2020 hemp growing season. The results presented within this chapter will include the plant tissue analysis, plant height, hemp yield, and soil analysis, as well as THC, CBD, and heavy metal analysis of the hemp flower bud. Additionally, the results and findings are expressed in tables and figures. Figure 3 shows the amount of precipitation experienced during the 2020 growing season.

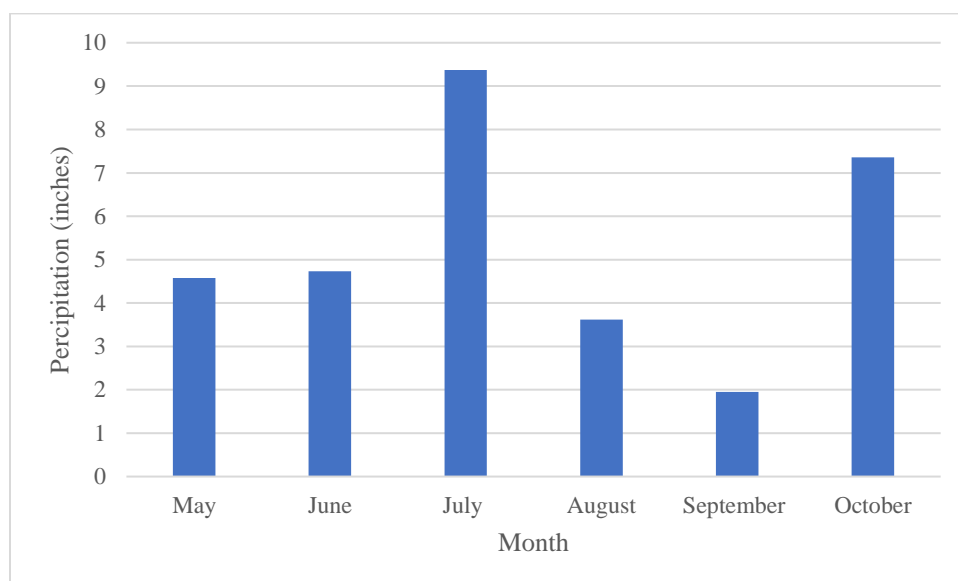


Figure 3. Monthly Precipitation During the 2020 Growing Season from Calloway Co. Kentucky Mesonet station located in near the Pullen Farm (Kentucky Mesonet, 2020).

Results for Objective 1

The main focus of objective one is to observe the effects of residual broiler litter on the nutrient make-up of the hemp plants, plant height, yield, soil nutrient levels, as

well as the THC, CBD, and heavy metal levels found in the hemp flower bud. The mean values of the plant tissue analysis reflect the amount of nutrients found in the hemp plants at the stage of flowering. Table 1 summarizes the mean macronutrient values from the plant tissue analysis of hemp between broiler litter treatments.

Table 1. Mean Hemp Tissue Analysis Values for Macronutrients

Broiler Litter Treatment (tons*ac ⁻¹)	N (%)	P (%)	K (%)	Mg (%)	Ca (%)	S (%)
0	4.66	0.491c	1.95e	0.381	2.33	0.335a
1	4.39	0.613ab	2.21d	0.386	2.22	0.323a
2	4.03	0.585b	2.25cd	0.377	2.11	0.291bc
3	4.21	0.594b	2.34cd	0.364	2.17	0.311ab
4	4.36	0.609ab	2.47bc	0.351	2.16	0.316ab
5	4.04	0.621a	2.63ab	0.350	2.16	0.287bc
6	4.07	0.650a	2.62ab	0.345	2.06	0.289bc
7	3.88	0.655a	2.76a	0.306	2.04	0.276c
Pr > F	0.1137	0.0010	0.0002	0.2006	0.4514	0.0427
LSD (P = 0.1)	n.s.	0.0527	0.242	n.s.	n.s.	0.0309
% CV	8.58	7.20	8.28	11.56	8.39	8.36

Note; n.s. indicates no significance between treatments. Values with a common letter are not statistically significant.

The plant tissue analysis revealed that the mean values for nitrogen ranged from 3.88% from plants treated with 7 tons B.L. * ac⁻¹ to 4.66% from plants treated with 0 tons B.L. * ac⁻¹. There was no statistically significant difference in nitrogen levels between broiler litter treatments. There was a statistically significant difference in mean phosphorus levels between broiler litter treatments. The mean phosphorus values ranged from 0.491% in plants treated with 0 tons B.L. * ac⁻¹ to 0.655% in plants treated with 7

tons B.L. * ac⁻¹. The plants from plots receiving no broiler litter had significantly lower phosphorus levels compared to plants in plots treated with any amount of broiler litter.

The mean plant tissue analysis values of potassium ranged from 1.95% from plants with 0 tons B.L. * ac⁻¹ to 2.76% from plants treated with 7 tons of B.L. * ac⁻¹. Similar to phosphorus, there was a consistent trend to higher potassium levels as the broiler litter treatments increased. The plants from plots treated with 7 tons B.L. * ac⁻¹ had significantly higher potassium levels compared to plants in plots treated with 4, 3, 2, and 1 ton B.L. * ac⁻¹.

The mean plant tissue analysis values of magnesium ranged from 0.381% from plants with 0 tons of B.L. * ac⁻¹ to 0.306% from plants treated with 7 tons of B.L. * ac⁻¹. No significant differences were observed in the plant uptake of magnesium between treatments. However, a trend was identified where magnesium levels increased with decreasing amounts of broiler litter.

The plant tissue analysis also revealed that the mean values of calcium ranged from 2.04% from plants treated with 0 tons B.L. * ac⁻¹ to 2.33% from plants treated with 7 tons. B.L. * ac⁻¹. No significant differences were observed between the plant uptake of calcium and broiler litter treatments. However, a trend was identified where calcium levels increased with increasing amounts of broiler litter.

There was a statistically significant difference in mean sulfur levels between broiler litter treatments. The mean sulfur values ranged from 0.276% in plants in plots treated with 7 tons of B.L. * ac⁻¹ to 0.335% in plants in plots treated with 0 tons of B.L. * ac⁻¹. The plants from plots receiving no broiler litter had significantly higher sulfur levels compared to plots treated with 2, 5, 6, and 7 tons B.L. * ac⁻¹.

The mean micronutrient levels from the plant tissue analysis of the hemp can be viewed in Table 2. The mean boron levels ranged from 53.75 ppm from plants in plots treated with 6 tons B.L. * ac⁻¹ to 59.50 ppm from plants in plots treated with 2 tons B.L. * ac⁻¹. There was no significant difference between boron levels and broiler litter treatments.

Table 2. Mean Hemp Tissue Analysis Values for Micronutrients

Broiler Litter Treatment (tons*ac ⁻¹)	B (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)
0	55.00	55.75	129.25	117.25	13.51
1	55.75	58.75	118.75	120.00	12.70
2	59.50	57.50	134.25	120.00	14.31
3	59.25	58.25	122.50	130.00	12.69
4	58.00	60.00	108.75	115.00	14.56
5	56.50	56.00	110.00	118.75	11.89
6	53.75	59.75	127.00	121.25	12.44
7	55.25	57.50	112.50	125.25	12.13
Pr > F	0.8737	0.9609	0.5496	0.7764	0.4633
LSD (P = 0.1)	n.s.	n.s.	n.s.	n.s.	n.s.
% CV	11.29	10.52	16.92	10.40	15.27

Note; n.s. indicates no significance between treatments

The mean plant tissue analysis values of zinc ranged from 55.75 ppm from plants in plots treated with 0 tons of B.L. * ac⁻¹ to 60.00 ppm from plants in plots treated with 4 tons B.L. * ac⁻¹. There was not a statistically significant difference in mean zinc values between broiler litter treatments.

Mean plant tissue analysis values for manganese ranged from 108.75 ppm from plants in plots treated with 4 tons B.L. * ac⁻¹ to 134.25 ppm from plants in plots treated with 2 tons B.L. * ac⁻¹. There was not a statistically significant difference in mean manganese levels between broiler litter treatments.

The mean plant tissue analysis values for iron ranged from 117.25 ppm in plants in plots treated with 0 tons of B.L. * ac⁻¹ to 130.00 ppm in plants in plots treated with 3 tons B.L. * ac⁻¹. No statistical differences were observed between iron levels and broiler litter treatments.

The plant tissue analysis also revealed that the mean values of copper ranged from 11.89 ppm in plants in plots treated with 5 tons B.L. * ac⁻¹ to 14.56 ppm in plants in plots treated with 4 tons B.L. * ac⁻¹. No statistically significant differences were observed between mean copper levels and broiler litter treatments. In addition, no trends were identified.

Plant height was taken in order to observe any possible relationship between height and the amount of residual broiler litter. Results can be found in Table 3. Differences were highly significant with plant height increasing in conjunction with increased broiler litter rates.

Hemp flower buds and bud leaves were weighed in order to determine yields. The mean hemp yield values from each broiler litter treatment are displayed in Table 4. Average hemp yield values ranged from 1968.85 lbs * ac⁻¹ in plots treated with 1 ton B.L. * ac⁻¹ to 2737.18 lbs * ac⁻¹ in plots treated with 6 tons B.L. * ac⁻¹. There were no statistically significant yield differences between broiler litter treatments. The amount of

variation (%CV) was very high, contributing to the lack of significance. However, there was a trend toward higher yield values at the higher broiler litter rates.

Table 3. Average Plant Height

Broiler Litter Treatment (tons*ac ⁻¹)	Plant Height (inches)
0	48.70
1	48.06
2	51.30
3	50.02
4	52.51
5	53.17
6	55.33
7	62.07
Pr > F	<.0001
% CV	15.49

Note; t-test mean comparisons are given in Appendix A.

Total CBD and THC content were analyzed in order to determine if there was a significant difference in these cannabinoid levels between broiler litter treatments. Total CBD and THC in the hemp flower bud are shown in Table 5.

Neither CBD nor THC was significantly influenced by broiler litter treatments. The total CBD values ranged from 10.00% from plants in plots treated with 0 tons B.L. * ac⁻¹ to 11.78% from plants in plots treated with 7 tons B.L. * ac⁻¹. No clear trends were observed between broiler litter treatments and CBD content. The total THC values ranged from 0.514% from plants in plots treated with 0 tons B.L. * ac⁻¹ to 0.612% from plants in plots treated with 3 tons B.L. * ac⁻¹.

Table 4. Mean Hemp Flower Yield

Broiler Litter Treatment (tons*ac ⁻¹)	Hemp Flower Yield (lbs*ac ⁻¹)
0	2443
1	1969
2	2413
3	2605
4	2281
5	2569
6	2737
7	2725
Pr > F	0.2891
LSD (P = 0.1)	n.s.
% CV	17.98

Note; n.s. indicates no significance

The hemp flower was analyzed in order to observe possible relationships between rates of broiler litter and the heavy metal content of the hemp flower bud. Results are summarized in Table 6 and Table 7. The mean manganese levels in the hemp flower bud ranged from 105.97 ppm from plants in plots treated with 7 tons B.L. * ac⁻¹ to 246.07 ppm from plants in plots treated with 2 tons B.L. * ac⁻¹. There were no statistically significant differences in manganese levels between broiler litter treatments.

Mean iron levels ranged from 97.670 ppm from plants in plots treated with 4 tons B.L. * ac⁻¹ to 139.99 ppm from plants in plots treated with 7 tons B.L. * ac⁻¹. No statistically significant differences were observed in iron levels between broiler litter treatments.

Table 5. Total CBD and THC in Hemp Flower Bud

Broiler Litter Treatment (tons*ac ⁻¹)	Total CBD (%)	Total THC (%)
0	10.00	0.514
1	11.01	0.604
2	10.64	0.559
3	11.66	0.612
4	10.99	0.571
5	10.31	0.524
6	10.49	0.552
7	11.78	0.594
Pr > F	0.4503	0.4928
LSD (P = 0.1)	n.s.	n.s.
% CV	11.48	13.09

Note; n.s. indicates no significance

The mean cobalt levels in the hemp flower bud ranged from 0.103 ppm from plants in plots treated with 0 tons B.L. * ac⁻¹ to 0.223 ppm from plants in plots treated with 2 tons B.L. * ac⁻¹. There were no statistically significant differences in cobalt levels between broiler litter treatments.

Mean copper levels ranged from 17.77 ppm from plants in plots treated with 4 tons B.L. * ac⁻¹ to 20.26 ppm from plants in plots treated with 6 tons B.L. * ac⁻¹. No statistically significant differences were observed in copper levels between broiler litter treatments. The mean zinc levels in hemp flower bud ranged from 70.57 ppm from plants in plots treated with 0 tons B.L. * ac⁻¹ to 76.51 ppm from plants in plots treated with 6

tons B.L. * ac⁻¹. There were no statistically significant differences in zinc levels between broiler litter treatments.

Table 6. Mean Metal Composition of Hemp Flower

Broiler Litter Treatment (tons*ac ⁻¹)	Mn (ppm)	Fe (ppm)	Co (ppm)	Cu (ppm)	Zn (ppm)
0	236.57	134.79	0.103	19.47	74.55
1	201.21	132.36	0.190	18.81	72.09
2	246.07	117.47	0.223	18.69	73.14
3	191.40	109.45	0.163	17.93	70.62
4	187.52	97.670	0.160	17.77	72.47
5	219.37	131.38	0.173	18.15	75.63
6	223.30	129.64	0.150	20.26	76.51
7	105.97	139.99	0.138	18.03	70.57
Pr > F	0.1093	0.2965	0.1070	0.7562	0.7098
LSD (P = 0.1)	n.s.	n.s.	n.s.	n.s.	n.s.
% CV	14.02	20.49	31.16	12.05	7.469

Note; n.s. indicates no significance

Mean arsenic levels (Table 7) in the hemp flower ranged from 0.006 ppm from plants in plots treated with 4 tons B.L. * ac⁻¹ to 0.014 ppm from plants in plots treated with 5 tons B.L. * ac⁻¹. No statistically significant differences were observed between broiler litter treatments and arsenic levels in the hemp flower.

The mean selenium levels in the hemp flower ranged from 0.058 ppm from plants in plots treated with 4 tons B.L. * ac⁻¹ to 0.104 ppm from plants in plots treated with 7 tons B.L. * ac⁻¹. There was a statistically significant difference between broiler litter

treatments and selenium levels in the hemp flower. Selenium levels were significantly higher in the 7 tons B.L. * ac^{-1} treatments compared to the 1, 2, 3, 4, and 5 tons B.L. * ac^{-1} treatments.

Mean molybdenum values in the hemp flower ranged from 0.396 ppm from plants in plots treated with 1 ton B.L. * ac^{-1} to 0.738 ppm from plants in plots treated with 7 tons B.L. * ac^{-1} . No statistically significant differences were observed between broiler litter treatments and molybdenum levels in the hemp flower.

The mean cadmium levels in the hemp flower ranged from 0.046 ppm from plants in plots treated with 6 tons B.L. * ac^{-1} to 0.114 ppm from plants in plots treated with 1 ton B.L. * ac^{-1} . There was no statistically significant difference between broiler litter treatments and cadmium levels in the hemp flower.

Mean lead levels in the hemp flower ranged from 0.119 ppm from plants in plots treated with 7 tons B.L. * ac^{-1} to 0.180 ppm from plants in plots treated with 1 ton B.L. * ac^{-1} . There was a statistically significant difference between broiler litter treatments and lead levels in the hemp flower. However, no general trend was observed, and the lowest lead levels were observed at the highest broiler litter application rate.

The soil nutrient analysis, as well as the pH analysis post-harvest, is summarized in Table 8 and Table 9. The mean pH levels of the soil ranged from 6.00 in plots treated with 0 tons B.L. * ac^{-1} to 6.20 in plots treated with both 3 and 6 tons B.L. * ac^{-1} . There was not a statistically significant difference in pH between broiler litter treatments. The average amount of organic matter found in the soil ranged from 1.67% in plots treated with 0 tons B.L. * ac^{-1} to 2.12% in plots treated with 6 tons B.L. * ac^{-1} . There was a statistically significant difference in organic matter levels between broiler litter

treatments. Organic matter levels were significantly lower in the plots treated with 0 tons B.L. * ac⁻¹ compared to plots treated with 1, 2, 4, 5, 6, and 7 tons B.L. * ac⁻¹.

Table 7. Mean Heavy Metal Composition of Hemp Flower

Broiler Litter Treatment (tons*ac ⁻¹)	As (ppm)	Se (ppm)	Mo (ppm)	Cd (ppm)	Pb (ppm)
0	0.013	0.082abc	0.696	0.051	0.164ab
1	0.011	0.060c	0.396	0.114	0.180a
2	0.010	0.061c	0.547	0.103	0.169ab
3	0.012	0.066bc	0.588	0.090	0.160ab
4	0.006	0.058c	0.572	0.073	0.138bc
5	0.014	0.064bc	0.653	0.058	0.143bc
6	0.009	0.090ab	0.690	0.046	0.140bc
7	0.011	0.104a	0.738	0.089	0.119c
Pr > F	0.7333	0.0145	0.1452	0.3095	0.0188
LSD (P = 0.1)	n.s.	0.0269	n.s.	n.s.	0.0328
% CV	53.92	24.98	26.91	56.78	14.72

Note; n.s. indicates no significance. Values with a common letter are not statistically significant.

The mean CEC levels (Table 8) of the soil ranged from 9.95 in plots treated with 0 tons B.L. * ac⁻¹ to 12.3 in plots treated 4 tons B.L. * ac⁻¹. There was a statistically significant difference in CEC levels between broiler litter treatments. CEC was significantly lower in plots treated with 0 tons B.L. * ac⁻¹ compared to plots treated with 1, 2, 4, 5, 6, and 7 tons B.L. * ac⁻¹. I

Mean soil phosphorus levels ranged from 13.00 lbs * ac⁻¹ in plots treated with 0 tons B.L. * ac⁻¹ to 126.0 lbs * ac⁻¹ in plots treated with 7 tons B.L. * ac⁻¹. There was a

statistically significant difference in phosphorus levels between broiler litter treatments. Soil phosphorus levels increased with increasing broiler litter application rates.

The mean soil potassium levels ranged from 115.50 lbs * ac⁻¹ in plots treated with 0 tons B.L. * ac⁻¹ to 238.75 lbs * ac⁻¹ in plots treated with 7 tons B.L. * ac⁻¹. There was a statistically significant difference in potassium levels between broiler litter treatments. Soil potassium levels increased with increasing broiler litter application rates. Soil potassium was significantly higher in plots treated with 7 tons B.L. * ac⁻¹ compared to plots treated with any lesser amount of broiler litter.

Mean soil magnesium values ranged from 294.50 lbs * ac⁻¹ in plots treated with 0 tons B.L. * ac⁻¹ to 432.75 lbs * ac⁻¹ in plots treated with 4 tons B.L. * ac⁻¹. There was a statistically significant difference in magnesium levels between broiler litter treatments. Soil magnesium levels were significantly lower in plots treated with 0 tons of B.L. * ac⁻¹ compared to plots treated with any amount of broiler litter.

The mean soil calcium values ranged from 2284.50 lbs * ac⁻¹ in plots treated with 0 tons B.L. * ac⁻¹ to 2816.00 lbs * ac⁻¹ in plots treated with 5 tons B.L. * ac⁻¹. There was a statistically significant difference in calcium levels between broiler litter treatments. Soil calcium levels were significantly lower in plots treated with 0 tons B.L. * ac⁻¹ compared to plots treated with 1, 2, 4, 5, 6, and 7 tons B.L. * ac⁻¹.

Mean sulfur levels in the soil ranged from 20.00 lbs * ac⁻¹ in plots treated with 1 and 5 tons B.L. * ac⁻¹ to 25.50 lbs * ac⁻¹ in plots treated with 7 tons B.L. * ac⁻¹. There was not a statistically significant difference in soil sulfur levels between broiler litter treatments.

Table 8. Mean Values of Soil pH, Organic Matter, and Macronutrients

Broiler Litter Treatment (tons*ac ⁻¹)	pH	O.M. (%)	CEC	P (lbs*ac ⁻¹)	K (lbs*ac ⁻¹)	Mg (lbs*ac ⁻¹)	Ca (lbs*ac ⁻¹)	S (lbs*ac ⁻¹)
0	6.00	1.67c	9.95e	13.00e	115.50d	294.50d	2284.50c	20.75
1	6.03	1.97ab	11.1bcd	39.25de	119.25d	378.75bc	2639.75ab	20.00
2	6.08	1.99ab	11.8ac	57.75cd	124.00cd	362.25bc	2638.75ab	21.50
3	6.20	1.77bc	10.5de	26.25e	127.00cd	352.75c	2549.50bc	23.25
4	6.18	1.99ab	12.3a	50.75d	156.50bc	432.75a	2915.75a	22.75
5	6.15	2.10a	11.9ab	72.25bc	181.75b	403.25abc	2816.00ab	20.00
6	6.20	2.12a	11.8ac	90.75b	184.25b	404.50ab	2766.00ab	21.25
7	6.13	2.04a	11.4ad	126.0a	238.75a	393.50abc	2667.75ab	25.50
Pr > F	0.5396	0.0221	0.0098	<.0001	<.0001	0.0062	0.0617	0.7193
LSD (P = 0.1)	n.s.	0.2214	0.9925	20.497	34.631	51.24	303.36	n.s.
% CV	2.71	9.31	7.19	28.31	18.26	11.15	9.37	21.46

Note; n.s. indicates no significance. Mehlich III extraction was used to analyze the soil samples.
Values with a common letter are not statistically significant.

Table 9 summarizes the mean micronutrient levels from the soil analysis between broiler litter treatments. The mean soil boron levels ranged from 0.80 lbs * ac⁻¹ in plots treated with 0 tons B.L. * ac⁻¹ to 1.08 lbs * ac⁻¹ in plots treated with both 6 and 7 tons B.L. * ac⁻¹. Mean soil boron levels were significantly higher in plots treated with 4, 5, 6, and 7 tons B.L. * ac⁻¹ compared to plots treated with 0, 1, 2, and 3 tons of B.L. * ac⁻¹. However, in general, soil boron levels tended to increase with increasing application rates of broiler litter.

The mean soil zinc levels ranged from 4.20 lbs * ac⁻¹ in plots treated with 0 tons B.L. * ac⁻¹ to 18.6 lbs * ac⁻¹ in plots treated with 7 tons B.L. * ac⁻¹. There was a statistically significant difference in soil zinc levels between broiler litter treatments. Soil zinc was significantly higher in plots treated with 7 tons B.L. * ac⁻¹ compared to plots treated with any lesser amount of broiler litter. In general, soil zinc levels increased with increasing application rates of broiler litter.

The mean soil manganese levels ranged from 412.25 lbs * ac⁻¹ in plots treated with 4 tons B.L. * ac⁻¹ to 612.00 lbs * ac⁻¹ in plots treated with 0 tons B.L. * ac⁻¹. There was a statistically significant difference in soil manganese levels between broiler litter treatments. Soil manganese was significantly higher in plots treated with 0 tons B.L. * ac⁻¹ compared to plots treated with 1, 4, 5, 6, and 7 tons B.L. * ac⁻¹.

Mean soil iron levels ranged from 247.75 lbs ac⁻¹ in plots treated with 0 tons B.L. * ac⁻¹ to 315.25 lbs * ac⁻¹ in plots treated with 5 tons B.L. * ac⁻¹. There was a statistically significant difference in soil iron levels between broiler litter treatments. However, no general trend was identified between soil iron levels and broiler litter applications.

Table 9. Mean Values of Soil Micronutrients

Broiler Litter Treatment (tons*ac ⁻¹)	B (lbs*ac ⁻¹)	Zn (lbs*ac ⁻¹)	Mn (lbs*ac ⁻¹)	Fe (lbs*ac ⁻¹)	Cu (lbs*ac ⁻¹)	Na (lbs*ac ⁻¹)
0	0.80c	4.20e	612.00a	247.75c	3.95g	21.75b
1	0.83bc	5.35e	454.50bc	271.75bc	6.28f	25.25ab
2	0.93b	7.50d	543.75ab	279.75ab _c	8.48e	29.00a
3	0.90bc	7.95d	565.00ab	258.50bc	9.63e	24.50b
4	1.05a	11.9c	412.25c	277.00bc	13.7d	28.75a
5	1.05a	13.3bc	436.75bc	315.25a	15.7c	25.50ab
6	1.08a	14.9b	510.75b	310.50a	17.5b	27.00a
7	1.08a	18.6a	459.50bc	304.25ab	19.9a	29.00a
Pr > F	0.0002	<.0001	0.0123	0.0425	<.0001	0.0994
LSD (P = 0.1)	0.1037	2.0987	91.158	37.256	1.764	4.4163
% CV	8.85	16.49	15.00	10.81	12.19	13.78

Note; n.s. indicates no significance. Mehlich III was used to analyze the soil samples. Values with a common letter are not statistically significant.

The mean soil copper levels ranged from 3.95 lbs ac⁻¹ in plots treated with 0 tons B.L. * ac⁻¹ to 19.9 lbs * ac⁻¹ in plots treated with 7 tons B.L. * ac⁻¹. There was a statistically significant difference in soil copper levels between broiler litter treatments. Soil copper levels increased with increasing application rates of broiler litter.

Mean soil sodium levels ranged from 21.75 lbs * ac⁻¹ in plots treated with 0 tons B.L. * ac⁻¹ to 29.00 lbs * ac⁻¹ in plots treated with 2 and 7 tons B.L. * ac⁻¹. There was a statistically significant difference in soil sodium levels between broiler litter treatments. Soil sodium levels were significantly lower in plots treated with 0 tons B.L. * ac⁻¹ compared to plots treated with 2, 4, 6, and 7 tons B.L. * ac⁻¹.

Results for Objective 2

The main focus of objective two is to observe the effects of residual copper on the elemental content of the hemp plants, hemp height, hemp yield, soil nutrient levels, as well as THC, CBD, and heavy metal analysis of hemp flower bud.

The mean values of the plant tissue analysis reflect the amount of nutrients found in the hemp plants at the stage of flowering. Table 10 summarizes the mean macronutrient values from the plant tissue analysis of hemp between copper treatments. The plant tissue analysis revealed the mean values of nitrogen ranged from 4.78% in plants from plots treated with 20 lbs Cu * ac⁻¹ to 5.03% in plants from plots treated with 10 lbs Cu * ac⁻¹. There was no statistically significant difference in nitrogen levels between copper treatments.

The plant tissue analysis revealed the mean values of nitrogen ranged from 4.78% in plants from plots treated with 20 lbs Cu * ac⁻¹ to 5.03% in plants from plots treated with 10 lbs Cu * ac⁻¹. There was no statistically significant difference in nitrogen levels between copper treatments.

Mean phosphorus levels ranged from 0.413% in plants from plots treated with 10 lbs Cu * ac⁻¹ to 0.428% in plants from plots treated with 0 lbs Cu * ac⁻¹. No statistically significant differences were found between phosphorus levels and copper treatments. The mean values of potassium ranged from 2.19% in plants from plots treated with 0 lbs Cu * ac⁻¹ to 2.32% in plants from plots treated with 20 lbs Cu * ac⁻¹. There were no statistically significant differences observed between the plant uptake of potassium and copper treatments.

Mean tissue magnesium levels ranged from 0.304% in plants from plots treated with 10 lbs Cu * ac⁻¹ to 0.356% in plants from plots treated with 0 lbs Cu * ac⁻¹. No statistically significant differences were observed in magnesium levels between copper treatments.

The mean values of calcium ranged from 2.77% in plants from plots treated with 20 lbs Cu * ac⁻¹ to 3.08% in plants from plots treated with 0 lbs Cu * ac⁻¹. Calcium levels were significantly higher in 0 lbs Cu ac⁻¹ treatment compared to any other treatment. In addition, calcium levels decreased with increasing copper applications.

Mean tissue sulfur levels ranged from 0.356% in plants from plots treated with 0 lbs Cu * ac⁻¹ to 0.345% in plants from plots treated with 20 lbs Cu * ac⁻¹. There was no statistically significant difference in tissue sulfur levels between copper treatments.

Table 10. Mean Hemp Tissue Analysis Values for Macronutrients

Copper Treatment (lbs*ac ⁻¹)	N (%)	P (%)	K (%)	Mg (%)	Ca (%)	S (%)
0	4.90	0.428	2.19	0.356	3.08a	0.356
10	5.03	0.413	2.23	0.304	2.79b	0.350
20	4.78	0.426	2.32	0.336	2.77b	0.345
Pr > F	0.1995	0.7786	0.6334	0.1486	0.0398	0.6136
LSD (P = 0.1)	n.s.	n.s.	n.s.	n.s.	0.1969	n.s.
% CV	3.46	7.81	7.97	9.65	4.98	4.23

Note; n.s. indicates no significance. Values with a common letter are not statistically different.

Table 11 summarizes the mean micronutrient levels from the plant tissue analysis of hemp between copper treatments. The mean boron levels ranged from 60.75 ppm from plants in plots treated with 20 lbs Cu * ac⁻¹ to 66.50 ppm from plants in plots treated with

0 lbs Cu * ac⁻¹. There were no statistically significant differences in plant tissue boron levels between copper treatments.

Mean zinc levels ranged from 43.50 ppm from plants in plots treated with both 0 and 10 lbs Cu * ac⁻¹ to 44.75 ppm from plants in plots treated with 20 lbs Cu * ac⁻¹. No statistically significant differences were observed in plant zinc levels and copper treatments. The mean manganese levels ranged from 75.25 ppm from plants in plots treated with 10 lbs Cu * ac⁻¹ to 80.00 ppm from plants in plots treated with 0 lbs Cu * ac⁻¹. There were no statistically significant differences in plant tissue manganese levels between copper treatments.

Table 11. Mean Hemp Tissue Analysis Values for Micronutrients

Copper Treatment (lbs*ac ⁻¹)	B (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)
0	66.50	43.50	80.00	112.50	12.95
10	64.00	43.50	75.25	111.50	13.26
20	60.75	44.75	79.25	110.75	13.75
Pr > F	0.2884	0.9203	0.3330	0.8149	0.5498
LSD (P = 0.1)	n.s.	n.s.	n.s.	n.s.	n.s.
% CV	7.29	11.33	5.67	3.47	7.45

Note; n.s. indicates no significance

Mean iron levels ranged from 110.75 ppm from plants in plots treated with 20 lbs Cu * ac⁻¹ to 112.50 ppm from plants in plots treated with 0 lbs Cu * ac⁻¹. No statistically significant differences were observed between plant iron levels and copper treatments. The mean copper levels ranged from 12.95 ppm from plants in plots treated with 0 lbs Cu * ac⁻¹ to 13.75 ppm from plants in plots treated with 20 lbs Cu * ac⁻¹. There were no

statistically significant differences in plant copper levels between copper treatments. However, plant copper levels did increase with increasing copper treatment rates.

Plant height was taken in order to observe any possible relationship between the amount of residual copper and plant growth. Results can be found in Table 12. The mean plant height ranged from 53.05 inches in plots treated with 10 lbs Cu * ac⁻¹ to 54.35 inches in plots treated with 20 lbs Cu * ac⁻¹. There were no statistically significant differences in plant height between copper treatments.

Hemp flower buds and bud leaves were weighed in order to determine yields. The mean hemp yield values from each broiler litter treatment are displayed in Table 13. Average hemp yield values ranged from 2196.95 lbs ac⁻¹ in plots treated with 0 lbs Cu * ac⁻¹ to 2557.10 lbs ac⁻¹ in plots treated with 10 lbs Cu * ac⁻¹. There were no statistically significant yield differences between copper treatments. However, the 10 lbs Cu * ac⁻¹ rate produced the highest hemp flower yield value.

Cannabinoid content was analyzed in order to observe possible relationships between the amount of residual copper in the soil and CBD and THC content. Total CBD and THC in the hemp flower bud are shown in Table 14. Neither CBD nor THC was significantly influenced by copper treatments. The total CBD values ranged from 11.40% from plants in plots treated with 0 lbs Cu * ac⁻¹ to 13.33% from plants in plots treated with 10 lbs Cu * ac⁻¹. No significant differences were observed in CBD levels between copper treatments. The total THC values ranged from 0.561% from plants in plots treated with 0 lbs Cu * ac⁻¹ to 0.649% from plants in plots treated with 10 lbs Cu * ac⁻¹. There were no significant differences in THC levels between copper treatments. However, both the CBD and THC values were higher in the ten pounds per acre treatment.

Table 12. Mean Hemp Plant Height

Copper Treatment (lbs*ac ⁻¹)	Average Plant Height (inches)
0	53.25
10	53.03
20	54.35
Pr > F	0.6186
LSD (P = 0.1)	n.s.
% CV	12.36

Note; n.s. indicates no significance

Table 13. Mean Hemp Flower Yield

Copper Treatment (lbs*ac ⁻¹)	Hemp Flower Yield (lbs*ac ⁻¹)
0	2197
10	2557
20	2179
Pr > F	0.4789
LSD (P = 0.1)	n.s.
% CV	20.21

Note; n.s. indicates no significance

The hemp flower was analyzed in order to observe possible relationships between rates of copper and heavy metal content. Results are summarized in Table 15 and Table 16. The mean manganese levels in the hemp flower bud ranged from 153.47 ppm from plants in plots treated with 0 lbs Cu * ac⁻¹ to 198.87 ppm from plants in plots treated with

10 lbs Cu * ac⁻¹. There were no statistically significant differences in manganese levels between copper treatments.

Table 14. Total CBD and THC in Hemp Flower Bud

Copper Treatment (lbs*ac ⁻¹)	Total CBD (%)	Total THC (%)
0	11.40	0.561
10	13.33	0.649
20	11.89	0.577
Pr > F	0.3196	0.3268
LSD (P = 0.1)	n.s.	n.s.
% CV	12.47	13.47

Note; n.s. indicates no significance

The mean iron levels ranged from 150.42 ppm from plants in plots treated with 0 lbs Cu * ac⁻¹ to 184.18 ppm from plants in plots treated with 20 lbs Cu * ac⁻¹. No statistically significant differences were observed in iron levels between copper treatments.

Mean cobalt levels in the hemp flower bud ranged from 0.140 ppm from plants in plots treated with 10 lbs Cu * ac⁻¹ to 0.215 from plants in plots treated with 20 lbs Cu * ac⁻¹. There were no statistically significant differences in cobalt levels between copper treatments. The mean copper levels ranged from 17.45 ppm from plants in plots treated with 0 lbs Cu * ac⁻¹ to 20.73 ppm from plants in plots treated with 20 lbs Cu * ac⁻¹. No statistically significant differences were observed in copper levels between copper treatments.

Table 15. Mean Metal Composition of Hemp Flower

Copper Treatment (lbs*ac ⁻¹)	Mn (ppm)	Fe (ppm)	Co (ppm)	Cu (ppm)	Zn (ppm)
0	153.47	150.42	0.190	17.45	64.52
10	198.87	150.97	0.140	19.75	76.74
20	183.78	184.18	0.215	20.73	75.17
Pr > F	0.1642	0.3871	0.1239	0.4736	0.3542
LSD (P = 0.1)	n.s.	n.s.	n.s.	n.s.	n.s.
% CV	16.44	22.61	24.20	18.92	16.55

Note; n.s. indicates no significance

Mean zinc levels in the hemp flower bud ranged from 64.52 ppm from plants in plots treated with 0 lbs Cu * ac⁻¹ to 76.74 ppm from plants in plots treated with 10 lbs Cu * ac⁻¹. There were no statistically significant differences in zinc levels between copper treatments. The mean arsenic levels (Table 6) in the hemp flower bud ranged from 0.019 ppm from plants in plots treated with 10 and 20 lbs Cu * ac⁻¹ to 0.021 ppm from plants in plots treated with 0 lbs Cu * ac⁻¹. No statistically significant differences were observed in arsenic levels between copper treatments. Mean selenium levels ranged from 0.076 ppm from plants in plots treated with 20 lbs Cu * ac⁻¹ to 0.095 ppm from plants in plots treated with 10 lbs Cu * ac⁻¹. There were no statistically significant differences in selenium levels between copper treatments.

The mean molybdenum levels in the hemp flower bud ranged from 0.804 ppm from plants in plots treated with 10 lbs Cu * ac⁻¹ to 1.379 ppm from plants in plots treated with 20 lbs Cu * ac⁻¹. No statistically significant differences were observed in molybdenum levels between copper treatments. Mean cadmium levels ranged from 0.039 ppm from plants in plots treated with 10 lbs Cu * ac⁻¹ to 0.048 ppm from plants in

plots treated with 20 lbs Cu * ac⁻¹. There were no statistically significant differences in cadmium levels between copper treatments.

Table 16. Mean Heavy Metal Composition of Hemp Flower

Copper Treatment (lbs*ac ⁻¹)	As (ppm)	Se (ppm)	Mo (ppm)	Cd (ppm)	Pb (ppm)
0	0.021	0.088	0.941	0.046	0.249
10	0.019	0.095	0.804	0.039	0.211
20	0.019	0.076	1.379	0.048	0.300
Pr > F	0.8240	0.9035	0.2078	0.7778	0.4419
LSD (P = 0.1)	n.s.	n.s.	n.s.	n.s.	n.s.
% CV	30.98	53.37	39.83	37.59	36.96

Note; n.s. indicates no significance

The mean lead levels in the hemp flower bud ranged from 0.211 ppm from plants in plots treated with 10 lbs Cu * ac⁻¹ to 0.300 ppm from plants in plots treated with 20 lbs Cu * ac⁻¹. No statistically significant differences were observed in lead levels between copper treatments.

The soil nutrient analysis, as well as the pH analysis post-harvest, is summarized in Table 17 and Table 18. The mean pH levels of the soil ranged from 6.58 in plots treated with 20 lbs Cu * ac⁻¹ to 6.88 in plots treated with 10 lbs Cu * ac⁻¹. There was not a statistically significant difference between copper treatments and soil pH.

The mean organic matter levels found in the soil ranged from 1.98% in plots treated with 20 lbs Cu * ac⁻¹ to 1.63% in plots treated with 0 lbs Cu * ac⁻¹. While there is not a statistically significant difference between copper treatments and organic matter levels, a clear trend was identified. Organic matter levels increased as the amount of copper per acre increased. Mean CEC levels ranged from 10.9 in plots treated with 20 lbs

Cu * ac⁻¹ to 11.5 in plots treated with 0 lbs Cu * ac⁻¹. There was not a statistically significant difference in the CEC between copper treatments; however, CEC decreased with increasing application rates of copper.

Mean soil phosphorus levels ranged from 18.00 lbs ac⁻¹ in plots treated with 20 lbs Cu * ac⁻¹ to 22.75 lbs * ac⁻¹ in plots treated with 0 lbs Cu * ac⁻¹. There was not a significant difference between copper treatments and soil phosphorus levels; however, phosphorus levels did decrease as the amount of copper per acre increased.

Mean soil potassium values ranged from 158.75 lbs * ac⁻¹ in plots treated with both 0 and 10 lbs Cu * ac⁻¹ to 170.00 lbs * ac⁻¹ in plots treated with 20 lbs Cu * ac⁻¹. There was no statistically significant difference between copper treatments and soil potassium levels.

The mean soil magnesium levels ranged from 356.00 lbs * ac⁻¹ in plots treated with 10 lbs Cu * ac⁻¹ to 390.50 lbs * ac⁻¹ in plots treated with 0 lbs Cu * ac⁻¹. No statistically significant differences were observed between copper treatments and soil magnesium levels.

Mean soil calcium values ranged from 2776.75 lbs * ac⁻¹ in plots treated with 20 lbs Cu * ac⁻¹ to 3167.25 lbs * ac⁻¹ in plots treated with 0 lbs Cu * ac⁻¹. There was no statistically significant difference between copper treatments and soil calcium levels. However, calcium levels did decrease with increasing rates of soil-applied copper.

The mean soil sulfur levels ranged from 25.00 lbs * ac⁻¹ in plots treated with 0 lbs Cu * ac⁻¹ to 26.25 lbs Cu * ac⁻¹ in plots treated with 20 lbs Cu * ac⁻¹. No statistically significant differences were observed between copper treatments and soil sulfur levels, but sulfur levels did increase with increasing rates of soil-applied copper.

The mean soil boron levels (Table 18) ranged from 0.83 lbs * ac⁻¹ in plots treated with 20 lbs Cu * ac⁻¹ to 1.00 lbs * ac⁻¹ in plots treated with 0 lbs Cu * ac⁻¹. There were no statistically significant differences in soil boron levels between copper treatments.

Mean soil zinc levels ranged from 4.53 lbs * ac⁻¹ in plots treated with 10 lbs Cu * ac⁻¹ to 4.80 lbs * ac⁻¹ in plots treated with 0 lbs Cu * ac⁻¹. No statistically significant differences were observed in soil zinc levels between copper treatments.

The mean soil manganese levels ranged from 325.25 lbs * ac⁻¹ in plots treated with 0 lbs Cu * ac⁻¹ to 383.25 lbs * ac⁻¹ in plots treated with 10 lbs Cu * ac⁻¹. Mean soil manganese levels were significantly higher in the 10 lbs Cu * ac⁻¹ treatment compared to the 0 lbs Cu * ac⁻¹ treatment.

Mean soil iron levels ranged from 212.50 lbs * ac⁻¹ in plots treated with 10 lbs Cu * ac⁻¹ to 225.00 lbs * ac⁻¹ in plots treated with 20 lbs Cu * ac⁻¹. There were no statistically significant differences in soil iron levels between copper treatments.

The mean soil copper levels ranged from 2.75 lbs * ac⁻¹ in plots treated with 0 lbs Cu * ac⁻¹ to 11.8 lbs * ac⁻¹ in plots treated with 20 lbs Cu * ac⁻¹. There was a statistically significant difference in soil copper levels between copper treatments. Soil copper levels significantly increased with increasing application rates of copper. Mean sodium soil levels ranged 19.25 lbs * ac⁻¹ in plots treated with 10 lbs Cu * ac⁻¹ to 21.75 lbs ac⁻¹ in plots treated with 20 lbs Cu * ac⁻¹. No statistically significant differences were observed in soil sodium levels between copper treatments.

Table 17. Mean Values of Soil pH, Organic Matter, and Macronutrients

Copper Treatment (lbs*ac ⁻¹)	pH	O.M. (%)	CEC	P (lbs*ac ⁻¹)	K (lbs*ac ⁻¹)	Mg (lbs*ac ⁻¹)	Ca (lbs*ac ⁻¹)	S (lbs*ac ⁻¹)
0	6.85	1.63	11.5	22.75	158.75	390.50	3167.25	25.00
10	6.88	1.70	11.1	19.75	158.75	356.00	3069.75	25.75
20	6.58	1.98	10.9	18.00	170.00	382.00	2776.75	26.25
Pr > F	0.1888	0.4893	0.3119	0.3762	0.5761	0.8915	0.3148	0.9518
LSD (P = 0.1)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
% CV	3.30	23.52	4.64	22.16	10.27	27.93	11.39	21.97

Note; n.s. indicates no significance. Mehlich III extraction was used to analyze the soil samples.

Table 18. Mean Values of Soil Micronutrients

Copper Treatment (lbs*ac ⁻¹)	B (lbs*ac ⁻¹)	Zn (lbs*ac ⁻¹)	Mn (lbs*ac ⁻¹)	Fe (lbs*ac ⁻¹)	Cu (lbs*ac ⁻¹)	Na (lbs*ac ⁻¹)
0	1.00	4.80	325.25b	216.25	2.75c	21.00
10	0.93	4.53	383.25a	212.50	6.48b	19.25
20	0.83	4.55	341.00ab	225.00	11.8a	21.75
Pr > F	0.2674	0.2260	0.0907	0.3159	<.0001	0.1767
LSD (P = 0.1)	n.s.	n.s.	42.977	n.s.	0.7763	n.s.
% CV	14.88	4.74	8.94	4.97	8.05	8.10

*Note; n.s. indicates no significance. Mehlich III was used to analyze the soil samples.
Values with a common letter are not statistically significant.*

Chapter 5

Conclusion and Recommendations

Introduction

Chapter five will discuss the conclusions that can be drawn from both the broiler litter and copper experiments based on the results from the previous chapter. In addition, recommendations will be given for future related research.

Conclusions for Objective 1

The soil analysis results from the broiler litter study revealed significant differences in the organic matter between broiler litter treatments even though the plots had not been treated with broiler litter since 2018. In general, increasing broiler litter applications increased soil organic matter levels. The CEC was statistically significant between broiler litter treatments and CEC increased with increasing rates of broiler litter. There was also a statistically significant difference in residual soil potassium levels between broiler litter treatments. Potassium increased with increasing application rates of broiler litter. These findings are similar to Adeli et al. (2010), who found that broiler litter increased CEC, soil total carbon, and soil potassium with increasing application rates. While Adeli et al. (2010) did not directly study organic matter in the experiment, soil total carbon is a component of soil organic matter, as organic matter is made up of 58% carbon (Edwards, 2021).

The soil analysis also revealed that phosphorus levels in the soil still showed a statistically significant difference between broiler litter treatments. Residual soil

phosphorus increased with increasing application rates of broiler litter. These observations are similar to Adeli et al. (2011), who, under a no-till regimen, still observed increased phosphorous levels compared to the control three years after applying broiler litter at rates greater than 1 ton * ac⁻¹. Based on current research, phosphorus levels in the soil were considered excessive for optimum hemp production in plots treated with 5, 6, and 7 tons B.L. * ac⁻¹ (Laboski, 2018). However, no noticeable visual changes were observed in plant health between broiler litter treatments. Due to the high levels of residual soil phosphorus from broiler litter applications, it would be beneficial for producers to base their broiler litter applications on yearly soil test results and the phosphorus needs of the hemp. These results also support Rasnake (1996) who concluded that alternating broiler litter use with nitrogen fertilizer year to year could negate increasing phosphorus levels while still providing enough residual phosphorus and potassium for a successful crop.

Soil magnesium and calcium levels were statistically significant between broiler litter treatments. No clear trend was observed between the amount of broiler litter added to the soil and residual soil magnesium and calcium; however, these nutrients tended to increase with increasing application rates of broiler litter. The soil boron levels were statistically significant between broiler litter treatments. Boron levels tended to increase with increasing application rates of broiler litter.

There was a statistically significant difference in residual soil zinc and copper levels between broiler litter treatments. Zinc and copper increased with increasing application rates of broiler litter. These findings are similar to Adeli et al. (2007), who saw significant increases in soil zinc and copper with increasing broiler litter

applications. Copper levels in the soil did not have a direct effect on the concentration of copper in leaves or hemp flower buds.

The plant tissue analysis results from the broiler litter study revealed a statistically significant difference in tissue phosphorus levels between broiler litter treatments. In general, phosphorus levels increased with increasing application rates of broiler litter. There was also a statistically significant difference in tissue potassium levels between broiler litter treatments. Potassium levels increased with increasing application rates of broiler litter. These findings are similar to the findings of the Lin et al. (2016) meta-analysis, where there was a significant positive effect on phosphorus and potassium uptake with the use of broiler litter.

The plant tissue analysis also revealed a statistically significant difference in tissue sulfur levels between broiler litter treatments. In general, sulfur levels in plant tissue decreased with increasing application rates of broiler litter. The average plant height between broiler litter treatments was statistically significant. Plant height tended to increase with increasing application rates of broiler litter.

The broiler litter treatments had no significant effect on hemp flower yield. However, there was a trend toward higher yields with increasing broiler litter application rates. There were no significant differences in total CBD or THC content between broiler litter treatments. The metal analysis of the hemp flower bud revealed a statistically significant difference in selenium levels between broiler litter treatments; however, no clear trends were observed. The metal analysis also revealed a statistically significant difference in lead levels between broiler litter treatments. No clear correlation was observed between the application rates of broiler litter and lead levels.

Conclusions for Objective 2

There was a statistically significant difference in residual soil copper levels between copper treatments even though the copper plots had not been treated with copper since 2009. Soil copper was lowest in the 0 lbs Cu * ac⁻¹ treatment and highest in the 20 lbs Cu * ac⁻¹ treatment. Even though soil copper was significant in the soil, it was not found to be significant in the hemp leaves or hemp flower bud. However, copper concentrations in these parts of the plant followed a similar trend.

The plant tissue analysis revealed a statistically significant difference in tissue calcium levels between copper treatments. Calcium levels decreased with increasing application rates of copper. These findings are similar to Osteras & Greger (2006), who saw decreases in two-year-old spruce seedlings' calcium levels with the addition of elevated concentrations of foliar-applied copper. The copper levels in the hemp leaves between copper treatments were not statistically significant; however, copper levels did increase with increasing application rates of copper.

The average hemp flower yields were not statistically significant between copper treatments. The 10 lbs Cu * ac⁻¹ treatment had the highest yield value, which is similar to previous research done on this plot with corn, where a significant difference in corn yields was observed when all six growing seasons were added together (Unpublished data). No significant differences were observed in major cannabinoid content and copper treatments. Total CBD and THC had the highest values under the 10 lbs Cu * ac⁻¹ treatment. This trend is similar to the trend observed in the hemp flower yield. The copper levels in the hemp flower bud were not statistically significant between copper treatments; however, copper values within the flower bud did increase with increasing

application rates of copper. The manganese levels in the hemp flower bud were not statistically significant between copper treatments; however, a trend was observed. Manganese levels were also highest at 10 lbs * ac⁻¹ rate. There was not much variation in soil fertility between copper treatments, with the exception of both manganese and copper. The soil manganese levels between copper treatments were statistically significant. Soil manganese was highest at the 10 lbs * ac⁻¹ rate, similar in trend to the manganese levels found in the hemp flower bud, the total CBD and THC content, and yield. These findings are similar to those of Radosavljevic-Stevanovic et al. (2014), who observed a positive correlation between soil manganese levels and THC production. This correlation should be researched further.

This data has the potential to help hemp producers by offering insight into typical heavy metal accumulation and content of hemp plants under varying soil conditions. In 2019, hemp contracts were negated because of the levels of various elements and metals in the hemp flower buds. In general, the hemp flower buds analyzed in this experiment did not have high levels of heavy metals. The heavy metal data obtained from this experiment provides insight into what the concentrations of metals are found under a variety of soil fertility levels. This information could be used to make equitable marketing decisions for all parties involved.

Furthermore, many studies have found hemp to be a bio accumulator of heavy metals (Angelova et al., 2004; Citterio et al., 2003) but we did not observe any substantial differences in heavy metal accumulation in flower bud or plant tissue between any of the copper or broiler litter treatments. However, other studies suggest that the majority of heavy metal accumulation in *Cannabis sativa* L. resides in the roots (Galić et al., 2019;

Linger et al., 2005). Collecting root samples, in addition to plant tissue and flower bud samples, for heavy metal analysis at various plant stages might provide better insight into hemp's (*Cannabis sativa* L.) role in the phytoremediation of heavy metals from the soil.

Overall Conclusions

In both the broiler litter and copper experiments trends were observed, yet not all were statistically significant. The experiments need to be repeated in order to see if the trends detected continue to be observed, then both years could be added into a single statistical analysis. Broiler litter contains a plethora of vital plant nutrients at a relatively low cost compared to commercial fertilizers. It also contains essential micronutrients and high levels of organic matter that commercial fertilizers do not typically supply.

The copper experiment, which was a spin-off project from the broiler litter experiment, continues to provide insight into this essential micronutrient. The roles of copper in plants are numerous, including being a cofactor in the plastocyanin and superoxide dismutase proteins which are important for photosynthesis and oxidative stress protection (Epstein & Bloom, 2005). Ciampitti & Vyn (2013) provide a good review of some roles that copper plays in maize crops. Likewise, the study reported here begins to evaluate the role of this micronutrient in hemp

Appendix A

T-Test Mean Comparisons for Plant Height in Broiler Litter Treatments

Comparison significant at the 0.1 level are indicated by ***.

trt Comparison	Difference Between Means	90% Confidence limits 325.25b		
7-6	6.744	3.862	9.626	***
7-5	8.898	6.047	11.749	***
7-4	9.563	6.647	12.478	***
7-2	10.779	7.913	13.645	***
7-3	12.051	9.200	14.902	***
7-0	13.371	10.489	16.253	***
7-1	14.014	11.132	16.896	***
6-7	-6.744	-9.626	-3.862	***
6-5	2.154	-0.680	4.989	
6-4	2.819	-0.080	5.718	
6-2	4.035	1.186	6.884	***
6-3	5.307	2.473	8.141	***
6-0	6.627	3.762	9.493	***
6-1	7.270	4.405	10.136	***
5-7	-8.898	-11.749	-6.047	***
5-6	-2.154	-4.989	0.680	
5-4	0.664	-2.204	3.533	
5-2	1.881	-0.937	4.698	
5-3	3.153	0.350	5.955	***
5-0	4.473	1.639	7.307	***
5-1	5.116	2.282	7.950	***
4-7	-9.563	-12.478	-6.647	***
4-6	-2.819	-5.718	0.080	
4-5	-0.664	-3.533	2.204	
4-2	1.216	-1.667	4.100	
4-3	2.488	-0.380	5.357	
4-0	3.808	0.909	6.708	***
4-1	4.452	1.552	7.351	***
2-7	-10.779	-13.645	-7.913	***
2-6	-4.035	-6.884	-1.186	***
2-5	-1.881	-4.698	0.937	
2-4	-1.216	-4.100	1.667	

Appendix A - Continued

T-Test Mean Comparisons for Plant Height in Broiler Litter Treatments

Comparison significant at the 0.1 level are indicated by ***.

trt Comparison	Difference Between Means	90% Confidence limits 325.25		
2-3	1.272	-1.546	4.090	
2-0	2.592	-0.257	5.442	
2-1	3.235	0.386	6.085	***
3-7	-12.051	-14.902	-9.200	***
3-6	-5.307	-8.141	-2.473	***
3-5	-3.153	-5.955	-0.350	***
3-4	-2.488	-5.357	0.380	
3-2	-1.272	-4.090	1.546	
3-0	1.320	-1.514	4.154	
3-1	1.963	-0.871	4.797	
0-7	-13.371	-16.253	-10.489	***
0-6	-6.627	-9.493	-3.762	***
0-5	-4.473	-7.307	-1.639	***
0-4	-3.808	-6.708	-0.909	***
0-2	-2.592	-5.442	0.257	
0-3	-1.320	-4.154	1.514	
0-1	0.643	-2.222	3.509	
1-7	-14.014	-16.896	-11.132	***
1-6	-7.270	-10.136	-4.405	***
1-5	-5.116	-7.950	-2.282	***
1-4	-4.452	-7.351	-1.552	***
1-2	-3.235	-6.085	-0.386	***
1-3	-1.963	-4.797	0.871	
1-0	-0.643	-3.509	2.222	

Bibliography

- Adeli, A., Sistani, K.R., Rowe, D.E. & Tewolde, H. (2005). Effects of broiler litter on soybean production and soil nitrogen and phosphorus concentrations. *Agronomy Journal*, 97, 314-321. <https://doi.org/10.2134/agronj2005.0314>
- Adeli, A., Sistani, K.R., Tewolde, H., & Rowe, D.E. (2007). Broiler litter application effects on selected trace elements under conventional and no-till systems. *Soil Science*, 172, 349-365. DOI: 10.1097/ss.0b013e318032ab7d
- Adeli, A., Tewolde, H., Sistani, K.R., & Rowe, D.E. (2010). Comparison of broiler litter and commercial fertilizer at equivalent N rates on soil properties. *Communications in Soil Science and Plant Analysis*, 41, 2432-2447. [10.1080/00103624.2010.511374](https://doi.org/10.1080/00103624.2010.511374)
- Adeli, A., Tewolde, H., Sistani, K.R., & Rowe, D.E. (2011). Continuous and residual effects of broiler litter application to cotton on soil properties. *Soil Science*, 176, 668-675. DOI: 10.1097/SS.0b013e3182343507
- Angelova, V., Ivanova, R., Delibaltova, V., Ivanov, K. (2004). Bio-accumulation and distribution of heavy metals in fibre crops (flax, cotton and hemp). *Industrial Crops and Products*, 19, 197-205. <https://doi.org/10.1016/j.indcrop.2003.10.001>.

- Arru, L., Rognoni, S., & Baroncini, M. (2004). Copper localization in *Cannabis sativa* L. grown in a copper-rich solution. *Euphytica*, 140, 33–38.
<https://doi.org/10.1007/s10681-004-4752-0>
- Carpenter, S. (2008) Phosphorus control is critical to mitigating eutrophication. *Proceedings of the National Academy of Sciences*, 105, 11039-11040. <https://doi.org/10.1073/pnas.0806112105>
- Ciampitti, I.A., & Vyn, T.J. (2013) Maize nutrient accumulation and partitioning in response to plant density and nitrogen rate: II, Calcium, magnesium, and micronutrients. *Agronomy Journal* 105, 1645-1657.
- Citterio, S., Santagostino, A., Fumagalli, P., Prato, N., Ranalli, P., & Sgorbati, S. (2003). Heavy metal tolerance and accumulation of Cd, Cr and Ni by *Cannabis sativa* L. *Plant and Soil*, 256, 243-252. <https://doi.org/10.1023/A:1026113905129>
- Chastain, J., Camberato, J., & Skewes, P. (n.d.) Poultry manure production and nutrient content. Clemson University Extension.
https://www.clemson.edu/extension/camm/manuals/poultry/pch3b_00.pdf
- Dunkley, C. S., Cunningham, D. L., & Harris, G. H. (2011). The value of poultry litter in south Georgia. University of Georgia Athens Extension.
<https://extension.uga.edu/publications/detail.html?number=B1386>.
- Edwards, T. (2021). Soil organic matter - frequently asked questions (FAQs). Department of Primary Industries and Development- Government of Western Australia.
www.agric.wa.gov.au/soil-carbon/soil-organic-matter-frequently-asked-questions-faqs#:~:text=Globally%20SOC%20is%20generally%20between,of%201.0%20g%20Fcm3.
- Epstein, E., Bloom, A.J. (2005) *Mineral nutrition of plants: Principles and Perspectives* (2nd Ed., pp. 212-213). Sunderland Massachusetts: Sinauer Associates, Inc.
- Galić, M., Perčin, A., Zgorelec, Ž. i Kisić, I. (2019). Evaluation of heavy metals accumulation potential of hemp (*Cannabis sativa* L.). *Journal of Central European Agriculture*, 20, 700-711. <https://doi.org/10.5513/JCEA01/20.2.2201>

- Gascho, G.J., Hubbard, R.K., Brenneman, T.B., Johnson, A.W., Sumner, D.R. & Harris, G.H. (2001). Effects of broiler litter in an irrigated, double-cropped, conservation-tilled rotation. *Agronomy Journal*, 93, 1315-1320. <https://doi-org.ezproxy.waterfield.murraystate.edu/10.2134/agronj2001.1315>
- Gupta U. & Yash P. K. (2006) Residual effect of copper and zinc from fertilizers on plant concentration, phytotoxicity, and crop yield response. *Communications in Soil Science and Plant Analysis*, 37, 2505-2511. [10.1080/00103620600822952](https://doi.org/10.1080/00103620600822952)
- Hoover N.L., Law J.Y., Long L.A.M., Kanwar R.S., & Soupir M.L. (2019). Long-term impact of poultry manure on crop yield, soil and water quality, and crop revenue. *Journal of Environmental Management*, 252. <https://doi.org/10.1016/j.jenvman.2019.109582>
- Kentucky Mesonet. (2020). Monthly climatological summary. Retrived July 22, 2021. http://www.kymesonet.org/historical_data.php
- Khan, M., Wajd, A., Noor, S., Khattak, K., Akhter, S., & Rahman I,U. (2008). Effect of soil contamination on some heavy metals content of *Cannabis sativa*. *Journal of The Chemical Society of Pakistan*, 30, 805-809.
- Laboski, C. (2018). NPK et cetera from the desk of Carrie Laboski. University of Wisconsin. npketc.soils.wisc.edu/2018/05/02/fertility-guidelines-for-hemp-in-wisconsin/.
- Lin, Y., van Santen, E., & Watts, D. (2016). The effect of poultry litter application on agricultural production: a meta-analysis of crop yield, nutrient uptake and soil fertility. *Conference on Applied Statistics in Agriculture*. <https://doi.org/10.4148/2475-7772.1494>
- Linger, P., Ostwald, A. & Haensler, J. (2005). *Cannabis sativa* L. growing on heavy metal contaminated soil: growth, cadmium uptake and photosynthesis. *Biologia Plantarum*, 49,567–576. <https://doi.org/10.1007/s10535-005-0051-4>
- MacDonald, J.M., Ribaud, M.O., Livingston, M.J., Beckman, J., & Huang, W. (2009). Manure use for fertilizer and for energy: report to Congress. U.S. Department of

Agriculture, Economic Research Service.

https://www.ers.usda.gov/webdocs/publications/42731/16744_ap037e_1_.pdf?v=0

Murdock, L. & Ritchey, E. (2012). 2012-2013 Lime and nutrient recommendations. *Agriculture and Natural Resources Publications*, 43.

https://uknowledge.uky.edu/anr_reports/43

Netthisinghe, A.M.P., Woosley, P.B., Gilfillen, R.A., Willian, T.W., Sistani, K.R. & Rowland, N.S. (2016). Corn grain yield and soil properties after 10 years of broiler litter amendment. *Agronomy Journal*, 108, 1816 - 1823. <https://doi.org/10.2134/agronj2016.02.0113>

Oldham, L. (2019). Secondary plant nutrients: calcium, magnesium, and sulfur. Mississippi State University Extension.

<http://extension.msstate.edu/publications/secondary-plant-nutrients-calcium-magnesium-and-sulfur>

Österås, A.H. & Greger, M. (2006). Interactions between calcium and copper or cadmium in Norway spruce. *Biologia Plantarum*, 50, 647.

<https://doi.org/10.1007/s10535-006-0101-6>

Paudel, K. & McIntosh, C. (2005). Country report: Broiler industry and broiler litter-related problems in the southeastern United States. *Waste Management*, 25, 1083-1088. doi: 10.1016/j.wasman.2005.01.023. PMID: 16243231.

Prasad, R. & Stanford, K. (2019). Nutrient content and composition of poultry litter. Alabama A&M and Auburn Universities Extension.

<https://www.aces.edu/blog/topics/crop-production/nutrient-content-and-composition-of-poultry-litter/>

Rasnake, M. (1996). Broiler litter production in Kentucky and potential use as a nutrient source. Bulletin AGR-168. University of Kentucky, Cooperative Extension Service.

http://afs.ca.uky.edu/files/broiler_litter_production_in_ky.pdf

SAS Institute. (2013). The SAS system. Release 9.4. SAS Institute, Cary, NC.

Radosavljevic-Stevanovic, N., Markovic, J., Agatonovic-Kustrin, S., & Razic, S. (2014). Metals and organic compounds in the biosynthesis of cannabinoids: a chemometric approach to the analysis of Cannabis sativa samples. *Natural Product Research*, 28, 511-516. DOI: 10.1080/14786419.2014.880912

Scott, J. (2010). Identifying factors affecting soybean productivity from previous broiler litter fertilization, including a possible copper nutrient effect. Thesis. Murray State University.

Syuhad, N., Jahan M.S., Khandaker, M., Mat N., Khairi, M., Nozulaidi, M., & Razali, M. (2014). Application of copper increased corn yield through enhancing physiological functions. *Australian Journal of Basic and Applied Sciences*, 8, 282-286.

United States Department of Agriculture. (2019). Economic Research Service fertilizer use and price. <https://www.ers.usda.gov/data-products/fertilizer-use-and-price.aspx>

United States Environmental Protection Agency. (n.d.). Agriculture nutrient management and fertilizer. <https://www.epa.gov/agriculture/agriculture-nutrient-management-and-fertilizer>

Upchurch, M. W. (2008). The residual effects of broiler litter on soybean yield and soil properties in western Kentucky. Thesis. Murray State University.

Yruela, I. (2009) Copper in plants: acquisition, transport and interactions. *Functional Plant Biology*, 36, 409-430. <https://doi.org/10.1071/FP08288>