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# Effectiveness of Biochar Addition in Reducing Concentrations of Selected Nutrients and Bacteria in Runoff

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**STUDENT AGREEMENT:**



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# EFFECTIVENESS OF BIOCHAR ADDITION IN REDUCING CONCENTRATIONS OF SELECTED NUTRIENTS AND BACTERIA IN RUNOFF

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THESIS

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A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in
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By

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# ABSTRACT OF THESIS

# EFFECTIVENESS OF BIOCHAR ADDITION IN REDUCING CONCENTRATIONS OF SELECTED NUTRIENTS AND BACTERIA IN RUNOFF

Land application and storage of horse manure and municipal sludge can increase nutrient and bacteria concentrations in runoff. Biochar increases soil nutrient retention when used as a soil amendment. The objectives of this study were to determine if biochar, when mixed with horse manure or sludge, affects runoff concentrations of total Kjehldahl nitrogen (TKN), ammonia-nitrogen ($NH_3$-N), nitrate ($NO_3$-N), total phosphorus (TP), dissolved phosphorus (DP), total suspended solids (TSS), chemical oxygen demand (COD), and fecal coliforms (FC). Horse manure and sludge were applied to 2.4 x 6.1 m fescue plots (six each), with three plots of each material amended with 5-8% biochar w/w. Simulated rainfall (101.6 mm/h) was applied to the 12 treatment plots and three control plots. The first 0.5 h of runoff was collected and analyzed for the above-listed parameters. The data were analyzed using an ANCOVA, with SCS runoff curve number (CN) used as the covariate. In general, CN was directly correlated to runoff concentrations of parameters. Plots with low CN values displayed no treatment differences for any measured parameter. Biochar reduced runoff concentrations of TKN and $NH_3$-N for municipal sludge treatments, and TKN, $NH_3$-N, TP, TSS, and FC for horse manure treatments.

KEYWORDS: Manure, Sludge, Biochar, Runoff, Nutrients

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February 23, 2016

EFFECTIVENESS OF BIOCHAR ADDITION IN REDUCING CONCENTRATIONS OF SELECTED NUTRIENTS AND BACTERIA IN RUNOFF

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This thesis is dedicated to my husband, Travis, who has supported me throughout this entire endeavor and been a constant source of encouragement, and to my parents, who always told me I could accomplish anything if I put my mind to it.

# TABLE OF CONTENTS

## LIST OF FIGURES

## LIST OF TABLES

# CHAPTER 1: INTRODUCTION

## 1.1 INTRODUCTION

Lexington is located in the center of the Bluegrass Region of the Commonwealth of Kentucky. Census data from 1900 put the population at 42,071 (Lexington-Fayette Urban County Government, 2012). In 2000, the population had increased to 260,512; only 10 years later the population was 295,803, a 13% increase over 2000 (LFUCG, 2012). The population projection for the year 2030 is 375,986, an increase of 27% over 2010 (LFUCG, 2012). A census has never been published that showed Lexington's population to decrease. If the city follows the trend of the past 100 years, it will continue to steadily grow in size. With a large population comes the need for waste management, particularly treatment of wastewater. Lexington has two treatment plants – West Hickman Creek and Town Branch. Together, both plants treat approximately 196 million liters of wastewater daily (LFUCG, 2011a, 2011b).

Another concern in terms of waste for Lexington involves the equine industry. Kentucky is known as the Horse Capital of the World. The equine industry is estimated to have an economic impact of nearly $3 billion annually (University of Kentucky College of Agriculture, 2013). The Bluegrass Region contains the highest concentration of horses in Kentucky, containing 39,000 (16%) of the state's equine population (University of Kentucky College of Agriculture, 2013). According to ASAE (American Society of Agricultural Engineers) Standard D384.2 (ASAE, 2005), a typical adult horse (500 kg) is estimated to produce 25 kg of manure daily (urine and feces combined). Taking into account the number of horses in the Bluegrass, approximately 975,000 kg of horse manure is produced daily.

With both horse manure and municipal sludge being found in large quantities in this area, runoff from these materials is of particular interest. Runoff quality is a major concern in regards to downstream water pollution, and nutrients in runoff can cause eutrophication in lakes, rivers, and streams (Chen et al., 2011; Smith et al., 1999). Eutrophication occurs when an excess of nutrients - typically nitrogen (N) and phosphorus (P) - enter a water body. This high nutrient content tends to encourage the growth of algae on the water's surface, which reduces the amount of light and oxygen that can reach the organisms living below the surface, thus harming aquatic ecosystems (Smith et al., 1999). In fact, eutrophication accounts for 60% of impaired streams in the United States (Smith et al., 1999). In Fayette County, there are almost 128 km of streams designated as impaired based on the 303(d) List of Waters (Kentucky Division of Water, 2013) with eutrophication as the source of the impairment accounting for 88 km of stream (Kentucky Division of Water, 2013). Also, pathogens have the potential to cause sickness in humans who come into contact with or ingest contaminated water (Bushee et al., 1998) through, for example, recreational use of lakes or streams. Fecal coliforms including *Escherichia* coli are also a source of impairment for 88 km of the streams on the 303(d) list (Kentucky Division of Water, 2013). For these reasons, it is important to find ways to mitigate the effects of horse manure and municipal sludge on water quality, particularly in Lexington, KY and other regions where these wastes are abundant and stream quality is poor.

### 1.1.1 Municipal Wastes

The treatment process at Town Branch consists of filtration of large debris and grit, followed by a biological treatment that converts remaining fines and dissolved organics into biological solids and removes ammonia. The solids are then settled out while the water is disinfected using chlorine. Chlorine is removed from water using sulfur dioxide, and then aerated and discharged to Town Branch Creek (LFUCG, 2011a). The West Hickman plant is classified as a two-stage activated sludge nitrification system and removes over 90% of incoming pollutants from the wastewater (LFUCG, 2011b). Byproducts from these processes, mainly sludge, are thickened and anaerobically digested to form a stable and dewatered product that is then transported to landfills for disposal (LFUCG, 2011a). According to the USEPA (2001), typical wastewater treatment produces 0.25 kg of biosolids per 1000 L. The plants in Lexington have the combined capacity to treat approximately 196 million liters a day, resulting in 49000 kg of sludge delivered to landfills daily.

Landfills are designed and regulated according to federal requirements to help protect the environment from being contaminated by the solid waste deposited there. The USEPA (2016) requires landfills to be located away from sensitive areas such as wetlands and floodplains and to have a lining placed over 0.6 m of compacted clay to reduce leachate to the soil and protect groundwater. Waste is frequently covered by a few inches of soil to reduce odor, insects and rodents, and to protect public health. The majority of federal regulations are designed to prevent the pollution of groundwater; however, surface water pollution from contaminated runoff is still a concern.

### 1.1.2 Equine Wastes

The average adult horse (500 kg) produces an estimated 25 kg of waste a day (ASAE Standard, 2005), which is traditionally disposed of in two ways: land application, either directly or composted, and storage or stockpiling (Komar et al., 2010; Wartell et al., 2012; Westendorf et al., 2010). The composition of animal manures can be influenced by the animal's diet (Dou et al., 2002; Ebeling et al., 2002; Velthof et al., 2005), the method of collection or storage (Barker and Zublena, 1996; Muck and Steenhuis, 1996; Nicholson et al., 2004), and the type of bedding used (Foulk et al., 2004; Miller et al., 2003; Wartell et al., 2012). Generally, horse manure has a high C content (and high C:N ratio) due to the proportion of bedding mixed with the waste (Natural Resources Conservation Service, 2007). Horse manure is, on average, near the low end of the range for N and P content of grazing animal manures; however, horse manure is still a significant source of nutrients for land application (Hubbard et al., 2004).

While manure characteristics are not uniform, there are potential negative impacts to the environment from any manure source, typically in the form of excess nutrients entering runoff or groundwater (Bushee et al., 1998; Hubbard et al., 2004; Komar et al., 2010; McLeod and Hegg, 1984; Pote et al., 2001). Heavy metals (Edwards et al., 1999; Moore et al., 1998) and pathogens (Bushee et al., 1998; Hubbard et al., 2004; Komar et al., 2010; Weaver et al., 2005) are also a concern for water quality. Mitigating the effects of horse manure on runoff quality, and in turn lake and stream quality, is important for environmental quality and public use of receiving waters.

### 1.1.3 Summary

The large quantities of human and equine waste produced in the Bluegrass Region of Kentucky can present environmental challenges in terms of runoff quality (Bushee et al., 1998; Chen et al., 2011b; Edwards et al., 1999; McCleod and Hegg, 1984). Manures and other wastes have a high nutrient content; when land applied or stored without cover (as in a landfill), they can promote significant nutrient and bacterial concentrations in runoff (Crane et al., 1983). Municipal sludge has specifically been found to have negative effects on runoff quality such as increased fecal coliforms (Bushee et al., 1998) and high concentrations of P (Chen et al., 2011b).

## 1.2 BIOCHAR AS AN AMENDMENT

The Commonwealth of Kentucky currently uses many different BMPs, or Best Management Practices, as defined by the Kentucky Agriculture Water Quality Act (KRS 224.71), to reduce the impacts of agriculture on water quality. The Act defines BMPs as the most effective and economical way for the State to reduce and prevent water pollution. Best Management Practices can be applied at any step in the agricultural production process. For example, BMPs can define the correct amount of fertilizers or pesticides necessary for a certain area and when to apply them, as well as how to properly store excess chemicals so that lesser quantities of nutrients are lost to runoff. Best Management Practices can also be applied downstream of agricultural production in the form of filter strips or riparian buffer zones to reduce the amount of nutrient-enriched runoff reaching waterways (Kentucky Division of Conservation, 2014). Given the importance of protecting the quality of lakes and rivers for the sake of public use and economic value, additional ways of reducing surface and groundwater pollution are worthy of study.

Biochar is a material that has only recently been studied as an environmental amendment. Biochar has long been used to date archaeological deposits due to its persistence in the environment (Lehmann, 2007), but only within the last 10 years has it been considered as a possible solution to nutrient losses from soil. Biochar is the byproduct of any type of biomass that has undergone pyrolysis (Mackie *et al.*, 2015), a process that converts biomass to a carbon-rich energy source by heating it to high temperatures in the absence of oxygen (Lehmann, 2007).

### 1.2.1 Properties of Biochar

Research into biochar has indicated possibilities for its use in increasing nutrient (Laird et al., 2010; Lehmann, 2007; Schnell et al., 2012; Zhai et al., 2015) and water retention

(Beck et al., 2008; Novak et al., 2009, Ulyett et al., 2014) in soils, filtering heavy metals (Park et al., 2011; Zhang et al., 2013), and reducing transport of microbes (Abit et al., 2014; Bolster and Abit, 2012; Mohanty et al., 2014). Biochar has also been posited as an option for reducing the impacts of climate change by carbon (C) sequestration (Laird, 2008; Lehmann et al., 2006; Lentz et al., 2014), by converting plant biomass to biochar and thus removing the stored C from the C cycle. Due to the recalcitrance of biochar in soil (Abit et al., 2012), sequestration could be a long-term solution for reducing $CO_2$ emissions to the atmosphere. These properties of biochar make it an attractive candidate for research relating to runoff quality.

#### *1.2.1.1 Soil Water Retention*

The water holding capacity (WHC) of a soil is highly affected by soil texture. Medium-textured soils such as silt loams and silty clay loams tend to have the greatest WHC due to the size and number of pores and amount of aggregation caused by the silt and clay particles (Plant and Soil Sciences eLibrary, 2016). Plant available water increases as WHC increases (typically estimated as 50% of WHC) (Plant and Soil Sciences eLibrary, 2016), which can improve plant growth.

Biochar's effects on water retention in soil are most likely due to its large pore size. Novak et al. (2009) found that a switchgrass biochar amendment to a loamy sand soil significantly increased the amount of water in the soil, with a higher pyrolysis temperature (500°C) resulting in greater retention. This corresponds with Uzoma et al. (2011), who also found that biochar heated at 500°C had greater water retention than when heated at 350°C. Novak et al. (2009) determined peanut hull biochar to produce a significant improvement in water retention as well; biochars produced from pecan shells and poultry litter, however, had no effect on soil WHC. Dugan et al. (2010) found that biochar amendments were most effective at increasing soil water in sandy soils relative to other soil textures, regardless of the feedstock. Other studies have also shown an increase in WHC in sandy soils after addition of a wood-based biochar (Basso et al., 2013; Uzoma et al., 2011) and attributed the increase to both increased pore spaces and greater surface area.

#### *1.2.1.2 Nutrient Retention*

Retaining nutrients in soil or fertilizer can reduce the impact of runoff pollution of waterways, potentially reducing both eutrophication and losses of applied nutrients. Biochar may be a viable solution to nutrient loss, thus reducing the nutrient concentrations in runoff. Biochar amendments can increase the cation exchange capacity (CEC) of a soil, increasing the potential for nutrient sorption to the surface of the biochar (Laird et al., 2010; Mackie et al., 2015). The ability to retain and exchange cations in a plant-available form is directly related to the nutrient retention capabilities of that material. Laird et al. (2010) showed an increase in N, organic C, P, K, Mg, and Ca in fine-loamy soil amended with hardwood biochar. A sorghum-based biochar also increased organic C and reduced mass loss of N, P, and K in runoff when incorporated into the soil (Schnell et al., 2012).

Concentrations of $NO_3$-N and $PO_4$-P were reduced in runoff from a waste wood biochar-amended soil column (Reddy et al., 2014) and from soil amended with an agricultural char (pecan, walnut, and coconut shells, and rice hulls) biochar (Beck et al., 2011). In addition to $NO_3$-N and $PO_4$-P, Beck et al. (2011) found reductions in TN, total phosphorus (TP), and total organic C.

A general trend was also found that with increasing application rates of biochar, there was an increase in nutrient retention in the soil (Zhai et al., 2015; Zhang et al., 2015). However, there has been a wide range of published application rates, and while high rates produce significant results, the economic impacts of these extreme rates were not considered.

### *1.2.1.3 Microbial Transport*

The presence of *E. coli* in soil and water has the potential to cause human sickness at very low concentrations. For example, Ziemer et al. (2010) found that a particular strain of *E. coli* requires only 5 to 10 cells to cause infection. Rainfall can cause bacteria to be infiltrated into the soil surface or transported by runoff across the surface. This danger to public health has prompted research into microbial transport through soil, for which biochar amendments may be a solution (Abit et al., 2012; Abit et al., 2014; Bolster and Abit, 2012).

Organic matter, pH, conductivity, and dissolved organic C were increased in the soil when poultry litter biochar was added to a sandy soil, resulting in decreased *E. coli* transport (Bolster and Abit, 2012). Each of these soil characteristics has been linked to bacterial transport through soil (Bolster et al., 2006; Harvey et al., 2011; Kim et al., 2009). Bolster and Abit (2012) also found that biochar application rate and pyrolysis temperature as well as *E. coli* surface characteristics play a role in the transport of *E. coli* through soil. The higher temperature biochar (700°C) showed a greater reduction in pathogen transport, probably due to the lesser negative surface charge of high temperature biochars. This finding was supported by Abit et al. (2012) and was further attributed to increased surface area of high temperature biochars, providing greater adhesion of *E. coli* cells.

The feedstock of biochar also plays a role in microbial transport. When a poultry litter biochar was compared to a pine chip biochar, the internal pore structure of the woody biochar was able to retain or sorb more bacteria (Abit et al., 2012). Abit et al. (2014) found that in addition to pyrolysis temperature and feedstock, the texture of the amended soil can also affect bacterial transport. Soils with a higher clay content showed less mobility of bacteria, possibly due to electrostatic attraction between the negatively charged microbes and the positively charged clay functional groups.

The effect of biochar on microbial transport through soil is dependent upon feedstock, temperature, and soil texture. The type of biochar used must be properly chosen to play a significant role in reducing bacterial transport. Biochar's ability to reduce pathogen transport through soil could also increase the bacterial population in runoff (Abit et al., 2012), necessitating further research into the effect of biochar on runoff concentrations of microbes.

#### *1.2.1.4 Heavy Metals*

Biochar has the potential to be used as a filter for heavy metals in contaminated soil through its ability to adsorb metals to its surface. Uchimaya et al. (2011) attributed the sequestration of Pb, Cd, Cu, and Ni by cottonseed hull biochar to the presence of functional groups (which determine the type of chemical reactions that can occur for that molecule) on the biochar's surface. As pH, volatile matter (VM), O:C (oxygen to C ratio), and N:C ratios in the biochar increased (all affected by functional groups) so did its capacity to sorb heavy metals. Concentrations of Cd and Pb in soil water were also reduced by a chicken manure biochar and a green waste biochar amendment; however, Cu increased in the soil water, possibly due to increased mobility via increased dissolved organic C (Park et al., 2011). Mackie et al. (2015) similarly reported no effect on extractable Cu from soil amended with hardwood biochar produced at 750°C.

Reddy et al. (2014) reported reductions of Cd, Cr, Cu, Pb, Ni, and Zi in runoff from a soil column amended with a wood-based biochar. Zhou et al. (2013) found that Cu, Cd, and Pb were removed from aqueous solution by addition of bamboo, sugarcane, hickory, and peanut hull biochars, with the bamboo feedstock being most effective. The increase in sorption was directly related to an increase in the pH of the solution, a relationship further characterized by Chen et al. (2011a), who reported an increase of Cu and Zn removal as pH increased when using hardwood and corn straw biochar prepared at 450°C and 600°C respectively. Long-term effects of biochar on heavy metal sorption are relatively unknown, but should be considered due to the recalcitrance of biochar.

### 1.2.2 Influence of Feedstock and Temperature on Properties of Biochar

The type of biomass, or feedstock, used and the pyrolysis temperature play a significant role in the chemical and physical properties of the biochar (Abit et al., 2012; Singh et al., 2010). Biochars produced at high temperatures (400-700°C) tend to have larger surface areas and a higher pH as well as lower N content but greater micronutrient content (Bolster and Abit, 2012; Gaskin et al., 2008). The increase in pH with increasing pyrolysis temperature is attributed to the higher ash content (Zhang et al., 2013). For the lower temperature range (250-400°C), there is a higher yield of biochar from the process, and the structural properties result in more nutrient exchange sites on the biochar (Bolster and Abit, 2012). There are also many different types of feedstock available for producing biochar, such as different species of wood, leaves, and manures. Biochars produced from woody feedstocks tend to have a higher C content and a lower percent makeup of other nutrients (Gaskin et al., 2008; Singh et al., 2010). Manures have a lower C content but greater amounts of TN and higher CEC (Singh et al., 2010). Even within these broad categories; however, there is still variation. It is important to characterize the properties of different biochars so that the most suitable material can be chosen for each desired outcome.

## 1.3 GAPS IN THE RESEARCH

The majority of research regarding biochar has been performed using the medium as a soil amendment to improve plant yields and nutrient retention within that soil. Little work has been done regarding application of biochar to reduce runoff contaminants, with nothing widely available that involves investigating biochar on a plot scale. The effects of horse manure and solid sludge on runoff quality are also relatively unknown compared to other common land applied substances (e.g. swine and dairy manure, inorganic fertilizers), and are relevant to the Bluegrass Region.

## 1.4 OBJECTIVES

Due to the limited amount of research regarding the effects of horse manure and solid sludge on runoff quality and the potential for biochar to mitigate these effects the following objectives were selected for a plot-scale experiment:

1. Determine whether biochar addition to horse manure will affect concentrations of total Kjehldahl nitrogen (TKN), ammonia-nitrogen ($NH_3$-N), nitrate ($NO_3$-N), total phosphorus (TP), dissolved phosphorus (DP), total suspended solids (TSS), chemical oxygen demand (COD), and fecal coliforms (FC) in runoff.

2. Determine whether biochar addition to municipal sludge will affect concentrations of the above-listed analytes in runoff.

The null hypothesis is that biochar addition will have no effect on any measures of runoff quality. However, if biochar is determined to be effective at reducing nutrients and bacteria in runoff from horse manure and municipal sludge, it could be used to lessen the environmental impacts of these wastes when they are either applied as a fertilizer, stored, or placed in landfills.

# CHAPTER 2: MATERIALS AND METHODS

## 2.1 SITE DESCRIPTION

The study site is located at the Maine Chance Research Farm of the University of Kentucky (38.12°N, 84.48°W) (Figure 1). The soil is mapped as a Maury silt loam (typic paleudalf) (NRCS, 2013) planted with tall fescue (*Festuca arundinaceus* Schreb.*)*. The area is divided into 75 identical plots, each measuring 2.4 by 6.1 m with a slope of 3% along the major axis. The plots are divided into 3 rows (B-D) of 10 plots each (1-10) separated by approximately 0.6 m, with a wide walkway of 2.5 m in the center of the rows, splitting the rows into two groups of five. The remaining 45 plots are split into five rows (L-P) with nine plots across. The plots in these nine columns are connected end-to-end to create nine long "super-plots" for studies evaluating distance from source as a variable. Each plot can still be used individually. Figure 2 shows the basic layout of the plots.

Each plot is surrounded on three sides by rustproof metal borders with an aluminum gutter at the bottom to collect the runoff. The runoff is then diverted from the gutter through a PVC pipe to facilitate sample collection. A wooden cover was placed over the gutter during the simulated rainfall to ensure only plot runoff, and not direct rainfall, was included in the samples.

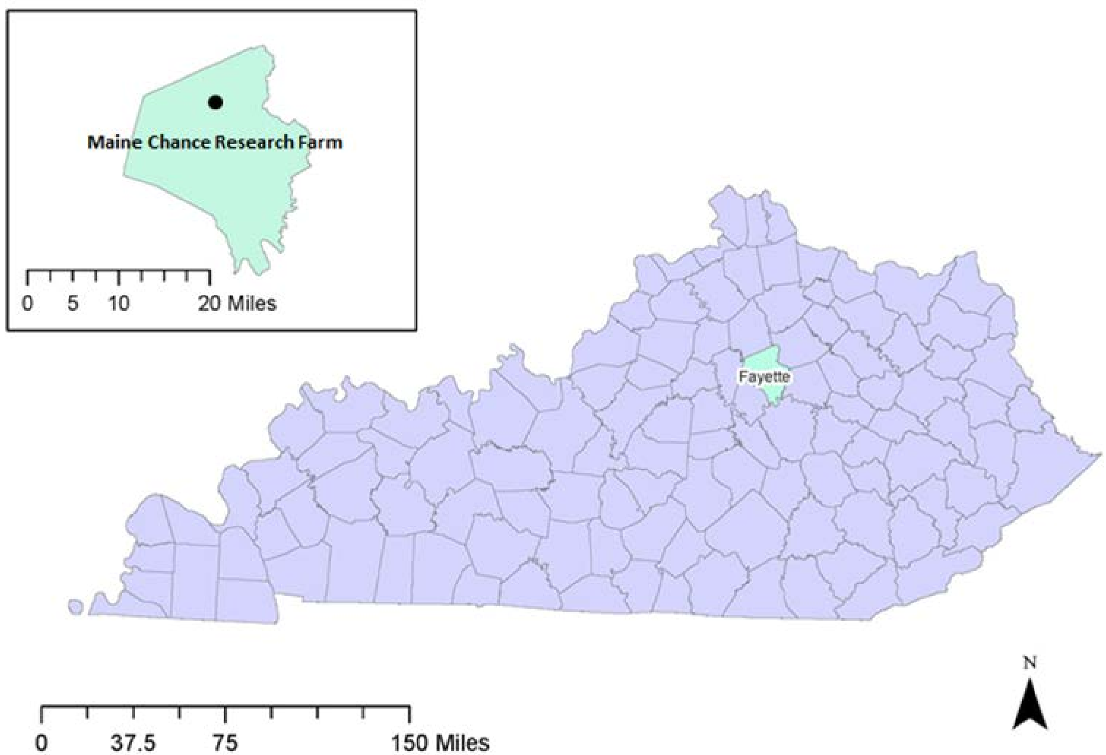


**Figure 1. Location of Maine Chance Research Farm in Fayette County, Kentucky.**

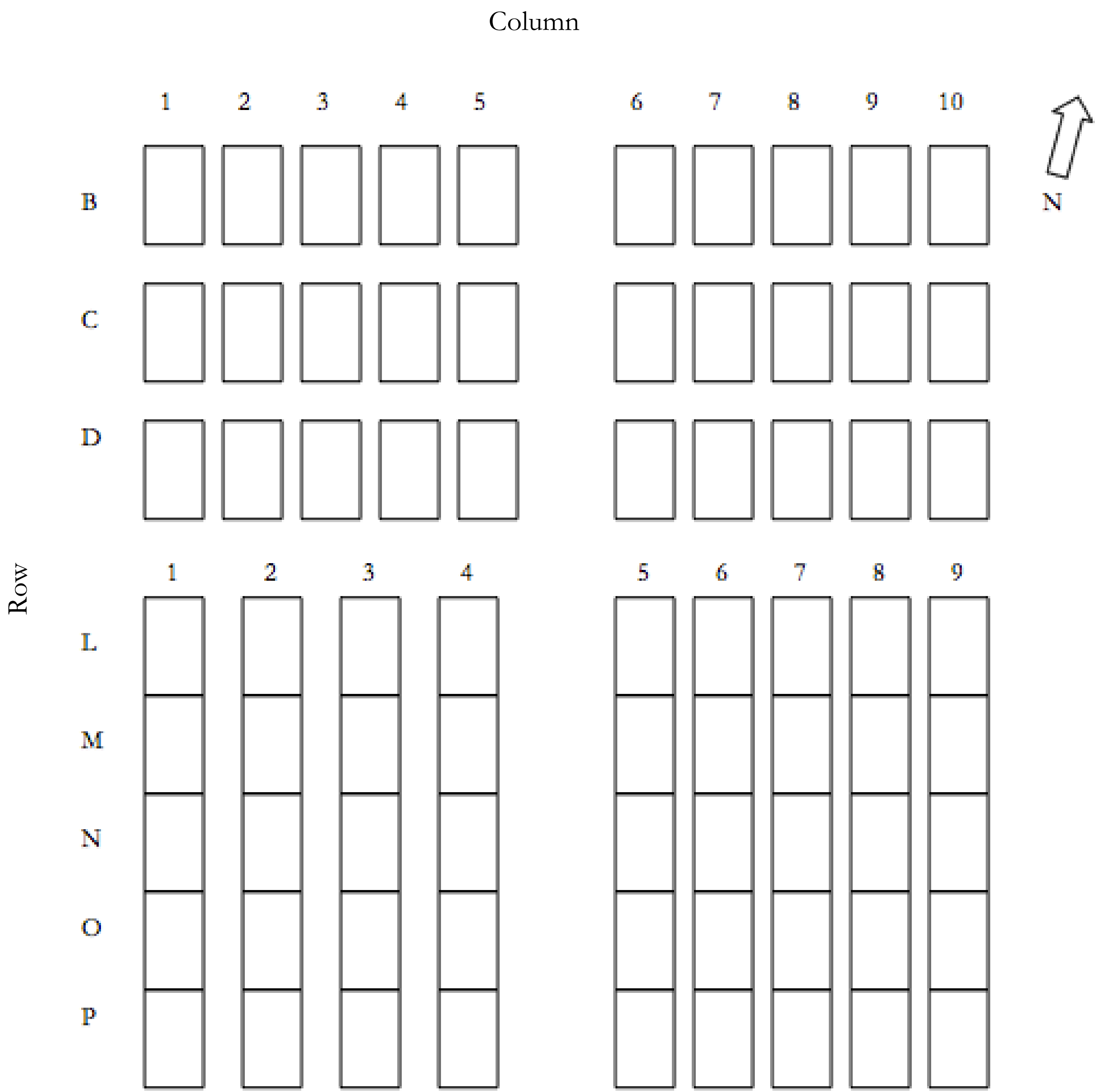


**Figure 2. Layout (not to scale) of rainfall simulator plots at Maine Chance Research Farm.**

## 2.2 MATERIALS

### 2.2.1 Horse Manure

Horse manure was collected from the muckwagon of a Maine Chance Research Farm equine research project on 13 July 2015. At that time, the project included a mix of yearling and adult horses ranging from 350-600 kg. Their diet consisted of a mixture of alfalfa and grass hay with a commercial feed concentrate. The horse stalls were cleaned daily, and manure was stored outside in the muckwagon until disposal (B. Cassill, personal communication, 10 February 2016). Fresh manure with pine chip bedding material was shoveled directly from the muckwagon into six large trash bags until they reached approximately 18 kg. The bags were then double-bagged, labeled as Manure 1-6, and stored in a walk-in cooler located in the Charles E. Barnhart Building (CEB) on the University of Kentucky's campus. Sub-samples were taken from each bag and individually analyzed at the University of Kentucky Regulatory Services (UKRS) Laboratory according to standard practices (Table 1, full results found in Appendix B). The bag contents were standardized to a uniform mass of 17.25 kg each (as-is), due to variation in bag weight from collection. Based on the varying moisture and N content of the different manure samples, each plot was treated with different N application rates, as shown in Table 1.

**Table 1. Characteristics of horse manure as analyzed by University of Kentucky Regulatory Services, and N application rate (kg/ha).**

| Sample | Moisture Content (Wet Basis) | Carbon (% dry) | Total Nitrogen (% dry) | Total Phosphorus (% dry) | Application Rate (kg N/ha) |
|---|---|---|---|---|---|
| Manure 1 | 34% | 46.7 | 0.73 | 0.18 | 56.2 |
| Manure 2 | 34% | 45.9 | 0.96 | 0.22 | 73.5 |
| Manure 3 | 35% | 46.8 | 0.78 | 0.24 | 59.0 |
| Manure 4 | 37% | 44.9 | 1.12 | 0.34 | 81.8 |
| Manure 5 | 37% | 45.9 | 0.92 | 0.28 | 67.1 |
| Manure 6 | 35% | 46.2 | 0.82 | 0.29 | 61.9 |
| **Mean** | 35% ± 1.3[1] | 46.1 ± 0.62 | 0.89 ± 0.13 | 0.26 ± 0.05 | 66.6 ± 8.8 |

[1]Standard Deviation

### 2.2.2 Municipal Solid Sludge

Sludge was collected from the Town Branch Wastewater Treatment Plant (38.06°N, 84.53°W) on 15 July 2015. The sludge was taken from the end of the conveyor belt just before it was loaded onto the truck designated for disposal. Six trash bags were filled to approximately 20 kg, double-bagged, and stored in the CEB walk-in cooler at 4°C. Each bag was labeled as Sludge 1-6. Sub-samples were taken from each bag, and the sub-samples of sludge 1 and 2 were mixed (sludge I), sludge 3 and 4 were mixed (sludge II), and sludge 5 and 6 were mixed (sludge III) as a cost-saving measure so that only three samples were sent to McCoy and McCoy Laboratories, Inc. (2456 Fortune Dr #160, Lexington, KY, 40509). The sub-samples of sludge I, II, and III were analyzed for TN and TP content, as well as the metals Zn, Cu, Fe, Cr, and Se (Table 2, full results in Appendix B). The bagged samples of sludge 1 and 2 were mixed (sludge I), 3 and 4 were mixed (sludge II), and 5 and 6 were mixed (sludge III) to be consistent with the analyzed sub-sample labeling and analysis. The combined bags of sludge I, II, and III were then each separated into two individual portions of 17.25 kg (as-is) each. These six portions were labeled sludge 1*-6*.

**Table 2. Characteristics of municipal sludge as analyzed by McCoy and McCoy Laboratories, and N application rate (kg/ha).**

| Sample | Sludge I | Sludge II | Sludge III | Mean[1] | S[2] |
|---|---|---|---|---|---|
| Moisture Content[3] (%) | 85 | 83 | 84 | 84 | 1.11 |
| Total Nitrogen[4] (%) | 5.29 | 4.68 | 5.11 | 5.03 | 0.31 |
| $NH_3$-N[4] (%) | 0.81 | 0.74 | 0.81 | 0.79 | 0.04 |
| $NO_3$-N[4] (mg/kg) | 1.3 | 1.4 | 1.8 | 1.5 | 0.26 |
| Total Phosphorus[4] (%) | 1.53 | 1.34 | 1.52 | 1.46 | 0.11 |
| N Application Rate (kg N/ha) | 92.6 | 93.8 | 94.8 | 93.0 | 0.98 |

[1]Mean of three samples
[2]Standard deviation of three samples
[3]Moisture content expressed as wet basis
[4]Expressed as percentage of dry weight

### 2.2.3 Biochar

Bison Biochar was used as the biochar amendment to the sludge and manure. This brand of biochar was used due to its commercial availability and its guarantee of consistency in quality and manufacturing procedure. Bison Biochar uses California Department of Food and Agriculture certified organic input material, or feedstock. The feedstock is yellow pine (*pinus*), pyrolyzed at 600°C, and then cooled slowly to produce large interior pore space within the product (D. Lemm, personal communication, 4 February 2016). Biochars derived from wood have been found to retain the original structure of the plant cells, resulting in pore sizes ranging from 5-10 $\mu$m (Abit et al., 2012). Higher pyrolysis temperatures (400-700°C) have also been linked to increased microporosity of the biochar as well as increased fine particles, both related to higher surface area (Abit et al., 2012). Water contained within micropores is considered stationary (Soil Science Society of America, 2008), which can help reduce nutrient leaching by immobilizing nutrients within these pore spaces so that they cannot contribute to runoff. Surface area is also related to nutrient retention - a larger surface area results in a greater capacity to sorb nutrients to the surface of the biochar. Woody biochars show a greater surface area than those produced from agronomic waste (Fryda and Visser, 2015), which, in addition to aiding in nutrient retention, is also optimum for retaining microorganisms (Abit et al., 2012; Mayer et al., 2014) and potentially reducing their presence in runoff from biochar-treated materials.

Three sub-samples of Bison Biochar were analyzed by UKRS for pH, conductivity, TC, TN, and plant-available P and $NO_3$-N (Table 3). The biochar was applied at 5% w/w (gross weight) to manure 1, 2, and 3, and 8% w/w (gross weight) to sludge 1*, 2*, and 3* (Table 4). This application rate was chosen based on both environmental and economic considerations. Higher application rates, while they may produce more pronounced effects, are also more expensive. Other studies testing the effects of biochar have used application rates for biochar ranging from 0.5% to 10% (w/w) (Abit et al., 2012; Abit et al., 2014; Beck et al., 2011; Bolster et al, 2012; Laird et al., 2010; Mohanty et al., 2014; Zhai et al., 2015), and 1.5 to 22.4 Mg/ha (Lentz et al., 2014; Schnell et al., 2012; Zhang et al., 2015). For studies that compared multiple rates of application, significant effects increased with increasing application rate (Bolster et al., 2012; Laird et al., 2010; Zhai et al., 2015; Zhang et al., 2015). The chosen rate of 5-8% was deemed high enough to display any potential effects of the biochar on the manure and sludge, while still being near the lower end of the range of published values for economical reasons.

Rather than purchasing a commercially-produced biochar, similar local alternatives are also available. The biochar used in this study was made from yellow pine, which encompasses a wide variety of pine species, including (but not limited to) lodgepole pine (*Pinus contorta)*, ponderosa pine (*Pinus Ponderosa)*, longleaf pine (*Pinus palustris)*, shortleaf pine (*Pinus echinata*), loblolly pine (*Pinus taeda*), and virginia pine (*Pinus virginiana*). These species are spread throughout the United States, primarily in the southeastern or northwestern part of the country (Natural Resources Conservation Service 2016). The species of shortleaf, loblolly, and virginia pine are found in Kentucky and could be used as a local source for biochar if commercial purchase is not a viable option.

**Table 3. Characteristics of biochar as analyzed by University of Kentucky Regulatory Services.**

| Parameter | Sub-sample | | | Mean[1] |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| pH | 9.9 | 9.9 | 9.9 | 9.9 ± 0[2] |
| Conductivity (dS/m) | 2.32 | 2.12 | 2.24 | 2.23 ± 0.10 |
| Total C (%) | 90.9 | 84.1 | 90.6 | 88.5 ± 3.8 |
| Total N (%) | 0.376 | 0.342 | 0.347 | 0.355 ± 0.02 |
| $NO_3$-N (mg/kg) | 3 | 2 | 2 | 2 ± 0.6 |
| Phosphorus (mg/kg) | 13.2 | 11.3 | 12.8 | 12.4 ± 1.0 |

[1] Mean of 3 samples
[2]Standard deviation

**Table 4. Biochar amendments based on 5% rate for manure and 8% rate for sludge. Gross mass = 17.25 kg for each plot.**

| **Sample** | **Biochar Amendment (kg)** |
|---|---|
| Manure 1 | 0.88 |
| Manure 2 | 0.90 |
| Manure 3 | 0.93 |
| **Manure Mean** | 0.9 ± 0.3[1] |
| Sludge 1* | 1.46 |
| Sludge 2* | 1.46 |
| Sludge 3* | 1.43 |
| **Sludge Mean** | 1.45 ± 0.02[1] |

[1]Standard deviation

## 2.3 METHODOLOGY

### 2.3.1 Plot Selection

Of the 75 available plots at Maine Chance, 15 plots were used: three control (C), three with applied horse manure (M), three with applied solid sludge (S), three with a horse manure and biochar combination (MB), and three with a solid sludge and biochar combination (SB). Plots were selected based on SCS runoff curve number (CN) results from eight previous studies performed at the site (Bushee et al., 1998; Bushee-Bullock, 1999; Enlow, 2014; Edwards, 1997; Edwards et al., 1997; Lim, 1997; Moss, 1998; Myers, 2001). A CN is used to estimate the amount of runoff that will be produced from a given area based on rainfall, soil, land use, hydrologic condition and antecedent moisture condition (McCuen, 1982). For a given area, CN can vary across rainfall events (McCuen, 2002). For this reason, plot selection was based on CN consistency as expressed by standard deviation.

Only plots in rows B-D (Figure 1) were considered; the majority of plots from rows L-P had only been used once, or not at all, so insufficient CN data were available. The method of CN calculation is discussed later in section 2.3.5. The standard deviations of all measured CN values for each plot were calculated (Table 5), and the 15 plots with the smallest standard deviations were chosen based on the assumption that small standard deviations in CN would provide more consistent results. Plots B1, B2, B3, B7, C1, C2, C3, C8, D1, D2, D4, D5, D6, D7, D9 were chosen using this method. One plot was discarded for the next alternate (D9) due to large bare spots present in the plot. The treatments were then randomly assigned to the plots (Figure 3).

**Table 5. Mean curve number (CN) and standard deviation for each plot used in this study based on values collected over 17 years from eight different studies.**

| Plot | Annual Mean CN | | | | | | Mean | S[1] |
|---|---|---|---|---|---|---|---|---|
| | 1996 | 1997 | 1998 | 1999 | 2001 | 2013 | | |
| B1 | 61 | 70 | -[2] | 68 | 58 | 77 | 67 | 8 |
| B2 | 74 | 66 | - | 82 | 68 | 96 | 77 | 12 |
| B3 | 56 | 61 | 39 | - | 52 | 55 | 53 | 8 |
| B7 | - | - | - | 69 | 63 | 74 | 69 | 6 |
| C1 | 51 | 65 | 58 | 74 | 69 | 90 | 68 | 14 |
| C2 | 54 | 57 | - | 75 | 68 | 70 | 65 | 9 |
| C3 | 46 | 69 | 54 | 67 | 57 | 60 | 59 | 8 |
| C8 | 50 | - | - | 55 | - | - | 53 | 4 |
| D1 | 58 | 63 | - | 79 | 67 | 83 | 70 | 11 |
| D2 | 41 | 67 | - | 76 | 73 | 82 | 68 | 16 |
| D4 | - | 67 | 45 | 69 | 59 | - | 60 | 11 |
| D5 | - | 63 | 47 | - | 69 | 61 | 60 | 9 |
| D6 | 49 | - | - | - | 51 | - | 50 | 6 |
| D7 | 45 | - | 51 | 54 | 45 | - | 49 | 5 |
| D9 | 57 | - | 44 | 57 | - | 84 | 60 | 17 |
| Overall | - | - | - | - | - | - | 63 | 8 |

[1] Standard deviation
[2] "-" Indicates no data available

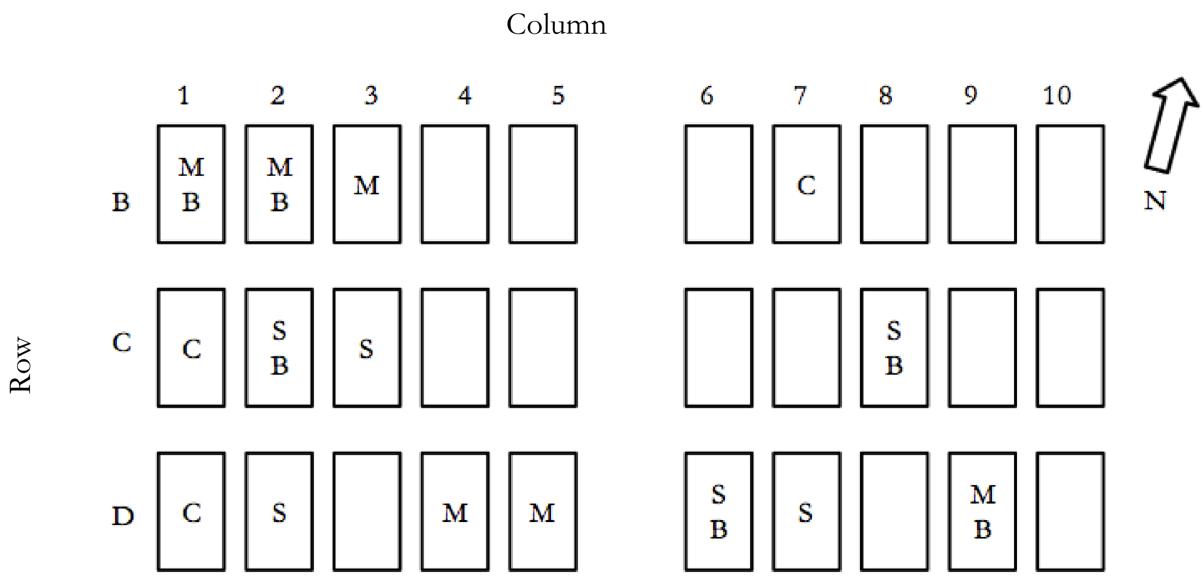


**Figure 3. Field layout of plots (not to scale) labeled with assigned treatment. C – control, M – manure, MB – manure + biochar, S – sludge, SB – sludge + biochar.**

### 2.3.2 Treatments

All plots were pre-wetted using a built-in sprinkler system for a minimum of 24 h prior to the study to reduce variability in soil moisture content between plots. Each bag of horse manure and sludge was transferred to its assigned plot and applied manually using rakes to ensure consistent coverage. Biochar was mixed with the manure and sludge in a wheelbarrow using rakes before land application.

### 2.3.3 Runoff Sample Collection

Three rainfall simulators were used, with each simulator placed directly over one plot. Additional description of rainfall simulators can be found in Bushee et al. (1998). Tarps were used on the four sides of the simulators to minimize drift due to wind. Rainfall intensity began at 101.6 mm/h for each plot and continued until 0.5 h of continuous runoff had occurred. This intensity was chosen to reliably produce runoff in a practical period of time, which was desirable for the focus of the study. For nearly half of the chosen plots (B1, C3, C8, D4, D6, D7, and D9), no runoff had occurred after 1 h at a rainfall intensity of 101.6 mm/h. For these plots, the intensity was increased to approximately 134.6 mm/h so that runoff would be available for sampling. Once the 0.5 h of continuous runoff was complete, the simulators were turned off and moved to the next plot.

Runoff samples were collected in plastic 1-L bottles at 2, 4, 8, 14, 22, and 30 min after continuous runoff began. Each bottle was autoclaved prior to experiment so that samples would remain sterile for evaluation of FC. Each sample was collected for 60 s or until the bottle was full with stopwatches used to keep time. Once collected, sample bottles from each plot were weighed and used to create flow-weighted composite samples. A portion of each composite was filtered with grade 40 filter paper (8 μm) into sterile collection cups to be analyzed for DP. Composites, filtered composites, and the remainder of the original samples were stored at 4°C in a refrigerator on site for up to 7 h until they could be transported back to the lab for analysis.

Experiments took place over multiple days due to weather and available manpower. Treatments for plots B7, C8, D1, D2, D4, D5, D6, D7, and D9 were completed on 23 September 2015. Plots B1, B2, B3, C1, C2, and C3 were completed on 8 October 2015.

### 2.3.4 Sample Analysis

Composite samples were analyzed for TKN, $NH_3$-N, $NO_3$-N, TP, DP, TSS, COD, and FC within 48 h of collection time. Analyses for FC were prepared within 6 h of the average collection time using Colilert-18 (USEPA, 1996). Analyses for COD were performed within 24 h using Hach High Range COD Digestion Vials. The Hach Method 8000 is approved by the USEPA (Eaton et al., 1998).

Analyses for $NH_3$-N, $NO_3$-N, and DP (Standard Methods EPA-129-B, EPA-127-B and EPA-115-B, and EPA-145-B) were performed using a Seal Analytical AQ1 Discrete Analyzer in the Biosystems and Agricultural Engineering Laboratory. The AQ1 automatically produces standard curves for each nutrient (Appendix E). Each composite sample was analyzed twice, and then the results were averaged. Analyses for TKN and TP were performed in the University of Kentucky Plant and Soil Sciences Laboratory.

A LISST machine (Laser *In Situ* Scattering and Transmissiometry) was used to analyze TSS in the samples using Standard Method ISO 13320 (International Organization for Standardization Technical Committee, 2009). Composites were thoroughly mixed, and a 175 mL portion of each was analyzed. Three 175 mL sub-samples of each composite were used so the results could be averaged.

### 2.3.5 Curve Number Calculation

For the SCS CN method, runoff depth (Q) is determined using Equations 1 and 2.

**Equation 1a**

$$Q = \frac{(R - I_a)^2}{(R - I_a) + S}, for\ R \geq I_a$$

**Equation 1b**

$$Q = 0, for\ R \leq I_a$$

**Equation 2**

$$I_a = \lambda S$$

In the equations above, R is rainfall depth, S is the storativity value, or maximum possible retention, and $I_a$ is initial abstraction, which must be satisfied before any runoff occurs. Infiltration, interception, and surface storage prior to initiation of runoff are included in $I_a$. The initial abstraction coefficient, λ, is typically taken as 0.2.

When both rainfall and runoff values are known, the CN can be back-calculated using Equations 3 and 4, where S is in mm. This was the method used to determine CNs for the plots used in the experiment and previously in the process of plot selection. Values for CN can fall anywhere between 0 and 100, with 100 representing the scenario in which absolutely no infiltration occurs and the amount of runoff is 100% the rainfall volume.

**Equation 3**

$$S = 5\ [R + 2Q - (4Q^2 + 5PQ)^{0.5}]$$

**Equation 4**

$$CN = \frac{25400}{S + 254}$$

## 2.4 STATISTICAL ANALYSIS

A one-way ANOVA was performed and determined to be a poor description of the data due to the heterogeneity of the experimental units (EU). Each EU, or plot, had a different CN (ranging from 13-79) that contributed substantial variation to the results. It was evident from the calculations that CN was a nuisance variable; i.e., an extraneous variable that is not a direct part of the study but affects the outcome of the dependent variable (runoff concentration). Experimental controls in the form of blocking could be used to control for this variable (CN); however, precise values for each plot's CN were unknown until after testing had been completed, which prohibited blocking. A statistical control was then used to explain the variation in the runoff concentrations by performing an ANCOVA (analysis of covariance) in which CN was used as the covariate.

The covariate in an ANCOVA is a continuous variable that enters the model as a regression variable (Pennsylvania State University Department of Statistics, 2015). The covariate, in this case CN, accounts for any trends that may occur due to the nuisance variable and essentially removes its effects on the dependent variable. In order for the ANCOVA to be effective, the CN must be linearly related with the runoff concentrations and unaffected by the treatment – as was found to be the case in this study, as discussed later.

To determine the "goodness of fit" of a statistical model, the mean square error value, or MSE, can be of use. The F-statistic used in an *F*-test is calculated as:

**Equation 5**

$$F = \frac{MSR}{MSE}$$

where MSR is the regression mean square. So as the MSE (the variance unexplained by the model) increases, the F-statistic decreases, making it more difficult for a significant determination to be made. If the MSE is made smaller by another model (in this case, switching from an ANOVA to an ANCOVA), the F-statistic will increase, making the calculated p-value smaller and increasing the likelihood of rejecting the null hypothesis. Based on this, if a regression equation evaluated by the ANCOVA is a poor fit of the data, it will be more difficult for the treatments to be classified as significantly different.

An ANCOVA can be performed using an equal slopes model or, in the case of unequal slopes among treatments, by regressing each treatment individually and then comparing the results for each treatment at different levels of the covariate. Each analysis constituent was tested for equal slopes of the regression between treatment and covariate. If an equal slopes model was not rejected, treatment means were compared at the average level of the covariate. For those that did not display equal slopes, the individual treatment regressions were compared at each level of the covariate, or at each CN, for a more complete description of the results. Effects for sludge and horse manure were analyzed separately. Analysis was performed using the PROC MIXED procedure in SAS 9.4 (Pennsylvania State University Department of Statistics, 2015) with $\alpha = 0.05$.

# CHAPTER 3: RESULTS AND DISCUSSION

## 3.1 BIOCHAR CHARACTERIZATION

The average results for the three sub-samples of yellow pine biochar used in the study and analyzed by UKRS are shown in Table 3. A report provided by the manufacturer characterizing a single sample is also provided in Appendix C. The average pH as determined in this study was 9.9, somewhat higher than results for pine chip biochar reported by Gaskin et al. (2008), perhaps due to their use of a pyrolysis temperature of 500°C. The average C content of this biochar is approximately the same as results from Rajkovich et al. (2011), while the N content is slightly higher (Gaskin et al., 2008; Rajkovich et al., 2011), resulting in a smaller C:N ratio.

## 3.2 CURVE NUMBER VARIATION

Using Equations 3 and 4, CNs were calculated for each plot (Table 6) based on R and Q values that can be found in Appendix A. When the calculated CNs are compared to the CNs found in the earlier studies (Table 4), there are noticeable differences. The original average CN of 63 ± 8 decreased to 42 ± 23, a large change accompanied by greater variability. This finding is possibly due to bioturbation, or changes in soil hydraulic properties due to plant and animal activity. Prior to the study, there was evidence of digging and burrowing activity by some type of rodents in the form of multiple large holes. None of these holes was found on or around any of the plots that were used in this study. However, it is still possible that moles or other animals have reworked the subsurface of the plots by creating macropores, increasing infiltration rate and water storage (Leonard et al., 2004; Ursic and Esher, 1988).

Due to the variability of the CNs and its effect on the dependent variable, the runoff concentrations of TKN, $NH_3$-N, $NO_3$-N, TP, DP, TSS, COD, and FC were compared statistically across treatments using an ANCOVA with CN as the covariate. When using an ANCOVA, it is beneficial to plot the covariate versus the outcome, or dependent variable, for each treatment group to evaluate the nature of the relationship(s). Dependent variables, or runoff concentrations, that are affected by the covariate will have a linear relationship. An ANCOVA is appropriate if at least one dependent variable displays this linear relationship with the covariate. Figure 4, which is presented as an example, demonstrates that higher CN values are linearly related to concentration of $NH_3$-N in the runoff for each treatment except C. In this example, the line slopes relating runoff $NH_3$-N concentration to CN were significant ($p < 0.05$) and nearly identical for each treatment except the control.

**Table 6. Calculated curve number (CN) values for the present study and previous studies.**

| Plot | Treatment | Present CN | Previous CN[1] |
|---|---|---|---|
| B7 | C | 36 | 69 |
| C1 | C | 76 | 68 |
| D1 | C | 79 | 70 |
| Mean | | 64 ± 24[2] | 69 ± 1 |
| B1 | MB | 25 | 67 |
| B2 | MB | 71 | 77 |
| D9 | MB | 27 | 60 |
| Mean | | 41 ± 26 | 68 ± 9 |
| B3 | M | 45 | 53 |
| D4 | M | 13 | 60 |
| D5 | M | 40 | 60 |
| Mean | | 33 ± 17 | 58 ± 4 |
| C2 | SB | 75 | 65 |
| C8 | SB | 28 | 53 |
| D6 | SB | 23 | 50 |
| Mean | | 42 ± 29 | 56 ± 8 |
| C3 | S | 28 | 59 |
| D2 | S | 50 | 68 |
| D7 | S | 16 | 49 |
| Mean | | 31 ± 17 | 56 ± 9 |
| **Overall Mean** | | 42 ± 23 | 63 ± 8 |

[1]Mean CN based on previous studies
[2]Standard Deviation

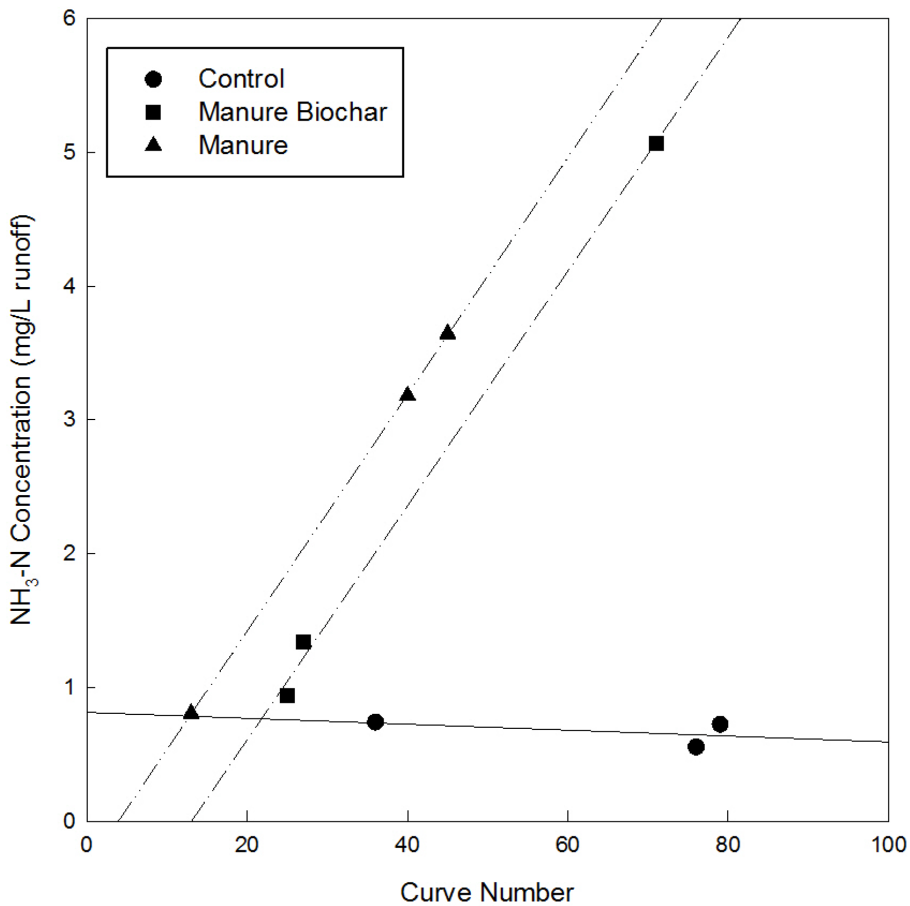


**Figure 4. Runoff $NH_3$-N concentrations as a function of treatment and covariate, curve number (CN).**

## 3.3 MUNICIPAL SLUDGE TREATMENTS

Mean runoff concentrations for municipal sludge treatments are shown in Table 7. As indicated, COD, $NO_3$-N, DP, and TP show little variation between treatments. This is not the case for FC and TSS. It appears that FC concentrations are decreased for the SB treatment; however, this is an artificial decrease due to one extreme value within the S treatment that raised the average significantly. Biochar addition seemed to increase TSS in runoff; based on average concentrations, TSS was greater in runoff from the SB treatment than from S.

At first glance, variation within treatments is large (relatively large standard deviation compared to the mean), which is in part due to the variation in CNs between the plots. When a one-way ANOVA was performed, disregarding CN, there was no significant difference between any treatment for any measured parameter. This finding is highly contradictory to published results (Bushee et al., 1998; Chen et al., 2011b; Edwards et al., 1999). Pote et al. (2001) found that areas of high infiltration will show decreased runoff concentrations of solutes. This, coupled with the high variation in CN value in this study, suggested the appropriateness of considering CN as a covariate and using ANCOVA as the statistical framework.

While outside the scope of this study, transport of solutes through the soil due to high infiltration rates is worth considering. For soil surfaces that infiltrate a large proportion of rainfall, any pollutants on land can be infiltrated with the rainfall into the soil (Rittenburg et al., 2015). These contaminants then have the potential to be transported through the soil to the water table and cause groundwater pollution (Walter et al., 1979).

**Table 7. Mean runoff concentrations of analytes for plots treated with municipal sludge.**

| Analyte | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | C | | SB | | S | |
| COD (g/L) | 2.6[1] ± | 0.2[2] | 2.6 ± | 0.2 | 2.5 ± | 0.2 |
| FC (MPN/100mL) | 5.5 ± | 7.9 | 26 ± | 37 | 150 ± | 247 |
| $NH_3$-N (mg/L) | 0.7 ± | 0.1 | 3.0 ± | 3.0 | 2.8 ± | 2.2 |
| $NO_3$-N (mg/L) | 0.6 ± | 0.4 | 0.4 ± | 0.6 | 0.6 ± | 0.4 |
| DP (mg/L) | 0.9 ± | 0.6 | 0.9 ± | 0.4 | 0.9 ± | 0.5 |
| TP (mg/L) | 0.7 ± | 0.1 | 0.8 ± | 0.2 | 0.8 ± | 0.3 |
| TKN (mg/L) | 2.4 ± | 1.3 | 5.0 ± | 4.7 | 4.9 ± | 3.8 |
| TSS (mg/L) | 29 ± | 4.6 | 145 ± | 152 | 88 ± | 38 |

[1]Mean of three samples
[2]Standard deviation of three samples

Further study into the relationship between infiltration rate and groundwater pollution, specifically to determine whether land applied amendments such as biochar could effect pollutant concentrations, is worth investigating.

The ANCOVA procedure produces regression equations for each treatment for each significant constituent. The values in Table 8 are estimated concentrations for each parameter, based on the ANCOVA regression equation, if it were to be analyzed in runoff from a plot at the given CN and treatment. The regression equations from the ANCOVA are listed in Table 9. There was no statistical difference ($p < 0.05$) between any treatment for COD, FC, $NO_3$-N, DP, or TP for municipal sludge, so they are not included in Table 8 or Table 9. This lack of significance indicates that addition of sludge did not increase concentrations of these parameters in the runoff. Bushee et al. (1998) found similar results for DP, in that municipal sludge addition did not increase DP concentrations in water. However, the same study found that COD and FC increased after sludge addition. The average FC concentration in Bushee et al. (1998) for municipal sludge was 650 mg/L, which is a much larger concentration than the average 150 MPN/100mL concentration of this study. This could indicate that the amount of FC in the sludge used in this study was much less than that in Bushee et al. (1998), which could explain the lack of significance of FC in runoff.

Results for TP and DP in this study were contrary to many other studies, where P was found to act similarly to N – it did not leach from soils into runoff after biochar addition (Laird *et al.*, 2010; Beck *et al.*, 2011; Reddy *et al.*, 2014; Schnell *et al.*, 2012; Zhai *et al.*, 2015). There are a few possible explanations for this. First, the amount of P in the sludge may not have been at high enough concentrations to appear in the runoff.

**Table 8. Estimated analyte concentrations for municipal sludge treatments (C – control, SB – sludge and biochar, S – sludge) at given curve number (CN) values based on ANCOVA regression.**

| Analyte | CN | Treatment | | |
|---|---|---|---|---|
| | | C | SB | S |
| $NH_3$-N (mg/L) | 16 | 0.78[a] | 0.27[a] | 0.76[a] |
| | 23 | 0.77[a] | 0.99[a] | 1.67[a] |
| | 28 | 0.75[b] | 1.51[b] | 2.32[a] |
| | 36 | 0.74[c] | 2.34[b] | 3.35[a] |
| | 50 | 0.71[c] | 3.79[b] | 5.16[a] |
| | 75 | 0.65[c] | 6.38[b] | 8.40[a] |
| | 76 | 0.65[c] | 6.48[b] | 8.53[a] |
| | 79 | 0.64[c] | 6.79[b] | 8.92[a] |
| TKN (mg/L) | 16 | 4.89[b] | 0.72[a] | 1.55[a] |
| | 23 | 4.51[a] | 1.87[b] | 3.07[a] |
| | 28 | 4.25[a] | 2.68[ab] | 4.16[a] |
| | 36 | 3.82[b] | 3.99[b] | 5.90[a] |
| | 50 | 3.08[c] | 6.29[b] | 8.94[a] |
| | 75 | 1.74[c] | 10.38[b] | 14.38[a] |
| | 76 | 1.69[c] | 10.55[b] | 14.60[a] |
| | 79 | 1.53[c] | 11.04[b] | 15.25[a] |
| TSS (mg/L) | 16 | 29.8[a] | 7.25[a] | 65.2[a] |
| | 23 | 29.6[a] | 44.3[a] | 75.8[a] |
| | 28 | 29.5[a] | 70.8[a] | 83.4[a] |
| | 36 | 29.3[a] | 113[a] | 95.5[a] |
| | 50 | 29.0[b] | 187[a] | 117[ab] |
| | 75 | 28.4[b] | 320[a] | 155[ab] |
| | 76 | 28.3[b] | 325[a] | 156[ab] |
| | 79 | 28.3[b] | 341[a] | 161[ab] |

*Within row values with the same superscript are not significantly different.

**Table 9. ANCOVA regression equations for each sludge treatment (C – control, SB – sludge and biochar, S – sludge) for each significant analyte.**

| Treatment | Analyte | | |
|---|---|---|---|
| | $NH_3$-N | TKN | TSS |
| C | -0.00*CN+0.73<br>$R^2$ = 0.20 | -0.05*CN+5.66<br>$R^2$ = 0.99 | -0.02*CN+30.16<br>$R^2$ = 0.01 |
| SB | 0.10*CN-1.39<br>$R^2$ = 0.99 | 0.16*CN-1.90<br>$R^2$ = 0.99 | 5.30*CN-77.52<br>$R^2$ = 0.99 |
| S | 0.13*CN-1.31<br>$R^2$ = 0.99 | 0.22*CN-1.93<br>$R^2$ = 0.99 | 1.52*CN+40.99<br>$R^2$ = 0.47 |

This is similar to two other studies also using municipal sludge from Lexington, KY. Neither study displayed an increase of P in runoff after sludge application (Bushee et al., 1998; Edwards et al., 1999). Another study using municipal sludge also found that TP was not affected by sludge application (McLeod and Hegg, 1984). The rate of TP applied by McLeod and Hegg (1984) was 67 kg/ha, even more than the 28 kg/ha of TP in this study, and still yielded no significant increase of P in runoff. Also, P sorbs easily to soil particles (Sharpley, 1995) and could have been retained in the soil before it could be transported to the runoff. It is also possible that due to the high intensity of the rainfall, P in the runoff was diluted (Edwards and Daniel, 1993; Fraser et al., 1999).

Nitrate was also at extremely low concentrations in the sludge, the highest being 1.8 mg/kg dry weight. These nutrient levels would contribute little to the runoff, explaining why there was no significant increase after sludge addition. This does not mean, however, that $NO_3$-N and P leaching are not a concern for sludge. Nitrate moves freely through the majority of soils and is the main form of N leachate found in groundwater (Follet, 1995). Soil and P have a high binding energy, meaning P adsorbs to soil particles readily (Sharpley, 1995). As P builds up in the soil, the potential for loss of P through runoff increases (Sharpley, 1995). So while P concentrations in runoff may not be immediately affected by sludge application, build up of P over time could still cause problems downstream in the form of excess P.

### 3.3.1 Effects of Curve Number

For $NH_3$-N, TKN, and TSS concentration, there is a greater potential for treatment effect at higher CN values. This is especially true for TSS, where only CNs above the average displayed any significant difference ($p < 0.05$) between treatments, and only then between the C and the SB treatment. There was no difference between the S and SB or the C and S treatments. Concentrations of TKN and $NH_3$-N were different between all treatments at average CNs and above. At lower CN values, treatment effects were less evident. This coincides with the study performed by Pote et al. in 2001, in which higher infiltration rates (lower CN) corresponded with lower runoff concentrations after manure was added. It appears that with high infiltration rates, solutes are transported into the soils to such a degree that the solutes are less available to be transported through runoff (Pote et al., 2001).

### 3.3.2 Effects of Biochar

While TSS was significantly different ($p < 0.05$) for treatments at high CNs, there was no effect found for biochar addition. At higher CNs, the biochar treatment actually showed a greater concentration of TSS in runoff as estimated by the ANCOVA regressions (Table 9). It is possible that the biochar actually contributed to TSS due to its loose particulate form and higher ash content (due to high pyrolysis temperature). For $NH_3$-N and TKN, water quality after biochar amendments was found to be significantly different ($p < 0.05$) than that of pure sludge at all but the lowest CNs. This indicates that adding biochar to sludge could decrease the runoff concentrations of $NH_3$-N and TKN, especially in more developed areas where CNs will be higher.

Many studies have found that biochar will either increase retention of N in the soil (Schnell *et al.*, 2012; Laird *et al.*, 2010) or decrease N in the runoff (Beck *et al.*, 2011; Reddy *et al.*, 2014). As the majority of N in the sludge was $NH_3$-N and TKN, it follows that these two constituents would be found at high concentrations in the runoff for sludge treatments and that those concentrations would be strongly affected by the presence of biochar.

### 3.3.3 Summary of Municipal Sludge Treatments

Municipal sludge treatments displayed no difference due to treatment or CN for COD, FC, $NO_3$-N, DP or TP. Mean concentrations for each, averaged over all treatments and replications, were 2.5 ± 0.2 g/L, 61 ± 142 MPN/100 mL, 0.53 ± 0.4 mg/L, 0.89 ± 0.4 mg/L, and 0.73 ± 0.2 mg/L, respectively. The CN had a significant effect on $NH_3$-N, TKN, and TSS concentrations. Plots with a low CN (and high infiltration rate) demonstrated no difference in runoff concentration for any treatment. As CN increased, significant treatment effects began to appear. This correlation between infiltration rate and runoff quality is similar to results published by Pote et al. (2001). Biochar addition to sludge resulted in lower runoff concentrations for $NH_3$-N and TKN at high CNs.

## 3.4 HORSE MANURE TREATMENTS

Flow-weighted mean concentrations of analysis parameters from runoff of manure treated plots are shown in Table 10. Similar to the results for municipal sludge, there is little variation in COD, $NO_3$-N, or DP between treatments, and there is large variation within treatments. Due to the variability of CN and infiltration rate between the plots (Pote *et al.*, 2001), the averages of runoff concentrations for each treatment do not fully describe the data. For this reason, the one-way ANOVA performed for manure treatments yielded the same results as those of municipal sludge treatments – no difference between any treatment for any measured parameter. After CN was used as a covariate for the data analysis (as discussed previously for sludge), the complication of infiltration rate variability was effectively removed so that treatment effects could be compared more accurately.

Much like the sludge, P only made up 1-3% of the manure when analyzed, which helps explain why DP was approximately the same for C, M, and MB treatments. Manure P is a combination of organic matter and DP (NRCS, 2007). The main source of P in this particular manure sample seems to have been organic matter to account for the significance of TP and not DP. The N in manure is also primarily organic compounds and ammonium, with little $NO_3$-N (NRCS, 2007). The amount of $NO_3$-N in this manure seems to have been insufficient to affect the runoff. As discussed previously, P and N can still be concerns for soil and groundwater quality (Follett, 1995; Sharpley, 1995) even when they do not have an effect on runoff quality.

**Table 10. Mean runoff concentrations of measured analytes for plots treated with horse manure (C – control, MB – manure and biochar, M – manure).**

| Analyte | Treatment | | |
|---|---|---|---|
| | C | MB | M |
| COD (g/L) | 2.6[1] ± 0.2[2] | 2.4 ± 0.3 | 2.5 ± 0.2 |
| FC (MPN/100mL) | 5.5 ± 7.9 | 168 ± 234 | 291 ± 249 |
| $NH_3$-N (mg/L) | 0.7 ± 0.1 | 2.5 ± 2.3 | 2.5 ± 1.5 |
| $NO_3^-$-N (mg/L) | 0.6 ± 0.4 | 0.8 ± 0.05 | 0.6 ± 0.4 |
| DP (mg/L) | 0.9 ± 0.6 | 1.9 ± 1.3 | 2.2 ± 1.1 |
| TP (mg/L) | 0.7 ± 0.1 | 2.2 ± 1.9 | 2.0 ± 1.3 |
| TKN (mg/L) | 2.3 ± 1.3 | 7.3 ± 6.6 | 5.8 ± 4.4 |
| TSS (mg/L) | 29 ± 4.6 | 83 ± 71 | 135 ± 99 |

[1]Mean of three samples
[2]Standard deviation of three samples

Regression equations produced by the ANCOVA procedure are shown in Table 11. These equations were used to generate the estimated values shown in Table 12 to display differences ($p < 0.05$) between treatments. There was no difference between any treatment for COD, $NO_3$-N, or DP; they are not included in Tables 11 or 12 for this reason.

### 3.4.1 Effects of Curve Number

Similar to results for municipal sludge, plots with low CN values demonstrated no significant difference ($p < 0.05$) in runoff quality between treatments for FC, TP, and TSS (Table 12, full statistical analysis shown in Appendix D). At low CN values, there was no difference between even the control and the pure manure application for these parameters. At these low CN values, runoff is dominated by the hydrologic properties of the soil, such as the soil texture, soil moisture, and infiltration rate. Such high levels of infiltration result in little runoff that has been greatly diluted by the amount of rainfall necessary to produce it.

Runoff concentrations of $NH_3$-N demonstrated a different reaction to CN than that displayed by the other constituents. There was a significant treatment effect at low CNs, however, at high CNs, the significance of treatment was no longer present. It is unclear why biochar lost its effect on $NH_3$-N at these CNs. It is possible that when runoff is produced quickly, as with high CNs, $NH_3$-N is not able to sorb to biochar before being washed away. The CN effect showed a stronger influence on TP concentrations than the other parameters – no treatment effects occurred until the CN reached slightly higher values than those of FC and TSS, although these were still below the average value of CN (42).

**Table 11. ANCOVA regression equations for significant analytes from manure treatments (C – control, MB – manure and biochar, M – manure).**

| Treatment | Analyte | | |
|---|---|---|---|
| | C | MB | M |
| $NH_3$-N | -.00*CN+0.73<br>$R^2 = 0.20$ | 0.09*CN-1.22<br>$R^2 = 0.99$ | 0.08*CN-0.34<br>$R^2 = 0.99$ |
| TKN | -0.05*CN+5.66<br>$R^2 = 0.99$ | 0.25*CN-2.8<br>$R^2 = 0.96$ | 0.24*CN-2.0<br>$R^2 = 0.84$ |
| TSS | -0.02*CN+30<br>$R^2 = 0.01$ | 2.72*CN-29<br>$R^2 = 0.99$ | 5.74*CN-52<br>$R^2 = 0.99$ |
| TP | 0.00*CN+0.68<br>$R^2 = 0.00$ | 0.08*CN-0.87<br>$R^2 = 0.99$ | 0.07*CN-0.39<br>$R^2 = 0.98$ |
| FC | 0.18*CN-5.98<br>$R^2 = 0.30$ | 14.33*CN-177<br>$R^2 = 0.98$ | 8.94*CN-198<br>$R^2 = 0.99$ |

**Table 12. Estimated analyte concentrations at given curve number (CN) values for each horse manure treatment (C – control, MB – manure and biochar, M – manure) using ANCOVA regression equations (all negative values reported as zero).**

| Analyte | CN | Treatment | | |
|---|---|---|---|---|
| | | C | MB | M |
| FC (MPN/100 mL) | 13 | 0.00[a] | 0.00[a] | 9.17[a] |
| | 25 | 0.00[ab] | 25.0[b] | 181[a] |
| | 27 | 0.00[b] | 42.9[b] | 210[a] |
| | 36 | 0.53[b] | 123[b] | 339[a] |
| | 40 | 1.25[b] | 159[b] | 396[a] |
| | 45 | 2.16[c] | 204[b] | 468[a] |
| | 71 | 6.86[c] | 436[b] | 841[a] |
| | 76 | 7.76[c] | 481[b] | 912[a] |
| | 79 | 8.31[c] | 508[b] | 956[a] |
| $NH_3$-N (mg/L) | 13 | 0.72[a] | 0.00[b] | 0.80[a] |
| | 25 | 0.71[b] | 1.01[b] | 1.86[a] |
| | 27 | 0.71[b] | 1.19[b] | 2.03[a] |
| | 36 | 0.70[c] | 1.99[b] | 2.83[a] |
| | 40 | 0.69[c] | 2.34[b] | 3.18[a] |
| | 45 | 0.69[c] | 2.79[b] | 3.62[a] |
| | 71 | 0.66[b] | 5.10[a] | 5.90[a] |
| | 76 | 0.65[b] | 5.55[a] | 6.34[a] |
| | 79 | 0.65[b] | 5.82[a] | 6.61[a] |
| TP (mg/L) | 13 | 0.69[a] | 0.11[a] | 0.57[a] |
| | 25 | 0.69[a] | 1.02[a] | 1.46[a] |
| | 27 | 0.69[a] | 1.17[a] | 1.61[a] |
| | 36 | 0.69[b] | 1.85[a] | 2.28[a] |
| | 40 | 0.69[b] | 2.16[a] | 2.58[a] |
| | 45 | 0.70[b] | 2.54[a] | 2.95[a] |
| | 71 | 0.70[b] | 4.51[a] | 4.88[a] |
| | 76 | 0.70[b] | 4.88[a] | 5.25[a] |
| | 79 | 0.70[b] | 5.11[a] | 5.48[a] |
| TKN (mg/L) | 13 | 4.99[a] | 0.42[a] | 1.10[a] |
| | 25 | 4.37[a] | 3.38[a] | 3.95[a] |
| | 27 | 4.26[a] | 3.87[a] | 4.42[a] |
| | 36 | 3.80[a] | 6.10[a] | 6.56[a] |
| | 40 | 3.59[a] | 7.08[a] | 7.51[a] |
| | 45 | 3.33[a] | 8.32[a] | 8.69[a] |
| | 71 | 1.99[a] | 14.7[b] | 14.9[ab] |

**Table 12. continued**

| Analyte | CN | Treatment | | |
|---|---|---|---|---|
| | | C | MB | M |
| | 76 | 1.73$^{a}$ | 16.0$^{b}$ | 16.0$^{ab}$ |
| | 79 | 1.57$^{a}$ | 16.7$^{b}$ | 16.8$^{ab}$ |
| TSS (mg/L) | 13 | 29.9$^{a}$ | 6.43$^{a}$ | 22.5$^{a}$ |
| | 25 | 29.6$^{b}$ | 39.1$^{b}$ | 91.3$^{a}$ |
| | 27 | 29.5$^{b}$ | 44.5$^{b}$ | 103$^{a}$ |
| | 36 | 29.3$^{c}$ | 69.0$^{b}$ | 154$^{a}$ |
| | 40 | 29.2$^{c}$ | 79.9$^{b}$ | 177$^{a}$ |
| | 45 | 29.1$^{c}$ | 93.5$^{b}$ | 206$^{a}$ |
| | 71 | 28.5$^{c}$ | 164$^{b}$ | 355$^{a}$ |
| | 76 | 28.4$^{c}$ | 178$^{b}$ | 384$^{a}$ |
| | 79 | 28.3$^{c}$ | 186$^{b}$ | 401$^{a}$ |

*Values within a single row that share the same superscript are not significantly different.

### 3.4.2 Effects of Biochar

There was no significant effect ($p < 0.05$) of biochar addition to manure for TP or TKN (or COD, $NO_3$-N, and DP, which showed no difference between any treatment). Concentrations of TP were only different between the C and M, and C and MB treatments; M and MB were not significantly different. Application of manure increased the TP in the runoff (but not DP, possibly due to the percentage of organic matter in the TP measurement); however, adding biochar to the manure did not reduce the TP concentrations. This is contrary to results from Laird et al. (2010) and Zhai et al. (2015), where soil retention of P was increased, leading to conclusions that P would not run off as much, and also with Beck et al. (2011), who found that biochar reduced TP in runoff.

Concentrations of TKN actually increased when biochar was added to plots with the highest CNs; there was no difference between C and M treatments, but there is a significant difference between C and MB. Interestingly, the biochar effect ($p < 0.05$) for $NH_3$-N that occurred at the majority of CNs was no longer effective at the same three values (CN = 71, 76, 79). As TKN is composed of organic N and $NH_3$-N, these results are most likely related. For each remaining level of the covariate, biochar addition decreased the $NH_3$-N concentration in the runoff; TKN was unaffected. The effect of biochar on these two forms of N in runoff is supported by Beck et al. (2011), Laird et al. (2010), Reddy et al. (2014), and Schnell et al. (2012). In these cases, either N had better soil retention after biochar was added or runoff concentrations of N were reduced.

For FC and TSS there was a biochar effect at all but the lowest CN (once again, the outlying CN), and also a significant difference ($p < 0.05$) between the control and pure manure treatment. This indicates that adding biochar to the manure before application can significantly decrease concentrations of FC and TSS in runoff. Mohanty et al. (2014)

reported similar results for bacteria. Few studies have investigated biochar's effect on TSS, but Schnell et al. (2012) found that biochar had a significant effect in reducing TSS by an average of 86%.

### 3.4.3 Summary of Horse Manure Treatments

Horse manure treatments displayed a significant effect due to CN for FC, $NH_3$-N, TP, TKN, and TSS. Plots with lower CNs had no treatment effects due to the high infiltration rate of those soils. A high capacity for the soil to accept water may lead to an increase of filtering and dilution of runoff which, in turn, could impact runoff quality through decreased analyte concentrations. High infiltration rates have been correlated to lower solute concentrations in runoff (Pote *et al.*, 2001).

Concentrations of COD, $NO_3$-N, and DP were unaffected by CN or biochar. Mean concentrations averaged over all treatments and replications were $2.5 \pm 0.2$ g/L, $0.66 \pm 0.3$ mg/kg, and $1.7 \pm 1.1$ mg/kg for each parameter, respectively. Biochar addition to horse manure significantly affected FC, $NH_3$-N, and TSS by decreasing the runoff concentrations compared to those of runoff from plots treated with manure only. This indicates that biochar could reduce the influence of these constituents in runoff from agricultural application or disposal of horse manure.

# CHAPTER 4: CONCLUSIONS

This experiment was conducted to characterize nutrient concentrations of TKN, $NH_3$-N, $NO_3$-N, TP, and DP, as well as FC, COD, and TSS in runoff from plots treated with horse manure and municipal sludge, and to evaluate the effects of biochar amendment to manure and sludge on runoff quality. Three plots each were treated with manure only, sludge only, manure with biochar, and sludge with biochar, with three control plots. Runoff was produced from rainfall simulators at an intensity of 101.6 mm/h (or 134.6 mm/h if no runoff was produced within 1 h) until continuous runoff occurred for 30 minutes, during which time frame samples were collected. Flow-weighted composites were prepared from collected samples and analyzed for TKN, $NH_3$-N, $NO_3$-N, TP, DP, FC, COD, and TSS.

Application of horse manure and sludge did not affect COD, $NO_3$-N, or DP in runoff; sludge also had no effect on runoff concentrations of FC and TP. Runoff concentrations for these constituents from manure and sludge treatments were not significantly different ($p < 0.05$) than the control concentrations. Biochar amendments reduced $NH_3$-N and TKN in runoff from sludge treatments, and increased TSS in runoff at high CNs. Biochar reduced $NH_3$-N, FC, and TSS for manure treatments. Biochar appeared to have no effect on P or $NO_3$-N, most likely due to the small presence of both in the manure and sludge and possible dilution of runoff due to high intensity rainfall. Biochar amendments to manure and sludge could reduce the presence of N and FC in runoff from agricultural fields, storage locations, and landfills.

# CHAPTER 5: FUTURE WORK

## 5.1 REEVALUATION OF EXPERIMENT

This experiment attempted to evaluate the effect of biochar addition to manure and sludge on runoff quality; throughout the process, however, some issues arose that could have affected the final results. Bacterial analysis of the sludge and horse manure prior to application would provide more clarity about the FC results in the runoff. It is unclear why municipal sludge did not contribute a significant amount of FC to the runoff while horse manure did. A more thorough characterization of the two materials could have delivered more explanation.

The variation in infiltration rates and CNs between the plots used made it difficult to analyze results within treatments. Preliminary rainfall-runoff tests performed on the plots in question might enable blocking of treatments before experimental analysis and potentially reduce the variation of runoff concentrations within each treatment. If this is not possible, or high variation of results still occurs, a greater number of replications for each treatment would increase the accuracy of the ANCOVA regressions. It is also possible that the subsurface of the rainfall plots at Maine Chance Research Farm is being altered in some way, potentially via bioturbation (plant and animal effects on soil), which would account for the difference in CN from this experiment and previous studies performed in the same location. It may be necessary to evaluate the effects of this on the rainfall-runoff relationship of the plots.

## 5.2 OTHER EXPERIMENTS

Biochar addition to municipal sludge appeared to have a more significant effect on N than it did when mixed with horse manure. This could be due to the higher application rate of biochar for sludge compared to manure. However, it could also be due to the higher N content in sludge (5.1 ± 0.3 %) than in the manure (0.89 ± 0.14 %). Further investigation into how N content of the applied material can affect N removal from runoff due to biochar addition could establish a relationship between N content and biochar effectiveness.

According to Town Branch Wastewater Treatment Plant technicians, one of the primary reasons municipal sludge in Lexington, KY isn't used as a fertilizer is the heavy metal content. Evaluating the effect of biochar on heavy metal concentrations in runoff, particularly when added to sludge containing large amounts of metals, may produce results that could reduce the amount of sludge being transported to landfills. Land application of municipal sludge would be a more efficient way of disposal than depositing it into landfills where it serves no useful purpose.

Due to the wide range of biochar products available (based on pyrolysis temperature and feedstock), choosing which kind of biochar to use can be an issue. Many studies have already been published characterizing biochars produced from different feedstocks and at different temperatures, as well as their potential environmental impacts. Continuing study into how these different types of biochar could affect runoff and soil quality would help consumers more efficiently determine which product would be most effective for different scenarios.

# APPENDIX A: RAW DATA

| | | | | |
|---|---|---|---|---|
| **Date** | 10/8/15 | | | |
| **Intensity:** | 4 in/hr | for | 56 | min |
| | 5.33 in/hr | for | 11.2 | min |
| **Simulator:** | 2 | | | |
| **Plot:** | B1 | | | |
| **Technician:** | Edwards | | | |
| **Comp. Vol:** | 1000 mL | | | |
| **Runoff Time:** | 67 min | 13 sec | | |

| Time | Time to Collect sec | Sample + Tare g | Tare g | Sample g | Flow Rate mL/sec | Increm. Volume mL | Composite Volume |
|---|---|---|---|---|---|---|---|
| 2 | 28.41 | 563.5 | 93.1 | 470.4 | 16.56 | 0.99 | 46 |
| 4 | 36.75 | 564.7 | 93.1 | 471.6 | 12.83 | 1.76 | 82 |
| 8 | 49.65 | 707.5 | 93.1 | 614.4 | 12.37 | 3.02 | 141 |
| 14 | 45.12 | 620.3 | 93.1 | 527.2 | 11.68 | 4.33 | 201 |
| 22 | 45.03 | 679.3 | 93.1 | 586.2 | 13.02 | 5.93 | 276 |
| 30 | 45.12 | 532.4 | 93.1 | 439.3 | 9.74 | 5.46 | 254 |
| | | | | | Sum: | 21.50 | 1000 |

| | | |
|---|---|---|
| Pre-Q Rain: | 4.73 | inches |
| Total Rain: | 7.39 | inches |
| Runoff | 0.06 | inches |
| Ratio: | 0.77 | % |

| | | |
|---|---|---|
| **Date** | 10/8/15 | |
| **Intensity:** | 4 in/hr | |
| **Simulator:** | 3 | |
| **Plot:** | B2 | |
| **Technician:** | Edwards/Williams | |
| | | |
| **Comp. Vol:** | 1000 mL | |
| | | |
| **Runoff Time:** | 12 min | 20 sec |

| Time | Time to Collect sec | Sample + Tare g | Tare g | Sample g | Flow Rate mL/sec | Increm. Volume mL | Composite Volume |
|---|---|---|---|---|---|---|---|
| 2 | 31.37 | 642.5 | 93.1 | 549.4 | 17.51 | 1.05 | 4 |
| 4 | 17.16 | 1002.7 | 93.1 | 909.6 | 53.01 | 4.23 | 16 |
| 8 | 4.94 | 822.9 | 93.1 | 729.8 | 147.73 | 24.09 | 94 |
| 14 | 4.72 | 914.6 | 93.1 | 821.5 | 174.05 | 57.92 | 225 |
| 22 | 4.72 | 931.5 | 93.1 | 838.4 | 177.63 | 84.40 | 328 |
| 30 | 4.5 | 899.5 | 93.1 | 806.4 | 179.20 | 85.64 | 333 |
| | | | | | Sum: | 257.33 | 1000 |

| | | |
|---|---|---|
| Pre-Q Rain: | 0.82 | inches |
| Total Rain: | 2.82 | inches |
| Runoff | 0.68 | inches |
| Ratio: | 24.15 | % |

| | | |
|---|---|---|
| **Date** | 10/8/15 | |
| **Intensity:** | 4 in/hr | |
| **Simulator:** | 3 | |
| **Plot:** | B3 | |
| **Technician:** | Edwards | |
| | | |
| **Comp. Vol:** | 1000 mL | |
| | | |
| **Runoff Time:** | 20 min | 30 sec |

| Time | Time to Collect sec | Sample + Tare g | Tare g | Sample g | Flow Rate mL/sec | Increm. Volume mL | Composite Volume |
|---|---|---|---|---|---|---|---|
| 2 | 45.12 | 632.4 | 93.1 | 539.3 | 11.95 | 0.72 | 27 |
| 4 | 45.06 | 740.3 | 93.1 | 647.2 | 14.36 | 1.58 | 60 |
| 8 | 44.9 | 800.1 | 93.1 | 707 | 15.75 | 3.61 | 138 |
| 14 | 44.78 | 814 | 93.1 | 720.9 | 16.10 | 5.73 | 219 |
| 22 | 45.31 | 765.5 | 93.1 | 672.4 | 14.84 | 7.43 | 284 |
| 30 | 46.69 | 774.7 | 93.1 | 681.6 | 14.60 | 7.07 | 270 |
| | | | | | Sum: | 26.13 | 1000 |

| | | |
|---|---|---|
| Pre-Q Rain: | 1.37 | inches |
| Total Rain: | 3.37 | inches |
| Runoff | 0.07 | inches |
| Ratio: | 2.06 | % |

| | | | |
|---|---|---|---|
| **Date** | 9/23/15 | | |
| **Intensity:** | 4 in/hr | | |
| **Simulator:** | 3 | | |
| **Plot:** | B7 | | |
| **Technician:** | Edwards | | |
| **Comp. Vol:** | 1000 mL | | |
| **Runoff Time:** | 44 min | 42 sec | |

| Time | Time to Collect sec | Sample + Tare g | Tare g | Sample g | Flow Rate mL/sec | Increm. Volume mL | Composite Volume |
|---|---|---|---|---|---|---|---|
| 2 | 29.99 | 617.66 | 93.1 | 524.56 | 17.49 | 1.05 | 25 |
| 4 | 35.71 | 773.15 | 93.1 | 680.05 | 19.04 | 2.19 | 52 |
| 8 | 41.14 | 793.94 | 93.1 | 700.84 | 17.04 | 4.33 | 102 |
| 14 | 27.61 | 1014.35 | 93.1 | 921.25 | 33.37 | 9.07 | 213 |
| 22 | 32.65 | 851.7 | 93.1 | 758.6 | 23.23 | 13.58 | 320 |
| 30 | 30.31 | 939.57 | 93.1 | 846.47 | 27.93 | 12.28 | 289 |
| | | | | | Sum: | 42.51 | 1000 |

| | | |
|---|---|---|
| Pre-Q Rain: | 2.98 | inches |
| Total Rain: | 4.98 | inches |
| Runoff | 0.11 | inches |
| Ratio: | 2.26 | % |

| | | |
|---|---|---|
| **Date** | 10/8/15 | |
| **Intensity:** | 4 in/hr | |
| **Simulator:** | 2 | |
| **Plot:** | C1 | |
| **Technician:** | Edwards | |
| **Comp. Vol:** | 1000 mL | |
| **Runoff Time:** | 10 min | 10 sec |

| Time | Time to Collect sec | Sample + Tare g | Tare g | Sample g | Flow Rate mL/sec | Increm. Volume mL | Composite Volume |
|---|---|---|---|---|---|---|---|
| 2 | 9.46 | 1076.6 | 93.1 | 983.5 | 103.96 | 6.24 | 21 |
| 4 | 5.44 | 874.22 | 93.1 | 781.12 | 143.59 | 14.85 | 49 |
| 8 | 5.03 | 1016.6 | 93.1 | 923.5 | 183.60 | 39.26 | 131 |
| 14 | 4.59 | 967.2 | 93.1 | 874.1 | 190.44 | 67.33 | 224 |
| 22 | 4.66 | 913.5 | 93.1 | 820.4 | 176.05 | 87.96 | 293 |
| 30 | 5.15 | 998.8 | 93.1 | 905.7 | 175.86 | 84.46 | 281 |
| | | | | | Sum: | 300.10 | 1000 |

| | | |
|---|---|---|
| Pre-Q Rain: | 0.68 | inches |
| Total Rain: | 2.68 | inches |
| Runoff | 0.79 | inches |
| Ratio: | 29.68 | % |

| | |
|---|---|
| **Date** | 10/8/15 |
| **Intensity:** | 4 in/hr |
| **Simulator:** | 4 |
| **Plot:** | C2 |
| **Technician:** | Edwards |
| **Comp. Vol:** | 1000 mL |
| **Runoff Time:** | 9 min 33 sec |

| Time | Time to Collect sec | Sample + Tare g | Tare g | Sample g | Flow Rate mL/sec | Increm. Volume mL | Composite Volume |
|---|---|---|---|---|---|---|---|
| 2 | 19.99 | 841.3 | 93.1 | 748.2 | 37.43 | 2.25 | 8 |
| 4 | 11.03 | 875.8 | 93.1 | 782.7 | 70.96 | 6.50 | 23 |
| 8 | 5.5 | 972.1 | 93.1 | 879 | 159.82 | 27.69 | 98 |
| 14 | 4.65 | 938.4 | 93.1 | 845.3 | 181.78 | 61.49 | 218 |
| 22 | 4.65 | 981.9 | 93.1 | 888.8 | 191.14 | 89.50 | 318 |
| 30 | 3.84 | 862.9 | 93.1 | 769.8 | 200.47 | 93.99 | 334 |
| | | | | | Sum: | 281.42 | 1000 |

| | | |
|---|---|---|
| Pre-Q Rain: | 0.64 | inches |
| Total Rain: | 2.64 | inches |
| Runoff | 0.75 | inches |
| Ratio: | 28.27 | % |

| | | | | |
|---|---|---|---|---|
| **Date** | 10/8/15 | | | |
| **Intensity:** | 4 in/hr | for | 62 | min |
| | 5.33 in/hr | for | 2.6 | min |
| **Simulator:** | 4 | | | |
| **Plot:** | C3 | | | |
| **Technician:** | Edwards | | | |
| **Comp. Vol:** | 1000 mL | | | |
| **Runoff Time:** | 64 min | 35 sec | | |

| Time | Time to Collect sec | Sample + Tare g | Tare g | Sample g | Flow Rate mL/sec | Increm. Volume mL | Composite Volume |
|---|---|---|---|---|---|---|---|
| 2 | 39.94 | 783.8 | 93.1 | 690.7 | 17.29 | 1.04 | 19 |
| 4 | 25.37 | 953.3 | 93.1 | 860.2 | 33.91 | 3.07 | 58 |
| 8 | 25.72 | 846.2 | 93.1 | 753.1 | 29.28 | 7.58 | 142 |
| 14 | 25.62 | 914.4 | 93.1 | 821.3 | 32.06 | 11.04 | 207 |
| 22 | 26.97 | 845.2 | 93.1 | 752.1 | 27.89 | 14.39 | 270 |
| 30 | 20.38 | 902.3 | 93.1 | 809.2 | 39.71 | 16.22 | 304 |
| | | | | | Sum: | 53.34 | 1000 |

| | | |
|---|---|---|
| Pre-Q Rain: | 4.36 | inches |
| Total Rain: | 7.03 | inches |
| Runoff | 0.14 | inches |
| Ratio: | 2.01 | % |

| | | | | |
|---|---|---|---|---|
| **Date** | 9/23/15 | | | |
| **Intensity:** | 4 in/hr | for | 30 | min |
| | 5.33 in/hr | for | 24 | min |
| **Simulator:** | 3 | | | |
| **Plot:** | C8 | | | |
| **Technician:** | Edwards | | | |
| **Comp. Vol:** | 1000 mL | | | |
| **Runoff Time:** | 94 min | 47 sec | | |

| Time | Time to Collect sec | Sample + Tare g | Tare g | Sample g | Flow Rate mL/sec | Increm. Volume mL | Composite Volume |
|---|---|---|---|---|---|---|---|
| 2 | 20.23 | 705.5 | 93.1 | 612.4 | 30.27 | 1.82 | 45 |
| 4 | 20.82 | 643.95 | 93.1 | 550.85 | 26.46 | 3.40 | 83 |
| 8 | 28.16 | 683.67 | 93.1 | 590.57 | 20.97 | 5.69 | 140 |
| 14 | 24.44 | 749.62 | 93.1 | 656.52 | 26.86 | 8.61 | 211 |
| 22 | 36.96 | 896.71 | 93.1 | 803.61 | 21.74 | 11.67 | 286 |
| 30 | 44.96 | 913.52 | 93.1 | 820.42 | 18.25 | 9.60 | 235 |
| | | | | | Sum: | 40.78 | 1000 |

| | | |
|---|---|---|
| Pre-Q Rain: | 4.13 | inches |
| Total Rain: | 6.80 | inches |
| Runoff | 0.11 | inches |
| Ratio: | 1.59 | % |

| | |
|---|---|
| **Date** | 9/23/15 |
| **Intensity:** | 4 in/hr |
| **Simulator:** | 2 |
| **Plot:** | D1 |
| **Technician:** | Whitney |
| **Comp. Vol:** | 1000 mL |
| **Runoff Time:** | 4 min 47 sec |

| Time | Time to Collect sec | Sample + Tare g | Tare g | Sample g | Flow Rate mL/sec | Increm. Volume mL | Composite Volume |
|---|---|---|---|---|---|---|---|
| 2 | 46.37 | 985.28 | 93.1 | 892.18 | 19.24 | 1.15 | 4 |
| 4 | 20.57 | 1023.91 | 93.1 | 930.81 | 45.25 | 3.87 | 14 |
| 8 | 7.12 | 1098.69 | 93.1 | 1005.59 | 141.23 | 22.38 | 82 |
| 14 | 5.03 | 1080.68 | 93.1 | 987.58 | 196.34 | 60.76 | 224 |
| 22 | 4.96 | 1013.58 | 93.1 | 920.48 | 185.58 | 91.66 | 338 |
| 30 | 5.09 | 1088.71 | 93.1 | 995.61 | 195.60 | 91.48 | 337 |
| | | | | | Sum: | 271.31 | 1000 |

| | | |
|---|---|---|
| Pre-Q Rain: | 0.32 | inches |
| Total Rain: | 2.32 | inches |
| Runoff | 0.72 | inches |
| Ratio: | 30.99 | % |

| | | | |
|---|---|---|---|
| **Date** | 9/23/15 | | |
| **Intensity:** | 4 in/hr | | |
| **Simulator:** | 2 | | |
| **Plot:** | D2 | | |
| **Technician:** | Saeid | | |
| | | | |
| **Comp. Vol:** | 1000 mL | | |
| | | | |
| **Runoff Time:** | 19 min | 20 sec | |

| Time | Time to Collect sec | Sample + Tare g | Tare g | Sample g | Flow Rate mL/sec | Increm. Volume mL | Composite Volume |
|---|---|---|---|---|---|---|---|
| 2 | 45.23 | 999.95 | 93.1 | 906.85 | 20.05 | 1.20 | 23 |
| 4 | 44.34 | 1096.09 | 93.1 | 1002.99 | 22.62 | 2.56 | 49 |
| 8 | 29.255 | 1026.04 | 93.1 | 932.94 | 31.89 | 6.54 | 124 |
| 14 | 24.8 | 1080.77 | 93.1 | 987.67 | 39.83 | 12.91 | 245 |
| 22 | 28.53 | 991.38 | 93.1 | 898.28 | 31.49 | 17.11 | 324 |
| 30 | 45.02 | 1004.83 | 93.1 | 911.73 | 20.25 | 12.42 | 235 |
| | | | | | Sum: | 52.74 | 1000 |

| | | |
|---|---|---|
| Pre-Q Rain: | 1.29 | inches |
| Total Rain: | 3.29 | inches |
| Runoff | 0.14 | inches |
| Ratio: | 4.25 | % |

| | | | | |
|---|---|---|---|---|
| **Date** | 9/23/15 | | | |
| **Intensity:** | 4 in/hr | for | 45 | min |
| | 5.33 in/hr | for | 212 | min |
| **Simulator:** | 2 | | | |
| **Plot:** | D4 | | | |
| **Technician:** | Saeid | | | |
| **Comp. Vol:** | 1000 mL | | | |
| **Runoff Time:** | 257 min | 17 sec | | |

| Time | Time to Collect sec | Sample + Tare g | Tare g | Sample g | Flow Rate mL/sec | Increm. Volume mL | Composite Volume |
|---|---|---|---|---|---|---|---|
| 2 | 2.2 | 844.8 | 93.1 | 751.7 | 341.68 | 20.50 | 34 |
| 4 | 2.34 | 1004.12 | 93.1 | 911.02 | 389.32 | 43.86 | 73 |
| 8 | 2.47 | 952.86 | 93.1 | 859.76 | 348.08 | 88.49 | 148 |
| 14 | 2.81 | 974.58 | 93.1 | 881.48 | 313.69 | 119.12 | 199 |
| 22 | 2.68 | 967.28 | 93.1 | 874.18 | 326.19 | 153.57 | 257 |
| 30 | 2.65 | 1127.43 | 93.1 | 1034.33 | 390.31 | 171.96 | 288 |
| | | | | | Sum: | 597.50 | 1000 |

| | | |
|---|---|---|
| Pre-Q Rain: | 21.86 | inches |
| Total Rain: | 24.52 | inches |
| Runoff | 1.58 | inches |
| Ratio: | 6.45 | % |

| | | |
|---|---|---|
| **Date** | 9/23/15 | |
| **Intensity:** | 4 in/hr | |
| **Simulator:** | 4 | |
| **Plot:** | D5 | |
| **Technician:** | Kameryn | |
| **Comp. Vol:** | 1000 mL | |
| **Runoff Time:** | 44 min | 30 sec |

| Time | Time to Collect sec | Sample + Tare g | Tare g | Sample g | Flow Rate mL/sec | Increm. Volume mL | Composite Volume |
|---|---|---|---|---|---|---|---|
| 2 | 60 | 901.92 | 93.1 | 808.82 | 13.48 | 0.81 | 10 |
| 4 | 50.03 | 1112.09 | 93.1 | 1018.99 | 20.37 | 2.03 | 25 |
| 8 | 35.4 | 1103.12 | 93.1 | 1010.02 | 28.53 | 5.87 | 71 |
| 14 | 20 | 1092.36 | 93.1 | 999.26 | 49.96 | 14.13 | 171 |
| 22 | 15.44 | 1096.13 | 93.1 | 1003.03 | 64.96 | 27.58 | 334 |
| 30 | 13.53 | 1023.19 | 93.1 | 930.09 | 68.74 | 32.09 | 389 |
| | | | | | Sum: | 82.51 | 1000 |

| | | |
|---|---|---|
| Pre-Q Rain: | 2.97 | inches |
| Total Rain: | 4.97 | inches |
| Runoff | 0.22 | inches |
| Ratio: | 4.40 | % |

| | | | | |
|---|---|---|---|---|
| **Date** | 9/23/15 | | | |
| **Intensity:** | 4 in/hr | for | 64 | min |
| | 5.33 in/hr | for | 14 | min |
| **Simulator:** | 4 | | | |
| **Plot:** | D6 | | | |
| **Technician:** | Kameryn | | | |
| **Comp. Vol:** | 1000 mL | | | |
| **Runoff Time:** | 78 min | | | |

| Time | Time to Collect sec | Sample + Tare g | Tare g | Sample g | Flow Rate mL/sec | Increm. Volume mL | Composite Volume |
|---|---|---|---|---|---|---|---|
| 2 | 60 | 664.4 | 93.1 | 571.3 | 9.52 | 0.57 | 27 |
| 4 | 60 | 771.2 | 93.1 | 678.1 | 11.30 | 1.25 | 58 |
| 8 | 60 | 872.06 | 93.1 | 778.96 | 12.98 | 2.91 | 136 |
| 14 | 60 | 890.71 | 93.1 | 797.61 | 13.29 | 4.73 | 221 |
| 22 | 60 | 887.21 | 93.1 | 794.11 | 13.24 | 6.37 | 298 |
| 30 | 60 | 688.44 | 93.1 | 595.34 | 9.92 | 5.56 | 260 |
| | | | | | Sum: | 21.39 | 1000 |

| | | |
|---|---|---|
| Pre-Q Rain: | 5.51 | inches |
| Total Rain: | 8.17 | inches |
| Runoff | 0.06 | inches |
| Ratio: | 0.69 | % |

| | | | | |
|---|---|---|---|---|
| **Date** | 9/23/15 | | | |
| **Intensity:** | 4 in/hr | for | 60 | min |
| | 5.33 in/hr | for | 120 | min |
| **Simulator:** | 4 | | | |
| **Plot:** | D7 | | | |
| **Technician:** | Som | | | |
| **Comp. Vol:** | 1000 mL | | | |
| **Runoff Time:** | 180 min | | | |

| Time | Time to Collect sec | Sample + Tare g | Tare g | Sample g | Flow Rate mL/sec | Increm. Volume mL | Composite Volume |
|---|---|---|---|---|---|---|---|
| 2 | 7.2 | 961.81 | 93.1 | 868.71 | 120.65 | 7.24 | 22 |
| 4 | 5 | 1121.76 | 93.1 | 1028.66 | 205.73 | 19.58 | 59 |
| 8 | 4.86 | 1023.41 | 93.1 | 930.31 | 191.42 | 47.66 | 145 |
| 14 | 3.84 | 928.5 | 93.1 | 835.4 | 217.55 | 73.62 | 223 |
| 22 | 5.8 | 1117.78 | 93.1 | 1024.68 | 176.67 | 94.61 | 287 |
| 30 | 5.12 | 1040.12 | 93.1 | 947.02 | 184.96 | 86.79 | 263 |
| | | | | | Sum: | 329.50 | 1000 |

| | | |
|---|---|---|
| Pre-Q Rain: | 14.66 | inches |
| Total Rain: | 17.33 | inches |
| Runoff | 0.87 | inches |
| Ratio: | 5.04 | % |

| | | | | |
|---|---|---|---|---|
| **Date** | 9/23/15 | | | |
| **Intensity:** | 4 in/hr | for | 54 | min |
| | 5.33 in/hr | for | 20 | min |
| **Simulator:** | 3 | | | |
| **Plot:** | D9 | | | |
| **Technician:** | Edwards | | | |
| **Comp. Vol:** | 1000 mL | | | |
| **Runoff Time:** | 74 min | | | |

| Time | Time to Collect sec | Sample + Tare g | Tare g | Sample g | Flow Rate mL/sec | Increm. Volume mL | Composite Volume |
|---|---|---|---|---|---|---|---|
| 2 | 14.45 | 693.4 | 93.1 | 600.3 | 41.54 | 2.49 | 26 |
| 4 | 12.7 | 827.4 | 93.1 | 734.3 | 57.82 | 5.96 | 63 |
| 8 | 10.58 | 825.75 | 93.1 | 732.65 | 69.25 | 15.25 | 160 |
| 14 | 14.06 | 846.42 | 93.1 | 753.32 | 53.58 | 22.11 | 232 |
| 22 | 15.93 | 951.81 | 93.1 | 858.71 | 53.91 | 25.80 | 271 |
| 30 | 13.76 | 709.35 | 93.1 | 616.25 | 44.79 | 23.69 | 249 |
| | | | | | Sum: | 95.29 | 1000 |

| | | |
|---|---|---|
| Pre-Q Rain: | 5.37 | inches |
| Total Rain: | 8.03 | inches |
| Runoff | 0.25 | inches |
| Ratio: | 3.14 | % |

# APPENDIX B: MANURE AND SLUDGE ANALYSIS RESULTS

**Table 13. Full analysis of horse manure for each sample, M1-M6.**

| | Sample | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Analyte** | M1 | M2 | M3 | M4 | M5 | M6 | Mean | St Dev[2] |
| Moisture Content[1] (%) | 34 | 34 | 35 | 37 | 37 | 35 | 35 | 1.3 |
| Carbon (%) | 46.7 | 45.9 | 46.8 | 44.9 | 45.9 | 46.2 | 46.1 | 0.68 |
| Nitrogen (%) | 0.73 | 0.96 | 0.78 | 1.12 | 0.92 | 0.82 | 0.89 | 0.14 |
| Phosphorus (mg/kg) | 0.18 | 0.22 | 0.24 | 0.34 | 0.28 | 0.29 | 0.26 | 0.06 |
| Potassium (mg/kg) | 1.34 | 1.46 | 1.23 | 1.53 | 1.55 | 1.69 | 1.47 | 0.16 |
| Calcium (mg/kg) | 0.77 | 0.83 | 0.79 | 0.97 | 0.85 | 1.24 | 0.91 | 0.18 |
| Magnesium | 0.22 | 0.23 | 0.22 | 0.29 | 0.25 | 0.38 | 0.27 | 0.06 |
| Zinc | 59 | 55 | 57 | 88 | 77 | 93 | 71 | 16.8 |
| Copper | 28 | 20 | 18 | 25 | 26 | 30 | 25 | 4.59 |
| Manganese | 138 | 119 | 140 | 169 | 210 | 164 | 157 | 32.0 |
| Iron | 967 | 944 | 859 | 1105 | 1139 | 947 | 994 | 106.8 |

[1]Moisture content expressed on wet basis
[2]Standard Deviation

**Table 14. Full analysis results of municipal sludge for samples S I - S III.**

| | Sample | | | | |
|---|---|---|---|---|---|
| **Analyte** | S I | S II | S III | Mean | St Dev[1] |
| Moisture Content (%) | 85 | 83 | 84 | 84 | 1.11 |
| Total Nitrogen (%) | 5.29 | 4.68 | 5.11 | 5.03 | 0.31 |
| Ammonia N (%) | 0.81 | 0.74 | 0.81 | 0.79 | 0.04 |
| Nitrate (mg/kg) | 1.3 | 1.4 | 1.8 | 1.5 | 0.26 |
| Phosphorus (%) | 1.53 | 1.34 | 1.52 | 1.46 | 0.11 |
| Zinc | 1040 | 936 | 1010 | 995 | 53.5 |
| Copper | 431 | 371 | 445 | 416 | 39.3 |
| Iron | 8130 | 6260 | 8460 | 7617 | 1186 |
| Chromium | 17.7 | 12.7 | 19.6 | 17 | 3.56 |
| Selenium | 5.5 | 4.29 | 5.19 | 5 | 0.63 |

[1]Standard Deviation

# APPENDIX C: LABORATORY ANALYSIS OF BIOCHAR

Control Laboratories

42 Hangar Way
Watsonville, CA 95076
www.biocharlab.com
Tel: 831 724-5422
Fax: 831 724-3188

Account No.:
8787
Batch
April 2015 M
CODE:
BioChar IBI

David Black
Waste to Energy, Inc. (WESI)
277 New Hinson Road
Slocomb, AL 36375

| | |
|---|---|
| Date Received: | 05/01/15 |
| Sample Id.: | Organic Bio-Char, Acti-Biochar, Bison Soils |
| Sample id. Number | 5050048 |

**International BioChar Initiative (IBI) Laboratory Tests for Certification Program**

| | Dry Basis Unless Stated: | Range | Units | Method |
|---|---|---|---|---|
| Moisture (time of analysis) | 56.4 | | % wet wt. | ASTM D1762-84 (105c) |
| Bulk Density | 13.9 | | lb/cu ft | |
| Organic Carbon | 85.5 | | % of total mass | Dry Combust-ASTM D 4373 |
| Hydrogen/Carbon (H:C) | 0.26 | 0.7 Max | Molar Ratio | H dry combustion/C(above) |
| Total Ash | 9.2 | | % of total mass | ASTM D-1762-84 |
| Total Nitrogen | 0.66 | | % of total mass | Dry Combustion |
| pH value | 9.49 | | units | 4.11USCC:dil. Rajkovich |
| Electrical Conductivity (EC20 w/w) | 0.523 | | dS/m | 4.10USCC:dil. Rajkovich |
| Liming (neut. Value as-CaCO3) | 8.7 | | %CaCO3 | AOAC 955.01 |
| Carbonates (as-CaCO3) | 3.8 | | %CaCO3 | ASTM D 4373 |
| Butane Act. | 11.2 | | g/100g dry | ASTM D 5742-95 |
| Surface Area Correlation | 489 | | m2/g dry | G |

| All units mg/kg dry unless stated: | | Results | Range of Max. Levels | Meth. Det. Limit (ppm) | Method |
|---|---|---|---|---|---|
| Arsenic | (As) | 1.3 | 13 to 100 | 0.13 | E |
| Cadmium | (Cd) | Below MDL | 1.4 to 39 | 0.0044 | E |
| Chromium | (Cr) | 4.8 | 93 to 1200 | 0.013 | E |
| Cobalt | (Co) | 0.87 | 34 to 100 | 0.22 | E |
| Copper | (Cu) | 15 | 143 to 6000 | 0.44 | E |
| Lead | (Pb) | 1.3 | 121 to 300 | 0.087 | E |
| Molybdenum | (Mo) | Below MDL | 5 to 75 | 0.52 | E |
| Mercury | (Hg) | 0.0002 | 1 to 17 | 0.00025 | EPA 7471 |
| Nickel | (Ni) | 2.6 | 47 to 420 | 0.044 | E |
| Selenium | (Se) | Below MDL | 2 to 200 | 0.22 | E |
| Zinc | (Zn) | 9.2 | 416 to 7400 | 0.087 | E |
| Boron | (B) | 14 | Declaration | 0.87 | TMECC |
| Chlorine | (Cl) | 97 | Declaration | 2.0 | TMECC |
| Sodium | (Na) | 1077 | Declaration | 4.4 | E |
| Iron | (Fe) | 3839 | Declaration | 0.44 | E |
| Manganese | (Mn) | 1137 | Declaration | 0.087 | E |

| Particle Size Distribution | Results | Units | Method |
|---|---|---|---|
| < 0.5mm | 40.0 | percent | F |
| 0.5-1mm | 37.7 | percent | F |
| 1-2mm | 16.9 | percent | F |
| 2-4mm | 3.3 | percent | F |
| 4-8mm | 2.0 | percent | F |
| 8-16mm | 0.0 | percent | F |
| 16-25mm | 0.0 | percent | F |
| 25-50mm | 0.0 | percent | F |
| >50mm | 0.0 | percent | F |
| **Basic Soil Enhancement Properties** | | | |
| Total (K) | 6563 | mg/kg | B |
| Total (P) | 2288 | mg/kg | B |
| Ammonia (NH4-N) | 15 | mg/kg | A |
| Nitrate (NO3-N) | 0.26 | mg/kg | A |
| Organic (Org-N) | 6599 | mg/kg | Calc. |
| Volatile Matter | 90.77 | percent dw | D |

Method A Rayment & Higginson
B Enders & Lehmann
C Wang after Rajan
D ASTM D1762-84
E EPA3050B/EPA 6010
F ASTM D 2862 Granular
G Butane Activity Surface Area Correlation Based on McLaughlin, Shields, Jagiello, & Thiele's 2012 paper: Analytical Options for Biochar Adsorption and Surface Area

Analyst: Megan Nutt

# APPENDIX D: WATER QUALITY ANALYSIS RESULTS

**Table 15. Full water quality analysis for each treatment and replication.**

| Plot | Treatment | CN | COD g/L | FC MPN | $NH_3$-N mg/L | $NO_3$-N mg/L | DP mg/L | TP mg/L | TKN mg/L | TSS mg/L |
|---|---|---|---|---|---|---|---|---|---|---|
| B7 | C | 36 | 2.74 | 1 | 0.74 | 0.75 | 0.97 | 0.67 | 3.82 | 29.0 |
| C1 | C | 76 | 2.32 | 1 | 0.56 | 0.88 | 0.26 | 0.58 | 1.65 | 33.1 |
| D1 | C | 79 | 2.66 | 15 | 0.73 | 0.08 | 1.45 | 0.80 | 1.57 | 23.9 |
| B3 | M | 45 | 2.34 | 435 | 3.64 | 0.91 | 2.33 | 3.06 | 10.3 | 205 |
| D4 | M | 13 | 2.64 | 3 | 0.81 | 0.78 | 1.01 | 0.60 | 1.43 | 22.3 |
| D5 | M | 40 | 2.56 | 435 | 3.18 | 0.09 | 3.24 | 2.39 | 5.61 | 178 |
| B1 | MB | 25 | 2.33 | 2 | 0.94 | 0.79 | 0.47 | 0.92 | 4.56 | 36.7 |
| B2 | MB | 71 | 2.06 | 435 | 5.07 | 0.87 | 2.91 | 4.45 | 14.8 | 164 |
| D9 | MB | 27 | 2.68 | 67 | 1.34 | 0.78 | 2.37 | 1.34 | 2.59 | 47.0 |
| C3 | S | 28 | 2.32 | 11 | 2.05 | 0.84 | 0.46 | 0.62 | 3.93 | 114 |
| D2 | S | 50 | 2.42 | 435 | 5.26 | 0.07 | 1.44 | 1.09 | 9.03 | 106 |
| D7 | S | 16 | 2.72 | 5 | 0.93 | 0.81 | 0.84 | 0.55 | 1.70 | 45.3 |
| C2 | SB | 75 | 2.40 | 69 | 6.37 | 1.18 | 0.35 | 0.70 | 10.4 | 321 |
| C8 | SB | 28 | 2.66 | 8 | 1.57 | 0.06 | 1.11 | 0.66 | 2.38 | 62.8 |
| D6 | SB | 23 | 2.71 | 3 | 0.94 | 0.08 | 1.13 | 0.94 | 2.14 | 51.6 |
| | | **Mean** | 2.50 | 128 | 2.28 | 0.60 | 1.36 | 1.29 | 5.06 | 96.0 |
| | | **S**[1] | 0.20 | 193 | 1.94 | 0.39 | 0.94 | 1.13 | 4.15 | 86.6 |

[1]Standard deviation

# APPENDIX E: FULL STATISTICAL ANALYSIS

There are two types of ANCOVA tests, equal slopes or unequal slopes. To begin, each constituent must be analyzed to determine which type of ANCOVA to run. If the treatment*covariate (in this case CN) interaction term is significant ($p < 0.05$), the slopes are significantly different among treatments, indicating unequal slopes (Table 16). For equal slopes, a common slope can be generated to compare responses (i.e. runoff concentration) between treatments at the mean value of the covariate. If $p < 0.05$, the treatments are significantly different than each other (Table 17, Table 18). When slopes are significantly different, a regression must be run for each treatment while comparing responses at different points along the covariate. In this case, each covariate value – or calculated CN – was used. Every $p < 0.05$ indicates that concentrations between those treatments at that CN are significantly different (Table 19, Table 20).

**Table 16. p-values for equal slopes analysis of horse manure and municipal sludge for each measured constituent.**

| Analyte | Manure Interaction | Sludge Interaction |
|---|---|---|
| COD | 0.902 | 0.987 |
| FC | 0.008* | 0.058 |
| $NH_3$-N | 0.000* | 0.001* |
| $NO_3^-$-N | 0.891 | 0.127 |
| DP | 0.577 | 0.461 |
| TP | 0.004* | 0.152 |
| TKN | 0.047* | 0.000* |
| TSS | 0.000* | 0.020* |

*< 0.05 considered significant.

**Table 17. p-values for treatment comparisons of each parameter with equal slopes among municipal sludge treatments.**

| Treatment Comparison | COD | FC | $NO_3$-N | DP | TP |
|---|---|---|---|---|---|
| C-SB | 0.26 | 0.26 | 0.95 | 0.94 | 0.82 |
| C-S | 0.72 | 0.77 | 1.00 | 0.92 | 0.84 |
| SB-S | 0.47 | 0.42 | 0.94 | 1.00 | 0.99 |

**Table 18. p-values for treatment comparisons of each parameter with equal slopes among horse manure treatments.**

| Treatment comparison | COD | $NO_3$-N | DP |
|---|---|---|---|
| C-MB | 0.30 | 0.99 | 0.18 |
| C-M | 0.13 | 0.82 | 0.26 |
| MB-M | 0.81 | 0.71 | 0.85 |

**Table 19. p-values for treatment comparisons of parameters with unequal slopes for municipal sludge treatments.**

| CN | Trt[1] Comparison | $NH_3$-N | TKN | TSS |
|---|---|---|---|---|
| 16 | C-S | 0.994 | 0.013* | 0.690 |
| | C-SB | 0.429 | 0.007* | 0.851 |
| | SB-S | 0.213 | 0.169 | 0.246 |
| 23 | C-S | 0.078 | 0.082 | 0.471 |
| | C-SB | 0.638 | 0.017* | 0.912 |
| | SB-S | 0.067 | 0.045* | 0.464 |
| 28 | C-S | 0.015* | 0.982 | 0.329 |
| | C-SB | 0.100 | 0.054 | 0.488 |
| | SB-S | 0.032* | 0.019* | 0.830 |
| 36 | C-S | 0.002* | 0.016* | 0.178 |
| | C-SB | 0.009* | 0.841 | 0.104 |
| | SB-S | 0.014* | 0.008* | 0.678 |
| 50 | C-S | 0.001* | 0.001* | 0.099 |
| | C-SB | 0.001* | 0.002* | 0.011* |
| | SB-S | 0.014* | 0.007* | 0.145 |
| 75 | C-S | 0.001* | 0.000* | 0.141 |
| | C-SB | 0.000* | 0.000* | 0.004* |
| | SB-S | 0.026* | 0.014* | 0.087 |
| 76 | C-S | 0.001* | 0.000* | 0.143 |
| | C-SB | 0.000* | 0.000* | 0.004* |
| | SB-S | 0.027* | 0.014* | 0.087 |
| 79 | C-S | 0.001* | 0.000* | 0.151 |
| | C-SB | 0.000* | 0.000* | 0.004* |
| | SB-S | 0.028* | 0.015* | 0.086 |

*< 0.05 considered significant.
[1]Treatment

**Table 20. p-values for treatment comparisons of parameters with unequal slopes for horse manure treatments.**

| CN | Trt[1] Comparison | FC | $NH_3$-N | TP | TKN | TSS |
|---|---|---|---|---|---|---|
| 13 | C-M | 0.980 | 0.905 | 0.945 | 0.546 | 0.671 |
| | C-MB | 0.538 | 0.051 | 0.358 | 0.451 | 0.120 |
| | MB-M | 0.294 | 0.017* | 0.317 | 0.958 | 0.143 |
| 25 | C-M | 0.075 | 0.009* | 0.125 | 0.985 | 0.004* |
| | C-MB | 0.874 | 0.254 | 0.529 | 0.926 | 0.407 |
| | MB-M | 0.042* | 0.007* | 0.182 | 0.944 | 0.002* |
| 27 | C-M | 0.046* | 0.005* | 0.074 | 0.998 | 0.002* |
| | C-MB | 0.689 | 0.086 | 0.303 | 0.987 | 0.173 |
| | MB-M | 0.031* | 0.006* | 0.166 | 0.946 | 0.002* |
| 36 | C-M | 0.008* | 0.001* | 0.011* | 0.469 | 0.000* |
| | C-MB | 0.116 | 0.003* | 0.026* | 0.568 | 0.009* |
| | MB-M | 0.011* | 0.005* | 0.142 | 0.950 | 0.000* |
| 40 | C-M | 0.005* | 0.000* | 0.006* | 0.263 | 0.000* |
| | C-MB | 0.052 | 0.001* | 0.011* | 0.300 | 0.003* |
| | MB-M | 0.010* | 0.005* | 0.159 | 0.961 | 0.000* |
| 45 | C-M | 0.003* | 0.000* | 0.004* | 0.139 | 0.000* |
| | C-MB | 0.022* | 0.001* | 0.005* | 0.127 | 0.001* |
| | MB-M | 0.010* | 0.007* | 0.204 | 0.975 | 0.000* |
| 71 | C-M | 0.002* | 0.000* | 0.002* | 0.054 | 0.000* |
| | C-MB | 0.004* | 0.000* | 0.001* | 0.018* | 0.000* |
| | MB-M | 0.021* | 0.054 | 0.614 | 0.999 | 0.000* |
| 76 | C-M | 0.002* | 0.000* | 0.003* | 0.054 | 0.000* |
| | C-MB | 0.004* | 0.000* | 0.001* | 0.018* | 0.000* |
| | MB-M | 0.023* | 0.073 | 0.677 | 1.000 | 0.000* |
| 79 | C-M | 0.002* | 0.000* | 0.003* | 0.055 | 0.000* |
| | C-MB | 0.004* | 0.000* | 0.001* | 0.018* | 0.000* |
| | MB-M | 0.025* | 0.085 | 0.709 | 1.000 | 0.001* |

*< 0.05 considered significant.
[1]Treatment

# APPENDIX F: STANDARD CURVES

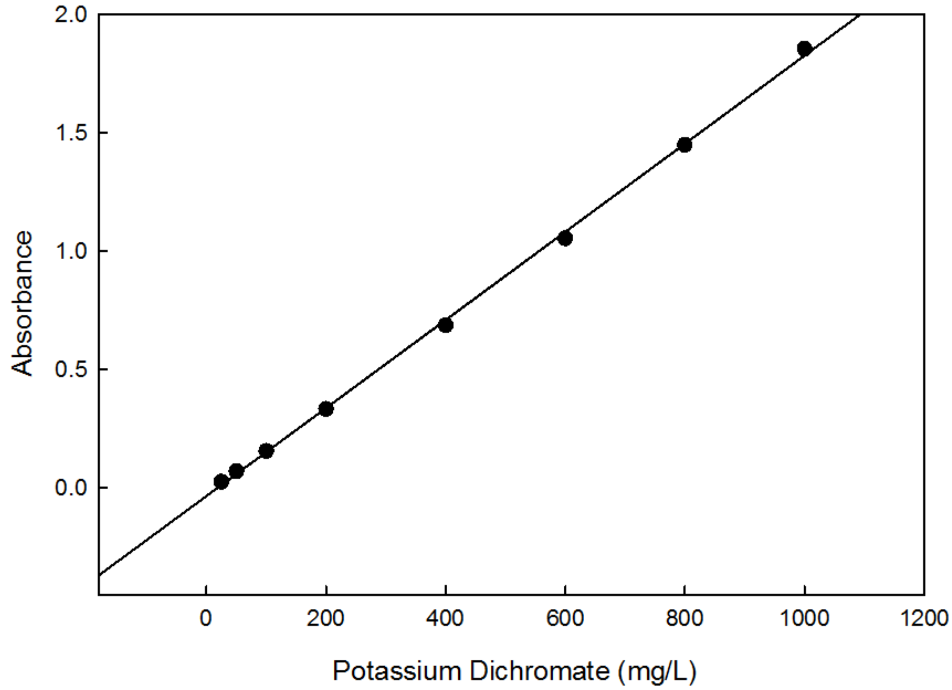


**Figure 5. Standard curve for chemical oxygen demand with $R^2$ = 0.99.**

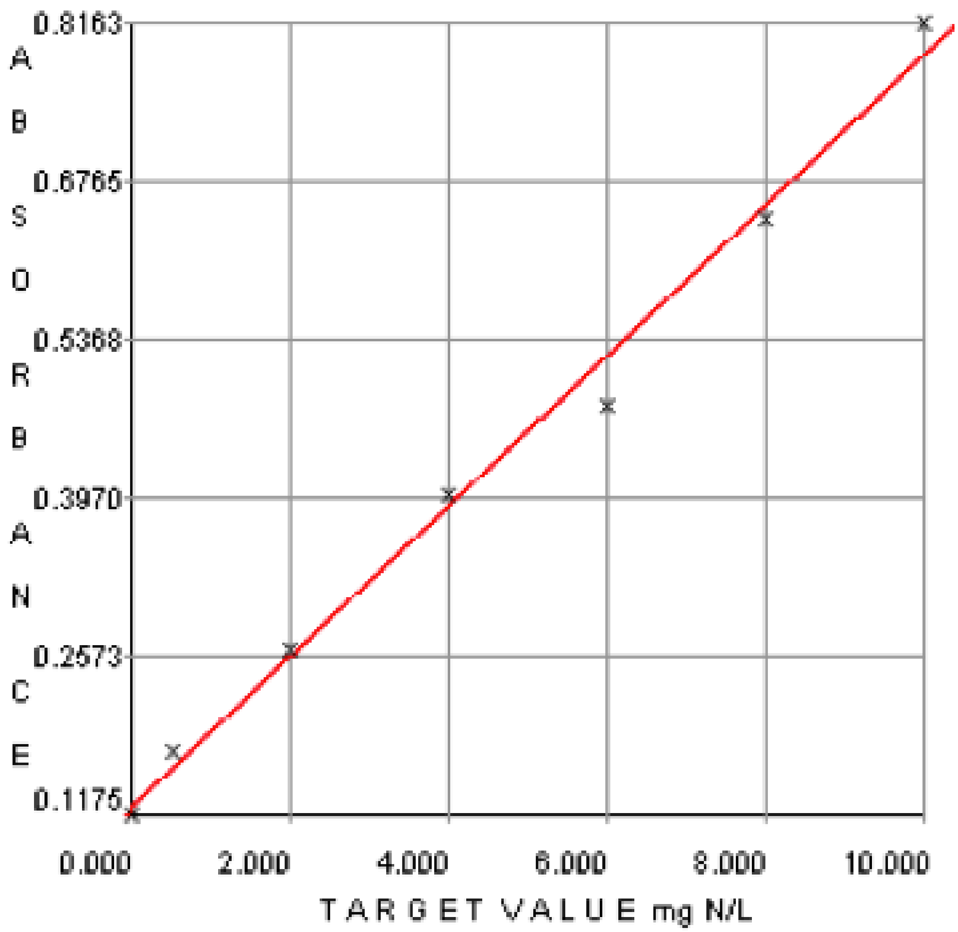


**Figure 6. Standard curve for ammonia ($NH_3$-N) supplied by AQ1 analyzer with $R^2$ = 0.996.**

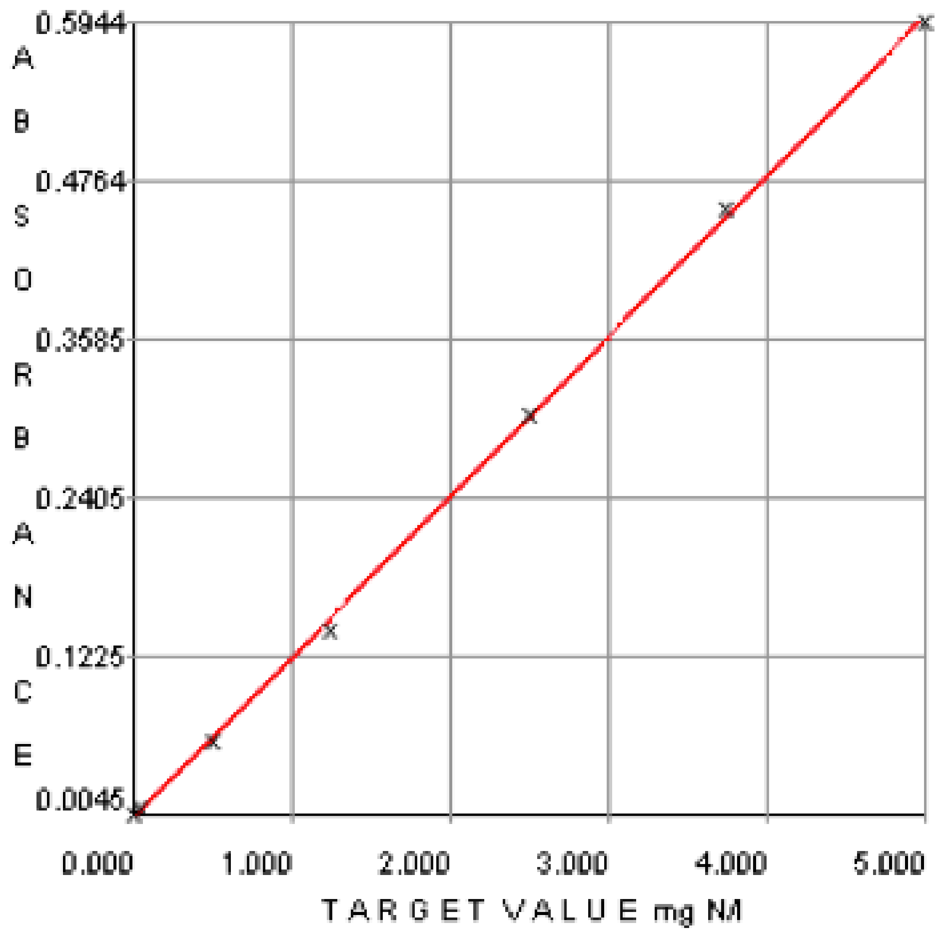


**Figure 7. Standard curve for nitrate ($NO_3$-N) as supplied by AQ1 analyzer with $R^2$ = 0.999.**

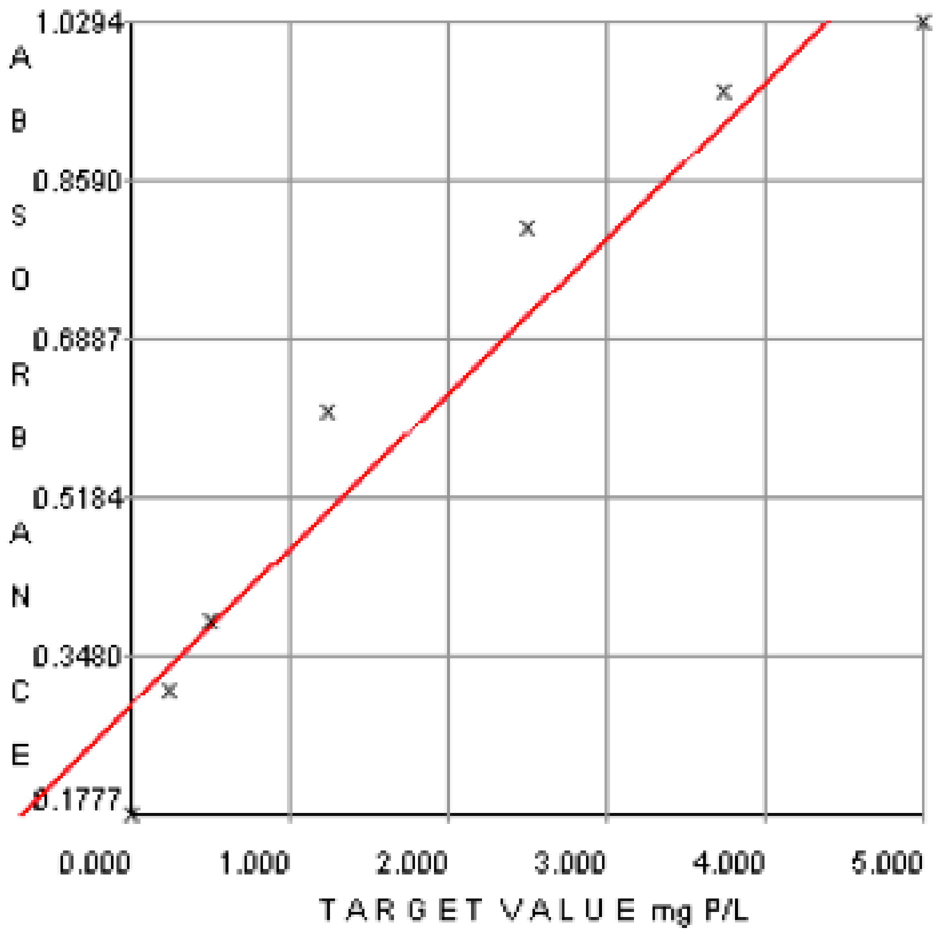


**Figure 8. Standard curve for dissolved phosphorus (DP) as supplied by AQ1 analyzer with $R^2$ = 0.965.**

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