NPB FINAL RESEARCH GRANT REPORT

Evaluation of Swine Carcass Disposal Benefits and Challenges Using Composting and Shallow Burial with Carbon (Project #22-073)

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Industry Summary. Diseases like porcine epidemic diarrhea virus (PEDV) and porcine reproductive and respiratory syndrome (PRRS) virus have challenged the U.S. swine industry for many years, and they continue to generate significant losses of animal lives when outbreaks occur. Land burial or composting of carcasses are often the chosen disposal methods, though questions remain about the most ideal solutions for disposal of infected carcasses to minimize disease transmission and introduction of contaminants to the environment. Shallow burial with carbon (SBC) has been evaluated as an alternative to, and somewhat of a cross between, traditional land burial and composting. Additionally, grinding of carcasses prior to establishing compost piles – in lieu of composting intact carcasses – has gained interest as a way to presumptively facilitate more rapid decomposition of mortalities and yield fewer large bones that require separate disposal. As the swine industry and its stakeholders seek to ensure preparedness for successful management of a foreign animal disease outbreak and manage existing losses of animals from catastrophic events and disease, important questions must be answered to guide efforts that ensure protection of animal and human health, minimize negative environmental impacts, and ensure feasibility of practice implementation by farmers. This project was conducted to address critical questions through the following objectives.

Objective 1. Determine the temporal variability in volume and characteristics of leachate generated during whole carcass composting (WCC), ground carcass composting (GCC), and shallow burial with carbon (SBC) when using wood mulch as the primary carbon source in each system, with and without addition of biochar.

Objective 2. Quantify the transport of contaminants in leachate generated during WCC, GCC, and SBC, with and without addition of biochar, through at least two different soil types.

Objective 3. Evaluate the potential effectiveness of WCC, GCC, and SBC on direct destruction of *E. coli* and modeled destruction of organisms responsible for Seneca Valley Virus (SVV), Foot and Mouth Disease (FMD), and African Swine Fever (ASF) based on temporal and spatial monitoring of conditions within the matrices of each disposal setting for a period of one year.

The study incorporated a yearlong carcass disposal study performed in disposal units equipped with impermeable liners and leachate collection systems. Internal temperatures in disposal units were monitored at eight locations in each disposal unit throughout the study and bi-weekly pumping of accumulated leachate was employed to determine leachate production volume. Analyses of collected leachates included pH, concentrations of nutrients and *E. coli*, and reactive nitrogen and oxygen species. A subsequent laboratory study employed soil columns receiving leachate treatments to monitor fate and transport of contaminants through a silty clay and a sandy clay loam soil with regular additions of water to simulate precipitation based on historical rainfall data for eastern Nebraska.

Questions about this research can be directed to Dr. Amy Millmier Schmidt at the University of Nebraska-Lincoln via e-mail to aschmidt@unl.edu.

Key Findings. Among the techniques analyzed – shallow burial with carbon (SBC), whole carcass composting (WCC), and ground carcass composting (GCC), each with and without the addition of biochar – **SBC treatments exhibited the most favorable overall performance in terms of both biosecurity and natural resource protection.** Specifically,

- Target internal disposal unit temperatures for pathogen destruction (40°C for at least 5 d and 55°C for at least 4 h) were exceeded in SBC treatments but were not consistently achieved in composting units.
- Significantly less leachate (0.3 liters/kg of carcasses) was generated by SBC treatments during the yearlong monitoring period compared to whole or ground carcass composting treatments (4.4 and 4.3 L/kg, respectively).
- While ground carcass composting provided more consistent distribution of pile contents (as evidenced by a more ideal C:N achieved), it does not appear to offer any advantages over whole carcass composting in terms of temperature goals or leachate volume produced, and it may provide greater risk of disease transmission due to the grinding process.
- Biochar addition to disposal units did not significantly impact any key performance metrics, but different biochar inclusion rates and methods may provide improved results.

Value of Research to the Swine Industry. This project directly addressed critical questions existing within the swine industry regarding effective and biosecure disposal methods for swine carcasses resulting from a catastrophic mortality event. Specifically, the research assessed three on-farm carcass disposal methods – composting of intact swine carcasses, composting of ground swine carcasses, and shallow burial with carbon using intact carcasses – all with and without the addition of biochar. Biochar was assessed for its effect on leachate retention and internal pile temperature. Leachate from field-scale mortality disposal units was used in a laboratory soil column study to assess contaminant fate and transport in a well-drained and poorly drained soil.

With equivalent masses of carcasses assigned to each carcass disposal unit, this research demonstrated that SBC required approximately 50% less carbon material than composting, generated only a fraction of the leachate produced during composting, and achieved higher internal unit temperatures for longer durations than composting units, contributing to lower nutrient and *E. coli* loads in leachate from SBC units.

While destruction of foreign animal diseases (FADs) like African swine fever (ASF) and foot and mouth disease (FMD) were not directly assessed in this study, the thermophilic temperatures achieved and sustained in SBC units suggests this carcass disposal method may be more effective at mitigating the transmission of foreign animal diseases (FADs) than composting.

Keywords: biochar, carcass disposal, composting, leachate, nutrients, pathogens, shallow burial with carbon

Scientific Abstract

The disposal of swine carcasses generated during disease outbreaks poses considerable biosecurity and ecological challenges. This research assessed leachate production and characteristics, disposal unit temperatures, and fate and transport of leachate contaminants for three carcass disposal techniques – whole carcass composting (WCC), ground carcass composting (GCC), and shallow burial with carbon (SBC) – with and without biochar addition. Six carcass disposal units measuring 4.3 x 6.7 m x 0.6 m were constructed, lined with 45 mil EPDM rubber pond liner, and equipped with perforated PVC pipe to convey leachate to vertical standpipes for collection. Wood chips were placed in each unit to a depth of 0.6 m. Twenty-five randomly assigned swine carcasses (2645 ±41.2 kg) were arranged in a single layer in the SBC and WCC units; carcasses assigned to GCC treatments were processed 1:1 with wood chips before being distributed in an even layer in GCC units. Four thermocouples were placed below and above carcasses along the centerline of each unit. One replicate of each treatment received 1.5 m^3 of biochar distributed across carcass layers. Each of the WCC and GCC treatments were covered with approximately 30 m^3 of wood chips while soil excavated to construct the SBC units was used to cover carcasses in those units. Temperature was logged every 15 min and leachate was collected from each unit every two weeks for the duration of the12-month study. Monthly leachate composites for each unit were analyzed for chemical characteristics, *E. coli* concentrations, and reactive oxygen and nitrogen species. Composites of leachate were created from units with (LB) and without biochar (L) addition. Soil columns (5 cm a 30 cm) were equipped with two common Nebraska soils – a silty clay and a sandy clay loam – to evaluate leachate contaminant fate and transport. Treatments (L, LB, or water (W)) were applied to columns in quadruplicate in a 2 x 3 factorial design for 33 d according to leachate production data from the field study. Regular additions of distilled water to columns modeled precipitation. The volume and characteristics of liquid discharged from soil columns were analyzed and soil samples from 0-5, 5-10, and 10-20 cm were obtained from each column for analysis upon study completion. Field study data revealed lower leachate production volumes for the two SBC units compared to the WCC and GCC units. SBC treatments reached and sustained internal temperatures of at least 40°C for five

consecutive days and at least 55°C for at least four hours, achieving the EPA thresholds for routine pathogen destruction in compost. Nutrient fate and transport in soil columns varied by soil texture, as expected, but no significant nutrient fate and transport concerns were identified. Findings suggest that SBC is the most efficient on-farm carcass disposal technique compared to composting of either intact or ground carcasses. Biochar inclusion had no discernible impact on measured characteristics of disposal units. These insights can guide best practices for swine carcass disposal, particularly in ecologically sensitive regions.

Introduction

Efforts to prepare state departments of agriculture, emergency management personnel, swine producers, and their advisors for responding to a foreign animal disease (FAD) outbreak in the swine industry created the impetus for this research. This research evolved as the culmination of a weeklong exercise in Nebraska supported by the National Pork Board to practice and understand the necessary steps in confirming an FAD, developing a response plan, depopulating a swine operation, and disposing of carcasses within the hypothetical farm boundary. The research reported here supports efforts to better describe the performances of three carcass disposal methods in terms of disposal unit temperatures and the quantity, characteristics, and transport of leachate in the soil environment.

Efficient carcass management is essential for controlling disease transmission and protecting biosecurity. However, while successful in pathogen deactivation, conventional disposal techniques like incineration, rendering, and landfilling frequently encounter practical challenges, including elevated operational expenses, environmental regulations, and resource demands (Kim & Pramanik, 2016). The off-farm disposal of carcasses will also not be permitted in the event of an FAD outbreak. Inadequate on-farm disposal of livestock carcasses can worsen environmental issues such as nutrient leaching, groundwater pollution, and greenhouse gas emissions, highlighting the necessity for sustainable solutions (Kalbasi et al., 2016).

Composting and shallow burial with carbon provide encouraging options to reconcile biosecurity with environmental responsibility. Swine carcass composting techniques have been investigated thoroughly to tackle the disposal of carcasses, especially those affected by ASFV. Composting is acknowledged as a biosecure approach capable of neutralizing ASFV in contaminated remains. Research using WCC and SBC methods has indicated that although ASFV DNA can be identified throughout the decomposed material, infectious virus particles are swiftly eradicated, with considerable inactivation occurring by day 3 to 5 when compost temperatures reach ≥55°C (Duc et al., 2022; Gabbert et al., 2023). Grinding of carcasses prior to compost pile construction has been suggested as a way to accelerate decomposition, potentially eradicating pathogenic material more rapidly than with traditional whole carcass composting. Therefore, the three disposal methods identified by the project team in consultation with the National Pork Board for evaluation in this study were whole carcass composting, ground carcass composting, and shallow burial with carbon.

Research to describe the variability in leachate production and characteristics over time during carcass decomposition in compost piles and shallow burial with carbon disposal units is scarce. Likewise, studies to assess the impacts of biochar on swine decomposition and leachate production are lacking despite evidence that biochar, a carbon-rich and exceptionally porous substance, can improve nutrient retention, stabilize organic carbon, and restrict the migration of detrimental pollutants in soil ecosystems (Huang et al., 2024). Finally, data describing the fate and transport of leachate contaminants in soil beneath compost piles and shallow burial with carbon disposal units is critical to fully evaluating the environmental impacts and biosecurity of these systems. Customizing disposal practices to local soil conditions is vital to reducing environmental threats.

This research examined temperature profiles within disposal units, and leachate volume and characteristics over time for whole carcass composting (WCC), ground carcass composting (GCC), and shallow burial with carbon (SBC) methods, with and without biochar enhancements, followed by assessment of leachate contaminant fate and transport in poorly drained and well-drained soils to collectively identify optimal strategies for achieving biosecurity and environmental protection during swine carcass disposal. By evaluating factors such as pathogen deactivation, leachate generation, and nutrient fate and transport, the study aspires to offer practical insights for producers, policymakers, and researchers in selecting methods that align with agricultural and ecological goals. These results contribute to establishing best practices in carcass management and promoting sustainable swine production in Nebraska and across the US.

Objectives

Objective 1. Determine the temporal variability in volume and characteristics of leachate generated during composting and shallow burial with carbon (SBWC) when using wood mulch as the primary carbon source in each system, with and without addition of biochar.

Objective 2. Quantify the transport of contaminants in leachate generated during composting and shallow burial with carbon (SBWC), with and without addition of biochar, through at least two different soil types.

Objective 3. Evaluate the potential effectiveness of composting and shallow burial with carbon (SBC) when using wood mulch as the primary carbon source in each system, with and without addition of biochar, on direct destruction of *E. coli* and modeled destruction of organisms responsible for Seneca Valley Virus (SVV), Foot and Mouth Disease (FMD), and African Swine Fever (ASF) based on temporal and spatial monitoring of temperature, pH, microbial community, and carcass decomposition over time within the matrices of each disposal setting.

Materials & Methods

Carcass Disposal Unit Construction. Six carcass disposal units measuring 4.3 x 6.7 m were established at the University of Nebraska-Lincoln's Eastern Research, Extension, and Education Center (ENREEC) near Mead, Nebraska (Figure 1). Soil was excavated to a depth of 0.6 m and basins were graded to collect leachate produced during carcass decomposition. Disposal units were lined with 45 mil EPDM rubber pond liner and equipped with perforated PVC pipe to convey leachate to vertical standpipes for collection (Figure 2). Wood chips ranging in size from approximately 1 to 6 cm were placed in each unit to a depth of 0.6 m (Figure 2). This carbon source was selected due to its high availability and relatively low cost in eastern Nebraska. Twenty-five randomly assigned swine carcasses (2645 ±41.2 kg) were arranged in a single layer in the SBC and WCC units while carcasses assigned to GCC treatments were processed with an equal part of wood chips before being distributed in an even layer in GCC units. One replicate of each treatment received 1.5 m^3 of biochar distributed evenly across carcasses. Each of the WCC and GCC treatments were covered with approximately 30 m^3 of wood chips as a "cap" while soil excavated to construct the SBC units was used to cover carcasses in those units with approximately 0.6 m of soil.

Carcass Disposal Unit Data Collection. Temperature was logged every 15 min for the duration of the 12-month study via type-T thermocouples evenly spaced along the centerline of each unit, with four below and four above carcasses, and connected to data loggers (Omega Engineering Inc., Norwalk, CT) (Figure 3). To directly assess the effectiveness of the tested disposal methods on bacterial pathogen survival, leachate was collected from each unit every two weeks throughout the study with a suction hose sterilized with 70% EtOH between units. The total volume of leachate collected from each unit was recorded, temperature and pH were measured on-site, and a 2-L sample of leachate was retained from each unit. Monthly subsamples were composited for analysis of total Kjeldahl nitrogen (TKN), ammonium-N, nitrate-N, phosphate (P_2O5), potash (K_2O), sulfur (S), calcium (Ca) , magnesium (Mg), sodium (Na), zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), boron (B), and total organic carbon (TOC). A 1-ml aliquot of each leachate sample was serially diluted and cultured on Chromagar ECC selective agar (DRG International, Springfield, NJ, USA), incubated at 37°C for 24 h, and enumerated for *E. coli* colonies. The microbiological procedures were conducted in a biosafety level 2 (BSL-2) laboratory in the Department of Biological Systems Engineering at the University of Nebraska-Lincoln.

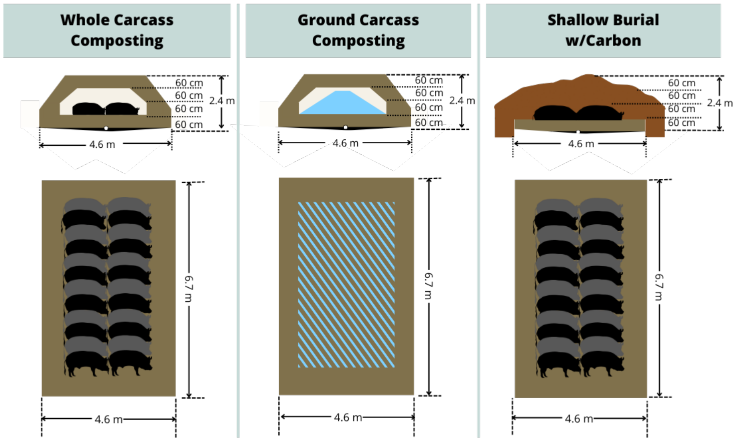

Figure 1. Schematics of carcass disposal units

Figure 2. Leachate collection system and wood chip base in disposal units

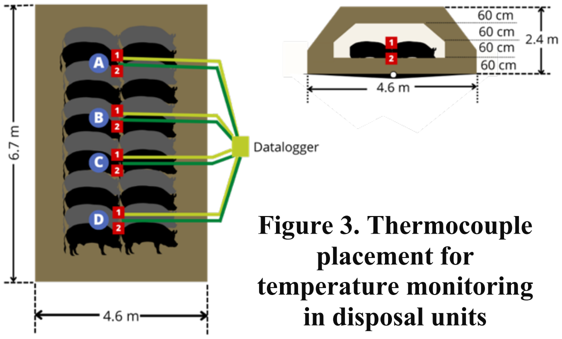

Figure 3. Thermocouple placement for temperature monitoring in disposal units

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) were assessed in monthly duplicate composite samples of leachate from each treatment unit using procedures developed for use with the ROS/RNS Assay Kit (ARG82769) (Arigo Biolaboratories, Zhubei City, Taiwan, ROC). The experimental procedures were conducted in a biosafety level 2 (BSL-2) laboratory in the Department of Animal Science at the Nebraska Center for Virology at the University of Nebraska-Lincoln. Samples were prepared by clarification using centrifugation and readings were performed using a fluorescent microplate reader (BioTek Synergy LX Multimode Reader) capable of reading 480 ηm (excitation) and 530 ηm (emission).

Soil Columns Establishment. Acrylic columns 5 cm in diameter and 30 cm in height were filled with dried and sieved silty clay or sandy clay loam soil to a depth of 25 cm (Figure 4). The initial gravimetric water content was adjusted to 12% by adding water and thoroughly mixing (Panday et al., 2020). The soils were packed into the column in 5 cm increments to achieve a uniform bulk density of 1.3 g/cm^3 for the silty clay soil and 1.65 g/cm^3 for the sandy clay loam soil. A porous ceramic plate with a strength of 105 Pa was placed in the bottom of each column and a filter paper (Whatman 42) was placed over the ceramic plate. Perforated foam caps were placed at the top of the column to ensure uniform liquid application. Columns were saturated from the bottom with deionized (DI) water using a slightly pressurized glass carboy. To ensure consistent packing between columns, the saturated conductivity of each column was assessed, and a chemical breakthrough curve test was performed using $CaCl_2$ tracer solution. Columns were then drained freely for six days prior to the start of the experiment.

Soil Column Treatments. A 2×3 factorial design with four replications of each treatment combination was used with the following factors and levels: Factor 1 (soil texture) had two levels: **silty clay (SC)** and **sandy clay loam (SCL)** soil. Factor 2 (leachate source) had three levels: water (W) as a control, leachate from disposal units without biochar (L) and leachate from disposal units with biochar (LB). Treatment combinations, therefore, were comprised of:

- sandy clay loam soil + leachate without biochar **(SCL+L)**
- sandy clay loam soil + leachate with biochar **(SCL+LB)**
- sandy clay loam soil + water **(SCL+W)**
- silty clay soil + leachate without biochar **(SC+L)**
- silty clay soil + leachate with biochar **(SC+LB)**
- silty clay soil + water **(SC+W)**

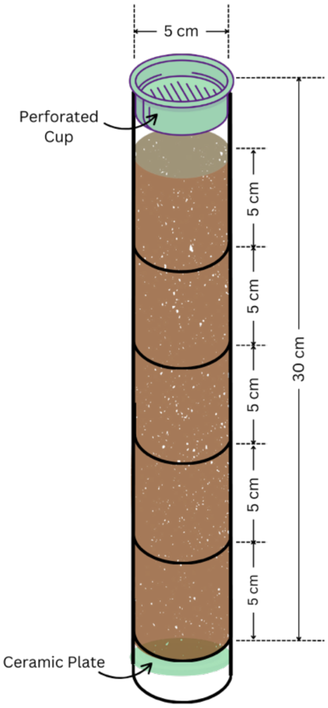

Figure 4. Soil column design

Leachate treatments were applied to columns every three days for a period of 33 d with volumes representing mean leachate production measured during the field study. All treatments received D.I. water added to columns throughout the study to simulate rainfall based on historical monthly precipitation data for eastern Nebraska from October 2014 through September 2015, a period of higher than average precipitation for the region (https://lincolnweather.unl.edu/data/monthly-precipitation.asp). Table 1 summarizes the volume of leachate treatments and precipitation applied during the study.

Table 1. Leachate (L and LB) and water application volumes by day

Day	**Leachate Volume** *(ml)*	**Precipitation Volume** *(ml)*
0	0	127
3	2	24
6	8	63
9	6	47
12	27	48
15	13	40
18	8	102
21	15	200
24	111	0
27	140	0
30	120	50
33	17	100
36	0	0
TOTAL	467	801

Analysis of Column Leachate. Column leachate was collected 24 hours after each irrigation event. Volume, pH, and EC were recorded (Accumet AP85 meter) prior to storing samples at 4°C. At the completion of the study, samples were composited by treatment combination for days 0 to 9, 10 to 15, 16 to 21, 22 to 27, and 28 to 33 (to ensure sufficient sample volume for laboratory analyses) and analyzed for ammonia (NH_3), nitrate (NO_3^-), total Kjeldahl nitrogen (TKN), total organic carbon (TOC), and total dissolved solids (TDS).

Analysis of Column Soils. Soil cores were taken from each column and cut into samples at depths of 0 to 5, 5 to 15, and 15 to 25 cm. The samples were stored at 4°C and sent to a commercial laboratory for analysis of pH, organic matter (OM), nitrate (NO_3^-), ammonium (NH_4), phosphate (P_2O_5), potash (K_2O), magnesium (Mg), manganese (Mn), calcium (Ca), iron (Fe), zinc (Zn), copper (Cu), boron (B), and sulfate (SO_4).

Statistical Analysis. Statistical analyses were conducted on leachate production and characteristics from disposal units by separating means for disposal method and biochar inclusion status. Temperature data for disposal units was assessed to determine minimum, maximum, and mean temperatures achieved in each disposal unit along with assessing the time over which mean target temperatures were sustained. Soil column data was analyzed using a three-factor factorial generalized linear model fitted with measured characteristics as the response variables (SAS Studio, 2024a). The fixed effect factors for the soil column study data analysis included soil type, treatment, and depth.

Results

Objective 1. Determine the temporal variability in volume and characteristics of leachate generated during composting and shallow burial with carbon (SBWC) when using wood mulch as the primary carbon source in each system, with and without addition of biochar.

Leachate Production and Characteristics. Biochar addition demonstrated no effect on the leachate volume produced. Cumulative leachate volume collected from disposal units varied considerably with mean leachate production for SBC units (835 L) being significantly less ($p<0.0001$) than mean volumes from whole (11,481 L) and ground (11,261 L) carcass compost units. Cumulative leachate production by among treatments totaled 578, 1,092, 6,269, 16,692, 15,179, and 7,342 L, respectively, for SBC, SBC+B, WCC, WCC+B, GCC, and GCC+B. The volume of leachate collected from each disposal unit per unit mass of carcasses is illustrated in Figure 5. The SBC units yielded 0.2 to 0.4 L/kg while WCC and GCC yielded 2.4 to 6.3 and 2.8 to 5.7 L/kg, respectively. Cumulative precipitation over the period of the field study totaled 60.83 cm, which was lower than historical total precipitation for this period for Mead, NE (76.45 cm). The utilization of wood chips as a carbon source in carcass disposal piles does not appear to be ideal, and future evaluation of alternative carbon sources is warranted.

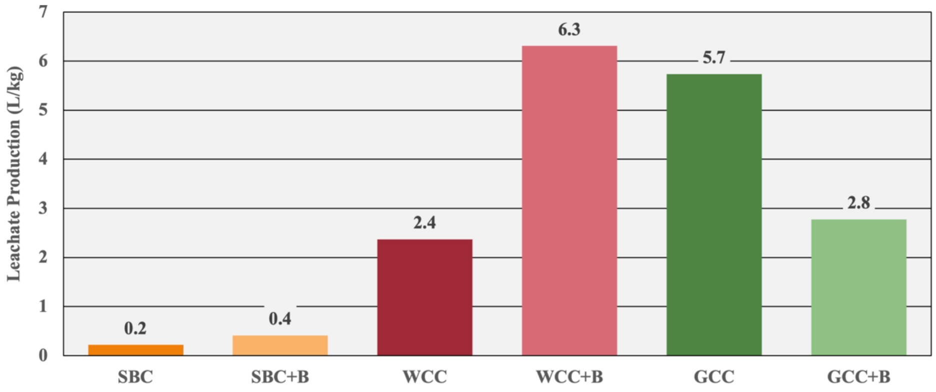

Figure 5. Leachate volume (liters) collected from disposal units per mass of carcasses (kilograms); SBC: shallow burial with carbon, WCC: whole carcass composting, GCC: ground carcass composting, B: biochar.

Mean leachate volume and nutrient concentrations were compared among disposal methods with or without biochar addition and no differences were found in any of the parameters at $a=0.05$. Total standardized chemical loads in leachate for each disposal unit were calculated by summing monthly chemical loads multiplied by monthly leachate volume production and dividing by total mass of carcasses. Reported loads are displayed in two separate graphs due to large variations in loads among groups of nutrients measured (Figures 6 & 7). All nutrient loads were numerically greater for WCC+B than all other disposal methods and biochar combinations, except nitrate (NO_3), shown in Fig. 7, which was greater for SBC than all treatment combinations except GCC. Loads of all other nutrients varied among WCC, WCC+B, GCC, and GCC+B, with no consistent pattern discernible between the WCC and GCC units, with or without biochar. Overall, WCC and GCC disposal units produced greater nutrient loads than SBC or SBC+B for every nutrient except nitrate.

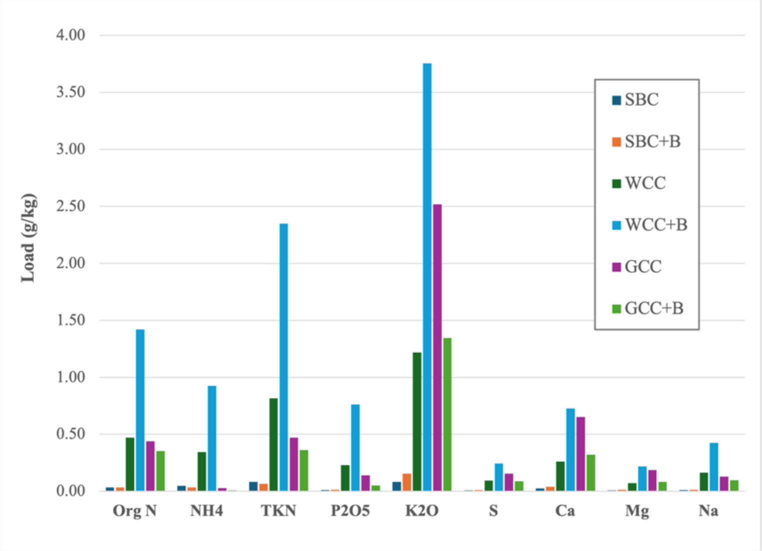

Figure 6. Mass of nutrients (g) in leachate per mass of carcasses (kg) by disposal unit; organic nitrogen (Org N), ammonium (NH_4), total nitrogen (TKN), phosphate (P_2O_5), potash (K_2O), sulfur (S), calcium (Ca), magnesium (Mg), and sodium (Na).

When comparing mean standardized loads of nutrients in disposal methods grouped by biochar inclusion, no statistically significant differences were identified. While this does not negate all effects of biochar on targeted metrics of performance, it does suggest that biochar inclusion did not have a discernible impact on performance in this study.

Mean standardized nutrient loads in leachate by disposal method *with biochar removed as a variable* are summarized in Table 2 with associated *p*-values for each nutrient. Because no differences in mean standardized loads were identified among disposal methods for NO_3, S, Ca, or Zn, these data are not displayed in Table 2. However, mean standardized loads for many nutrients were significant and are further illustrated in Figures 8 and 9.

Mean nutrient loads for K_2O, S, Ca, and Mg (Fig. 8) and for Zn, Mn, Cu, and B (Fig. 9) were significantly lower from the SBC units than WCC or GCC. In addition, loads of organic N, NH_4, TKN, P_2O_5, and Na (Fig 8) and for Fe (Fig. 9) were lower for SBC than WCC. While NO_3 loads from SBC were numerically greater than WCC, WCC+B, GCC, and GCC+B (Fig. 7), mean loads of NO_3 were not different among disposal methods when biochar inclusion status was ignored. While WCC and GCC collectively did not perform as well as SBC when comparing mean loads of nutrients, it is worth noting that mean loads of NH_4, TKN, P_2O_5, and Na (Fig. 8) were lower for GCC than for WCC.

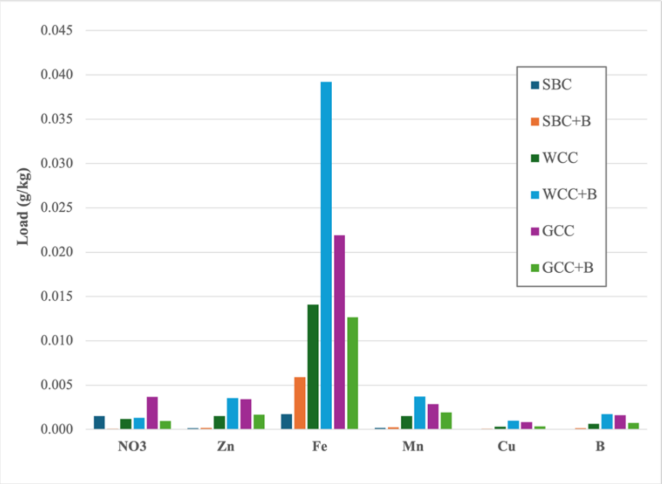

Figure 7. Mass of nutrients (g) in leachate per mass of carcasses (kg) by disposal method and biochar inclusion, October 2022 to October 2023; nitrates (NO_3^-), zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), and boron (B).

Table 2. Mean nutrient loads from leachate by disposal method

Treatment	Org N	NH_4	NO_3	TKN	K_2O	P_2O_5	Na	Mg	Mn	Cu	B
SBC	0.003^{a}	0.004^{a}	0.0001^{a}	0.007^{a}	0.01^{a}	0.001^{a}	0.001^{a}	0.0009^{a}	0.00002^{a}	0.00001^{a}	0.00001^{a}
WCC	0.086^{b}	0.058^{b}	0.0001^{a}	0.144^{b}	0.23^{b}	0.045^{a}	0.027^{b}	0.0131^{b}	0.00024^{b}	0.00006^{b}	0.00011^{b}
GCC	0.036^{ab}	0.002^{a}	0.0002^{a}	0.038^{b}	0.18^{b}	0.009^{a}	0.010^{a}	0.0122^{b}	0.00022^{b}	0.00005^{b}	0.00011^{b}
p-value	*<0.001*	*<0.01*	*>0.05*	*<0.01*	*<0.01*	*<0.01*	*<0.001*	*<0.01*	*<0.01*	*<0.01*	*<0.01*

Values within a column sharing a superscript are not significantly different.

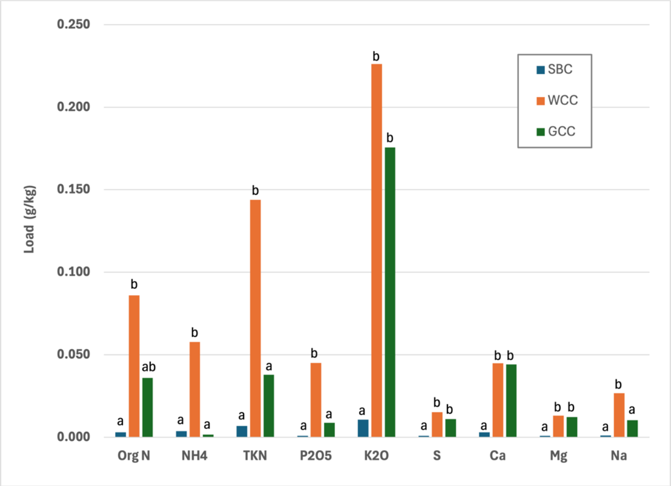

Figure 8. Mean monthly mass of nutrients (g) produced in leachate per mass of carcasses (kg) by disposal method; organic nitrogen (Org N), ammonium (NH_4), total nitrogen (TKN), phosphate (P_2O_5). potash (K_2O), sulfur (S), calcium (Ca), magnesium (Mg), and sodium (Na); columns within a nutrient sharing a superscript are not significantly different ($p < 0.05$).

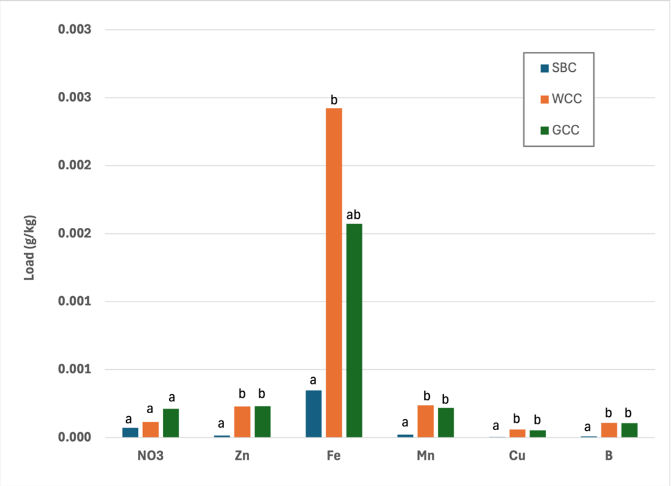

Figure 9. Mean monthly mass of nutrients (g) produced in leachate per mass of carcasses (kg) by disposal method; nitrate (NO3), zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), and boron (B); columns within a nutrient sharing a superscript are not significantly different ($p < 0.05$).

***E. coli* Enumeration.** Cumulative concentrations of *E. coli* (CFUs/mL) in samples from all six disposal units over the course of the yearlong study are displayed in Figure 10. The SBC treatment yielded numerically lower concentrations of *E. coli* than other treatments, though no *E. coli* results were significant among disposal methods nor disposal method x biochar inclusion. Numerically, the cumulative load of *E. coli* from WCC+B was greater than for all other disposal units, while the load from GCC was greater than from WCC, GCC+B, SBC, and SBC+B.

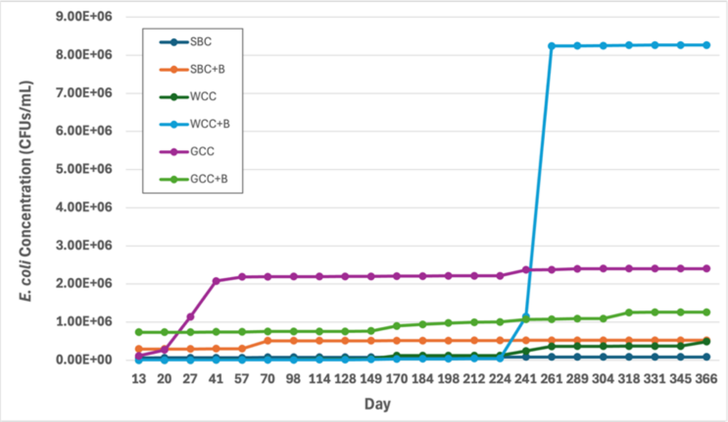

Figure 10. Cumulative *E. coli* colony forming units by disposal unit, October 2022 to October 2023

Standardizing the *E. coli* loads to reflect total colony forming units (CFUs) leached from each unit over the yearlong study as a function of the mass of carcasses in each unit (kg), provides a more accurate comparison of risk associated with pathogen discharge from disposal units. As reflected in Figure 11, standardizing the loads of *E. coli* from each disposal unit further illustrates the diversity in performance. The loads from SBC (1.84×10^7 CFUs/kg) and SBC+B (2.14×10^8 CFUs/kg) are at least one log lower than the loads from all other units. The loads from WCC and GCC+B (1.15×10^9 and 3.47×10^9 CFUs/kg, respectively) are one log lower than the loads from WCC+B and GCC (5.18×10^{10} and 1.37×10^{10} CFUs/kg, respectively).

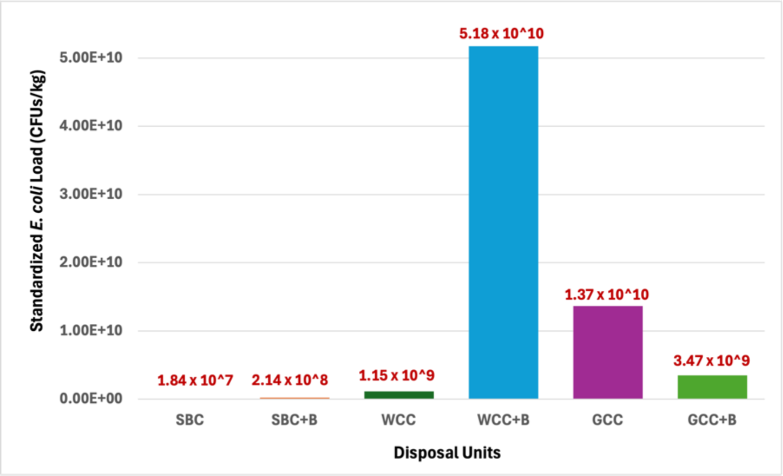

Figure 11. Standardized *E. coli* loads (CFUs/kg of carcasses) by disposal unit, October 2022 to October 2023

Objective 2. Quantify the transport of contaminants in leachate generated during composting and shallow burial with carbon (SBWC), with and without addition of biochar, through at least two different soil types.

Column Leachate Volume. Leachate production from columns occurred beginning on day 6 and continued throughout the study termination (Figures 12a and 12b). As expected, daily leachate production was typically greater for SCL than SC soil, though no evidence of a three-way interaction among soil type x leachate x day was observed. Mean volume of leachate by soil type and leachate source (Figure 13) reflected some significant interactions between soil type and leachate source. Mean leachate volume for silty clay (SC) soil receiving water only was numerically less than mean volumes for SC receiving L or LB treatments, though not statistically significant ($p < 0.05$). Conversely, the volume of leachate produced by SCL soil receiving water only was significantly greater than the volume for the same soil receiving L, and numerically greater than SCL soil receiving LB. The inclusion of biochar in disposal units may contribute to characteristics of leachate that alter flow dynamics in soil beneath disposal units, but not enough evidence exists to promote the use of biochar based upon this data.

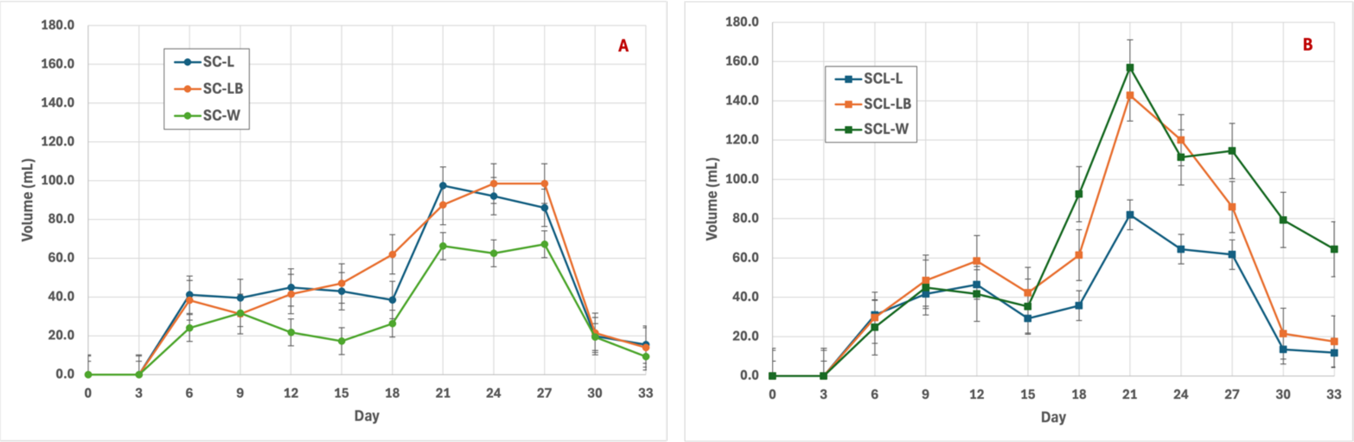

Figure 12. Mean daily volume of leachate collected from soil columns for (A) silty clay soil and (B) sandy clay loam soil; error bars represent standard errors.

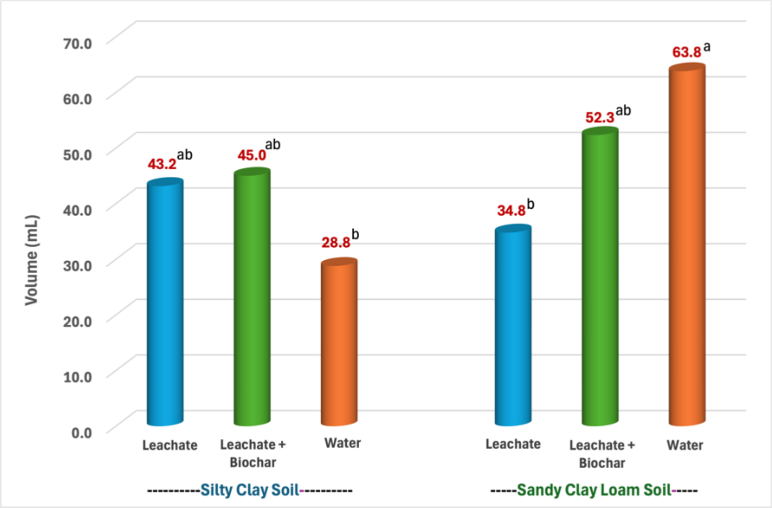

Figure 13. Mean daily volume of leachate collected from soil columns; columns sharing a superscript are not significantly different ($p < 0.05$)

Column Leachate Characteristics. A significant interaction between treatment and day ($p<0.05$) was observed for pH of column leachate among treatments. No evidence of treatment effects was found for EC, though an interaction was observed between soil type and day ($p<0.05$). Concentrations of NO_3, TKN, TOC, and TDS in leachate peaked on day 9 and decreased steadily thereafter. As expected, concentrations of NO_3, TKN, and TDS in leachate from SC soil were notably lower than from SCL soils, reflecting improved nitrogen retention with greater soil organic matter concentration. TOC concentrations were not significantly affected by treatment or soil type. While TDS concentrations were greater in leachate from SCL soil, leachate treatment did not significantly impact TDS concentration.

Retention of Contaminants in Column Soil. A *three-way interaction* (soil x treatment x depth) was observed only for K, Mn, Cu, and S (Table 3). Potassium concentration decreased significantly with depth in both SC and SCL soils receiving L or LB treatments. No effect was observed by depth or soil type where water was the treatment applied to columns. The three-way interaction was only significant for Mn concentrations in the 0-5 and 15-25 cm layers of SC soil receiving leachate (L). No other differences were observed for Mn.

Copper concentration was only significantly different between the top (0-5 cm) and bottom (15-25 cm) layers of soil in columns having SC soil and receiving leachate (L) or leachate + biochar (LB). Sulfur concentration followed a similar pattern. While some nutrients (e.g., Mn) moved more readily through SCL soil than SC soil, and others (e.g., K, Cu, and S) were retained more readily in the top (0-5 cm) layer of the SC soil receiving L or LB treatments, none of these nutrients were found in concentrations that are concerning, particularly give the relatively normal pH (e.g., pH = 7.0) of the soils used in the study.

Table 3. LS Means of nutrients for which a three-way interaction was observed

Soil x TRT x Depth	K *(mg/kg)*	Mn *(mg/L)*	Cu *(mg/kg)*	S *(mg/kg)*
Silty Clay (SC) Soil				
Leachate				
0 – 5 cm	731.0^{d}	176.8^{a}	2.13^{ab}	12.5^{ab}
5 – 15 cm	327.8^{a}	104.0^{abc}	1.60^{bcd}	9.3^{abcde}
15 – 25 cm	297.8^{a}	72.8^{bcd}	1.38^{cd}	7.3^{cdef}
Leachate + Biochar				
0 – 5 cm	872.8^{e}	119.0^{ab}	2.20^{a}	13.0^{a}
5 – 15 cm	318.5^{a}	75.0^{bcd}	1.65^{bcd}	9.0^{abcde}
15 – 25 cm	296.3^{a}	86.8^{bcd}	1.53^{cd}	7.0^{cdef}
Water				
0 – 5 cm	289.8^{a}	31.3^{cd}	1.18^{cd}	4.3^{f}
5 – 15 cm	287.0^{a}	27.0^{cd}	1.25^{cd}	5.0^{ef}
15 – 25 cm	283.5^{a}	68.5^{bcd}	1.28^{cd}	6.0^{def}
Sandy Clay Loam (SCL) Soil				
Leachate				
0 – 5 cm	481.0^{bc}	72.8^{bcd}	1.10^{cd}	8.8^{abcde}
5 – 15 cm	357.8^{ab}	88.5^{bcd}	1.35^{cd}	11.0^{abc}
15 – 25 cm	345.3^{a}	91.3^{bcd}	1.30^{cd}	10.8^{abc}
Leachate + Biochar				
0 – 5 cm	520.5^{c}	75.0^{bcd}	1.23^{cd}	8.5^{bcdef}
5 – 15 cm	342.5^{a}	88.5^{bcd}	1.38^{cd}	10.0^{abcd}
15 – 25 cm	333.3^{a}	90.5^{bcd}	1.25^{cd}	10.3^{abcd}
Water				
0 – 5 cm	273.5^{a}	90.5^{bcd}	1.25^{cd}	8.0^{cdef}
5 – 15 cm	325.0^{a}	85.8^{bcd}	1.33^{cd}	10.8^{abc}
15 – 25 cm	344.5^{a}	86.8^{bcd}	1.35^{cd}	10.5^{abc}
p-value	*<0.0001*	*<0.01*	*<0.05*	*<0.05*

Soil x depth interactions were observed for B, Zn, Fe, and EC (Figure 14). Regardless, none of these chemical characteristics were measured in concentrations that present a concern for plant toxicity among crops commonly grown in Nebraska, nor do they present a concern for groundwater quality impairment.

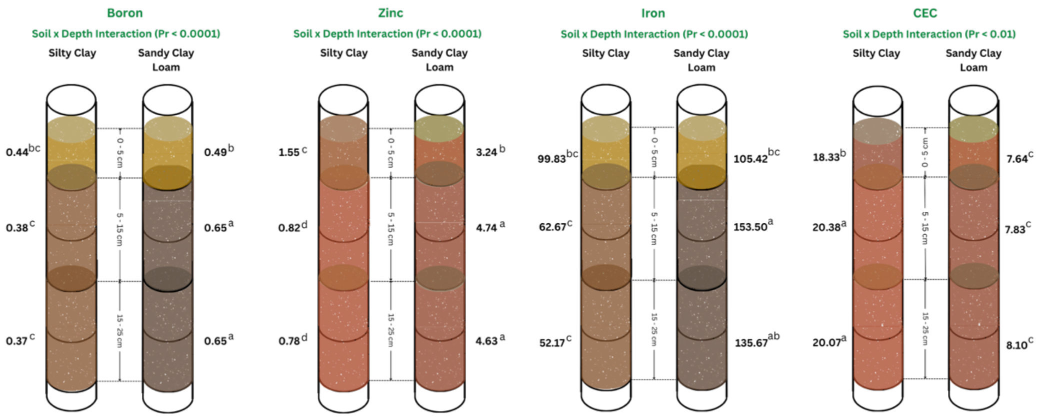

Figure 14. Concentrations of boron (B), zinc (Zn), and iron (Fe), and cation exchange capacity (CEC) by soil type and depth; values within a pair of columns having the same superscript are not different ($p < 0.05$).

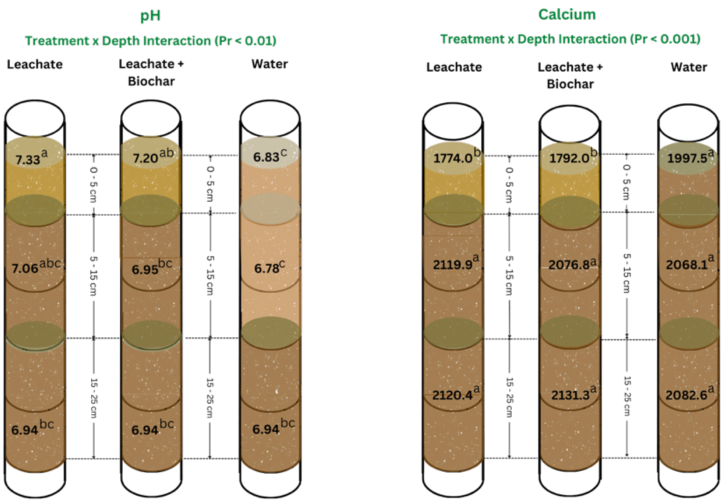

Figure 15. pH and concentration of calcium (Ca) by treatment and depth; values within a set of columns having the same superscript are not different ($p < 0.05$).

Soil x treatment interactions were observed for pH and Ca (Figure 14). pH decreased significantly with depth with the application of leachate without biochar, but did not differ for LB or W treatments. Calcium concentrations in the top 0-5 cm of soil were significantly lower for the L and LB treatments compared to the W treatment, and Ca migrated downward in soils regardless of treatment. As with other soil chemical parameters that were measured, none of these chemical characteristics present a concern for plant toxicity among crops commonly grown in Nebraska, nor do they present a concern for groundwater quality impairment. Interestingly, no significant difference in nitrate (NO_3) concentration was observed among soil types, depths, or leachate treatments, which is critically important given the significant detriment to groundwater quality that can result from nitrogen leaching through soils. A longer study may better represent the fate of nitrogen compounds in soil as a function of carcass disposal system and modeling of nitrogen fate and transport will be conducted using the data collected in this study.

Objective 3. Evaluate the potential effectiveness of composting and shallow burial with carbon (SBWC) when using wood mulch as the primary carbon source in each system, with and without addition of biochar, on direct destruction of *E. coli* and modeled destruction of organisms responsible for Seneca Valley Virus (SVV), Foot and Mouth Disease (FMD), and African Swine Fever (ASF) based on temporal and spatial monitoring of temperature, pH, microbial community, and carcass decomposition over time within the matrices of each disposal setting.

While the direct impacts of pile characteristics (temperature, pH, etc.) on the persistence of the causative agents of SVV, FMD, and ASF were not evaluated, the collection of pile characteristics data provides a basis from which to predict the effectiveness of the tested disposal methods on the destruction of these critically important pathogens. While achieving target temperatures for pathogen destruction in the disposal units is one goal, the time over which those temperatures are sustained provides a more accurate prediction of the capability for pathogen destruction. Standards published by the U.S. Environmental Protection Agency (USEPA) under 40 CFR Part 503 describe the pathogen and vector attraction reduction requirements that must be met for the use or disposal of Class B sewage sludge biosolids to protect public health. Because similar standards do not exist for livestock manure or mortality compost, the EPA's "503b" standard is often referenced when considering mortality compost temperature targets. Under this rule (40 CFR Part 503b), achieving a significant reduction in pathogens during composting requires that the compost be maintained at or above 40°C for at least 5 days, with temperatures exceeding 55°C for at least 4 hours during this period.

The minimum, maximum, and mean (n=8) temperatures recorded in each disposal unit (Table 4) demonstrate better overall performance of the SBC units (regardless of biochar inclusion) than the WCC or GCC units, with mean maximum temperatures achieved in the SBC units exceeding the "503b" standard of 55°C. Likewise, the time (days) over which a mean temperature of at least 55°C was maintained in each unit reflects that SBC units achieved higher temperatures for much longer periods of time than the WCC or GCC units. While a greater maximum temperature (65.7°C) was achieved in the SBC treatment with biochar addition (SBC+B) than without biochar addition (SBC) (64.6°C), there is insufficient evidence to conclude that the biochar addition was the causative factor.

Table 4. Minimum, maximum, and mean temperatures (± SD) recorded and time over which a temperature of ≥55°C was maintained in disposal units

Treatment	**Min Temp** (°C)	**Max Temp** (°C)	**Mean Temp** (°C)	**Time ≥ 55°C** *(days)*
SBC	30.0 ± 4.6	64.6 ± 2.7	39.4 ± 7.4	24.1
SBC+B	28.9 ± 2.9	65.7 ± 1.9	38.3 ± 7.6	27.3
GCC	-13.0 ± 5.6	51.3 ± 20.1	19.9 ± 16.4	7.0
GCC+B	0.3 ± 12.2	51.9 ± 18.5	22.2 ± 14.7	6.4
WCC	-2.3 ± 4.6	47.3 ± 20.2	19.3 ± 13.1	8.4
WCC+B	0.3 ± 3.3	69.9 ± 1.8	29.6 ± 19.6	27.6

The mean temperature profile (n=8 monitoring locations) inside each disposal unit throughout the yearlong monitoring period are displayed in Figure 14. Temperatures remained steadier throughout the yearlong study for SBC and SBC+B disposal units while WCC, WCC+B, GCC, and GCC+B units experienced greater daily fluctuations in temperature. While the WCC+B unit experienced higher temperatures than the WCC unit in the final four months of the study, there is no indication that biochar was responsible for this temperature difference.

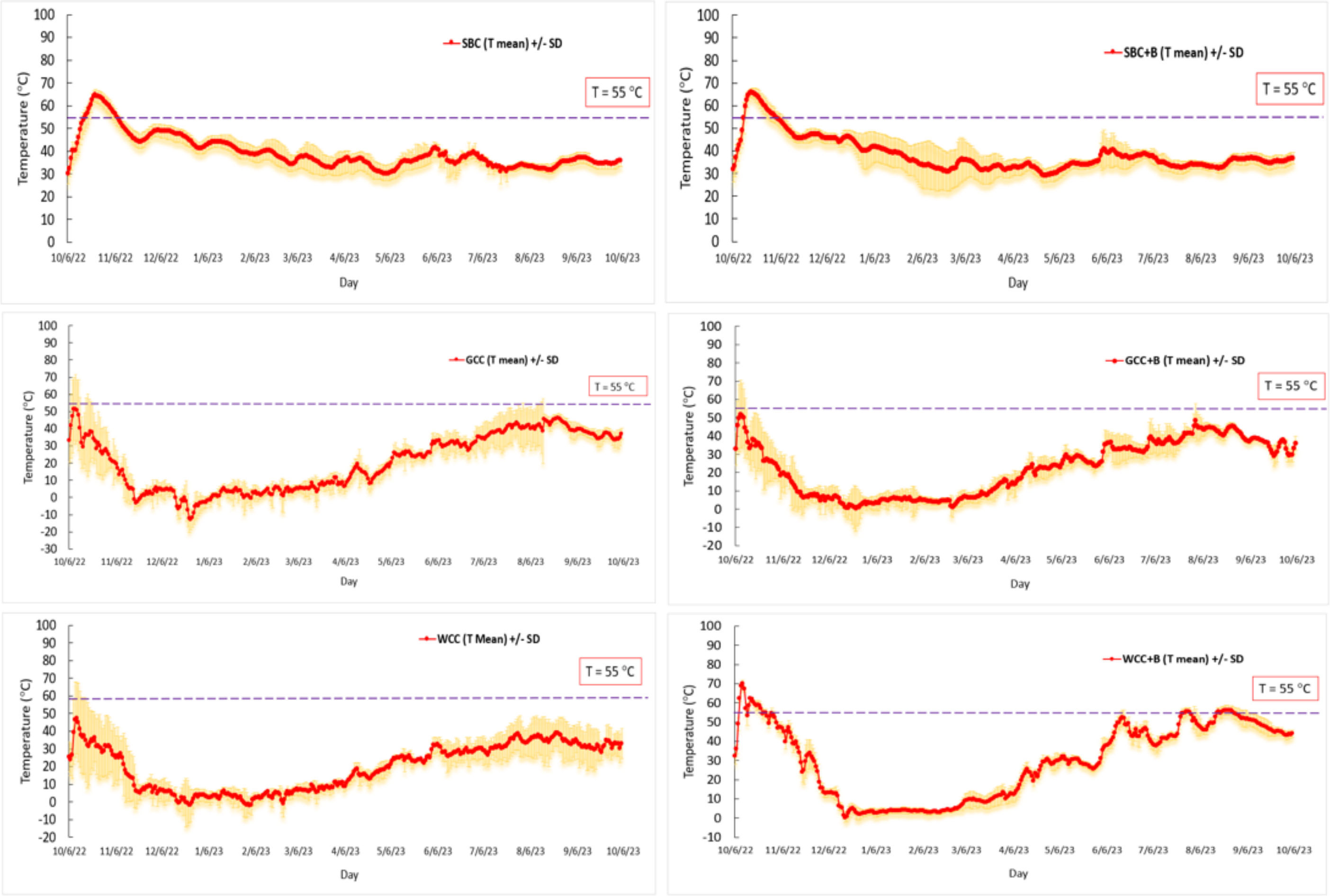

Figure 16. Mean temperatures within each disposal unit throughout the yearlong study; SD shown in yellow

Reactive oxygen species (RONS) such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH) are potent antimicrobial agents that induce oxidative stress, leading to damage to cellular macromolecules like DNA, lipids, and proteins, ultimately causing microbial cell death (Alfei et al., 2024). The highest mean RONS concentration (382.1 µM) was observed for leachate samples from the GCC unit while the lowest concentration (1.2 µM) was observed for leachate samples from the GCC+B unit, though the very large SDs in all of the means negates drawing any strong conclusion about the role of the disposal method in RONS concentration (Table 5).

Table 5. Mean H_2O_2 concentrations (±SD) in leachate samples (n=11) by disposal unit

Mean H_2O_2 (µM)					
SBC	**SBC+B**	**WCC**	**WCC+B**	**GCC+B**	**GCC**
98.7 ± 309.8	101.0 ± 267.9	95.5 ± 199.7	124.5 ± 288.1	1.2 ± 3.9	382.1 ± 640.2

SBC: shallow burial with carbon; WCC: whole carcass composting; GCC: ground carcass composting; B: biochar

Discussion

Leachate generation varied considerably across treatments, with SBC and SBC+B units producing the lowest cumulative volumes (624 and 1,138 L, respectively). **Lower leachate volumes in SBC treatments suggest that this carcass disposal method poses less risk for nutrient leaching and groundwater pollution than either of the composting systems.** In contrast, the WCC and GCC methods appear to present much more substantial environmental risk, particularly in areas with high precipitation, shallow groundwater tables, and/or well-drained soils.

The **SBC units** (regardless of biochar inclusion) **experienced higher mean temperatures for much longer periods of time than the WCC or GCC units**, with mean maximum temperatures achieved in the SBC units exceeding the "503b" standard of 55°C.

The SBC and SBC+B disposal units yielded numerically lower mean concentrations and standardized loads of *E. coli* than other treatments, though no *E. coli* results were significant among disposal methods nor disposal method x biochar inclusion.

Gleaning value from the analysis of reactive oxygen and nitrogen species (RONS) proved difficult given large standard deviations in these measurements for all disposal units. While this characteristic of carcass disposal units may aid in predicting the effectiveness of disposal methods for mitigating ASFV transmission, the complexity of the interaction with viruses and the need for their application to be targeted indicate the further need for research to optimize their use as indicators of potential ASFV reduction. Understanding these dynamics will be paramount in devising efficient management strategies against ASFV and other viral infections.

The soil column investigation uncovered variations in nutrient transport through silty clay (SC) and sandy clay loam (SCL) soils and some differences in transport as a function of leachate treatment. Lower transport rates of nutrients in the SC soil compared to SCL soil indicate a lower risk for groundwater contamination from carcass disposal in poorly drained soils than well-drained soils, as expected. In contrast, retention of some chemicals in the top layer of soil (0-5 cm) could present a toxicity risk to crops if a single location is used repeatedly for carcass disposal. In general, disposal via SBC appears to be suitable in both poorly- and well-drained soils given the significantly lower volume of leachate generated by SBC units in this study.

Implications for Sustainable Carcass Disposal. Among the techniques analyzed – shallow burial with carbon (SBC), whole carcass composting (WCC), and ground carcass composting (GCC), each with and without the addition of biochar – **SBC treatments exhibited the most favorable overall performance in terms of both biosecurity and natural resource protection.** SBC treatments reached and maintained higher mean temperatures for longer periods of time than WCC or GCC units, with temperatures exceeding "503 b" standards for pathogen reduction in compost. Prolonged thermophilic conditions in SBC treatments coupled with significantly lower leachate production compared to WCC and GCC methods demonstrates that SBC is a favorable carcass disposal method during routine mortality events and may offer desired biosecurity during disposal of carcasses resulting from a foreign animal disease (FAD). Carcass disposal via SBC is advised for areas with well-drained soils and/or elevated groundwater levels as the data collected during this study suggests much lower risk to groundwater contamination from this disposal method compared to whole or ground carcass composting.

Limitations and Future Research. Due to resource limitations, replication of the six treatments was not possible. However, the six disposal units constructed for this study remain operable and could accommodate future studies with two replications of SBC and up to four replications of composting. Further evaluation of ground carcass composting is not recommended based upon the results of the current study. Likewise, addition of biochar to swine carcass disposal units does not warrant further evaluation unless significant availability of biochar at a near-zero price point emerges. The results of this project suggests that future inquiries should focus on WCC vs. SBC with replications of disposal methods and utilization of different carbon sources to determine if higher pile temperatures and lower leachate production can be accomplished with either disposal method.

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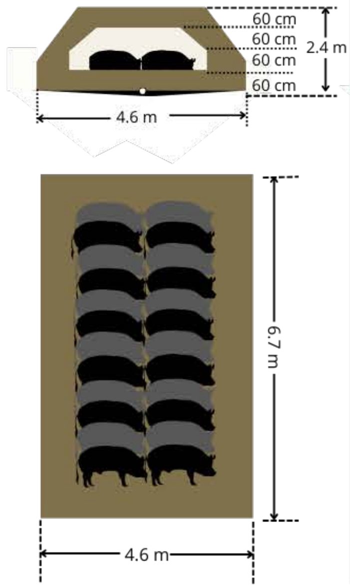
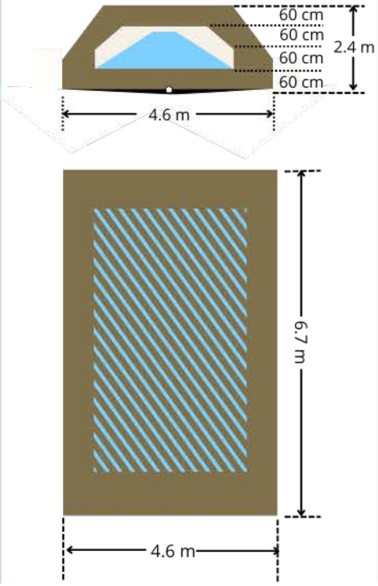
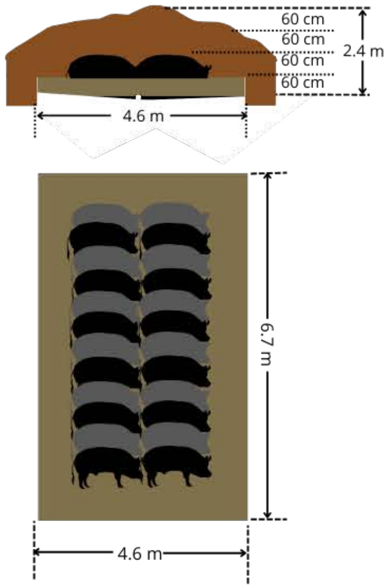
Whole Carcass Composting



Ground Carcass Composting

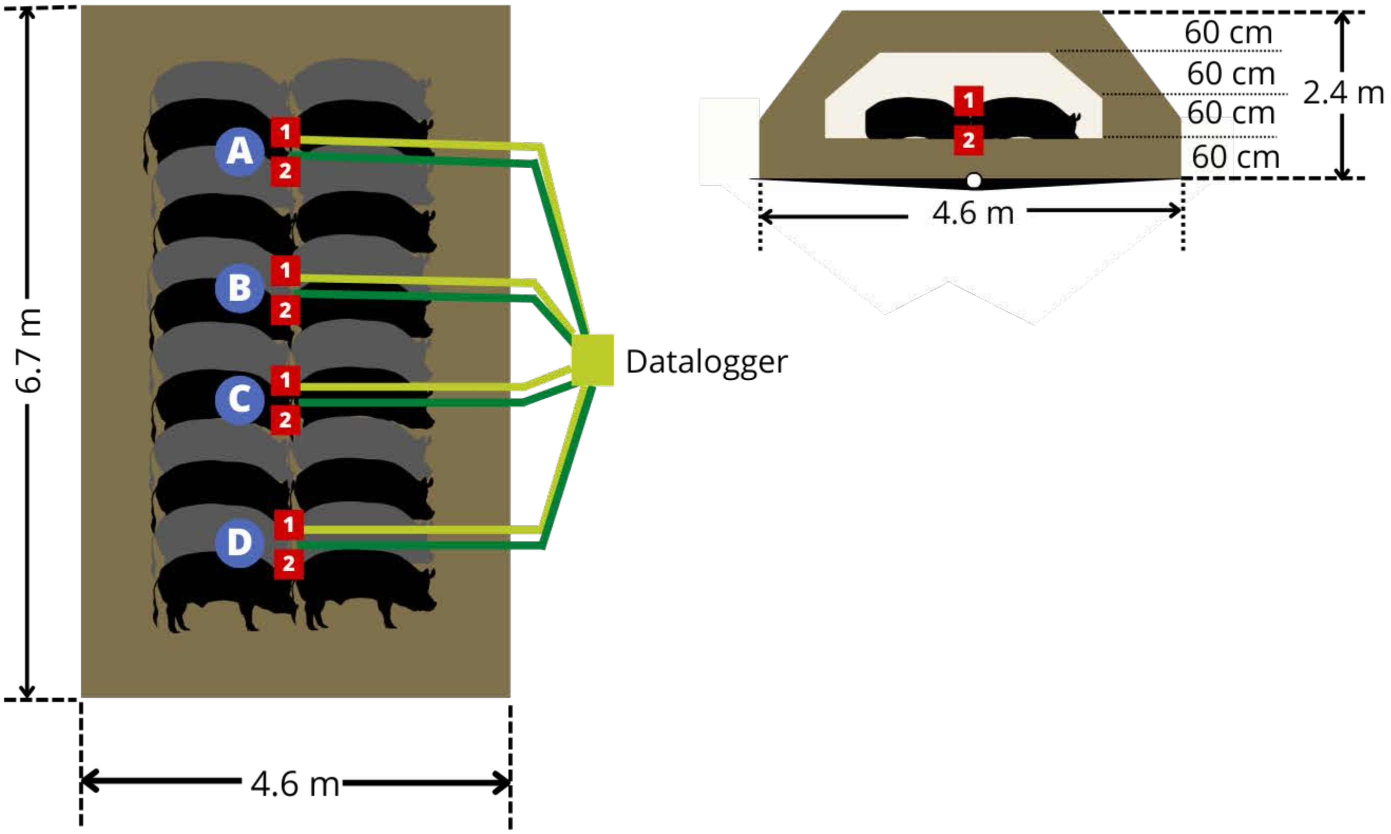


Shallow Burial w/Carbon



Composting Units

TC Wires, evenly spaced down roughly the centerline of the disposal units, one set of four wires below and one set of four wires on top of the carcasses

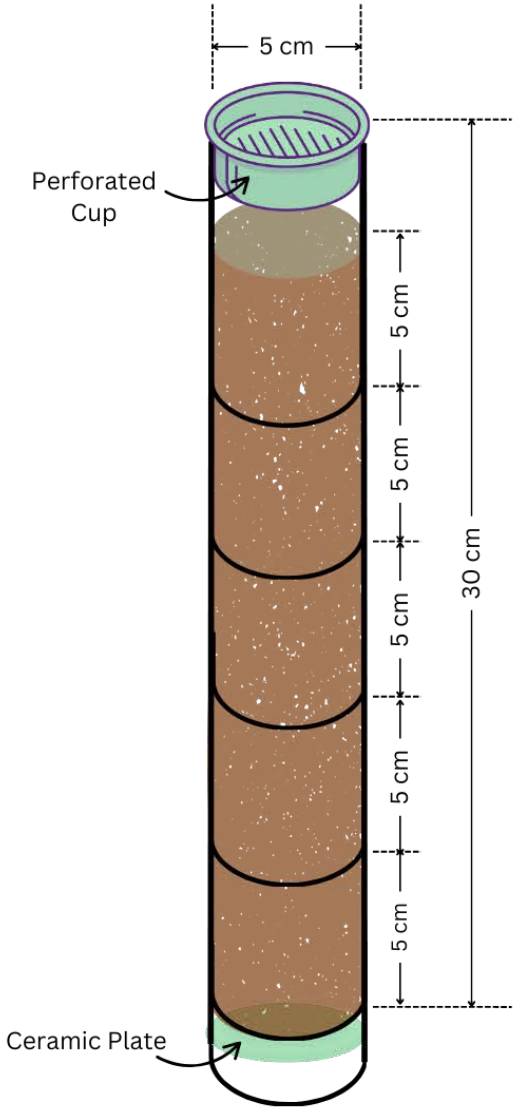


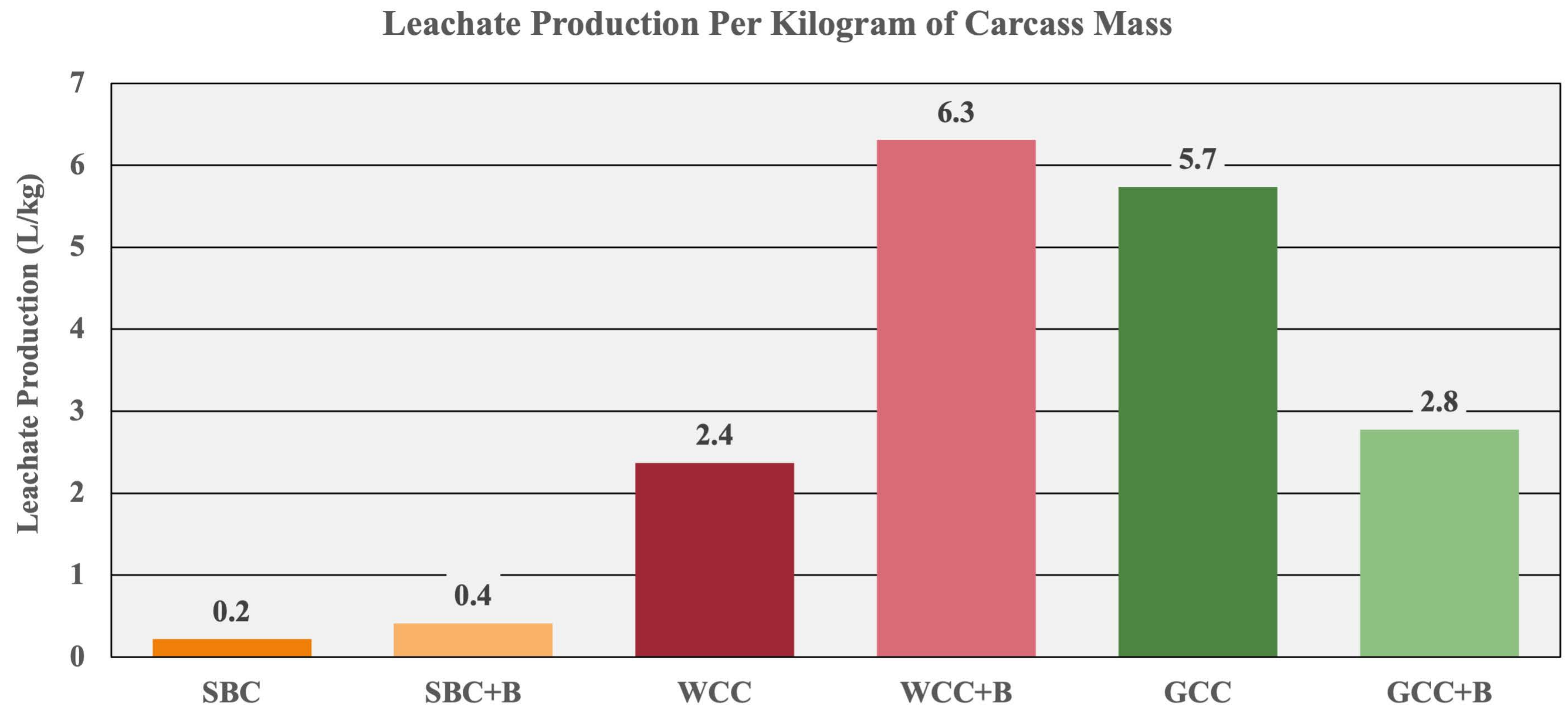
▨ Wood Mulch

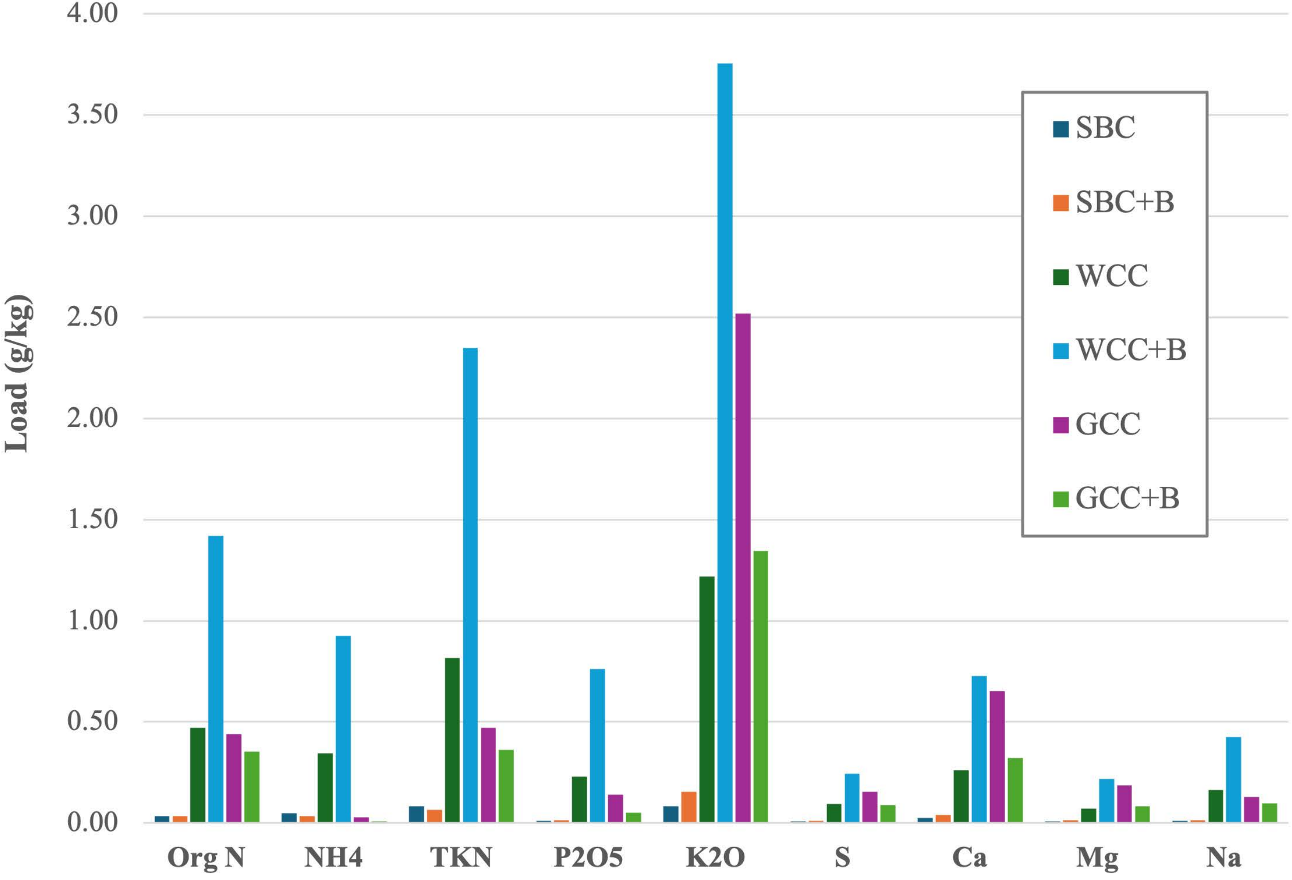
○ Leachate Collection Pipe

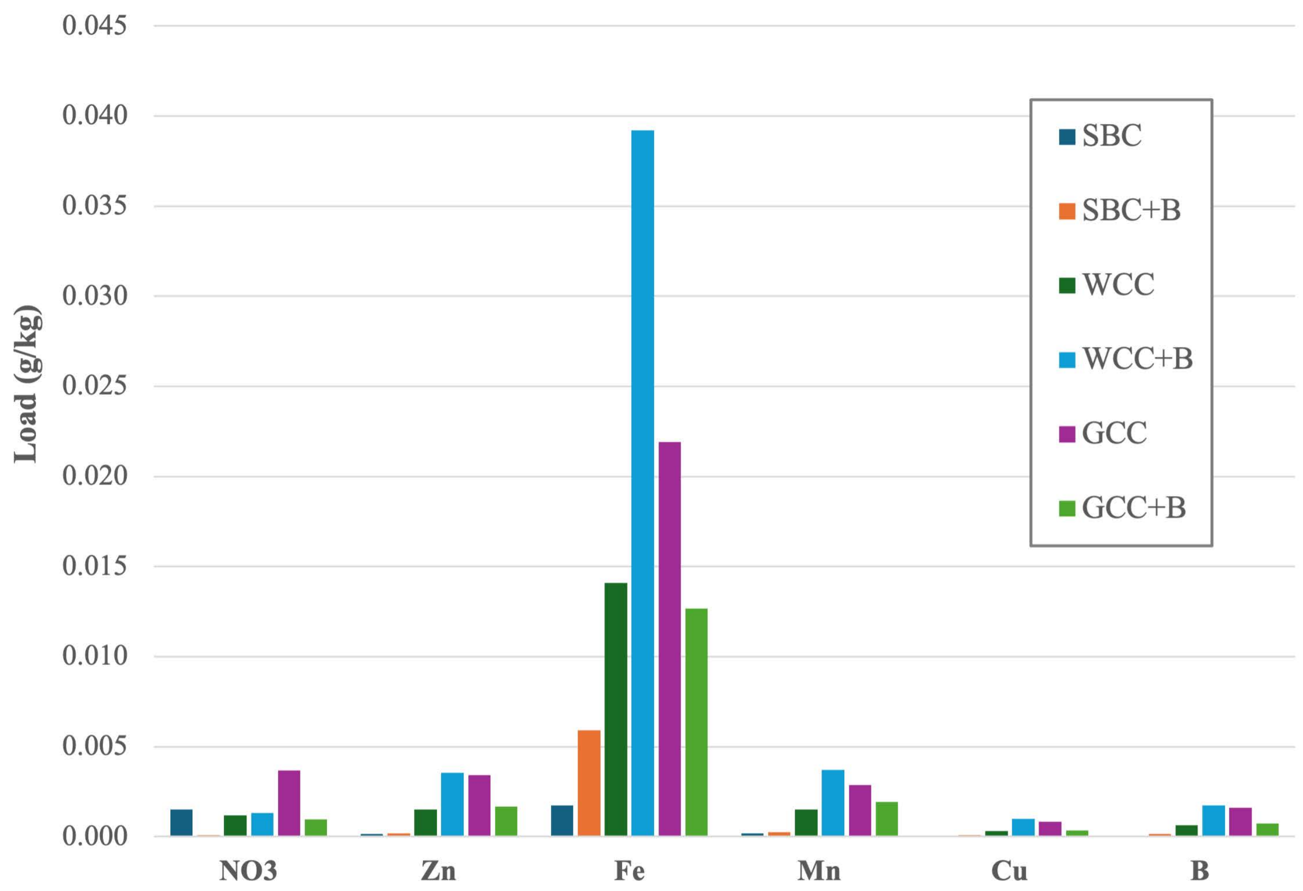
Notes:

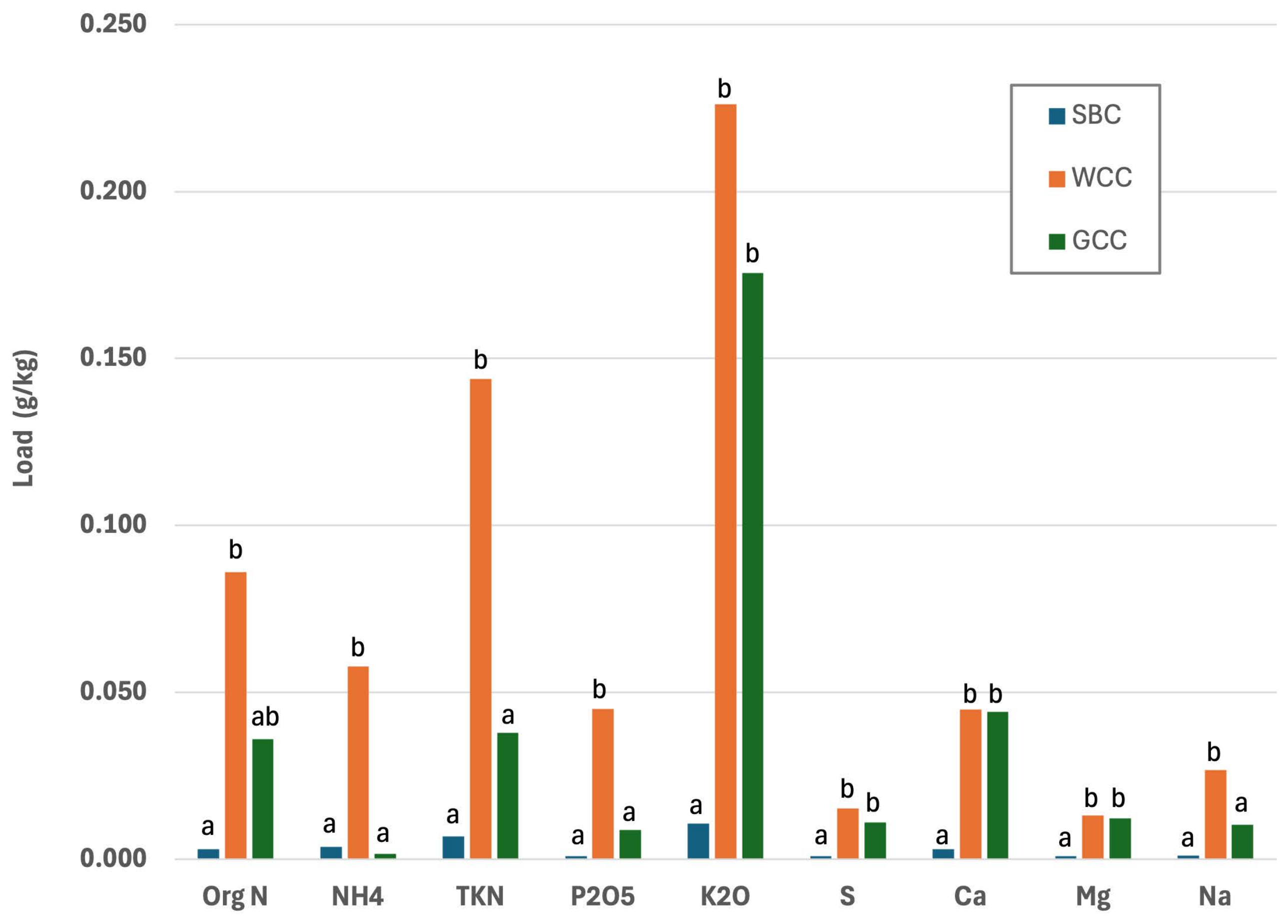
1. Grade soil beneath compost piles to drain leachate from perimeter to center line of piles and to south end of piles.
2. Install perforated pipe along bottom centerline of prepared base and cover with at least 6 inches of crushed limestone.


5 cm
Perforated Cup
5 cm
5 cm
5 cm
5 cm
5 cm
30 cm
Ceramic Plate


Leachate Production Per Kilogram of Carcass Mass
Leachate Production (L/kg)
7
6
5
4
3
2
1
0
0.2
0.4
2.4
6.3
5.7
2.8
SBC
SBC+B
WCC
WCC+B
GCC
GCC+B


4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Load (g/kg)
Org N
NH4
TKN
P2O5
K2O
S
Ca
Mg
Na
SBC
SBC+B
WCC
WCC+B
GCC
GCC+B


Load (g/kg)
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
NO3
Zn
Fe
Mn
Cu
B
SBC
SBC+B
WCC
WCC+B
GCC
GCC+B


Load (g/kg)
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0.200
0.150
0.100
0.050
0.000
Org N
NH4
TKN
P2O5
K2O
S
Ca
Mg
Na
SBC
WCC
GCC
a
b
ab
a
b
a
a
b
a
a
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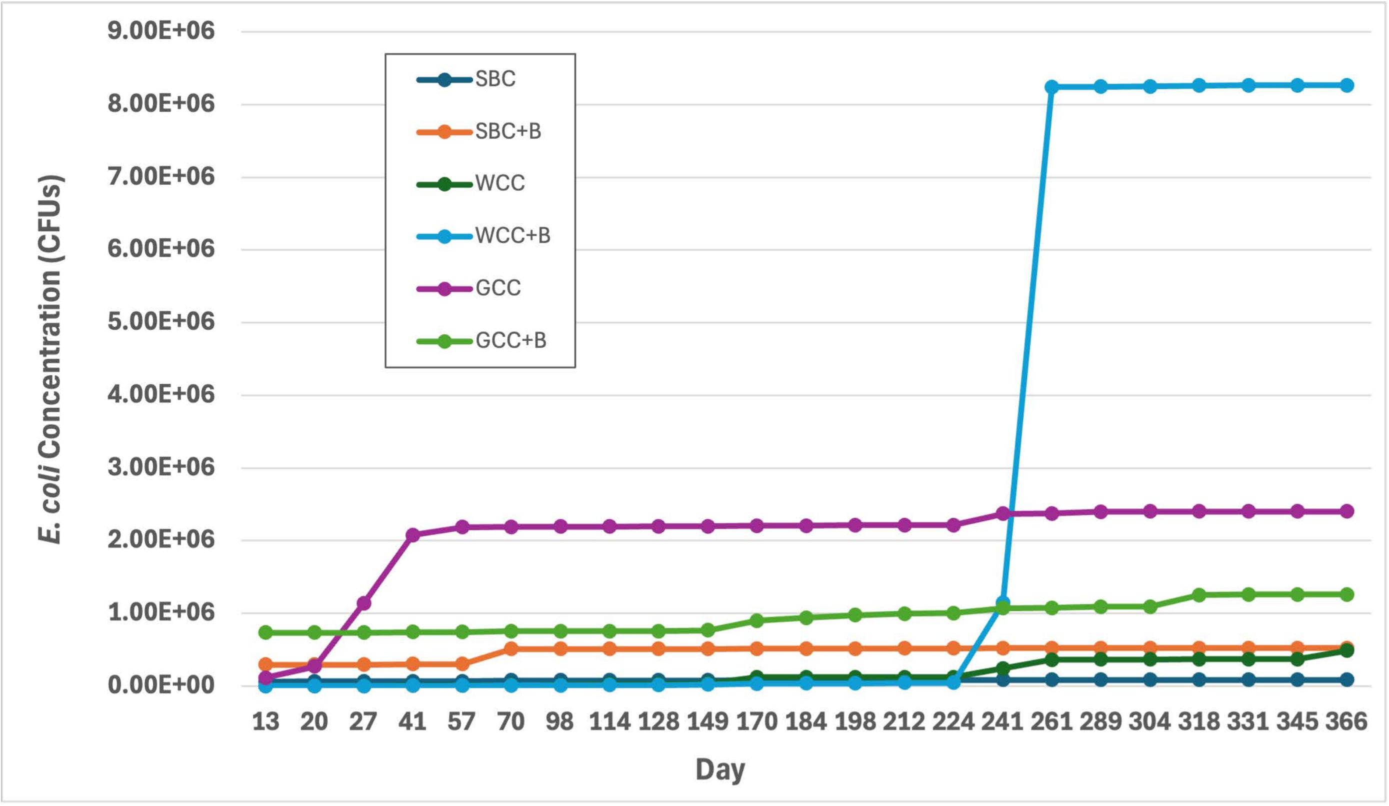
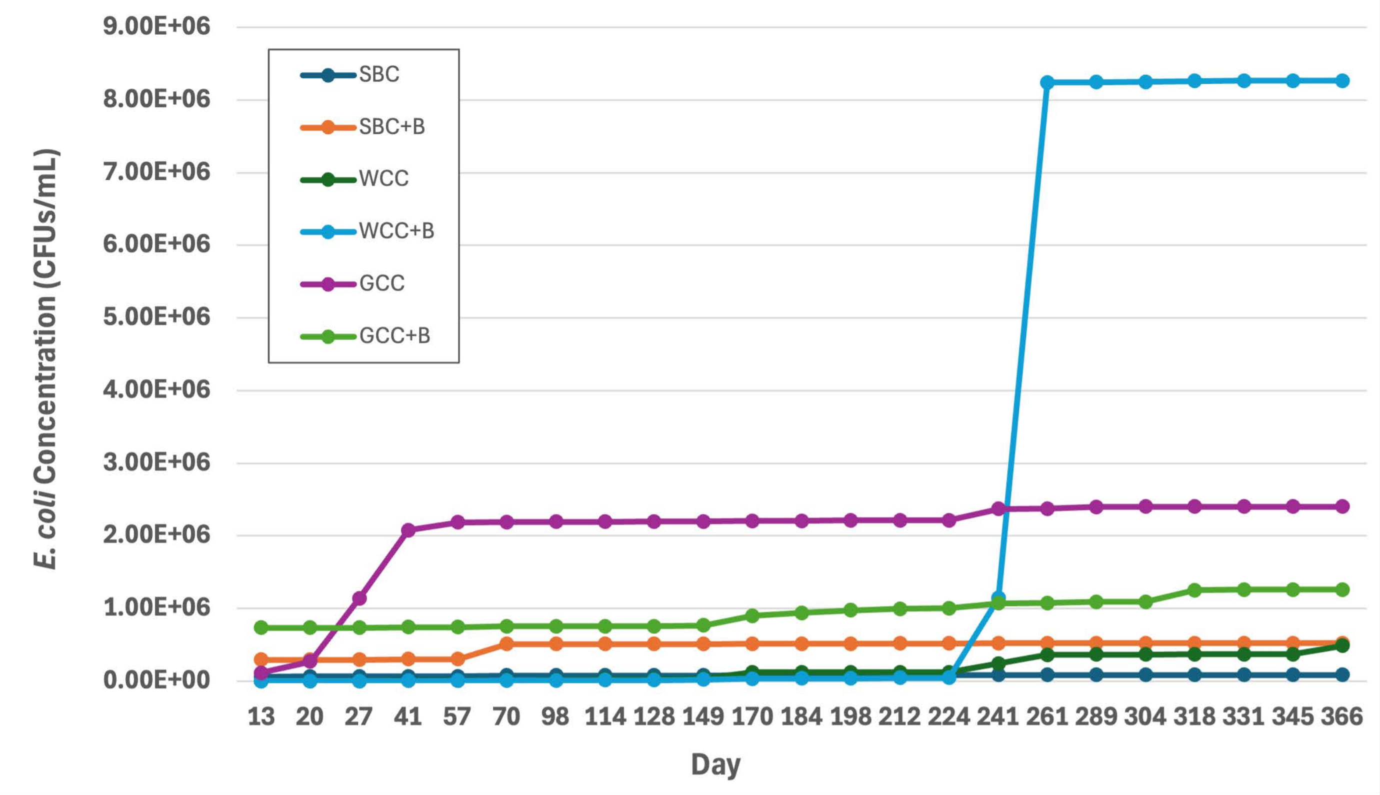
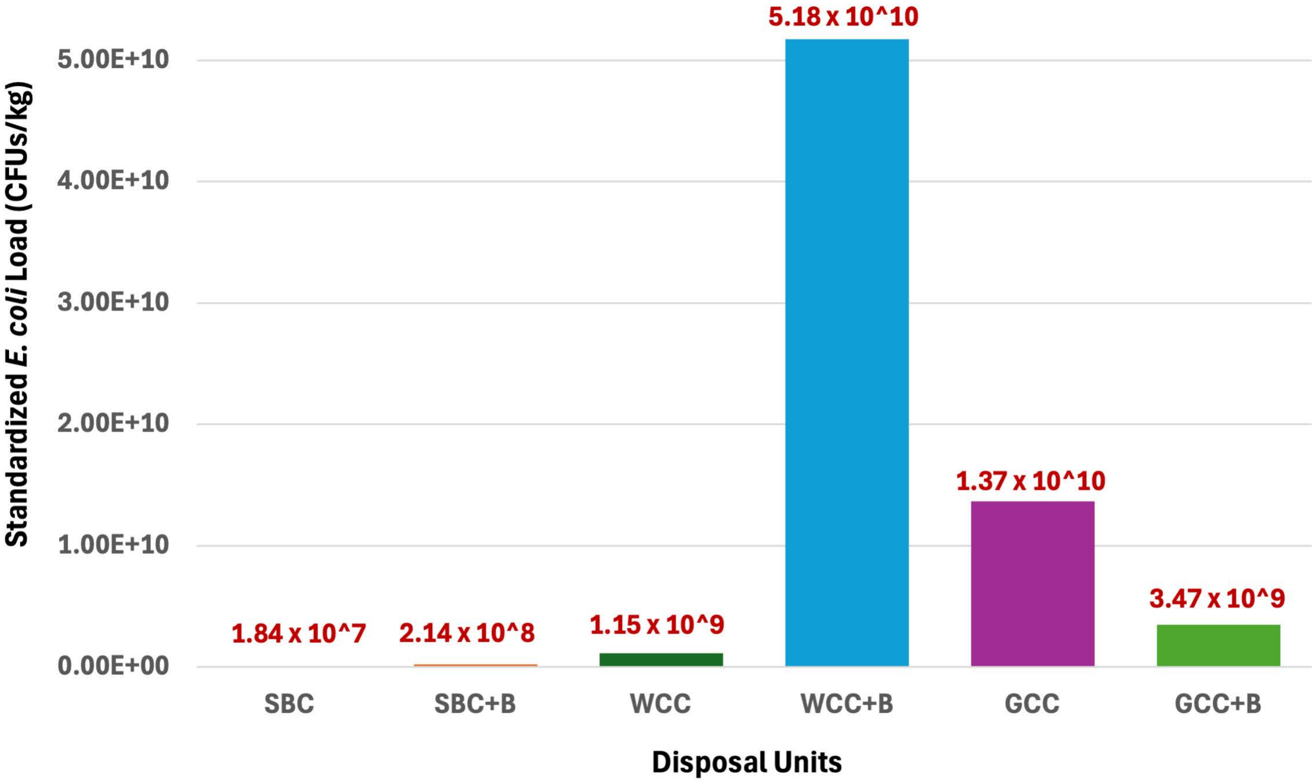
b
a


9.00E+06
8.00E+06
7.00E+06
6.00E+06
5.00E+06
4.00E+06
3.00E+06
2.00E+06
1.00E+06
0.00E+00
E. coli Concentration (CFUs)
SBC
SBC+B
WCC
WCC+B
GCC
GCC+B
13
20
27
41
57
70
98
114
128
149
170
184
198
212
224
241
261
289
304
318
331
345
366
Day


9.00E+06
8.00E+06
7.00E+06
6.00E+06
5.00E+06
4.00E+06
3.00E+06
2.00E+06
1.00E+06
0.00E+00
E. coli Concentration (CFUs/mL)
SBC
SBC+B
WCC
WCC+B
GCC
GCC+B
13
20
27
41
57
70
98
114
128
149
170
184
198
212
224
241
261
289
304
318
331
345
366
Day


Standardized *E. coli* Load (CFUs/kg)
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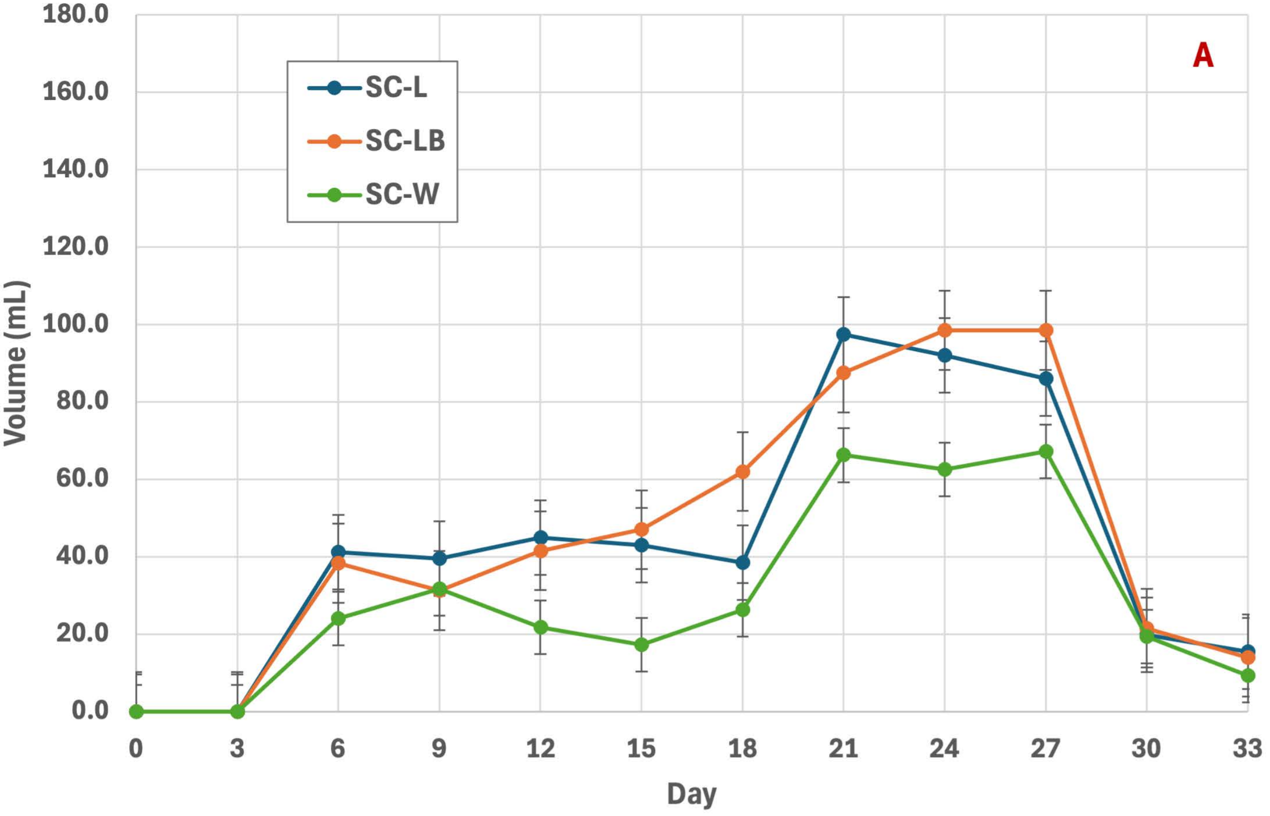
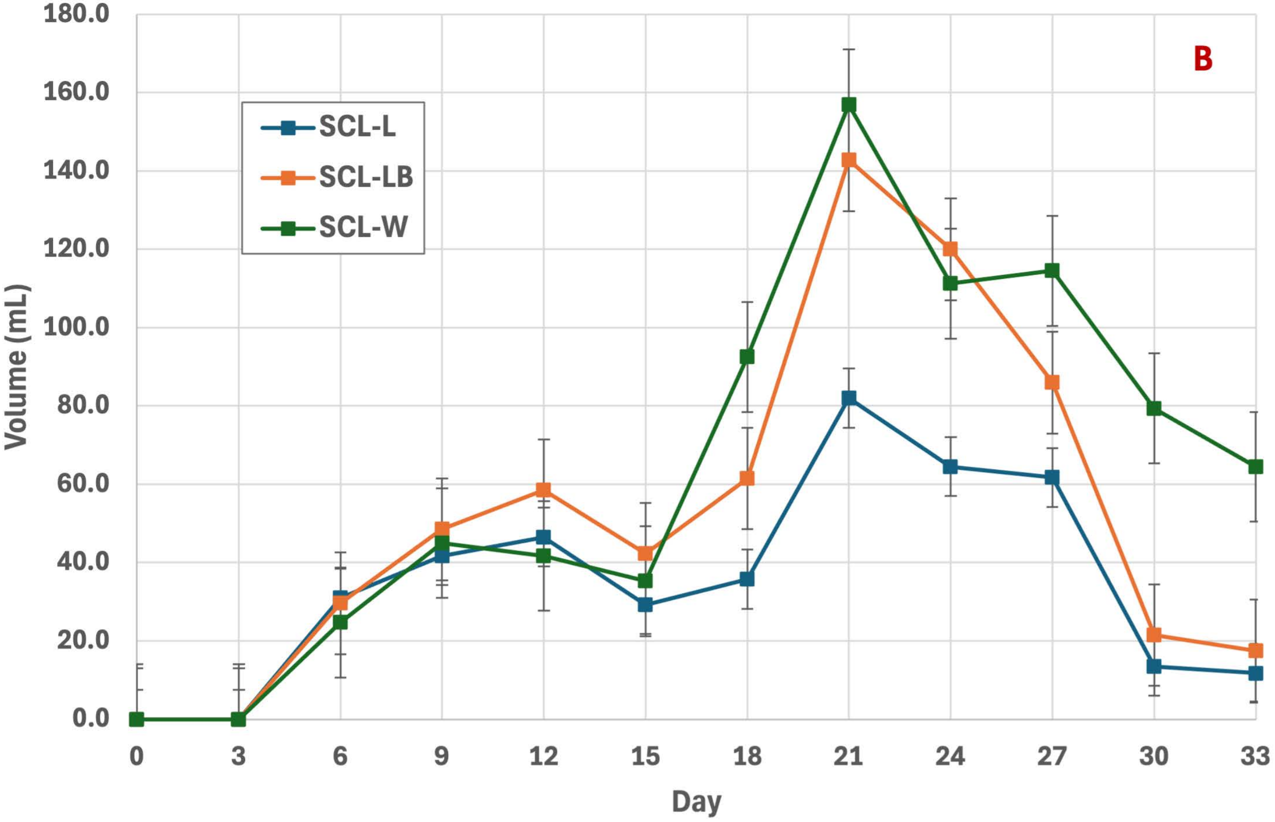
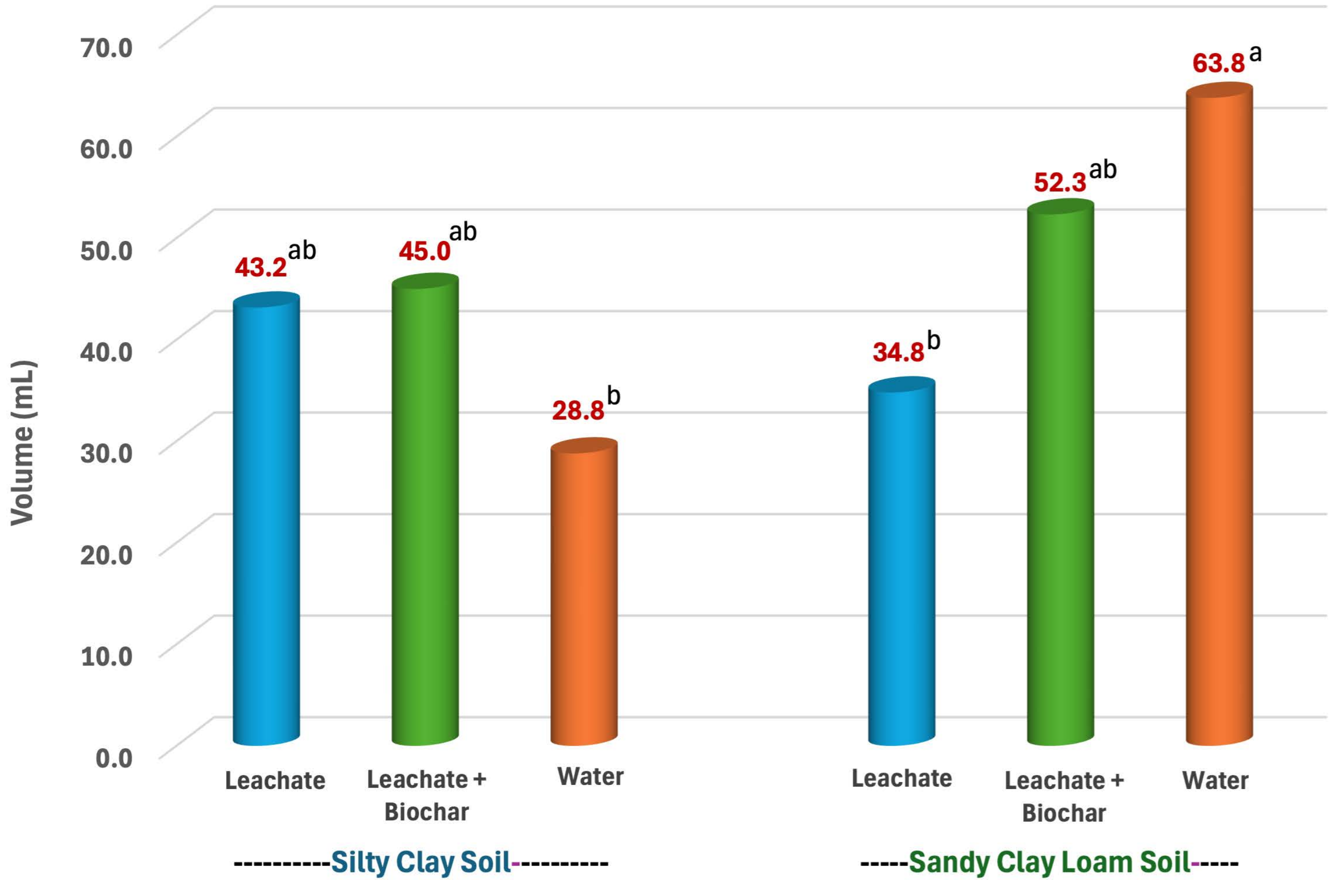
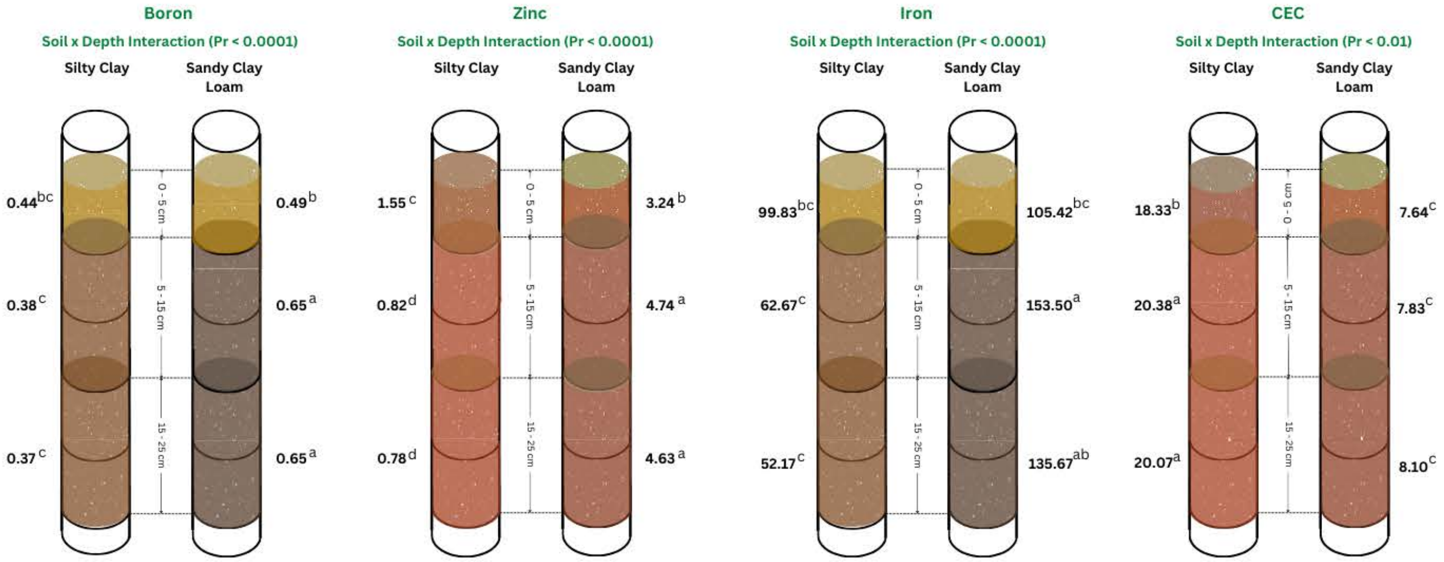
4.00E+10
3.00E+10
2.00E+10
1.00E+10
0.00E+00
1.84 x 10^7
2.14 x 10^8
1.15 x 10^9
5.18 x 10^10
1.37 x 10^10
3.47 x 10^9
SBC
SBC+B
WCC
WCC+B
GCC
GCC+B
Disposal Units


A
SC-L
SC-LB
SC-W
Volume (mL)
180.0
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Day
0
3
6
9
12
15
18
21
24
27
30
33


B
SCL-L
SCL-LB
SCL-W
Volume (mL)
180.0
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Day
0
3
6
9
12
15
18
21
24
27
30
33


Volume (mL)
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
43.2^ab
45.0^ab
28.8^b
34.8^b
52.3^ab
63.8^a
Leachate
Leachate + Biochar
Water
Leachate
Leachate + Biochar
Water
----------Silty Clay Soil----------
-----Sandy Clay Loam Soil-----


Boron
Soil x Depth Interaction (Pr < 0.0001)
Silty Clay
Sandy Clay Loam
0.44bc
0.38c
0.37c
0.49b
0.65a
0.65a
0 - 5 cm
5 - 15 cm
15 - 25 cm
Zinc
Soil x Depth Interaction (Pr < 0.0001)
Silty Clay
Sandy Clay Loam
1.55c
0.82d
0.78d
3.24b
4.74a
4.63a
0 - 5 cm
5 - 15 cm
15 - 25 cm
Iron
Soil x Depth Interaction (Pr < 0.0001)
Silty Clay
Sandy Clay Loam
99.83bc
62.67c
52.17c
105.42bc
153.50a
135.67ab
0 - 5 cm
5 - 15 cm
15 - 25 cm
CEC
Soil x Depth Interaction (Pr < 0.01)
Silty Clay
Sandy Clay Loam
18.33b
20.38a
20.07a
7.64c
7.83c
8.10c
0 - 5 cm
5 - 15 cm
15 - 25 cm