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The Transport and Persistence of *Escherichia coli* in Leachate from Poultry Litter Amended Soils

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Abstract

Fecal coliform bacteria such as *Escherichia coli* (*E. coli*) are one of the main sources of groundwater pollution. An assessment of the transport and Persistence of *E. c*oli in poultry litter amended Decatur silty Clay soil and Hartsells Sandy soil was conducted using soil columns and simulated groundwater leaching. Enumeration of initial *E. coli* was determined to range from 2.851 × 10^3 to 3.044 × 10^3 CFU per gram of soil. These results have been used in a batch study to determine the persistence rate of *E. coli* in Decatur silty Clay soil and Hartsells Sandy soil. Results prove that *E. coli* survival growth rate increases for clay soil later than and at a higher rate than sandy soil. The column study has determined that *E. coli* was transported at a rate of 3.7 × 10^6 CFU for Decatur silty loam and 6.3 × 10^6 CFU for Hartsells sandy per gram of soil. Further, linear regression analysis predictions show higher porosity and soil moisture content affect transport, and Hartsells sandy soil has higher transport of *E. coli* due to its higher porosity and lower volumetric water content.

Keywords

Transport, Leachate, Persistence, Poultry Litter, *E. coli*

1. Introduction

Groundwater is of high importance and should not be jeopardized by pathogenic bacteria such as Escherichia coli (*E. coli*). Moreover, efforts should be made to attain a quality of groundwater that is as clean as possible for drinking [1]. *E. coli* is commonly found in the fecal matter of animals, and it is often used as a fertilizer [2]. Poultry litter, for example, is commonly used in the southeastern

United States as a low-cost fertilizer [3]. It is well known that poultry litter residues contain *E. coli* [4]. Once poultry litter is broadcasted on fields and crops rainfall induced recharge can cause the transport of *E. coli* vertically into the soil and into the groundwater supplies [5]. This can affect the water quality of groundwater systems.

The high tonnage of poultry litter produced by the state of Alabama calls for the use of best waste management practices. One common waste management practice for poultry litter is the spreading of poultry litter onto cropped fields and pastures. The Alabama Department of Environmental Management (ADEM), along with other agencies (Natural Resources Conservation Services, Environmental Protection Agency) have guidelines for proper handling and disposal of poultry litter. Environmental officials report that the standards for each government agency are being met around the state. However, in a 2014 report on water quality in Alabama, agriculture practices were cited as being responsible for 515 miles of impaired rivers and streams [6].

The major issue of impaired waterways stems from the application of poultry litter to croplands and pastures. It is believed that groundwater contamination could be occurring along with the impairment of rivers and streams in Alabama [1] [7]. In fact, recently the poultry litter industry has grown in Northern Alabama. The heaviest concentration of poultry farms is now in the northern part of the state in Cullman, DeKalb, and Marshall counties [6]. *E. coli* found in poultry litter can be life threatening when they are present in groundwater systems at high concentrations [1] [7]. Many areas across the United States have been impacted by the hazardous effects of the use of poultry litter as a fertilizer [8]. Specifically, when poultry litter is applied to crops for nutrients such as nitrogen, phosphorus, and potassium, fecal indicator bacteria such as *E. coli* or *Salmonella* are transported in surface water and can adversely impact water quality [8]. For example, some *E. coli* bacteria are harmless and live in the intestines of healthy humans and animals. However, several strains can produce powerful toxins and cause severe illness in humans when consumed from contaminated water sources. Importantly, *E. coli* can cause a wide variety of diseases including urinary tract infections and meningitis. The *E. coli* O157:H7 strain, which is responsible for an estimated 73,000 cases of infection and 61 deaths in the United States each year, has garnered global media coverage. These devastatingly high numbers have made the *E. coli* O157:H7 strain the most pathogenic of all bacteria [9].

In addition to human health and water quality issues, even broader environmental concerns such as ecosystem health can be influenced when poultry litter is applied to crops for nutrients. For example, when nitrogen, phosphorus, and potassium exceed plant needs, or when they are applied just before it rains, they can wash into aquatic ecosystems. They can also cause algae blooms, which can prevent swimming and boating opportunities, create foul taste and odor in drinking water, and kill fish by removing oxygen from the water. High concentrations of nitrates in drinking water can cause methemoglobinemia, a poten-

tially fatal disease in infants, also known as blue baby syndrome [10].

Thankfully there are some helpful remediations for managing risks. To combat nutrient losses, farmers implement nutrient management plans that help maintain high yields save money on fertilizers, and effectively manage nutrient needs [10]. Moreover, The Alabama Department of Environmental Management (ADEM), along with other agencies (Environmental Protection Agency (EPA), USDA-Natural Resources Conservation Services), have guidelines for proper handling and disposal of poultry litter. The goal of these Environmental officials is to meet the standards for each government agency and to maintain those standards around the state [6] [11].

To gain an understanding of the environmental risks associated with poultry litter amendments this research has assessed the factors that affect the transport and persistence of *E. coli* from poultry litter amended soils into the groundwater systems in the state of Alabama. As such, an understanding of the persistence and transport of *E. coli* in the soil and in leachate can be gained by first identifying some characteristics of soil types since soil type is an inherent quality that influences persistence and transport. Some observable soil characteristics are soil depth, soil layer thickness, soil moisture, soil texture, soil consistency, soil color, soil cracks, and soil pH [12]. Two characteristics, soil moisture and soil texture, are soil properties that appear to have the greatest impact on bacterial survival. Moisture retention is linked to particle size distribution and organic matter content [13]. Therefore, it is perceived that soil moisture content and soil texture are likely to have effects on the survival of *E. coli* in leachate from poultry litter amended soils. This research will examine soil types based on their texture and moisture holding capacity in order to determine the persistence and transport of *E. coli.*

2. Materials and Methods

2.1. Soil Column Assembly

Each column was constructed from raw materials. A total of 9 columns were constructed using 4 in. × 10 ft. PVC sewer and drainpipes with a drain assembly covered in mesh wire. A total of 2 feet of PVC sewer drainpipe was used to construct the columns. The bottom of each PVC column was fitted with a thin metal screen to prevent soil loss. Each column was filled with experimental soil. A 2-inch space was allowed at the top of each column to hold the poultry litter and E. coli inoculum. Hooks were drilled into the top of the soil column on each side at a 2-inch drop from the top. Then each soil column was hung vertically from rope to a horizontal beam inside of the metal frame ceiling of the Stillman College greenhouse. This hanging methodology allowed direct simulation of rainfall to occur over the soil surface of the columns to create vertical leaching inside each column.

2.2. Bacteria Strains and Culture Conditions

Isolates of *E. coli* ATCC 25922 were used in the soil column experiment. *E. coli*

ATCC 25922 isolates were labeled with a green, fluorescent marker and an ampicillin-resistant marker according to the method described by Sambrook *et al.* [14]. When viewed under a handheld dark reader UV lamp, transformed colonies were bright green. To maintain the plasmid in the isolates, all labeled isolates were individually grown at 37˚C for 24 hours on tryptic soy agar (TSA; Acumedia, Lansing, MI, USA) supplemented with 100 $mg \cdot ml^{-1}$ ampicillin (Roche Diagnostics, Indianapolis, IN, USA) (TSA-Amp). Preparation of inoculum involved each isolate consecutively being sub-cultured individually on TSA-Amp plates for 24 hours at 37˚C. From these plates, individual colonies were transferred into 100 ml TSB-Amp and incubated at 37˚C for 24 hours with agitation (150 rpm).

To recover the cells from the broth culture, the mixture was subjected to centrifugation (4050 g, 15 min, 4˚C), and the pellet was washed and suspended with 1 mg ml^{-1} peptone water. This operation was repeated three more times, and the final pellet was suspended in 1 $mg \cdot ml^{-1}$ peptone water to give c. 10^5 $CFU \cdot ml^{-1}$ (optical density of c. 0.5 at 630 nm). Suspensions of all *E. coli* ATCC 25922 isolates were combined in equal portions by volume, and this inoculum mixture was diluted in sterile deionized water for subsequent spraying and mixing into the poultry litter [15]. The dry weight of *E. coli* inoculated into 32 g of poultry litter was 5 log $CFU \cdot g^{-1}$.

2.3. Leachate Collection & *E. coli* Analysis

Leachate samples were collected from the container placed underneath the soil column. Additionally, at the laboratory an enrichment of each sample was conducted to determine concentrations of indicator *E. coli* [16] [17]. An analysis of indicator *E. coli* from the leachate samples was conducted using commercial Colilert® kits and the semi–automated most probable number (MPN) methodology (IDEXX, Atlanta, GA) [3] [8] [18]. This methodology used a 100 ml sample of leachate from the simulated rainfall that was created on the surface of the soil with poultry litter in the column. Enrichment broth on 100 ml of each sample was used. The samples were then poured into a Quanti-Tray®, sealed, and incubated for 24 hours at 35.5˚C for Colilert®. The Quanti-Trays were analyzed for fluorescence in a dark room underneath a UV-6-volt light to confirm the presence of *E. coli* per 100 ml of leachate and per g soil and litter were derived. Leachate samples analyzed by Colilert® methodology that result in no cells detected were considered to have a concentration of at most 0.5 MPN $g \cdot soil^{-1}$ or 0.5 MPN 100 $ml \cdot leachate^{-1}$ [3]. These commercial Colilert® kits represent a defined substrate technology [16] [17]. **Figure 1** illustrates the soil column setup and how the transport of *E. coli* will take place.

Greenhouse Rainfall Simulation

The nine experimental soil columns were evaluated using a constant intensity rainfall pattern in a rainfall simulation system. Soils were pre-wet to control for antecedent moisture. A piece of a furnace filter was placed on the soil surface to

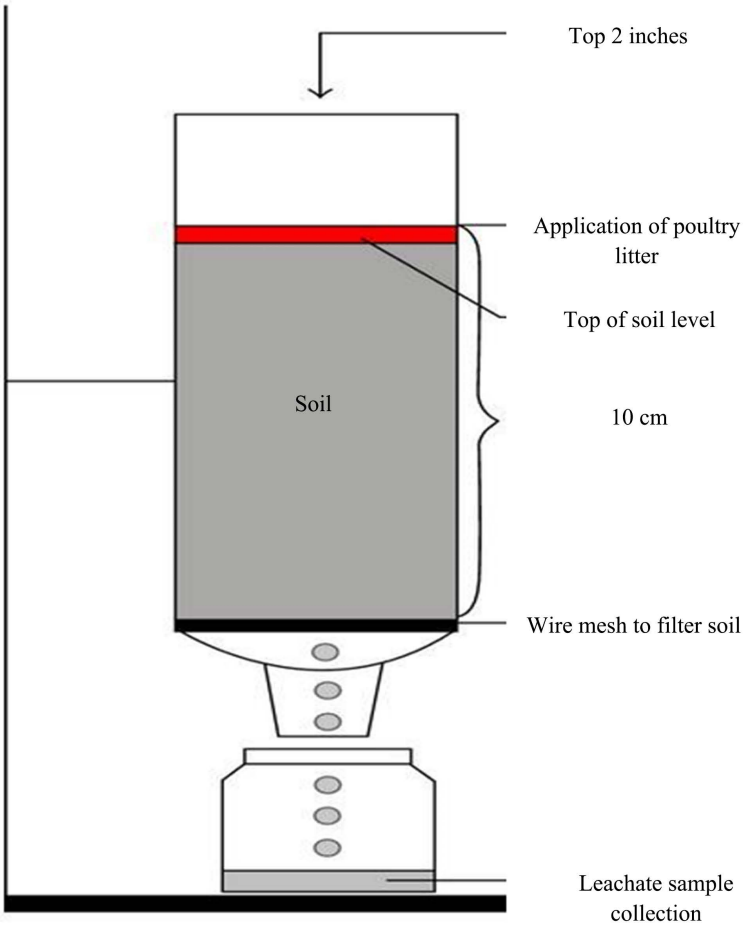

Figure 1. Column setup for *E. coli* transport study [19].

protect the soil from raindrop impact, simulating crop cover. The furnace filter was removed, and the soil was saturated using the rainfall simulator. Saturated soils were left to drain for 24 - 36 hours. (covered with plastic) until field capacity was achieved. Volumetric soil moisture content was determined by theta probe. Each soil was evaluated under both a "pre-wetted" and "air-dried" condition (no pre-wetting). The soil columns had a rainfall simulation system consisting of a Melnor 33 inch 8-pattern watering wand. The wand was centered above the soil columns 3 m (9.8 ft.) high and was connected to a metal frame. A low-pressure regulator was used in combination with a liquid–filled pressure gauge to insure that a 28 kPa (4.1 psi) sprayer head pressure was maintained. An in-line filter was placed in the flow stream to prevent foreign particles from clogging the regulator and the sprayer head. A garden hose supplied water to the simulator. Rainfall was simulated for 30 minutes as a continuous flow rain event with an intensity of 70 $mm \cdot h^{-1}$ (2.8 $in \cdot h^{-1}$) [20].

Water for all simulations was obtained from the public water supply and passed through reverse osmosis filters [3]. Before simulating rainfall, soil samples from each soil sample site were taken with a flame sterilized soil corer, placed in sterile plastic bags, mixed thoroughly, and taken to the lab for analysis.

2.4. Soil Physical Data Collection

In addition, measurements of soil physical properties were collected. For example, soil moisture m^3/m^3 volumetric water content (VMC), soil temperature ˚C and soil pH. Both VMC and soil temperature measurements were taken before and after the simulated rainfall was applied. Measurements were collected using Em50 Series Data Collection System (Decagon Devices, Inc, Pullman, WA). Measurements were recorded in 30-minute intervals from the soil columns with poultry litter applied to them. Organic matter estimates present in a soil sample was conducted by measuring the weight lost by an oven-dried (105˚C) soil sample when it was heated to 400˚C; this is known as "loss on ignition", essentially the organic matter is burnt off [21]. Porosity calculations were calculated to determine soil texture effects.

Gravimetric water content Equation:

$$\theta_g = \frac{m_{water}}{m_{soil}} = \frac{m_{wet} - m_{dry}}{m_{dry}} \quad (1)$$

Air dry Soil Moisture Content (MC) Equation:

$$MC = \frac{\text{Wet soil} - \text{Oven Dry soil}}{\text{Oven Dry soil} \times 100\%} \quad (2)$$

The soil moisture content calculations were determined by taking 100 ml of each soil type and measuring the initial mass. Next, the soil was dried in an oven for 24 hours and the mass was measured. By following Equation (1), the MC (moisture content) was determined to be lowest in Hartsells Sandy soil.

3. Presentation and Analysis of Results

Table 1 shows the air-dry soil moisture content calculations for both Decatur silty clay loam soil and Hartsells sandy soil. Table 2 shows the water content based on 10 ml of inoculum added to both soils. Table 3 shows the mean values of the CFUs for the soil samples, represented by clay soil (CS) and sandy soil (SS). CFU/ml denotes *E. coli* per 1 ml of soil water sample. Figure 2 shows a graph with the soil moisture content and enumeration of *E. coli* in sandy soil samples for each column. Figure 3 shows a graph with the soil moisture content and enumeration of *E. coli* in clay soil samples for each column. Figure 4 shows a graph of the mean values of *E. coli* in both clay soil and sandy soil. CFU/ml denotes *E. coli* per 1 ml of soil water sample. Table 4 shows a paired T-Test for sandy soil enumeration data and soil moisture content. Table 5 shows descriptive statistics for sandy soil enumeration data and soil moisture content. Table 6 shows the coefficients of the linear regression analysis for sandy soil enumeration data and soil moisture content. Figure 5 shows a graph of partial linear regression of sandy soil MPN·CFU·ml^{-1}-Y (dependent variable) based on MC m^3/m^3 VWC-X (independent variable). Figure 6 shows a graph of the linear regression of sandy soil for MPN·CFU·ml^{-1} data of *E. coli* with the MC m^3/m^3 VWC data providing the slope, y-intercept, and R^2-value.

Table 1. Air dry soil moisture content calculations.

Soil Type	Wet Soil (g)	Oven Dry Soil (g)	Moisture Content (%)
Decatur Silty Clay Loam soil	138.36 g	131.22g	5.65%
	138.81 g	140.40 g	−1.13%
	138.97 g	131.56 g	5.63%
Hartsells Sandy soil	137.73 g	134.64g	2.29%
	137.23 g	133.71g	2.63%
	137.87g	134.61g	2.42%

Table 2. Water Content based on 10 ml of inoculum added.

Soil Type	Moisture (%)
Decatur Silty Clay Loam soil	15.64%
Hartsells Sandy soil	12.44%

Table 3. Enumeration of *E. coli* from clay and sandy soil for each column.

Column	CS	SS
1	2.12916×10^3 CFU·g^{-1}	4.12916×10^3 CFU·g^{-1}
2	1.291×10^3 CFU·g^{-1}	4.291×10^3 CFU·g^{-1}
3	2.32578×10^3 CFU·g^{-1}	4.32578×10^3 CFU·g^{-1}
4	1.02916×10^3 CFU·g^{-1}	4.02916×10^3 CFU·g^{-1}
5	2.12456×10^3 CFU·g^{-1}	3.12456×10^3 CFU·g^{-1}
6	0.92118×10^3 CFU·g^{-1}	3.92118×10^3 CFU·g^{-1}
7	1.22416×10^3 CFU·g^{-1}	4.22416×10^3 CFU·g^{-1}
8	2.18016×10^3 CFU·g^{-1}	4.18016×10^3 CFU·g^{-1}
9	2.12916×10^3 CFU·g^{-1}	3.92926×10^3 CFU·g^{-1}

Mean values of the CFUs for the soil samples, represented by clay soil (CS) and sandy soil (SS). CFU·g^{-1} denotes *E. coli* per 1 ml of soil water sample. Mean values are based on 3.0×10^3 CFU·g^{-1} and 1:1000 dilution.

Table 4. Paired T-Test for sandy soil enumeration data and soil moisture content.

Paired Samples T-Test					
Measure 1		Measure 2	t	df	*p*
m^3/m^3 VMC	–	Log5 CFU/ml^{-1}	−8.029	8	<0.001

Note. Student's T-Test.

Table 5. Descriptive statistics for sandy soil enumeration data and soil moisture content.

Descriptive Statistics		
	m^3/m^3 VMC	Log5 CFU/ml^{-1}
Valid	9	9
Missing	0	0
Mean	0.184	4.017
Std. Deviation	0.027	0.365
Minimum	0.117	3.125
Maximum	0.210	4.326

Table 6. Coefficients of the linear regression analysis for sandy soil enumeration data and soil moisture content.

Coefficients						
Model		Unstandardized	Standard Error	Standardized	t	*p*
H_0	(Intercept)	0.184	0.009		20.521	<0.001
H_1	(Intercept)	−0.076	0.053		−1.421	0.198
	Log5 CFU/ml^{-1}	0.065	0.013	0.879	4.873	0.002

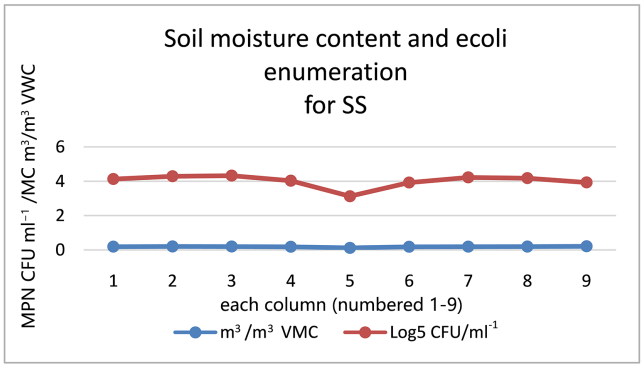

Figure 2. A graph showing the soil moisture content and enumeration of *E. coli* in sandy soil samples in each column.

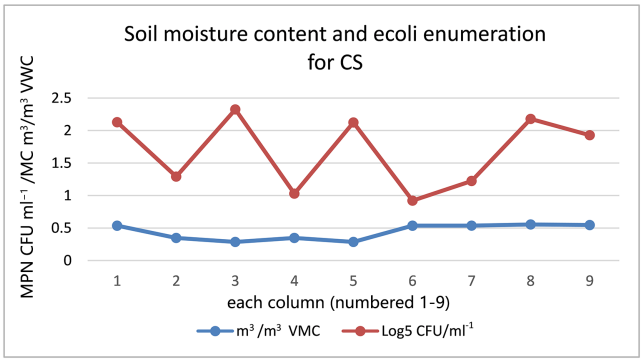

Figure 3. A graph showing the soil moisture content and enumeration of *E. coli* in clay soil samples in each column.

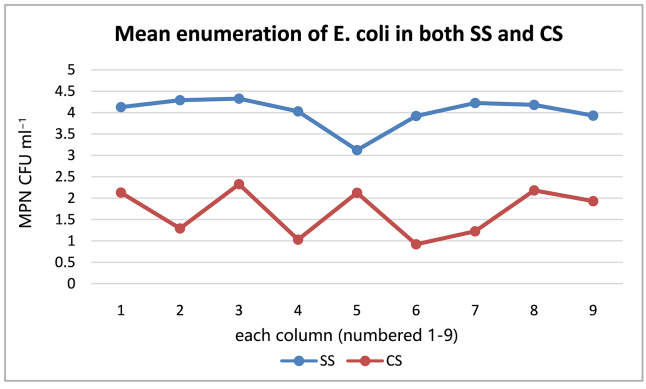

Figure 4. A graph of the mean values of *E. coli* in both clay soil and sandy soil. CFU/ml denotes E. coli per 1 ml of soil water sample.

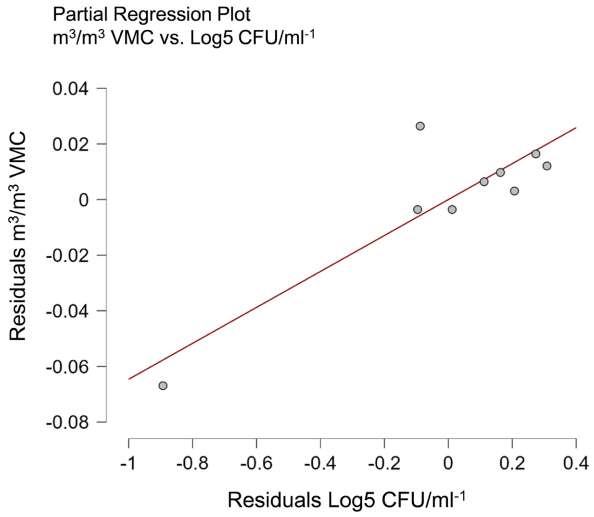

Figure 5. Partial linear regression of sandy soil MPN CFU ml^{-1}-Y (dependent variable) based on MC m^3/m^3 VWC-X (independent variable).

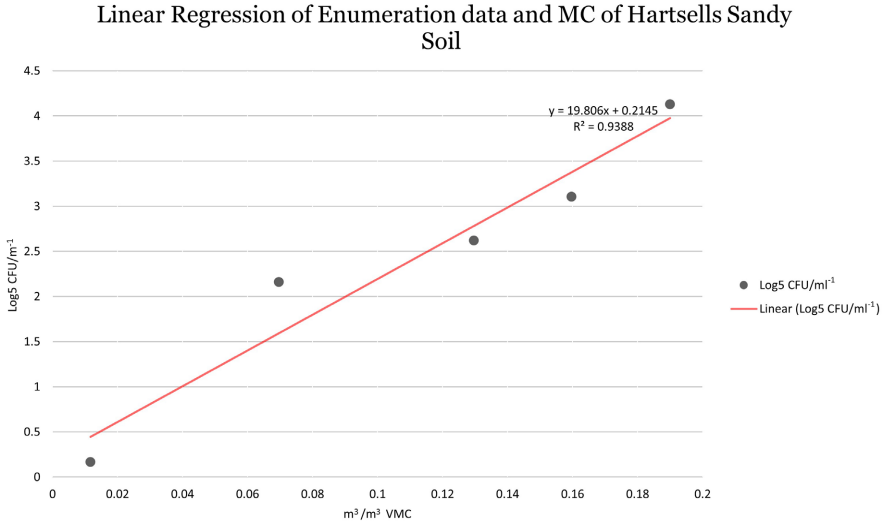

Figure 6. Linear regression of sandy soil for MPN CFU ml^{-1} data of *E. coli* with the MC m^3/m^3 VWC data.

The graph in **Figure 4** shows mean scores for both sandy and clay soils. Mean values are based on a 3.0×10^3 CFU·g^{-1} initial inoculation concentration. The clay line on the chart (**Figure 3**) is clearly different from the sandy line. The sandy soil has higher values. As such, there is an indication that *E. coli* transport rate is higher for sandy soil. **Figure 2** confirms that sandy soil has the lower soil moisture content and the higher enumeration value, which proves that soil moisture content affects transport. Soils with lower soil moisture content will have higher transport, where soil with higher soil moisture content will have lower transport of *E. coli.* Soil moisture content was shown to positively affect persistence and transport of *E. coli* in the leachate of poultry litter amended soils.

Assessments show that clay soil has higher moisture content than sandy soil (Figure 3, Table 1); however, the higher moisture content does not constitute a better growth rate or survival rate for *E. coli.* In fact, it is possible that the Decatur silty clay loam soil could have too much water present for *E. coli* survival. Having a MC of 15.64%, the persistence could be suppressed.

Studies by Ibekwe *et al.* [22] suggest that transport is significantly affected by soil type. The research conducted on the persistence of *E. coli* in contrasting soils results is greater in clay soil in a longer time frame than in sandy soil. However, shorter-term persistence occurred at a lower rate in sandy soil than in clay soil. It is believed that due to properties of clay soil such as soil texture, pore space and protozoa, the transport rates were affected. According to Ibekwe *et al.* [22], there was more variability in mobility based on a comparison of finer-textured (clayey) soils and coarser-textured (sandy) soils. Comparing these two soils resulted in prolonged survival of *E. coli* because of higher availability of protective pore spaces against feeding by soil fauna like protozoa [22].

Paired Samples T-Test and Descriptive Statistics for SS

To identify if the data were highly statistically significant a T-Test was conducted using both the sandy soil moisture content data and the sandy soil enumeration data. The results of the T-Test are shown in Table 4. Table 4 shows a p-value of <0.001 which indicates the data are highly statistically significant.

Correlation for SS

In addition to the T-Test a linear regression analysis test was conducted. Table 5 shows the results of the linear regression. Table 5 shows that the sandy soil enumeration data was able to have a significant positive correlation with the soil moisture content. The coefficients of linear regression analysis for sandy soil enumeration data and soil moisture is shown in Table 6. Table 6 shows that there is a p-value was <0.001 but <0.005 indicating that there is statistical significance.

In addition, a linear regression model (Figure 5) was created to predict the trend in data from sandy enumeration and soil moisture content. The MPN·CFU·ml^{-1} data of *E. coli* was plotted on the graph with the MC m^3/m^3 VWC data. The straight-line fits into the data points to predict the trend in the data. The trend line tells us where our graph is trending, which proves that our trend can be predicted well.

Moreover, the linear regression (Figure 6) model predicts the trend in the sandy soil. The MPN·CFU·ml^{-1} data of *E. coli* was plotted on the graph with the MC m^3/m^3 VWC data. The straight-line fits into the data points to predict the trend in the data. The trend line tells us where our graph is trending. The equation shows that our slope is 19.9 and the Y-intercept is 0.214. The R^2 value is 0.939. This is a high R^2 value, which proves that our trend can be predicted well.

4. Discussion

The correlation analysis of the sandy soil moisture content and the MPN CFU indicate that as moisture content increases, so does CFU MPN in the leachate.

Also, as moisture content decreases, so does the CFU MPN of *E. coli* in the leachate. Although this analysis does not show which variable influences the other, it indicates that as one variable increases, so does the other. This proves that there is a positive correlation between these variables as seen in **Figure 5** by the positive regression plot. As such, the linear regression analysis predictions show that higher porosity and soil moisture content affects transport, and Hartsells sandy soil has higher transport of *E. coli* due to its higher porosity and lower volumetric water content.

The infiltration of water affects the amount and rate of leaching through the soil [23]. The rate of infiltration is the rate at which water enters the soil at the surface and is controlled by surface conditions. The transmission rate is the rate at which the water moves through the soil and is controlled by the soil layers [24]. In general, when the rate of infiltration and transmission through the soil is higher, the volume of leachate is lower. Because of low infiltration and transmission rates, fine textured soils such as clay produce a higher leachate volume than coarse textured soils, such as sand [25].

Clay loam soils have slow infiltration and transmission rates and high leachate volume when wet. They are distinguished by a layer that obstructs downward movement of water i.e. leaching. Predominantly clay soils with a high swelling potential or a permanent high-water table have the slowest infiltration and transmission rates and the highest holding capacity for microbial transport vertically [24]. As such, this comparison of higher transport and persistence of *E. coli* due to higher porosity and lower volumetric water content is accurate based on this experimental design.

However, the objective was carried out by exposing packed soil columns and simulating a soil profile to partial environmental conditions. As such, this study was limited in not having exact environmental conditions such as plant roots, soil fauna, and real-world variations and various factors that might influence results. As such, such limitations have been considered in the preparation for future recommended field-based studies. In order to validate our laboratory finding subjecting the columns at a sample site to naturally occurring field conditions of weathering periods is proposed. During the weathering period, the columns will be subjected to 5 freeze/thaw cycles and 6 wet/dry cycles. The columns will be buried vertically into the soil, such that their top surface will be level with the field surface. The lower column interfaces will be in contact with the underlying soil, permitting natural drainage [26]. This method will better simulate this experiment and further validate our laboratory findings.

5. Conclusion

Hartsells sandy soil, when compared to Decatur silty clay loam soil, has lower moisture content and higher porosity. As moisture content increases, so does leachate, and vice versa. Although the statistical analysis doesn't show which variable influences the other, it indicates that as one increases so does the other. In conclusion, the assessment of the transport potential of *E. coli* into leachate us-

ing a soil column from two poultry litter amended highly weathered soils for dry and moist soil conditions indicates that *E. coli* has a higher survival rate in the leachate from the soil type with lower moisture contents and higher porosity. There is a higher survival rate of *E. coli* in Hartsells sandy soil when compared to Decatur silty clay loam soil and this is indicated by the highly statistically significant p-value < 0.001. In closing, by conducting this study to explore soil texture, soil moisture and microbial interactions and their roles in microbial transport and survival we have provided a theoretical framework to support these findings and guide future research directions.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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