ABSTRACT

STALL, CHRISTOPHER JAMES. Microbial Fate and Transport in a Seasonally Saturated North Carolina Coastal Plain Soil. (Under direction of A. Amoozegar and D. L. Lindbo.)

Infiltrative surfaces of dispersal trenches or beds are required to be installed at least 30 cm above soil wetness conditions in loamy or finer soils. Insufficient separation between infiltrative surface and the water table (WT) may allow untreated wastewater, containing enteric bacteria, to reach groundwater. Due to high demand for housing development in areas not served by public sewer systems, some septic systems may be installed in marginally suitable soils with a seasonal high water table (SHWT). The objectives of this study were to evaluate: (1) fate of *Escherichia coli* (*E. coli*) at various WT depths, (2) bacterial transport by a falling WT (3) the efficiency of treatment in saturated conditions by SHWT, and (4) the effect of resting on the efficiency of soil treatment of wastewater. Seventeen columns measuring 15 cm in diameter and 75 cm in length were constructed and packed with Norfolk sandy loam (Typic Kandiudults) material that had been air dried and sieved. Water tables were established at 30, 45, and 60 cm below the soil surface in the columns with Mariotte bottle systems, and two control columns with WT at 30 cm below the soil surface. Two hundred mL of artificial wastewater inoculated with approximately 10^6 colony forming units (CFU) of *E. coli* were applied daily on top of each column, except control columns, which received 200 mL of sterilized artificial wastewater, simulating a loading rate of 0.3 gallons foot^{-2} day^{-1}. A 100-mL sample was collected from the top of the WT and analyzed for *E. coli*. To maintain a constant WT, another 100 mL sample was collected from the bottom of each column and discarded. After the breakthrough of microbes in the 30 cm depth treatment, the WT in these columns was lowered to 60 cm below the soil surface and the experiment was continued to day 65. Subsequently, the WT was brought to the soil surface in selected columns to represent SHWT. Application of wastewater and collection/analysis of the samples were continued for another 55 days. Then, no wastewater was applied to the columns for 30 d followed by 30 d of application and monitoring of *E. coli*. *E. coli* concentrations for the 30 cm of separation distance were significantly higher than 45 cm of separation, and microbial counts for both 30 and 45 cm of separation distances were statistically higher than 60 cm of separation in the first part of the experiment. Overall,

only 60 cm of separation showed sufficient bacterial treatment. Dropping the WT from 30 to 60 cm resulted in significant reduction in microbial count at the top of the WT. Statistically higher treatment was achieved when the WT remained at 60 cm depth, and 45 cm of separation distance was significantly less effective than 60 cm of separation distance in removing *E. coli*. Treatment capacity of the soil was significantly reduced when the WT reached the soil surface in the column for all treatments. The capacity of the soil to attenuate *E. coli* was improved by stopping daily application of wastewater (i.e., by resting the soil). However, effects of resting did not last within 30 days after restarting wastewater application. Results show that 30 cm of separation distance between the bottom of the trenches and SHWT may not be sufficient for the removal of enteric bacteria from septic tank effluent. Also, bacterial contamination may not move downward when WT drops, but little treatment is provided when WT rises and the soil under the trenches becomes saturated. Finally, a 30-d break in wastewater application to the soil may be beneficial, but the effects may be short-term.

Microbial Fate and Transport in a Seasonally Saturated North Carolina Coastal Plain Soil

by
Christopher J. Stall

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

Soil Science

Raleigh, North Carolina

2008

APPROVED BY:

Dr. Aziz Amoozegar Co-chair of Advisory Committee	Dr. David Lindbo Co-chair of Advisory Committee
Dr. Alexandria Graves	Dr. Diana Rashash

BIOGRAPHY

Christopher James Stall is from Pittsfield, MA. He received his Associate of Arts and Sciences degree from Paul Smith's College of the Adirondacks in Ecological and Environmental Technology in 2004. He received his Bachelor of Science degree in Environmental Science from SUNY Plattsburgh in 2006. In 2003-2006 he worked as an undergraduate research assistant on a joint project between Paul Smith's College and SUNY Plattsburgh on a grant from the National Science Foundation examining differences between managed and preserved watersheds of the upland forests of the Adirondack Park. In the fall of 2006, he moved to Raleigh, NC to enroll in the Master of Science program at North Carolina State University (NCSU) with a major in Soil Science under the direction of Dr. Aziz Amoozegar and Dr. David Lindbo. After graduating from NCSU, he plans to join NewFields Agricultural and Environmental Resources, LLC in Sacramento, CA as a soil scientist.

ACKNOWLEDGEMENTS

I would like to express the deepest gratitude to my adviser Dr. Aziz Amoozegar for his belief in my abilities as a researcher and student. Also, I would like to thank him for all his help with the research process, as he went above and beyond many times to help me. I would also like to thank my co-adviser, Dr. David Lindbo, without whom my thesis and presentations would not have been possible. Dr. Lindbo persuaded me into many conference presentations that I would not have had the confidence to register for myself. I would also like to express my appreciation to Dr. Alexandria Graves, another member of my committee, not only for her invaluable advice regarding microbial analysis, but also for the use of her laboratory space and equipment. I consumed almost half of her lab space for almost six months and never got anything less than a smile. I greatly appreciate the help from the final member of my committee, Dr. Diana Rashash, with statistical analysis and upbeat look towards the overwhelming subject of non-parametric statistics.

I would like to thank Dr. Jeff Thompson for helping with the statistical results for my Master's research. Brad Robinson, research specialist in Dr. Grave's lab, kept me smiling and awake at 6:30 in the morning during the data collection for my study with stories and jokes. Chris Niewoehner was also a great help with physical measurements of soil characteristics. I would like to thank the entire Soil Science Department at NCSU, including faculty, staff, and student in for a very enjoyable experience. I would especially like to thank my co-students Sergio Abit, Deanna Babcock, Carlin Conk, and David Wall for advice and support during my project.

Lastly, I would like to thank my girlfriend, Shannon and my family, especially my mother. Finishing this project and degree would have been much more difficult without their love and unwavering support over the last two years.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

1. Artificial Wastewater .... AWW
2. Air-Water Interface .... AWI
3. Colony Forming Units .... CFU
4. Centimeters .... cm
5. Coastal Plain .... CP
6. Days.. .... d
7. *Escheria coli* .... *E. coli*
8. Gallons per Day .... gpd
9. Liters L
10. Meters .... m
11. Milliliters .... mL
12. Most Probable Number .... MPN
13. 5-methylumbelliferyl-β-D-glucuronide .... MUG
14. Number of Samples Taken per Treatment Period .... n_i
15. Number of Treatments .... k
16. On-site Wastewater Systems .... OSWS
17. Average Rank of Data .... r_i
18. Seasonal High Water Table. .... SHWT
19. Septic Tank Effluent .... STE
20. Water Table .... WT
21. Alpha/Probability Level .... α

INTRODUCTION

According the U.S. Census Bureau (2006), 21% of homes in the United States use on-site systems (also referred to as septic systems) for managing their household sewage. In North Carolina, the use of septic systems is even more prevalent, with close to 50% of domestic households using this method of sewage disposal. The population of North Carolina has increased from 6,628,637 in 1990 to 8,856,505 in 2006, a 33.6% increase (US Census Bureau, 2006). Based on current trends, North Carolina has a large population that will continue to grow. This will, in turn, put more stress on developers to find more sites that may contain marginally suitable soils for on-site septic systems.

On-Site Wastewater Systems

On-site wastewater systems (OSWS) are used in rural and suburban areas where centralized sewage treatment facilities are not available. These systems offer economically and environmentally sound alternatives to public sewage treatment systems and may be used for managing sewage from single family dwellings to apartments and condominiums, office complexes, shopping centers, schools and other large facilities.

Basic ideas for OSWS have been in place since the 1870's in France, and similar concepts were being used in the United States by the 1880's (Cotteral and Norris, 1969; Canter and Knox, 1985; UC Cooperative Extension, 2003). Originally, these systems were employed to dispose of wastewater from residential dwellings. They were considered an improvement over the direct discharging of sewage to a ditch or field because they deposited the waste underground, minimizing primary contact (Reynolds, 2004).

There are a number of different types of OSWS. A conventional system is operated by gravity flow to convey wastewater from component to component. Conventional systems are comprised of mainly three components, the source (i.e., the plumbing system in the dwelling), the septic tank, and the drainfield (hereafter referred to as soil treatment area). The septic tank is an underground receptacle that holds wastewater for primary treatment and is designed for maintaining anaerobic conditions. In the septic tank, primary treatment occurs when most of the solids present in the sewage settle out to the bottom of the tank and fats, oils, and greases float to the top of the liquid in the tank as the scum layer. It is often constructed from reinforced concrete and measures approximately 3 m long by 1.5 m wide by 1.5 m tall (8' x 4' x4'). Typical size for a residential septic tank in NC is 1000 to 1500 gallons (3800 to 5700 L). A second type of treatment occurs in the septic tank as anaerobic bacteria degrade solids and scum layer. Wastewater containing dissolved and suspended contaminants, referred to as septic tank effluent (STE), then flows out of the tank through an effluent screen below the wastewater level on the opposite side of the inflow pipe. Septic tank effluent contains numerous contaminants; including nitrogen, phosphorus, organic matter, and enteric pathogens. (USEPA, 1980; Hoover, 1994; North Carolina Cooperative Extension Service, 1996). In case of septic system failure, this bacterial laden wastewater could pose a threat to the potability of groundwater (DallaValle and Jones, 1940; Crane and Moore, 1984).

Wastewater from the septic tank is dispersed into the environment by application below the soil surface through a series of trenches (or drip lines) in the soil treatment area. In North Carolina, the most common soil treatment area for conventional systems is the gravity trench, typically measuring 90 cm (36") wide (with the infiltrative surface (trench bottom) between 30 to 100 cm (12" to 40") below ground surface) and a minimum of 270 cm (108") of spacing between the centers of neighboring trenches. In each of the trenches, there is a 30 cm (12") layer of coarse material (e.g., washed gravel, stone, tire chips) placed around the distribution pipe (perforated 4-in diameter pipe). When the STE is introduced into the trenches in the soil treatment area, it slowly infiltrates into the soil where the tertiary treatment occurs (USEPA, 1980).

Soil in the soil treatment area must be suitable for wastewater disposal. Suitable soils include permeable soils with wetness conditions (indicated by ≤2 chroma colors), and/or other unsuitable horizons that are deep enough to allow an unsaturated (i.e., aerobic) zone to be maintained below the point where wastewater is applied (i.e., bottom of the trenches or drip lines). In the soil treatment area, the aerobic soils below the trenches should convert ammonia-N to nitrate-N, reduce the biochemical oxygen demand (BOD) of the STE, and reduce its harmful bacteria that have survived in the septic tank. If the soil treatment area is installed in an unsuitable soil due to excessive permeability, low hydraulic conductivity, wetness conditions, or other limitations; bacteria may survive and contaminate ground water (US EPA, 1999).

According to North Carolina State rules; 15 A NCAC 18A .1900, Laws and Rules for Sewage Treatment and Disposal Systems (NCDENR, 2008), suitable sites for individual septic systems (up to 3000 gpd or more than 1500 gpd per acre soil treatment area) in North Carolina must contain adequate area with suitable soils that allow a minimum of 45 cm (18") of separation between the bottom of septic system trenches and any restrictive layer in group I soils (sandy soils) and a minimum of 30 cm (12") of separation in groups II (coarse loamy soils), III (fine loamy soils), and IV soils (clayey soils). One of the restrictive layers considered for these separation distances is the seasonal high water table (SHWT), which must be identified by direct observation of water table or be determined by soil morphological properties (i.e., redoximorphic features). According to the regulations, "a soil wetness condition shall be determined by the indication of colors of chroma 2 or less (Munsell Color Charts) at ≥2% of soil volume in mottles or matrix of a horizon or horizon subdivision." (The specific rules concerning SHWT are presented in Appendix A). Severson (2005) showed that the SHWT may come up above these Redoximorphic (redox) features of chroma 2 or less at some time during the year. This may create a smaller vertical separation than is required by state rules and has the potential for bacterial contamination.

In the United States, state and local regulations concerning the separation distance between the bottom of the trenches (or drip lines) and restrictive layers vary from 15 to 150 cm (6" to 60") (Table 1). For example, Idaho (Idaho DEQ, 2002) and Indiana (Purdue University, 1998) have 60 cm (24") of separation between the trench bottom and SHWT, whereas Delaware mandates 90 cm (36") of vertical separation (State of DE-DNERC, 2005). Is the separation that North Carolina requires adequate to effectively eliminate microbes present in wastewater disposed through septic systems?

Table 1. Minimum vertical separation distance between the bottom of the septic trench for individual residences and seasonal high water table in various states in the US and Province of Ontario, Canada.

State	Separation Distance, inches	Source
North Carolina	18/12 (Sands/other soils)	NCDENR, 2008
Delaware	36	State of DE-DNERC 2005
Florida	24	State of Florida, 1985
Idaho	24	Idaho DEQ, 2002
Indiana	24	Indiana SDH, 1990
Massachusetts	60/48 (Sands/other soils)	Commonwealth of Massachusetts, 2006
Minnesota	36	State of Minnesota, 2008
Montana	48	State of Montana, 1991
New York	24	State of New York, 1990
Ontario, Canada	36	Ontario Rural Wastewater Centre. 2006
Virginia	24/18 (Sands/other soils)	VA Dept. of Health, 1994
Washington	36/24 (Sands/other soils)	Deparment of Natural Resources, 1997

A seasonal high water table decreases the amount of aerobic and unsaturated soil between the bottom of the septic trench and the top of the water table (WT). Studies have shown that with decreasing depth to WT, there is decreasing treatment of pathogens (Powelson and Gerba, 1994; McLeod et al., 2001; Karathanasis et al., 2006). Also, with fluctuating water tables, the changing water contents have been shown to dislodge cells adsorbed to solid surfaces and increase the suspended cell concentration in soil solution (Powelson and Mills, 1998), possibly leading to increased contamination of the ground water.

Originally, it was assumed that soils effectively filter and remove all contaminants under all conditions; this however, may not be the case in some unsuitable areas (Reynolds, 2004). In a properly functioning soil treatment area, viruses, bacteria, and other pathogens are removed by physical filtration, adsorption to soil surfaces, and competition and predation by native soil microorganisms (North Carolina Cooperative Extension Service, 1996). The degree of treatment of pathogenic microorganisms in the soil depends on a variety of factors, including pH, porosity (f), organic matter content, texture, particle size distribution, clay mineralogy, temperature, adsorption and filtration capacities, availability of nutrients, and moisture content (Crane and Moore, 1984).

Under proper operating conditions, soils in the soil treatment area should remain aerobic and unsaturated because aerobic conditions create hostile environments for sewage borne organisms and pathogens (Cogger, 1988). Continuous unsaturated conditions are best for the removal of anaerobic microbes such as *Escheria coli* (*E. coli*) (Powelson and Mills, 2001) because the active indigenous microbial biomass in a well aerated soil is four to five times greater than in a comparable saturated soil. Also, a well aerated soil contains significantly greater numbers of nematodes and protozoa than saturated soils (USEPA, 2006). These organisms prey on smaller anaerobic bacteria, but they are generally absent or reduced in number in saturated soils. A number of studies have shown that active aeration of soil treatment areas increases the efficacy of removal of nitrogen, fecal coliform (including *E. coli*), and organic matter (Potts et al., 2004; Amador et al., 2006). Application of septic tank effluent can also cause soils to become anaerobic if soil oxygen cannot be replenished from the atmosphere or if the soil becomes saturated. This is a result of oxygen consumption by microbes in response to the increase in the amount of dissolved organic matter from the effluent.

Water content of the soil is an important factor influencing the distance that anaerobic microorganisms can be transported. In unsaturated soils, there is more efficient removal of pathogens because of their close proximity to soil particles (Wong and Griffin, 1976). This is a result of flow being restricted to small pores and water films lining particles in the soil matrix. This gives increased opportunities for physical and chemical attenuation to occur at the interfaces with soil particles (Griffin and Quail 1968; Wong and Griffin 1976; and Bitton et al.1974).

Unsaturated conditions of properly functioning soil treatment area contain more air-water interfaces (AWI) and triple point contacts (TPC). An AWI is an area where air in a pore space and soil solution meet and a TPC is where soil solids, air, and soil solution meet. Wan et al. (1994) and Cattaneo et al. (1997) showed that AWI or TPC accumulate microorganisms as a colloid and microbes are excluded from the soil solution. Proper aeration in the soil encourages AWI and TPC. However, if the soil is saturated these conditions do not exist due to the lack of air filled pores, therefore, there may be less microbial retention.

System failure from a health standpoint is defined as anything that reduces the efficiency of the soil treatment area to treat STE (Lee et al., 2005; USEPA, 2005b) . Other perspectives, such as the legal definition or the definition by environmental firms may be different, but, altruistically, it is desirable to avoid contamination of water in all cases. In the eye of the owner of a dwelling served by a septic system, a failed system is a financial burden that must be addressed. There are many possibilities for a system failure; including soil clogging, surface discharge, and seasonal high water table.

Unlike many other chemical parameters of concern in STE applied to soil (e.g., nitrate-N, BOD), groundwater contamination regulations do not include microbial contamination (McQuillan, 2004), but it is very desirable to ascertain pathogenic bacterial fate once they are introduced into the environment (Allen and Geldreich, 1975). It is important to know bacterial fate in the environment because it may impact water resources, cause disease, or cause a number of detrimental environmental impacts (e.g., shellfish water closure). It is important to know if bacteria are sufficiently treated when introduced into the soil environment before they come into contact with people, animals or drinking and recreational waters.

Importance of Treatment

There are over 100 types of disease causing organisms in untreated sewage (Paul et al., 1995) including *Salmonella spp., Campvlobacter spp., Helicobacter pvlori, Vibrio cholerae, and Escherichia coli* (MacConnell and Coburn 2000). Septic systems have been cited as the most frequent source of ground water contamination (McCoy and Hagedorn, 1979; Powelson and Gerba, 1994; Reynolds, 2004). Contaminated ground water causes almost half of the outbreaks of water borne diseases in the United States (US EPA, 1977; Craun, 1979, 1984; Hagedorn et al., 1981; Pye et al., 1983; Sevebeck and Kroehler, 1992; Reynolds, 2004). As indicated earlier, in a functioning soil treatment area, pathogens are eliminated when transported through soils under unsaturated and aerobic conditions. However, with a seasonally high water table, the transport of the pathogens through the saturated soil is rather rapid, with little treatment (i.e., die-off) or filtering (McCoy and Hagedorn, 1979). Once bacteria reach the ground water (relatively highly conductive zone), long distances are needed for further reduction of bacterial density (McCoy and Hagedorn, 1979).

The soil treatment area of septic systems installed in unsuitable soils with insufficient separation distance between the bottom of the trenches and zone of saturation or water table have been identified as a major source of coliform contamination in coastal waters. According to Duda and Cromartie (1982), only one conventional septic system should be installed per seven acres of watershed in coastal environments. They also stated that densities higher than this value may cause a significant increase in bacterial concentrations in surrounding waters to a level high enough to cause the closure of shellfish waters.

Microbial Movement-Studies

There have been many studies exploring the fate and movement of microbial pathogens in the soil (Caldwell and Parr, 1937; Butler et al., 1954; Tate, 1978; Faust, 1982; Cattaneo et al., 1997; Crabill et al., 1998; Ausland et al., 2002; Celico et al., 2004; Karathanasis et al., 2006). Results, however, have lead to conflicting conclusions. Duncan et al. (1994) showed that 15 cm of separation distance between the application point and water table may be enough for complete removal of bacteria from solution when a dosing regime is employed. Other researchers have shown that efficient removal occurs in 60 to 90 cm of unsaturated flow through fine sand when social dosing (applying wastewater as large pulse, as opposed to small doses over a period of time) is used (Gilbert et al., 1976). Table 2 lists the separation distances that have determined to be effective in attenuating/removing microbes

Table 2. Threshold distance of unsaturated flow for removal of anaerobic bacteria.

Source	**Soil type**	**Distance**
Caldwell, 1937; Caldwell and Parr, 1937	"Permeable strata"	150 to 200 cm
McCoy, 1969	Sand	35 cm
Romero, 1970	Sandy loam	90 to 200 cm
Bouma et al., 1972	Sand	60 cm
Bouwer et al., 1974	Sandy	60 cm
Gilbert et al., 1976	Sandy loam	60 to 90 cm
Brown et al., 1979	Sandy loam, Clay loam, Clay	120 cm
Ziebell *et al.,* 1974; Viraraghavan and Warnock, 1975; Brown et al., 1980	All suitable soils	100 to 200 cm
Cogger et al., 1988	Fine sand	60 cm
Ho et al., 1991	Red-mud amended sand	65 cm
Van Cuyk et al., 2001	Sand	60-90 cm
Gagliardi and Karns, 2002	Loamy sand	60 cm
Karathanasis et al., 2006	Coarse textured soil	60 cm

Under unsaturated conditions, bacteria are subjected to a straining effect in smaller pores (Hagedorn et al., 1981). Krone et al. (1958) suggested that in the soil, there are three main processes for the filtration of bacteria from soil solution. First, there is the actual filtration by the solid matrix. Bacteria are excluded from pores and are removed from the soil solution. Second, in low flow systems, the bacteria may actually settle out of soil solution. Sedimentation may be a major contributor to bacterial removal in soil solution. Third, 'bridging' between soil particles may occur, meaning that bacteria accumulate at the soil pore openings and physically make the pore openings smaller and increase the soil's ability for physical filtration.

Gerba et al. (1975) found that 92 to 97% of bacteria were eliminated in the first centimeter of unsaturated flow when applied to soil. McCoy (1969) found that there was total removal within 35 cm of application surface. They also noted that there was a decreased efficiency of removal of bacteria with increased sand content. This could be due to the presence of larger pores and less contact time with less charged soil particles in sand-textured soils than finer texture soils. In a column study utilizing sand and clay soil materials, clayey soil material was 99% efficient at removing bacteria from solution added to the columns (Glotzbecker and Novello 1975). Clay materials were much more efficient because of the increased adsorption capacity of the particle surfaces. Also, clays may be more efficient at removal because of the micropore flow as a result of small particles. This micropore flow increases the contact time with soil particles as well as the retention time of the soil solution in the soil matrix.

According to Ziebell et al. (1974), Viraraghavan (1976), and Brown et al. (1980), if the soil treatment area remains unsaturated, bacteria will meet water quality standards in most cases within one meter of unsaturated flow in both horizontal and vertical directions from a sewage discharge point. To include safety margins, these three studies gave recommendation of two meters to ensure proper treatment of microbial contamination in wastewater.

Bouwer et al. (1974) found most pathogenic microbes were removed in the first 60 cm directly following application. However, elevated concentrations of bacteria were detected in the soil after a flooding event, which increases the soil water content in the soil and increases survival (Bouwer et al. 1974). Brown et al. (1979) found with an in situ study with lysimeters that there were occasional positive samples for coliform through 120 cm of unsaturated flow beneath a septic trench. Summarizing the results of an extensive study in Whittier and Azuza, California, Romero (1970) noted that drinking water standards were met after surface application and one to two meters of unsaturated flow through sand. Also in California, Butler et al. (1954) concluded that filtration of bacteria was sufficient in 1.5 m of a sandy loam. This unsaturated soil distance would sufficiently filter STE for regulation purposes, but coliform bacteria were still present in reduced numbers after four meters of unsaturated flow (Butler et al., 1954). Karathanasis et al. (2006) found that in intact soil cores from coarse textured soils in Kentucky, microbial removal may not be adequate in the first 30 cm of unsaturated flow. They concluded from the data that 30 cm was not adequate for the removal of bacteria, but 60 cm may be the critical threshold for maximum discharge limits.

Soil water content is seen to be the most important factor in the transport and survival of microbes in soils (Gerba et al. 1975; Kibbey et al 1978; Tate 1978; Crane et al. 1981; Reddy et al. 1981; Faust 1982; Entry et al. 2000; Mubiru et al., 2000). Cattaneo et al. (1997) found microbial survival in unsaturated columns to increase with increasing water content. They found that in unsaturated columns above 40% degree of saturation, there was a four-order of magnitude increase of bacterial population. Kibbey et al. (1978) concluded that with increased soil moisture conditions, there is an increase in survival rates for bacteria traveling through soil. In another column study, Bitton et al. (1974) found that bacterial movement in soil moisture conditions less than field capacity was insignificant. They attributed this to more tortuous and turbulent flow paths under unsaturated conditions. Concurring with these conclusions, Wong and Griffin (1975) found that there is little to no microbial movement at soil water pressure heads of less than -150 cm. They assumed that most microbial movement occurs under conditions that have a mass flow of water into the soil, such as an irrigation or rain event. Wan et al. (1994) reported that 30 cm of unsaturated flow decreased cell counts while 30 cm of saturated flow did not decrease cell counts. In an in-situ study of the survival of a nonpathogenic strain of *E. coli* and *E. coli* 0157:H7, Mubiru et al. (2000) concluded that there were lower dieoff rates of the bacteria due to lower soil matric potentials (i.e., wetter conditions). Concurring with these results, Kibbey et al. (1978) found higher dieoff rates of *Streptococcus faecalis* (fecal borne indicator organism) under low soil moisture conditions. Changing the soil water status of the soil may also impact microbial survival. In an alternating application system, where the soil treatment area is able to rest without wastewater application, the treatment should improve after a resting period because the soil has an opportunity to re-aerate (Washington State Department of Health, 2007). It is essential, therefore, to maintain unsaturated conditions to remove pathogenic microorganisms from STE.

Soil water holding capacity and status is highly correlated with soil texture and organic matter content. Soil texture plays a role in water retention, pore size distribution, and water velocity through the soil. In general, soils with a higher percentage of sand have a lower total porosity than other soils, but contain a higher percentage of large macropores (Brady and Weil 2002). Mubiru et al. (2000) investigated the differences in survival of *E. coli* in two soils. Both soils were classified as silt loam soils, but one soil had twice as much clay as the other. Results show that the soil with higher clay content had decreased survival. They attribute this to the higher tension that the microbes had to overcome for survival due to smaller pore size and greater surface area. There has also been found to be a high variability with types of soils and the efficiency of removal of bacteria from the soil matrix

In soils, adsorption plays a major role in removing bacteria from soil water. In laboratory studies, adsorption to clay and organic matter were efficient mechanisms in removing bacteria from liquid suspensions (McGauhey and Krone, 1967; Garcia and McKay, 1970; Brady, 1974). Average bacterial adsorption rate coefficient to clay was 18 times greater under unsaturated conditions, as compared to saturated conditions (Powelson and Mills, 1998). This increased retention time may lead to more efficient filtering and retention of pathogens under unsaturated conditions. Longer retention times and more efficient filtering increase the efficiency of the soil treatment area. Tate (1978) investigated microbial survival in organic histisol soils. He found that *E. coli* survival was the greatest under sterilized saturated or flooded conditions in these histisols. There was a two-fold increase of bacteria over ten days when these saturated soils were incubated. Also, Tate (1978) found that eight days after a manure application bacterial survival was three-fold greater in an organic soil than a sandy soil. This was attributed mainly to the organic soil's ability to maintain moisture conditions for survival of bacteria.

Inconclusive evidence has been found about the effect of a fluctuating water table on groundwater contamination by microbes. If the water table comes closer to the surface, becomes contaminated by bacteria, and then falls, "does the bacterial contamination move with the falling water table?" Rahe et al. (1978) indicated that SHWTs may inundate soil treatment areas very rapidly and have the potential to transport microbes. In the North Carolina Coastal Plain region, large storms are fairly common (Elsner and Kara, 1999). Major storm events producing a large amount of precipitation in a short period of time may cause a quick rise in the WT (Newman, 2006).

In an early study investigating the effects of water content on the movement of bacteria in soils, Stiles and Crohurst (1923) found that *Bacillus coli* bacterial contamination was restricted to a thin layer of the upper part of the ground water. Their results showed no bacterial contamination in nearby deep cased wells. They hypothesized that the bacterial transport is relegated to periods of high water tables and when the water tables recede in periods of low rainfall, bacterial contamination is stranded in the unsaturated zone and capillary fringe and dies off. Hagedorn et al (1978) found the largest bacterial number peaks in the WT after large rainfall events. Increasing the water content of the soils due to these rain events tended to increase the amount of bacteria that survived and reached ground water wells. Hagedorn and McCoy (1979) also found that microbial dieoff is only important during long retention periods in the soil, such as those with alternating periods of saturated and unsaturated flow.

Cogger et al. (1988) found that fluctuating WT levels in a sandy soil were very important for the removal of bacteria from STE. When the average separation between the bottom of the septic trench and water table was approximately 70 cm, there was adequate separation for the soil to remain aerobic. They reported complete nitrification and fecal coliform counts remaining below 60 CFU/100 mL when the water table was more than 70 cm below the septic trench. In a separate treatment in their study, the average separation distance was 39 cm, and the soils became anaerobic, nitrogen was found predominantly as ammonium, and coliform bacteria were detected at levels of 25,000 CFU/100 mL in the WT. They concluded that 60 cm of separation provides sufficient treatment and safety margins.

It is believed that once bacteria reach the saturated zone, they have the potential to move laterally quickly to create more problems. Stiles and Crohurst **(**1923) found *Bacillus coli* traveling up to 20 m laterally after being added to the saturated zone in fine sand (effective grain size of 0.13 mm) under an outhouse latrine trench in only 27 days. Bacteria was found to be contaminating a fishing and swimming basin after flow through 450 m of sand and gravel from the point of inoculation in a study by Merrel et al. (1967) in a Santee, California aquifer. In Fort Devins, MA, Schaub and Sorber (1977) found that enteric indicator bacteria were readily detected in the ground water 183 m laterally from the injection site. Coliform bacteria have been reported to move from 0.9 to 456 m laterally in varying soils (Gerba et al., 1975). These bacteria were detected because they were capable of traveling vertically through the unsaturated zone under a land surface application site of wastewater. Once they reached the ground water, they were highly mobile laterally. Table 3 summarizes a number of studies that have addressed transport of microbes under saturated flow conditions. In other studies, viruses have shown the otential to move even farther through soils (Gerba et al., 1975; Schaub and Sorber, 1977).

Table 3. A summary of studies on lateral bacterial transport through soils (Adapted from McCoy and Hagedorn, 1979).

Authors	**Medium**	**Distance Traveled**
Stiles and Crohurst, 1923	Fine Sand	19.8 m
Warrick and Muegge, 1930	Fine Sand	70.7 m
Caldwell, 1937	Fine and coarse sand	24.4 m
Caldwell and Parr, 1937	Fine and medium sand	10.7 m
Butler et al., 1954	Fine sandy loam	0.6 to 4 m
McGauhey and Krone, 1954	Aquifer	30 m
Baars, 1957	Sand	3.1 m
Merrell et al., 1967	Coarse gravel	457.2 m
Krone et al., 1958	Sand and pea gravel aquifer	30.5 m
Wesner and Baier, 1970	Fine to coarse aquifer	30.5 m
Annan'ev and Demin, 1971	Sand and gravel	830 m
Young, 1973	Fine to medium Sand	6.1 m
Bouwer et al., 1974	Fine loamy sand to gravel	9.1 m
Schaub and Sorber, 1977	Silty sand and gravel	183 m
Reneau and Pettry, 1975	Fine loamy soil	6.1 to 13.5 m
McCoy and Hagedorn, 1979	Silty clay loam	20 m

It is also important to understand the distribution of microbes attached to soil particles under a septic trench. Bacteria that are retained by soil and able to survive are a reservoir for further contamination during rain events or mass flow of water in the soil (Burton et al., 1987; Crabill et al., 1998). Unlike anions, microbes may be trapped in small pores or on soil surfaces and a fraction of them may be subsequently released over a long period of time, acting as a source for contamination (Wollum and Cassel, 1978). Brown et al. (1979) found uneven distribution of Coliforms in the soil after their experiment on separation distance had run for two years. As a result of preferential flow through root channels and interped spaces that the septic trench may intercept, more flow along larger pores deposited more viable colony forming units (CFU) in concentrated areas in the soil. Less flow along smaller pores deposited fewer microbes in intraped voids. There was prevalent coliform in the soil within 35 cm of application but limited coliform below 35 cm.

Objectives

The general objective of this study was to assess the fate and transport of microbes present in septic tank effluent applied to a Coastal Plain soil with short transport distances to a seasonal high water table. This was accomplished by a laboratory column study. There were four specific objectives to this study as related to the general objective:

i. Evaluate depth to water table for treatment of bacteria.

ii. Determine if microbial contamination moves with the fluctuating water table.

iii. Assess the effects of a saturated septic trench on fate of microbes.

iv. Assess the impact of resting the soil treatment area on treatment capacity of the soil for microbial attenuation.

MATERIALS AND METHODS

A series of laboratory column experiments using soil material collected from a field site mapped as Norfolk series in the lower CP of North Carolina (Figure 1) was conducted under controlled conditions. Vertical transport of microbes was assessed under different vertical separations between the septic trench bottom and water tables and soil moisture conditions. This laboratory column study had four separate treatment experiments applied over 6 ½ months.

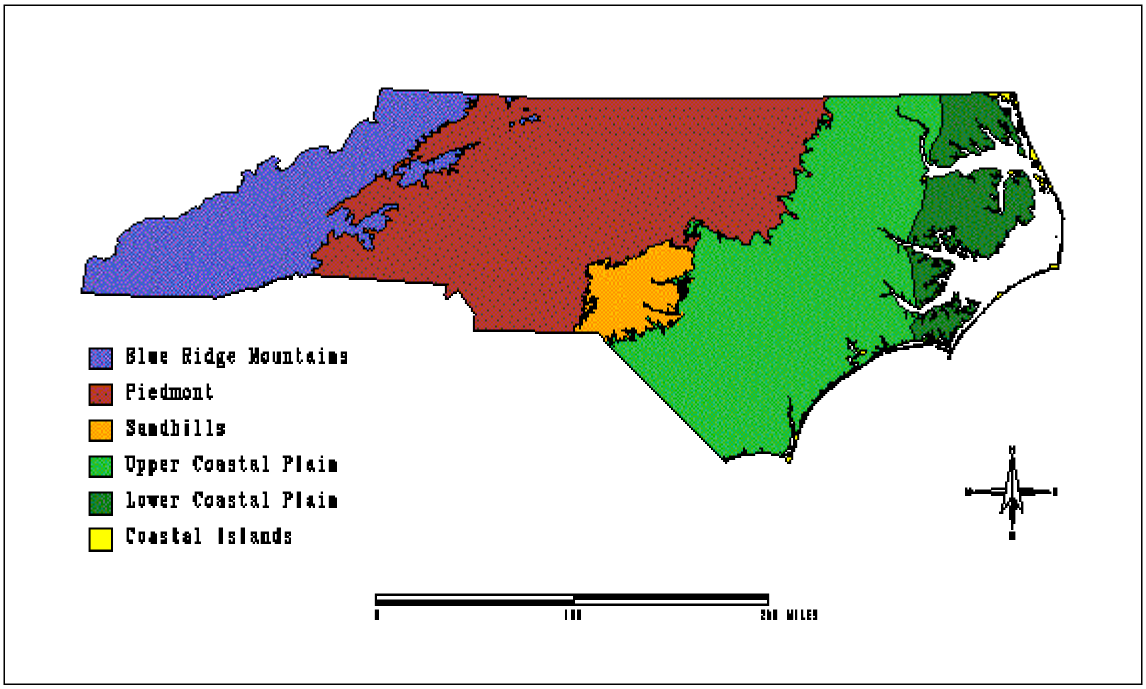


Figure 1. Map of physiographic regions of North Carolina, including the upper and lower coastal plains (Matson and Fels, 1996)

Coastal Plain Region

The general area of interest of this study is the North Carolina Coastal Plain (CP) region. The CP of North Carolina is part of the Atlantic CP which stretches from New Jersey to Florida bordering the Atlantic Ocean (USGS, 2000). The North Carolina CP makes up approximately 45% of the state's area. Alluvial and marine sediments comprising the CP were deposited 130 million years ago by rivers and ocean. More specifically, the lower CP sediments are dominated by marine deposited material lying east of the Surry Scarp (Daniels et al., 1970;1972). Within these deposited sediments, there are many coarse-loamy textured soils, with the sand being siliceous in mineralogy (Smith et al., 1976). In the lower CP, there is less local relief than in the upper or middle CP. This leads to wider interstream divides and larger areas of soils that are poorly or very poorly drained. These interstream divides in the lower Coastal Plain may have elevation changes of less than 1.5 meters over 3 to 4 kilometers (Daniels et al., 1999).

Soil Material

Soil materials were collected from an area mapped as Norfolk loamy sand (Fine-loamy, kaolinitic, thermic Typic Kandiudults) 0-2 percent slopes within the Peanut Belt Agricultural research station near Lewiston-Woodville (36°7′8″N, 77°10′56″W) in the lower CP region of North Carolina. The soil collected was from an area mapped as Norfolk soil occurs on wide level ridges and is classified as well drained (Kleiss et al., 1982). Approximately half of a cubic meter of soil was collected from approximately 30 cm below the surface from the E/Bt horizon. Soil particle size distribution was analyzed using the pipette method as described by Gee and Or (2002). The Norfolk soil material used was 64% sand, 30% silt and 6% clay with 0.27% organic matter by weight. A generalized profile description for Norfolk series soil is given in the Appendix B.

Soil Columns

Seventeen columns (Figure 2) were constructed from 75-cm long sections of 6-inch (15 cm inside diameter) polyvinyl chloride (PVC) pipe. To prevent preferential wall flow between the soil and the PVC pipe, the inside of the columns were covered with silicon caulk and medium sand (0.25 to 0.5 mm). This was accomplished by spreading a thin layer of silicon caulk on the inside of the PVC pipe by hand and covering the caulk with dry medium sand. A 20 cm by 20 cm section of 3/8" (~9.5 mm thick) PVC sheet was affixed to the bottom of each column with three screws and sealed with Marine-Tex® epoxy. Three outlets were installed on the bottom plate and connected to Tygon tubing for collecting outflow as well as regulating the level of the water table in each column.

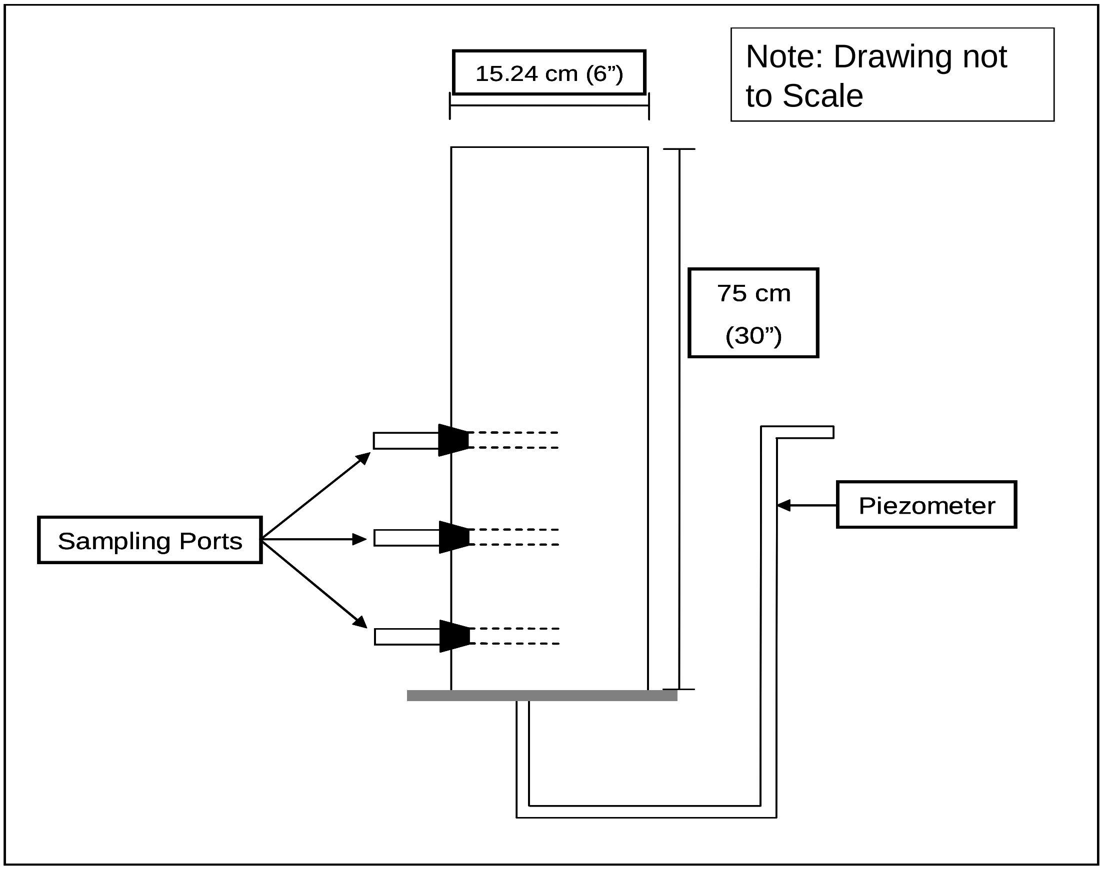


Figure 2. Schematic diagram of PVC columns used in laboratory study.

Soil materials were air dried, crushed and passed through a number 10 (2-mm) sieve to remove large particles and plant materials. A circular piece of fiberglass cloth was placed at the bottom of the interior of the column on top of the PVC plate. Approximately 3 cm of well-packing sand was placed over the glass wool cloth and another circular piece of fiberglass cloth was positioned on top of the sand. This arrangement was to ensure that the outlets and Tygon tubing did not clog by fine soil particles. Approximately 1300 g of the sieved Norfolk soil material was then placed on top of the glass wool cloth and packed to a depth of 4.5 cm by tamping the soil using a long plexiglass dowel. After gently disturbing the top of the packed soil in the column, approximately 1300 g of soil was added to the column and packed to a depth of 4.5 cm. This procedure was repeated until 18.6 kg of soil was packed to a depth of 60 cm in each column. This method of packing resulted in a uniform density of 1.57 g/cm^{-3}. A 2.5 cm thick layer of coarse quartz sand was placed on top of the packed soil in each column in order to provide a more uniform distribution of wastewater and to prevent scouring of the soil material. The top of the soil in the column represented the bottom of the septic trench.

Three sampling ports were drilled in the side of each column at 40, 55, and 70 cm below the top of the column (at 30, 45, and 60 cm below the soil surface in the column). A 7.5-cm long perforated plastic tube, wrapped in cheese cloth, was then inserted into the middle of the column through each port using a #1 rubber stopper. The plastic tubing extended half way through the column and was perforated with 3/32" (~2.5 mm) holes to ensure a representative soil solution sample. Figure 3 represents a schematic diagram of the soil columns establishing a WT at 30 cm below the soil surface.

To establish water table in the columns, water was introduced into each column using an individual Mariotte bottle system attached to the outlets on the PVC plate at the bottom of the column. Water table was introduced from the bottom of the column to ensure that no air pockets were entrapped in the soil. The water level was then regulated with the Tygon tubing as an outlet, with the water level in the column occurring at the same level as the outlet tube (Figure 3). Water tables were established at 30, 45, and 60 cm below the soil surface in the columns using five replications. Two columns were used as control with water table at 30 cm below the soil surface. Once the water table was established at the correct depth below the soil surface, the Mariotte bottle was removed and the connecting Tygon tubing was clamped off. Columns were allowed to remain stagnant for three weeks to ensure anaerobic conditions formed.

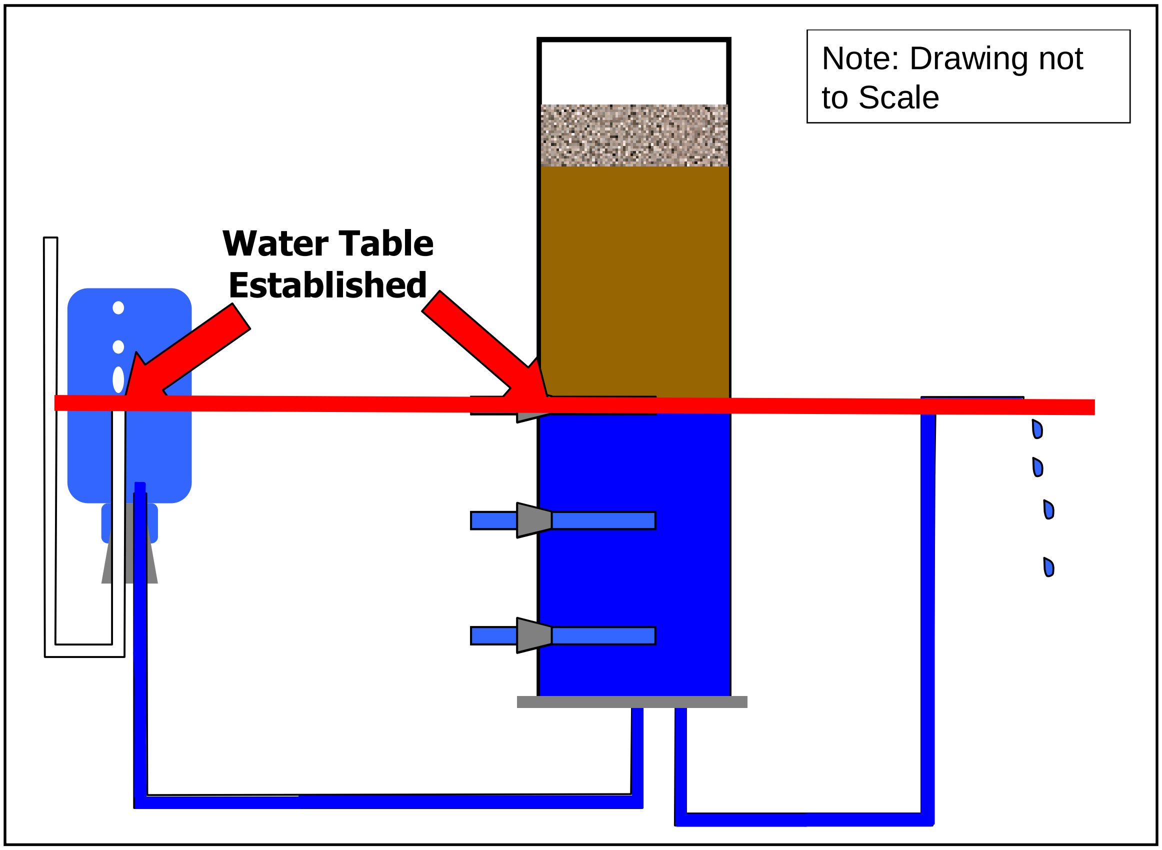


Figure 3. Mariotte bottle setup for establishing the WT in soil columns (example of WT at 30 cm)

Wastewater

Artificial wastewater (AWW) was used in this column experiment because it was more homogeneous, predictable, and safer to handle than actual STE. The AWW formula (Table 4) was adapted from Powelson and Mills (2001). According to Powelson and Mills (2001), this artificial wastewater closely mimics the survival of coliform bacteria when compared to actual STE. This combination of inorganic salts and nutrient broth was developed by Powelson and Mills (2001) and tested against five other formulas for survival of bacteria as compared to actual STE.

Table 4. Components of AWW, adapted from Powelson and Mills (2001)

Component	Concentration (mg/L)
KH_2PO_4	8.5
K_2HPO_4	21.75
Na_2HPO_4	17.7
$CaCl_2$	27.5
$MgSO_4$	11
NaCl	15
Nutrient Broth	60

Escheria coli (*E. coli*) isolated from human urine (ATCC #: 11775, Obtained from ATCC: The Bioresource Center) was used in the study. These indicator microbes were selected for their similar characteristics of indicator organisms found in STE in the field (USEPA, 2005a). Also, these microbes have a biosafety level of 1 meaning the organisms would only cause illness in individuals with a compromised immune system. Therefore, were safer to work with than those in raw sewage.

Each day, seventeen 200 mL aliquots of AWW were autoclaved in 250-mL Erlenmeyer flasks with their mouths covered by aluminum foil. Fifteen of these sterilized aliquots were then inoculated with the indicator organism. Subsequent to inoculation, flasks were incubated for 24 hours at 37°C. Each 200 mL dose of AWW had between 9.4×10^4 and 9.7×10^6 CFU of bacteria/100 mL which is typical of actual STE (Ausland et al., 2001; Prasad et al., 2006).

The AWW *E. coli* concentration was enumerated by a plating technique daily. A serial dilution from 10^{-4} to 10^{-6} were grown on 12 mFC agar plates. These 12 mFC agar plates were incubated in a water bath at 44.5° C for 24 hours. After the incubation period, blue colonies that formed on the mFC plates were enumerated so the concentration of *E. coli* applied to the columns each day was known. From these mFC plates, the next day's AWW was inoculated with the same strain of *E. coli*.

Sample Collection and Analysis

At the same time each day 200 mL of AWW (which had been incubated for 24 hours) was applied to the top of each column. Immediately following the application of the AWW, a 100 mL sample was taken from the top of the WT. The sampling port in the side of the column was opened and allowed to drain freely and drip into a sampling vessel. For example, the sample for columns with 30 cm of aerobic soil was taken at 30 cm sampling port in the side of the column. These samples were collected in light blocking bottles to ensure sterilization from natural UV light did not occur and samples were analyzed within 30 minutes of collection. To maintain a constant depth to water table, another 100 mL sample was collected from an outlet at the bottom of the column and discarded after sterilization.

The 100 mL samples collected from the top of the water table were enumerated for *E. coli* using the Colilert procedure (USEPA, 1997, EPA method# 9223B) (IDEXX-Atlanta, GA.). This method correlates well with the membrane filter technique and multiple tube fermentation, commonly used in environmental microbiology assessments (Edberg et al., 1989, 1990, 1991; Eckner, 1998). In this procedure, 5-methylumbelliferyl-β-D-glucuronide (MUG) was metabolized by *E. coli* and fluoresced under long wave ultraviolet light. This test measures biochemical changes in the media rather than colony formation. One-hundred mL of the sample was combined with indicator nutrients containing MUG and placed into a well tray (Figure 4).

The tray was then incubated at 37°C for 24 hours and fluorescent wells (indicative of the presence of *E. coli*) were enumerated (Figure 5). These positive wells are compared to a most probable number (MPN) chart and a MPN/100 mL is obtained. According to IDEXX (document enclosed with sample trays), the upper detection limit for this procedure is approximately 2419.6 CFU/100 mL. If this detection limit was reached, dilutions of the soil solution were preformed by adding a known volume of soil solution to a volume of sterilized deionized water. The same Colilert procedure was followed with the dilutions. All sets of analyses for *E. coli* determination included blanks and replicate samples for purposes of quality control.

Figure 4. IDEXX Colilert MPN tray.

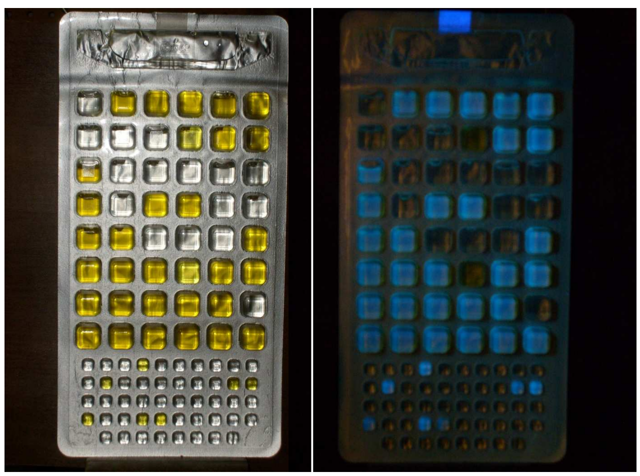


Figure 5. IDEXX Colilert MPN tray, yellow wells positive for fecal coliform (left) and fluorescent wells positive for *E. coli* (right).

Experimental Design

There were five replications of each WT treatment for the first objective of the study. The treatments consisted of 30 cm of separation, 45 cm of separation, and 60 cm of separation between the top of the soil in the column (representing the bottom of the septic trench) and the top of the water table. Two columns were maintained as control, and received 200 mL of non-inoculated, sterilized AWW each day. The WT in the control columns was maintained at 30 cm of separation. Four separate experiments were conducted in the following orders. In Appendix C, there is a schematic flowchart of the treatments for ease of understanding.

Experiment 1

Application of wastewater inoculated with *E. coli.* was continued daily until either there was a complete breakthrough of microbial contamination where the microbial concentration of the sample collected from top of the water table was equal to the inflow AWW concentration or the duration of 65 days, whichever was less.

Experiment 2

After nine days, the outflow concentration for the 30 cm separation treatment was equal to the inflow concentration for all five replications, indicating complete breakthrough of *E. coli*. The WT for all five replications was then lowered 30 cm to increase the separation distance to 60 cm. This was accomplished by lowering the outflow piezometer approximately 5 cm every hour until the desired level was achieved. A one-hundred mL sample was collected from the saturated zone (resembling the ground water) in each of the five columns as the water table was lowered. These samples were analyzed for bacterial concentrations by the Colilert procedure described earlier. This treatment simulates a fluctuating WT. When the WT rises in the soil and then falls, it is desirable to know if biological contamination travels with the WT.

The water table lowering took approximately six hours to complete with the drop of five cm per hour. Although this rate of drop maybe quicker than what is observed under natural conditions, it may resemble the rapid drop of the water table following ground water mounding after wastewater application in a dose to a drainfield (e.g., under a low pressure pipe distribution system in sandy soils). The second part of the experiment was conducted from day 10 to day 65, with applications of AWW to the top of each column. The same procedure of wastewater application, leachate sampling, and analyzing the samples was continued for all treatments through the second part of the experiment. For this part of the study, there were five columns with 60 cm of separation for the duration of the 65 days, five columns with 45 cm of separation for the duration of 65 days, five columns with 60 cm of separation for 54 days (that had been lowered from 30 cm of separation), and two control columns with 30 cm of separation receiving only sterilized artificial wastewater.

Experiment 3

On day 65, three of the columns that had 60 cm of separation for the entire duration of the experiment, and three of the columns that originally had 30 cm of separation and the saturated zone was lowered to 60 cm were selected for the next treatment. The outlet port for each of these columns was raised to the same level as the top of the soil surface in the columns, so the columns could be saturated. To accomplish saturation (i.e., bringing the simulated water table to the surface), approximately 50 mL/hour of tap water (to simulate rain water) was applied to the surface of the column and allowed to percolate toward the saturated zone. This was continued until the water table in the column reached the soil surface (which was set to be the bottom of the trenches). This simulation was designed to mimic a quick WT rise due to a large precipitation event.

The daily procedure for the application of AWW to these saturated trench columns remained the same. In the initial days after the trench being saturated, 100 mL samples were collected from the 30 cm port. After *E. coli* was detected from the 30 cm port, samples were collected from the 45 cm port. A 100 mL sample was collected from the 30 cm port and a 100 mL sample was collected from the 45 cm port. This was continued until *E. coli* was detected at the 45 cm port. Once *E. coli* had been detected at the 45 cm port, all three ports were sampled. A 50 mL sample was taken from the 30 cm port, a 50 mL sample was collected from the 45 cm port, and a 100 mL sample was taken from the 60 cm port.   The sum of the samples never exceeded the rate of AWW application and samples were diluted as needed.

In addition to the above columns in this part of the study, there were two columns with 60 cm of separation and five columns with 45 cm of separation for 130 days, two columns with 30 cm of separation for 9 days and 60 cm of separation for 121 days, and two control columns with 30 cm of separation receiving only sterilized artificial wastewater for the duration of the study. Application of AWW to these columns and collection and analysis of the samples from top of the saturated zone were continued as before (see Experiment Part 2) for all these replicates.

Experiment 4

For the final part of the study, application of AWW to all columns was ceased on day 130 and no column received AWW for 30 days. This resting period was intended to mimic an alternating soil treatment area system. On day 160, application of AWW to all columns started following the same procedure as mentioned in the third part of the study (see Experiment Part 3), and continued for another 30 days.

E. coli and Water Distribution in the Soil Columns

At the termination of the AWW applications (day 195), the columns were dissected and sampled for determining the distributions of microbes and water content in the soil material. To collect samples for determining water content and microbial population, the coarse sand that was placed on top of each column was removed and a 5 cm diameter and 3.5 cm long metal cylinders was pushed into the soil. The sampling cylinder was then carefully excavated from the column and the soil material was placed in a pre-weighed moisture can and weighed for determining its water content. In addition to the intact sample, two 1-g soil samples were collected with a sterilized scoop from the 0 to 5 cm interval of each column. In a similar manner, one intact and two 1-g samples were collected from 5 to 10 cm depth interval. Additional intact and disturbed samples were then collected from 15, 30, 45, and 60 cm below the surface of the soil material in each column. All the samples collected in the cylinders were immediately placed in pre-weighed moisture cans and weighed. After drying these samples at 105° C for 24 hours, the water content and bulk density of the samples were calculated by determining the oven-dried weight of the samples.

For the disturbed samples, the first one gram sample of soil was deposited in a glass bottle with 99 mL of sterilized distilled water for a water extraction. The second one gram sample of soil was deposited in a glass bottle with 99 mL of sterilized nutrient broth solution (Fisher Scientific #: DF0003-17-8, concentration: 8 g/L). The water extraction bottles were placed on a table shaker and agitated for 20 minutes prior to plating. The water extraction method was to plate a serial dilution on mFC agar. Dilutions from 10^{-1} to 10^{-6} were plated in triplicate on mFC agar plates within two hours of sampling. These plates were incubated in a water bath at 44.5° C for 24 hours and enumerated.

The nutrient extraction included incubating the nutrient broth bottles with one gram samples of soil material for 24 hours at 37° C. After this incubation period, the mixture was agitated on a table shaker for 20 minutes prior to plating. Similar to water extraction method, the nutrient extraction method was to plate a serial dilution on mFC agar. Dilutions from 10^{-1} to 10^{-6} were plated in triplicate on mFC agar plates within two hours of sampling. These plates were incubated in a water bath at 44.5° C for 24 hours and enumerated.

Physical Properties

Saturated Hydraulic Conductivity (K_{sat})

In total, six cores (7.6 cm diameter, 7.6 cm height) were taken from three columns. Six cores of the same size were also packed with soil material at the same bulk density of 1.57 g cm^{-3}. Another metal cylinder with a barb installed on its side was attached to the top of the sample cylinder with a wide rubber band, and the bottom of each core was cored with cheesecloth to prevent the soil from falling out. The cores were then placed on a metal screen in a tub. Water was then added to the tub to saturate the cores from the bottom. After saturation, the cores were transferred to a funnel, and a Mariotte bottle system was attached to the barb to maintain a constant head of water on top of the soil surface. This setup is shown in Figure 6. Water flowing through each core was collected with time. Using Darcy's law, saturated hydraulic conductivity was calculated after the rate of water flow through cores reached steady-state.

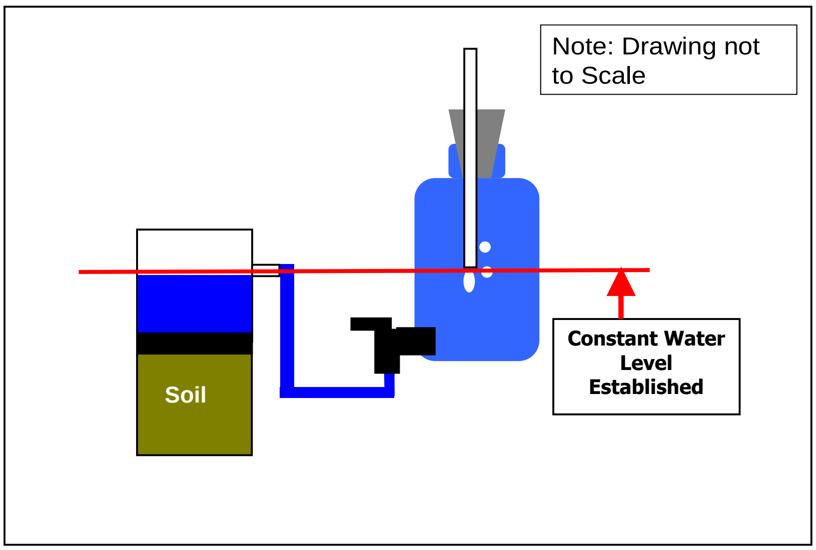


Figure 6. Schematic diagram of the setup for K_{sat} measurement using a Mariotte bottle system

Moisture Release

After K_{sat} measurements, the top metal cylinder and cheesecloth were removed, and each core was placed in a Buchner funnel (i.e., a funnel with a porous plate) and resaturated from the bottom. After saturating the column, the extra water from the funnel was removed and the top of the funnel was sealed for applying air pressure to the column. An empty graduated cylinder was placed under the bottom of the funnel and parafilm was used to seal the opening to prevent evaporation of water coming from the funnel. The saturated cylinders were first allowed to drain under gravity for 48 hours before a reading was made to simulate gravitational water. Then, air was applied to the closed Buchner funnels under 10 cm of pressure and the outflow was measured after 24 hours. The pressure was then increased to 20, 40, 80, 150, and 300 cm in 48-hour periods and the outflow under each pressure was measured. At the end of this process, the cores were removed from the funnel and weighed immediately before drying them in an over at 105 ^{o}C for 24 hours. After calculating the volumetric water content at the end of measurements (after draining under 300 cm of pressure), the amount of water retained by the soil at the end of each pressure increment was recalculated. The data were then used to construct the soil water characteristics between 0 and 300 cm of pressure head.

Redox Potential

To ensure that the saturated zone in the soil columns became anaerobic, one column was selected to assess the aerobic status of the soil (i.e., availability of oxygen) using redox probes. Redox probes, constructed from metal wire attached to a platinum tip, were installed through the side of the PVC column at depths of 10, 20, 30, 40, 50, and 60 cm below the soil surface and the reference electrode was installed approximately 10 cm below the soil surface. A baseline reading was taken with a voltmeter and no WT in the column was taken on the first day. Once the baseline aerobic soil reading was taken, water table was established at 30 cm below the soil surface in the column using a Mariotte bottle system attached to the outlet on the PVC plate. Once the water table was established at the correct depth below the soil surface, the Mariotte bottle was removed and the connecting Tygon tubing was clamped off. Daily readings of the redox potential were measured in milliamps with a voltmeter. Columns were allowed to sit stagnant for three weeks to become anaerobic. After this three week period, the same AWW inoculated with *E. coli* was applied to the top of the column and redox measurements were taken. After two weeks of AWW application and water table becoming highly anaerobic, the water table was lowered from 30 cm to 60 cm to see how quickly the soil would reestablish itself as aerobic. Application of AWW and measurements of redox potential continued for 22 days. Lastly, the water table was brought to the surface to observe how quickly the soil became anaerobic in the worst case scenario. Application of AWW and measurements of redox potential continued for five days.

Statistical Analysis

Column treatments in all four experiments were compared with a non parametric one way Kruskal-Wallis Test (Hollander and Wolfe, 1973). A Kruskal-Wallis Test was used because it is a non parametric test, or distribution free tests, making no assumptions about the normality of the data. The Kruskal-Wallis test fits these data because of the high variability involved. The Kruskal-Wallis procedure tests greater than two independent groups of sampled data. In these statistics, the null hypothesis being tested is that the samples come from identical populations versus the alternative hypothesis that the samples come from different populations.

The first part of the statistical analysis was completed using the SAS statistical program (SAS Institute, 1989; 1994). The NPAR1WAY Procedure was used to find if there was a difference between treatments in the soil columns. If there was a statistical difference between the soil column treatments, comparison with the Kruskal-Wallis test was performed (See appendix D-G for SAS output).

A follow-up procedure was used to make pair-wise comparisons between two treatments at a time. Due to the limits of the Kruskal-Wallis procedure, the exact procedure could not be implemented for the multiple comparisons. The conservative procedure as described in Hollander and Wolfe (1973) could not be used because of the large sample sizes. The large sample approximations that they suggest are for equal sample sizes only. So, the procedure that was used was a modified version as offered by Dunn (1964) in Hollander and Wolfe (1973). Dunn's procedure was modified for a one-sided multiple comparison. Initially for all pair-wise comparisons, a probability (alpha or α) level of 0.05 was used for initial tests. Because of the conservative nature of this procedure, the probability level was increased to find any potential significant differences among treatments.

RESULTS AND DISCUSSION

Safe drinking water standards for coliform concentration are less than one CFU/100 mL (USEPA, 2003). Primary contact water is designated as water that people swim in (i.e., swimable water), and the secondary contact water is reserved for fishing and boating. Since there are no NC state regulations as to ground water concentration of *E. coli* or total coliforms, the level for comparison in this study was arbitrarily set at the primary contact level of 200 CFU/100 mL. Therefore, for all the analyses, the outflow concentrations will be compared to the standard for primary contact water.

Experiment 1

Part one of the study was to evaluate depth to water table for treatment, and assess duration of saturation for removal of microbes. This was achieved with 65 days of continuous AWW application or until there was complete breakthrough of microbes in the water table with outflow concentrations on the same order of magnitude as the inflow concentrations. The overall results indicate that microbial population declined significantly with separation distance to water table.

Although there was variability among the columns, within the first nine days of initial application, the number of bacteria detected on top of water table at 30 cm depth below the wastewater application surface (assumed to be the bottom of the trenches) was near the inflow concentration of the artificial wastewater for all five columns (Figure 7). By day two, three out of the five replications had concentrations above the threshold value of 200 CFU/100 mL, and by day six, all five columns had concentrations greater than this primary contact level. On the 9th day, concentrations of samples taken from the top of the saturated zone (i.e., simulated water table) reached approximately 2.2×10^6 while the inflow concentration of the AWW was 3.9×10^6. Comparing the geometric mean of CFUs passing through 30 cm of unsaturated soil to the 200 CFU/100 mL standard (Figure 8), the 30 cm separation distance did not provide sufficient removal of *E. coli*. This may be a result of not having adequate retention time in the unsaturated zone to efficiently filter the contaminants from the wastewater. As stated earlier, according to NC State regulations, sandy soils are required to have 45 cm (18 inches) of separation distance between the bottom of trenches and the seasonal high water table for single family home septic systems. All other soils are required to have 30 cm (12 inches) of separation distance. This Norfolk soil is classified as a sandy loam, which does not fall into the sandy textures group. For actual septic systems, a soil similar to the one used in our study would require only 30 cm of separation.

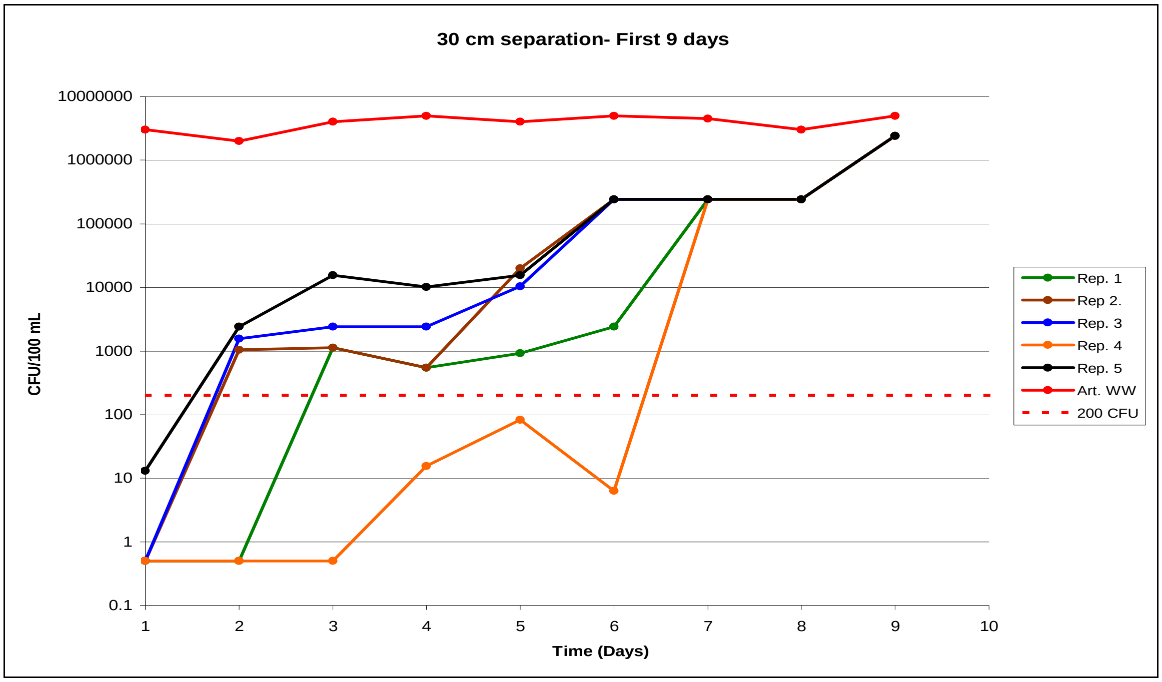


Figure 7. Concentration of *E. coli* bacteria detected at 30 cm depth in soil columns after passing AWW through 30 cm of unsaturated soil.

Columns simulating 45 cm of separation between the water table and the trench bottom acted as a marginally effective filter. There was, however, high variability within this treatment (Figure 9). Outflow collected from the top of the saturated zone at 45 cm depth in only one column reached a maximum of 7 x 10^5 CFU/100 mL in 65 days. During the same period, the remaining four columns had spikes on the order of 10^3 CFU/100 mL. Evaluating 45 cm of aerobic soil to achieve the primary contact standard, it can be concluded that this separation distance may have inadequacies. Geometric means of the concentrations of *E. coli* at the top of the water table for all five columns during 65 days of continuous application (Figure 10) shows that the primary contact standard was reached or exceeded seven times and was between 100 and 200 CFU/100 mL four other occasions. Although no complete breakthrough was observed, *E. coli* concentrations greater than 200 CFUs/100 mL were observed occasionally in these columns, which may be a result of inadequate separation between the trench bottom and seasonably high water table to allow physical filtration and adequate retention time for removal of microbes. As shown by the moisture release curve for this soil material (Figure 32), soil pores are near saturated at low tensions. Estimating 90% saturation on the moisture release curve results in a 17 cm capillary fringe. This 17 cm capillary fringe would realistically only leave 13 cm of truly unsaturated soil in the 30 cm of separation between the bottom of the trench and the WT and result in 28 cm of unsaturated flow for the treatments with 45 cm of separation for the microbes to be eliminated.

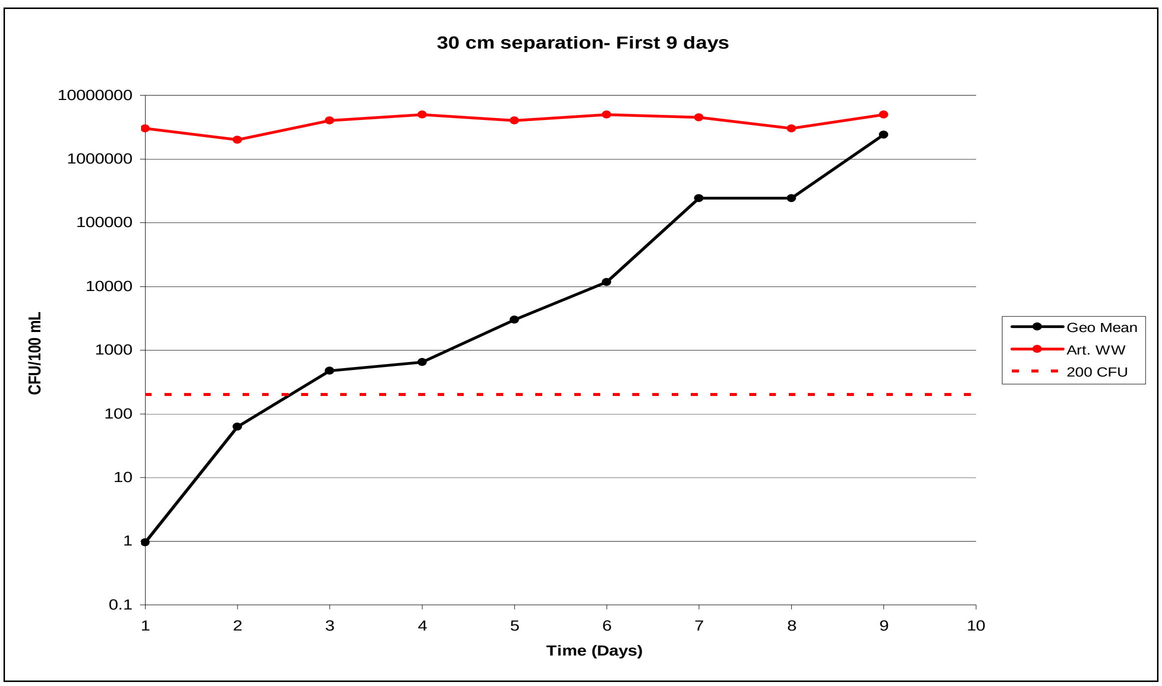


Figure 8. Geometric mean of the concentration of *E. coli* bacteria detected at 30 cm depth in soil columns after passing AWW through 30 cm of unsaturated soil.

Figure 9. Concentration of *E. coli* bacteria detected at 45 cm depth in soil columns after passing AWW through 45 cm of unsaturated soil.

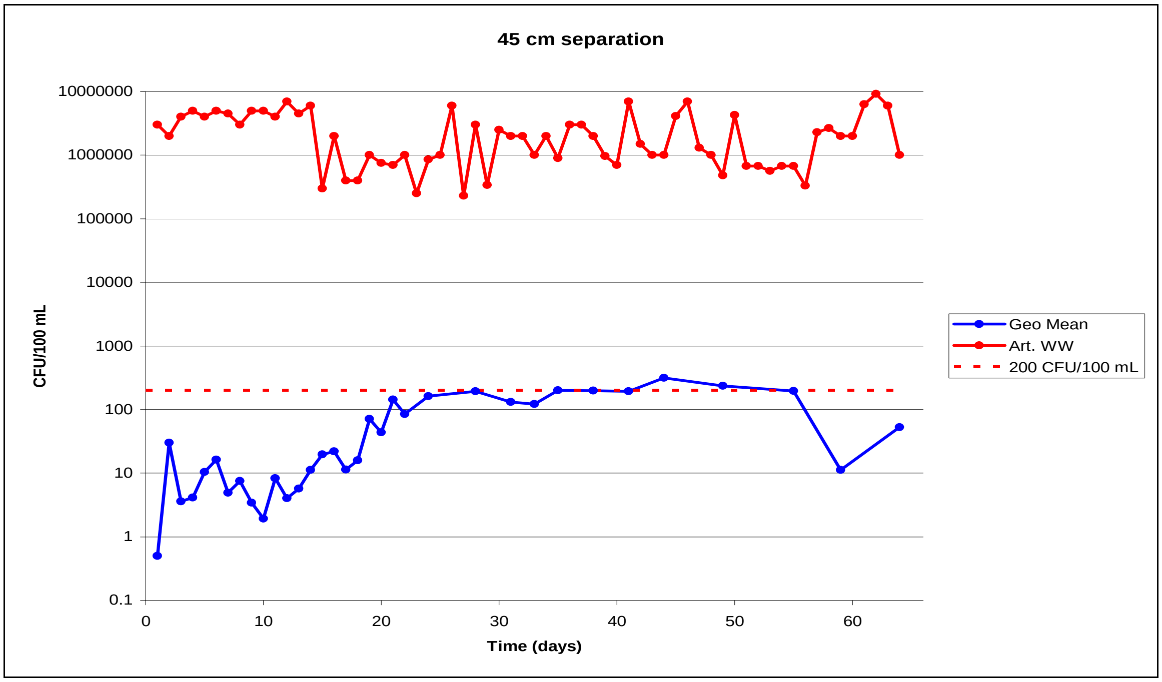


Figure 10. Geometric mean of the concentration of *E. coli* bacteria detected at 45 cm depth in soil columns after passing AWW through 45 cm of unsaturated soil.

When the highest value column is removed, the geometric mean does not exceed the 200 CFU/100 mL standard. Three of the replications do not exceed the 200 CFU/100 mL standard and two replications do exceed it. The highest value column skews the data slightly, which is why the non parametric statistical procedures were used. These soil columns were packed in the same way to ensure there would be no macropore flow or interped voids, as there would be under field conditions. Despite uniform packing, these data show the high variability of the treatments.

Sixty cm of separation between the trench bottom of a septic field and the seasonably high water table may be the critical value. One of the five replicates for the 60-cm separation distance treatment showed slightly elevated concentrations of bacteria (up to 34 CFU/100 mL), one column had detectable concentrations twice (two CFU/100 mL or less), and the other three columns never showed the presence of *E. coli* (Figure 11). Over 65 days, the primary contact standard was not violated in this treatment. Geometric means of concentrations stayed well below the 200 CFU/100 mL standard applied (Figure 12). It appears that during 65 days of continuous wastewater application, 60 cm of separation distance provided sufficient filtration and die off under aerobic conditions to attenuate bacteria contaminants present in the artificial wastewater. When the capillary fringe distance is added to the WT in this treatment, there is still 43 cm of truly unsaturated flow for the microbes to be eliminated.

The differences among the three separation distance treatments are statistically significant. According to the Kruskal-Wallis non parametric test, with two degrees of freedom, there is a strong statistical evidence that not all treatments were from the same population (Appendix D). In other words, there was a statistical difference between the three treatment groups. Looking more in depth at these data and doing a pairwise comparison, there are statistical differences between separation distances of 30 cm vs. 60 cm and separation distances of 45 cm vs. 60 cm at the probability level of 0.05. At this significance level, the CFUs for 30 cm separation are greater than 60 cm of separation, and the CFUs for 45 cm separation are greater than 60 cm. There seems to be no statistically significant difference between 30 and 45 cm treatments even though the numerical average concentration of *E. coli* for 30 cm separation distance is substantially greater than the 45 cm separation distance. At the probability level of 0.075, all three treatment comparisons are statistically significant with the shallower separation distance containing significantly greater CFUs than the greater separation distance.

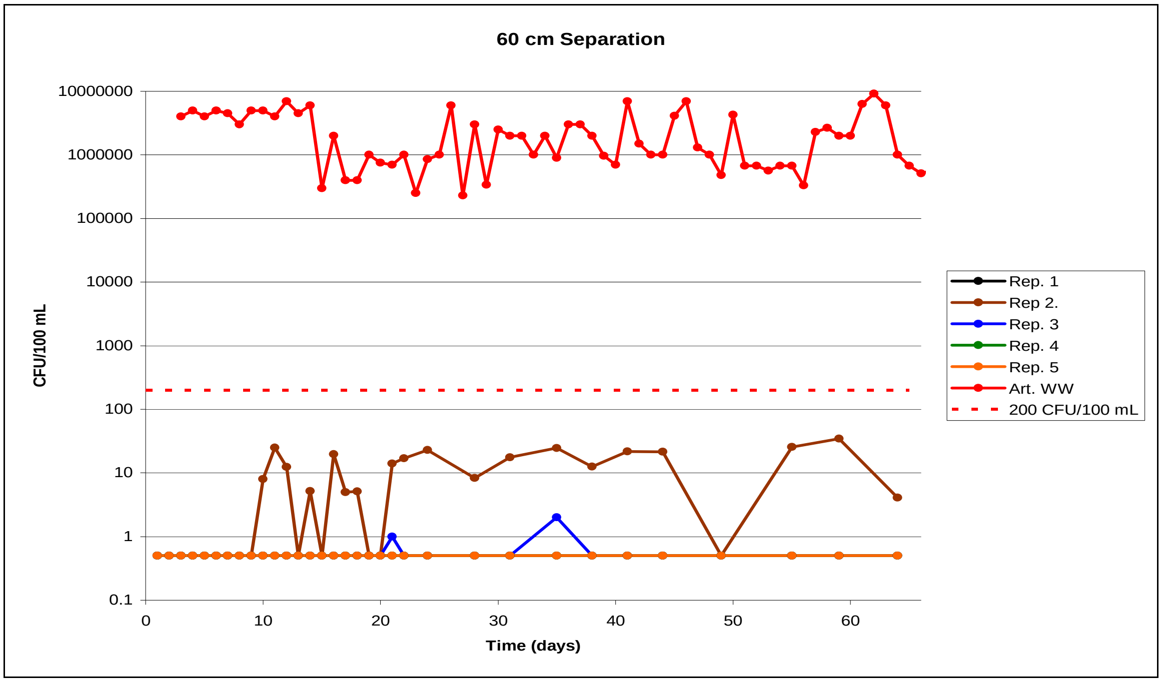


Figure 11. Concentration of *E. coli* bacteria detected at 60 cm depth in soil columns after passing AWW through 60 cm of unsaturated soil for the first 65 days of the experiment.

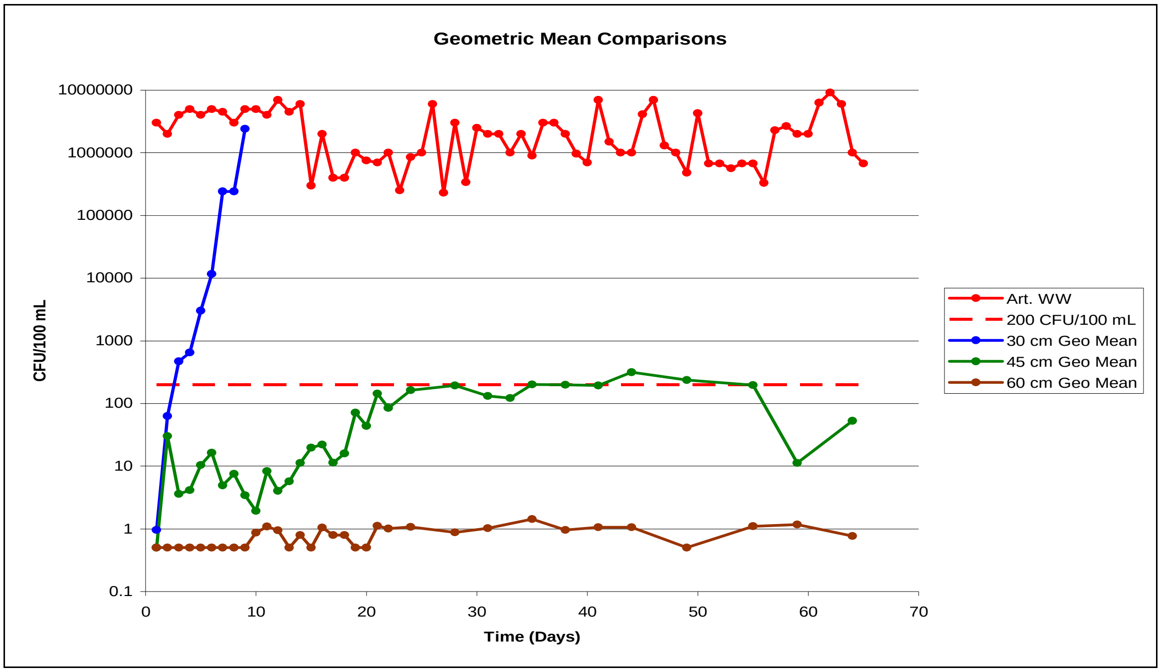


Figure 12. Comparison of the geometric means of the concentration of *E. coli* bacteria detected at 30, 45, and 60 cm depths in soil columns after passing AWW through 30, 45, and 60 cm of unsaturated soil, respectively. Note that the line for the 30 cm treatment terminates at day nine as a result of complete breakthrough.

Experiment 2

The second part of this study dealt with microbial transport with a fluctuating water table. Assuming that the ground water becomes contaminated by insufficient vertical separation distance, does the microbial contamination move with the falling water table, or does contamination become stranded in the unsaturated soil and become eliminated by natural processes?

As indicated earlier, complete breakthrough of microbial contamination occurred under 30 cm of unsaturated flow within nine days of AWW application (Figs. 7 and 12). After *E. coli* reached the zone of saturation at 30 cm below the AWW application surface, the WT was lowered to 60 cm in the columns, and AWW application continued. When the WT was lowered, concentration of bacteria in the samples collected from the top of the saturated zone at 60 cm depth decreased substantially in all five replications

(Figure 13). In fact, the concentrations of *E. coli* remained below the 200 CFU/100 mL standard for 54 days after the water table was lowered.

Based on these results, bacteria do not move with the falling WT. This finding concurs with Stiles and Crohurst (1923) that bacterial contamination in the upper part of an aquifer is stranded in the unsaturated and capillary fringe when the water table recedes. This is perhaps because an inhospitable environmental condition is created for *E. coli* and other enteric bacteria to cause their elimination when the WT recedes and the soil becomes unsaturated.

For the columns with water table remaining continuously at 60 cm below the application surface, the maximum concentrations remained below 34 CFU/100 mL of *E. coli* as described before. For the columns that the water table was lowered, however, the maximum concentrations were slightly higher and reached 138 CFU/100 mL (Figure 14). Comparisons of results for the columns with lowered water table with the 45 cm of separation show that even though the concentration of microbes was extremely high when the WT was originally at 30 cm, once the WT was lowered, better treatment was achieved for 60 cm than 45 cm of separation distance.

According to the overall Kruskal-Wallis non parametric statistical test, there was at least one treatment taken from a different population (Appendix E) meaning that there was a significant difference among treatments. Looking further and using a pairwise comparison statistical method, the differences among all microbial transport for the three treatments are statistically significant. Treatments that began with 30 cm of separation and were lowered to 60 cm of separation had higher CFU values than the treatments when water table remained at 60 cm of separation. Treatments with 45 cm of separation had greater values of CFUs than both of the other treatments after day 10.

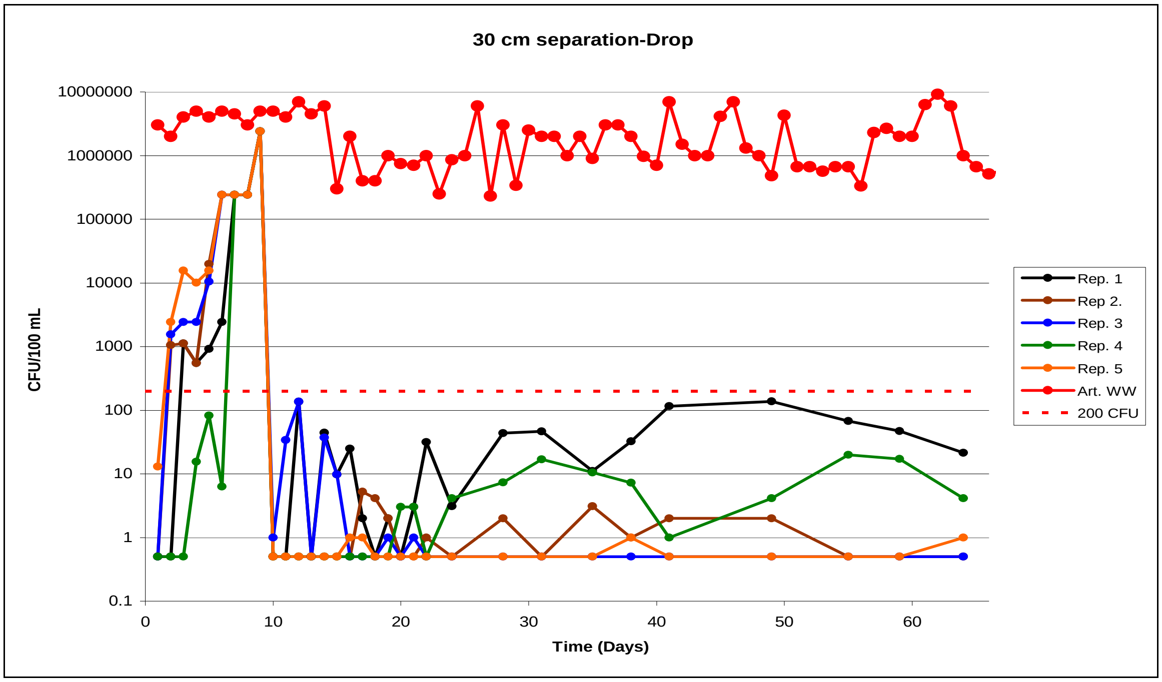


Figure 13. Concentration of *E. coli* bacteria detected at 60 cm depth in soil columns after lowering the water table from 30 cm to 60 cm.

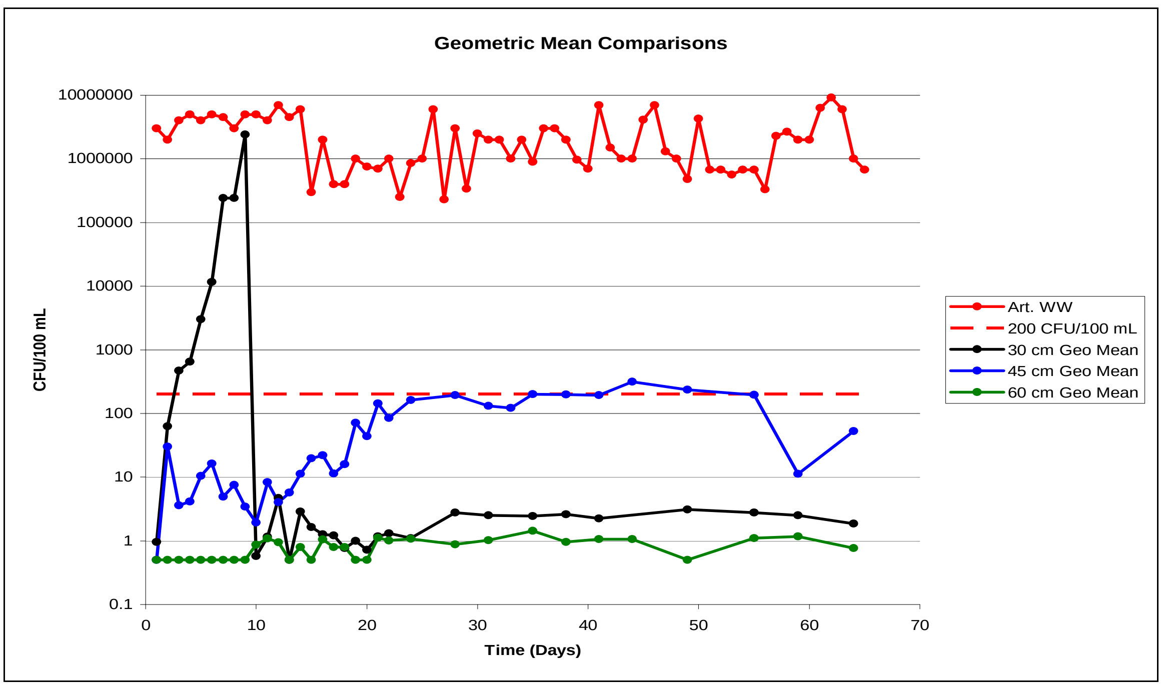


Figure 14. Geometric mean for the microbial count for all treatments during the first 64 days of the study.

Experiment 3

The third part of the study was to duplicate a worst case scenario, in which the soil under the trench bottom may become saturated under extreme events. Briefly, this condition was replicated by raising the WT to the soil surface in three of the columns with WT originally at 60 cm of separation and three of the columns that had a WT originally at 30 cm and lowered to 60 cm. The WTs in two of the columns with 60 cm of separation and two of the columns that began at 30 cm and were lowered to 60 cm remained at the same depth (60 cm). Also, the five columns with 45 cm of separation between the top of the soil and the WT continued with the same treatment. Application of wastewater was continued while collecting 100 mL samples from the 30, 45 and 60 cm depths.

Microbial population through 30 cm of saturated flow remained above an acceptable level, indicating little to no treatment (Figure 15). The inflow concentrations over the time period for this part of the study were approximately 1.78 x 10^6 CFU/100 mL and the average concentration of samples taken from the 30 cm sampling port for the same period was approximately 8.0 x 10^5 CFU/100 mL (Fig. 16). The results show that there was little to no treatment of AWW through the 30 cm of saturated flow regardless of their prior water table depth. Three of the columns were on the same order of magnitude as the inflow concentration, showing no treatment, but three columns provided some treatment. The concentrations of *E. coli* for all six replicates remained above the 200 CFU/100 mL for the duration of the treatment.

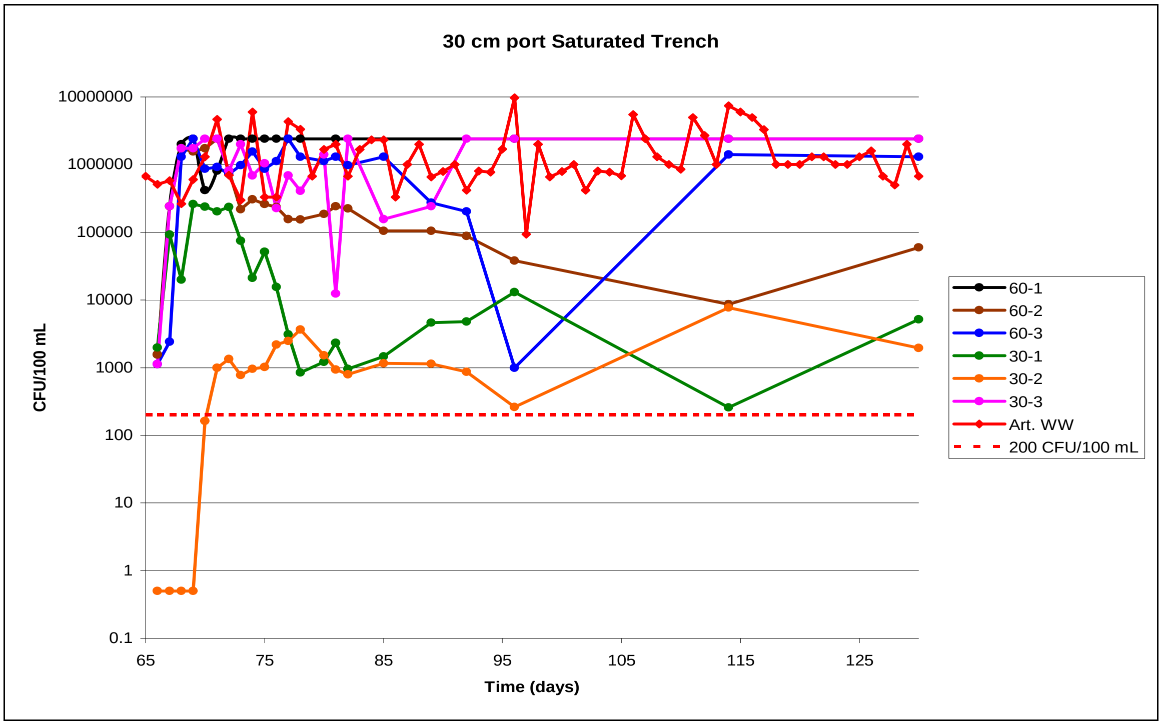


Figure 15. Concentration of *E. coli* bacteria detected at 30 cm depth in soil columns after passing AWW through 30 cm of saturated soil when WT was raised to soil surface in the columns. The 60-1, 60-2, and 60-3 refers to the three columns for the 60-cm treatment and 30-1, 30-2, and 30-3 refer to the three columns in which water table was originally at 30 cm.

One surprising observation was that concentrations flowing through 30 cm of saturated flow were less than 30 cm of unsaturated flow (compare Figures 12 and 16). This may be a result of slower pore water velocity under saturated flow. Under saturated flow, pore water velocity was 2.475 cm/day. Under unsaturated conditions, pore water velocity was calculated to be 3.65 cm/day, based on average water content in the top 30 cm. Unsaturated pore water velocity was 1.5 times greater than under saturated conditions, so there may be less time for filtration and treatment. Under slower pore water velocity, bacteria may have more time and probability to be trapped in smaller pores and die off naturally. Also, under saturated conditions, dilution may be more of a factor. Concentrations of microbes per 100 mL sample may be less because instead of sampling at the top of a saturated zone, samples were taken 30 cm below the surface of the saturated zone, leading to more dilution.

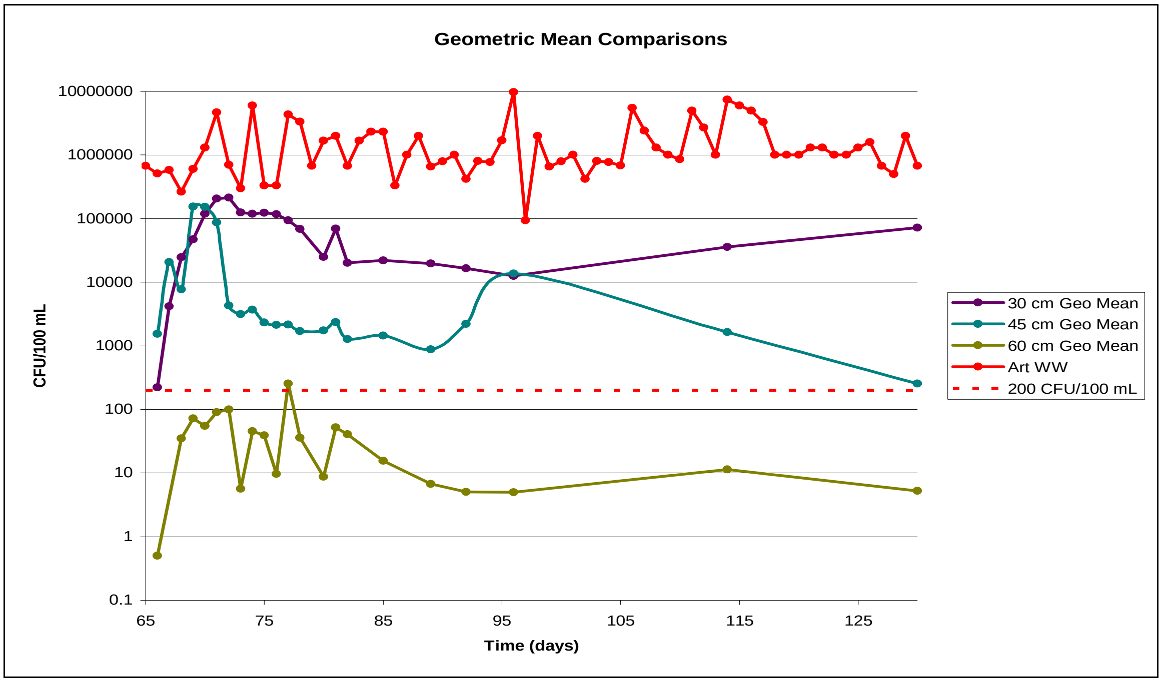


Figure 16. Geometric mean for the microbial count for all saturated trench treatments during days 66-130 of the study.

Colony forming units of *E. coli* decreased through 45 cm of saturated flow. The average concentrations of the outflow samples collected at the 45 cm sampling port were approximately 2.13 x 10^4 CFU/100 mL, while the average inflow concentration remained at 1.78 x 10^6 CFU/100 mL (Figure 17). This is equivalent to two order of magnitude reduction. Four out of the six treatment replications, all three treatments that originally had 60 cm of separation and one of the replications that began at 30 cm of separation and was lowered to 60 cm of aerobic soil, exceeded the 200 CFU/100 mL standard. Overall, the geometric mean of the replications show there was inadequate treatment in the 45 cm of saturated flow (Figure 16).

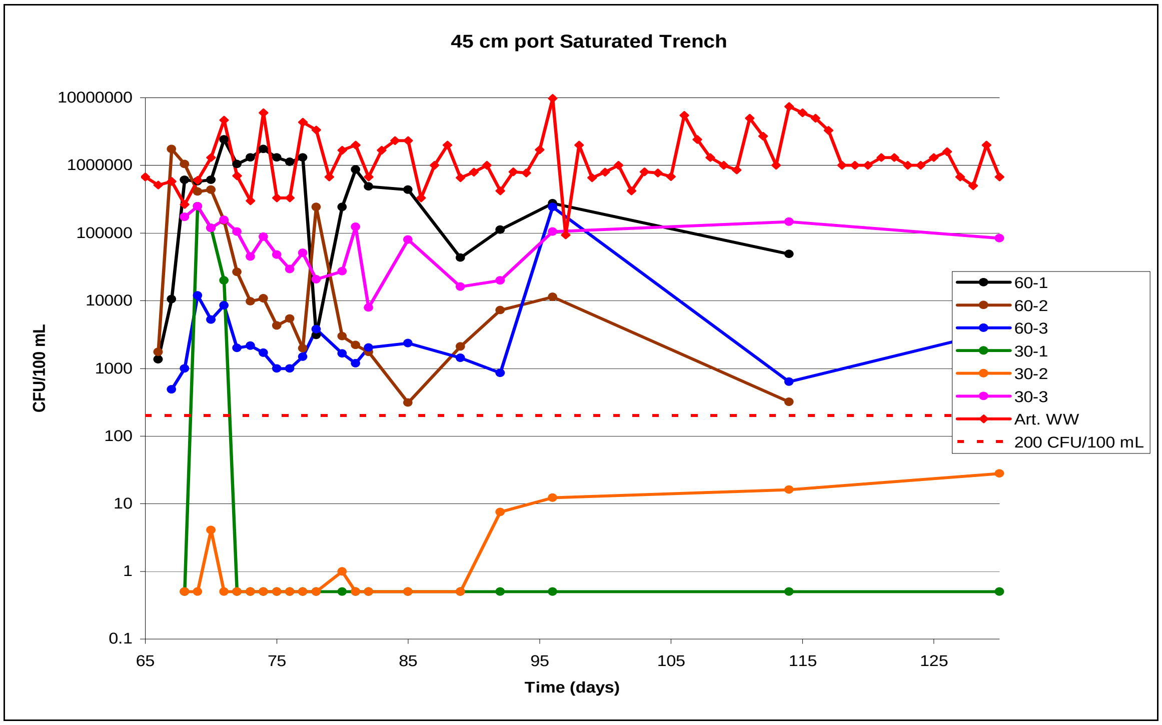


Figure 17. Concentration of *E. coli* bacteria detected at 45 cm depth in soil columns after passing AWW through 45 cm of saturated soil when WT was raised to soil surface.

For the most part, concentrations of *E. coli* after 60 cm of saturated flow were reduced to less than 200 CFU/100 mL, taken to be an acceptable level in this study, with five of the six replications did not exceed the 200 CFU/100 mL standard (Figure 18). One replication, however, exceeded this standard and remained above it for the duration of the experiment. Average concentrations of *E. coli* collected from the 60 cm sampling port after 60 cm of saturated flow were 42 CFU/100 mL, a five fold reduction in the microbial count of the AWW applied to the top of the column (see Figure 16).

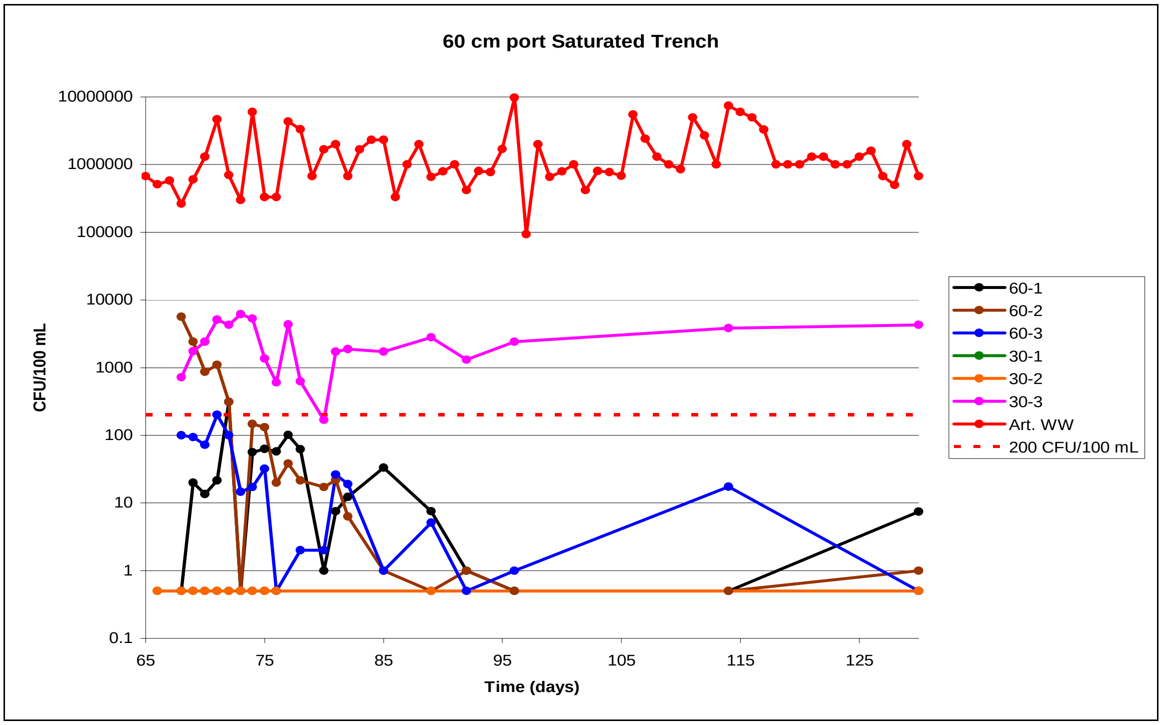


Figure 18. Concentration of *E. coli* bacteria detected at 60 cm depth in soil columns after passing AWW through 60 cm of saturated soil when WT was raised to soil surface.

Although there was some treatment, 60 cm of saturated flow did not treat bacterial concentrations to the same level as 60 cm of unsaturated flow (Figure 19). Unsaturated flow through 60 cm of soil in days 66 through 130 continued to provide adequate treatment, even though colony forming units sampled from the top of the WT were elevated at some points (Figure 20). However, concentrations of *E. coli* never exceeded the standard of 200 CFU/100 mL and the geometric mean of these values remained below the arbitrary value assigned for success (Figure 21).

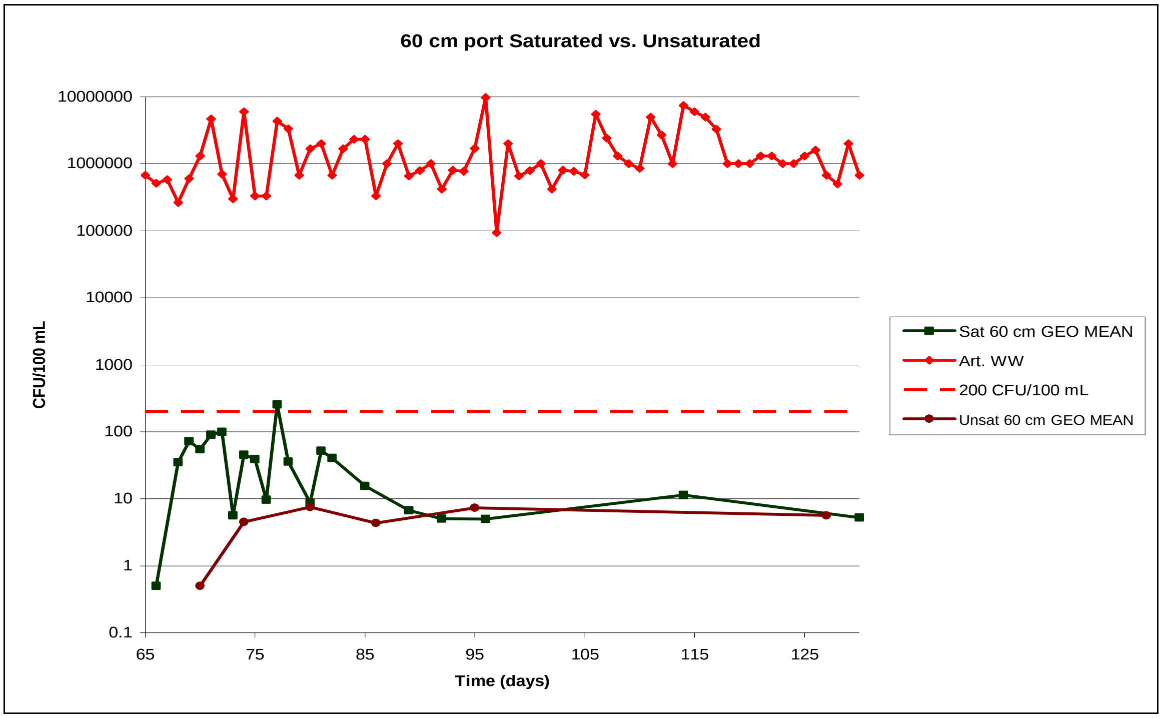


Figure 19. Comparison of 60 cm of saturated and unsaturated flow on *E. coli* removal.

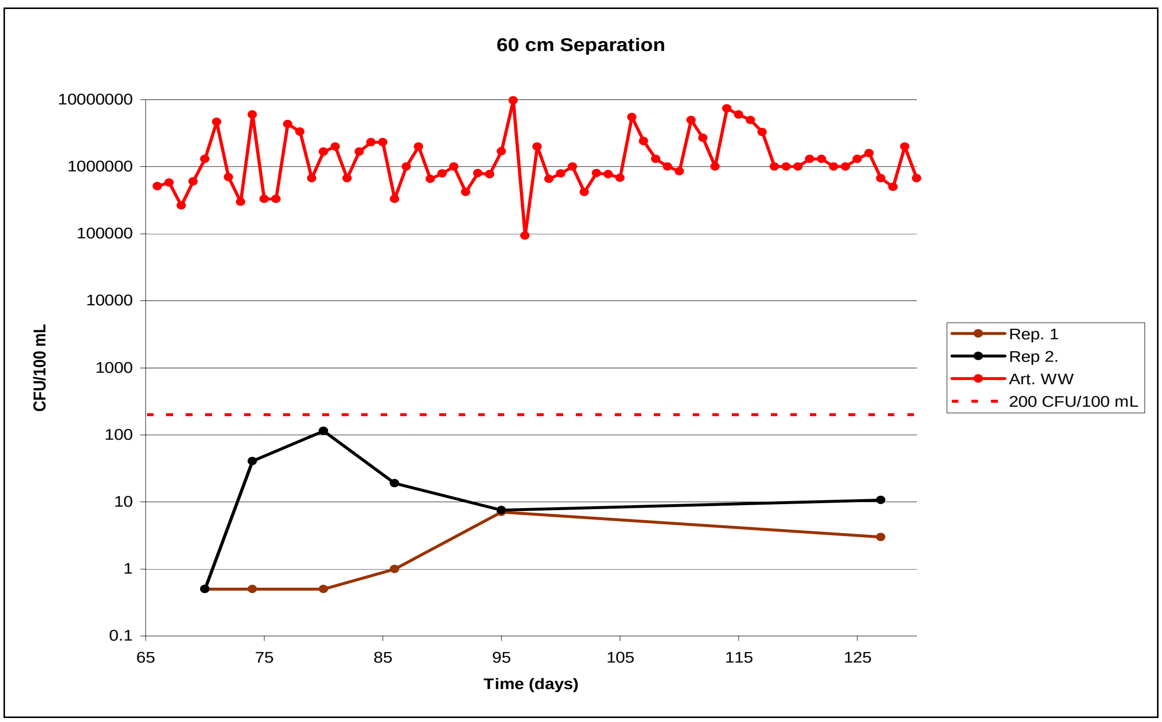


Figure 20. Geometric mean of the concentration of *E. coli* bacteria detected at 60 cm depth in soil columns after passing AWW through 60 cm of unsaturated soil for days 60-135.

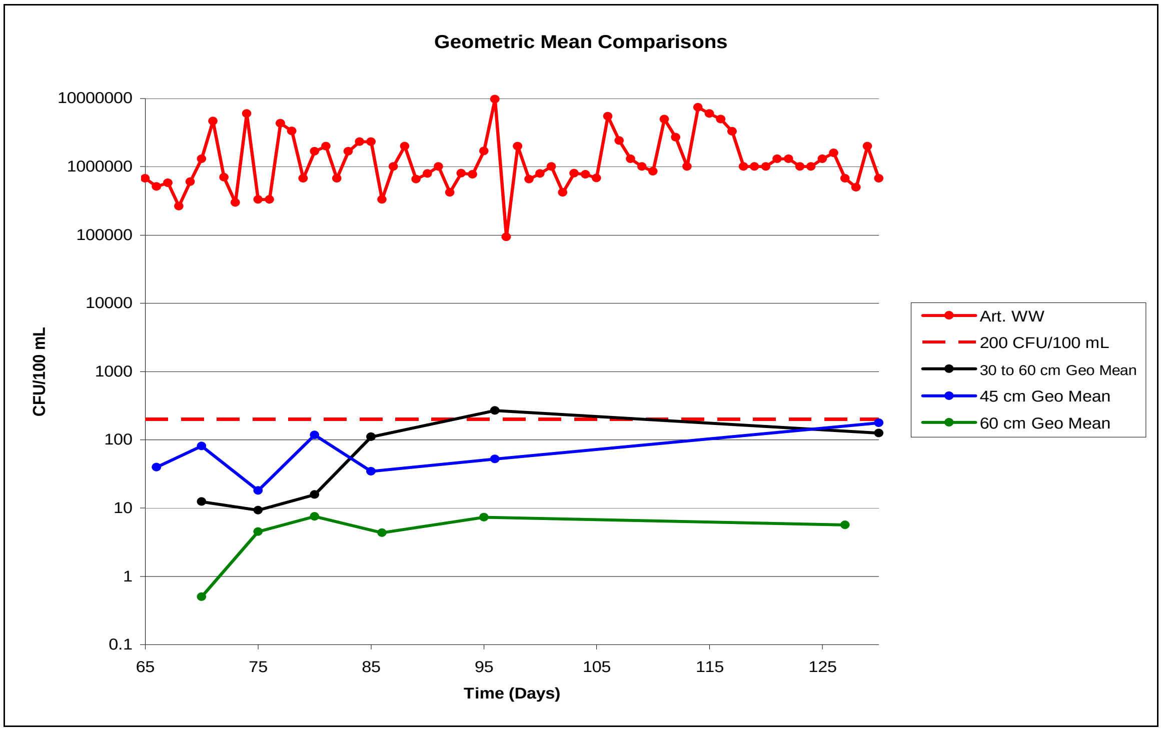


Figure 21. Geometric mean for the microbial count for all unsaturated trench treatments during days 66-130 of the study.

Concentrations of *E. coli* reaching the WT during this time period (days 66-130) through 45 cm of unsaturated flow were highly variable (Figure 22). One replication of this treatment was consistently above the 200 CFU/100 mL limit for this time period. Two of the other replications were close to this cutoff with values above and below the threshold value. The remaining two replications stayed below the critical value for this time period. As a result of the high variability, the geometric mean is very useful in predicting whether this amount of unsaturated flow would be an effective distance for removal. A shown in Fig. 21, the geometric mean of the CFUs passing through 45 cm of unsaturated flow remains below 200 CFU/100 mL for the entire time period during experiment 3. At the end of this treatment period, the concentrations of *E. coli* approached the 200 CFU/100 mL standard.

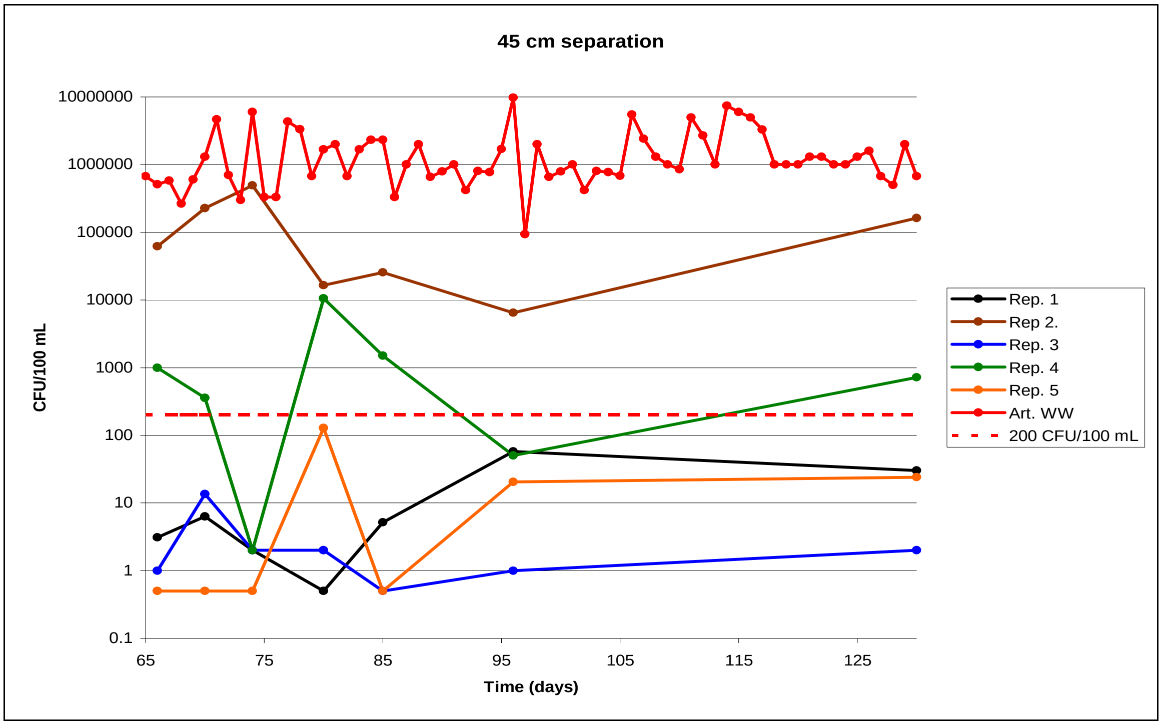


Figure 22. Concentrations of *E. coli* bacteria detected at 45 cm depth in soil columns after passing AWW through 45 cm of unsaturated soil for days 65 through 130.

Concentrations of *E. coli* flowing through columns with the treatment of originally 30 cm of separation lowered to 60 cm of separation are given in Figure 23. One replication of the treatment remains on the order of 10^3 to 10^4 CFU/100 mL. The other replication in this treatment has concentrations of 1 to 10 CFUs/100 mL. The geometric means (Figure 21) of these concentrations of this treatment are greater than concentrations in the treatment that was originally had 60 cm of separation.

Testing the hypothesis that all treatments were taken from the same population versus the alternative hypothesis that there was at least one treatment taken from a different population, there is strong statistical evidence that there was at least one treatment taken from a different population (Appendix F). Trends can be established from examining all 15 pair wise comparisons. At an alpha level of 0.05, CFUs for 30 cm of saturated flow are statistically greater than CFUs for column treatments of 30 cm lowered to 60 cm of separation, 45 cm of separation, 60 cm of separation, and 60 cm of saturated flow. Also proving to be significant at this level 60 cm of unsaturated flow was more effective at removing *E. coli* than 45 cm of saturated flow and 60 cm of saturated flow was more effective than 30 cm of saturated flow.

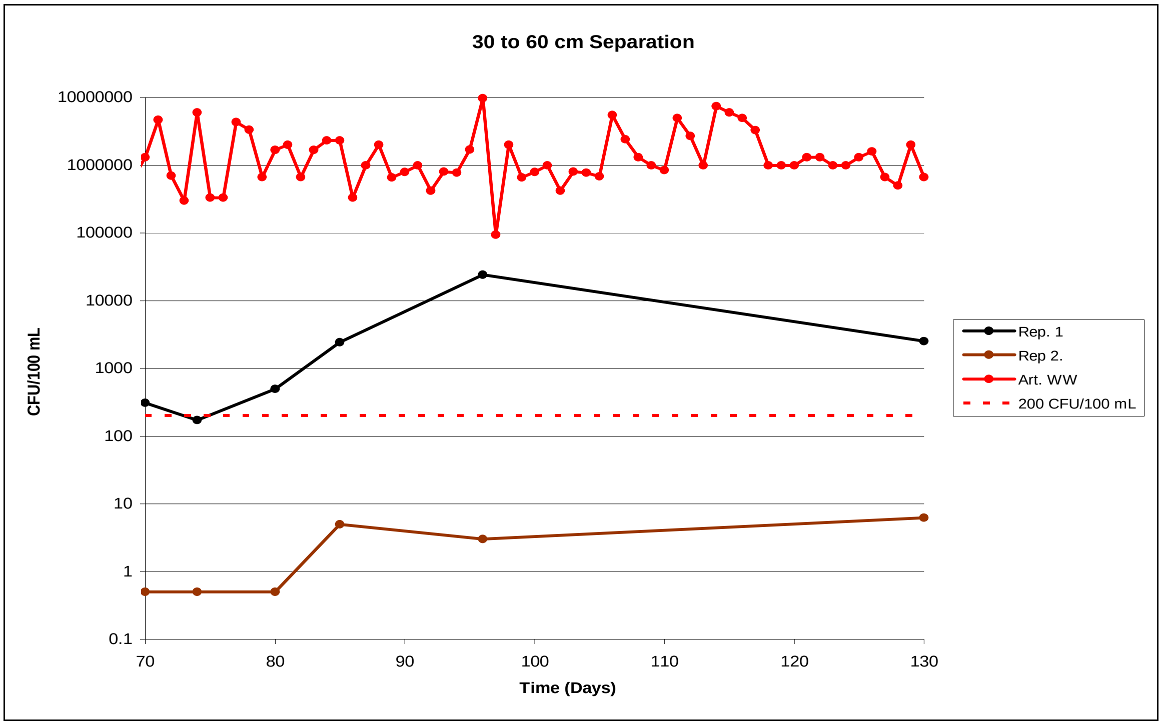


Figure 23. Concentration of *E. coli* bacteria detected at 60 cm depth in soil columns after passing AWW through 60 cm of unsaturated soil when WT was lowered from 30 cm to 60 cm.

Experiment 4

The final part of the study was to mimic an alternating soil treatment area system in which wastewater application is alternated between two drainfields allowing one soil treatment area to rest for a period of time, while the other receives all the wastewater from the dwelling.   When no STE is applied to the soil, does the soil rejuvenate enough in 30 days to improve treatment of STE?

Overall, after stopping application of AWW for 30 days, treatment was improved initially. However, 30 days after restarting wastewater application, CFUs in the WT were elevated to the same level as before the 30-day break. This leads to the conclusion that although there is some immediate benefit in some cases for resting the soil treatment area, there may not be any positive long term effects.

After the 30-day break, all six replications for the treatment with 30 cm of saturated flows had a lower concentration of *E. coli* (Figure 24). Two of the replications went from 10^3 to the lower detection limit with the first measurement after this break on day 165. Fifteen days later, on day 180, all bacterial measurements had increased. With the third and final measurement, on day 194, all concentrations had closely mimicked the concentration of *E. coli* in the WT before the 30-day break had commenced. Under 30 cm of saturated flow following a 30-day break, treatment is not sufficient as demonstrated by the geometric mean in Figure 25.

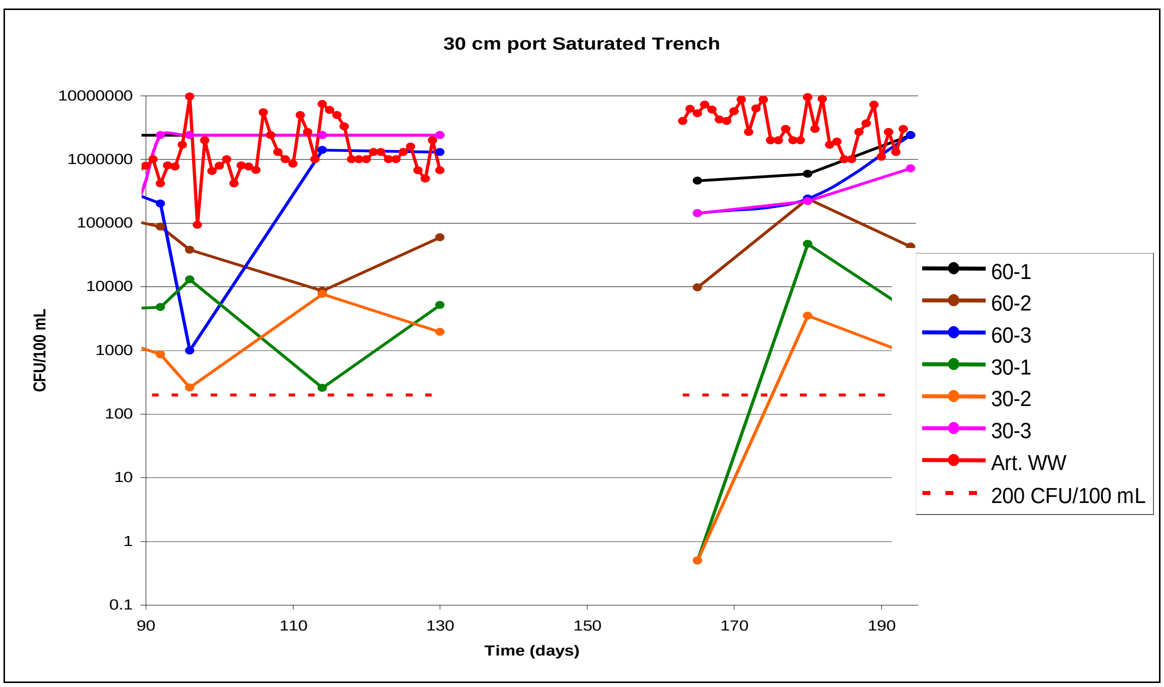


Figure 24. Concentration of *E. coli* bacteria detected at 30 cm depth in soil columns after passing AWW through 30 cm of saturated soil following a 30-day break.

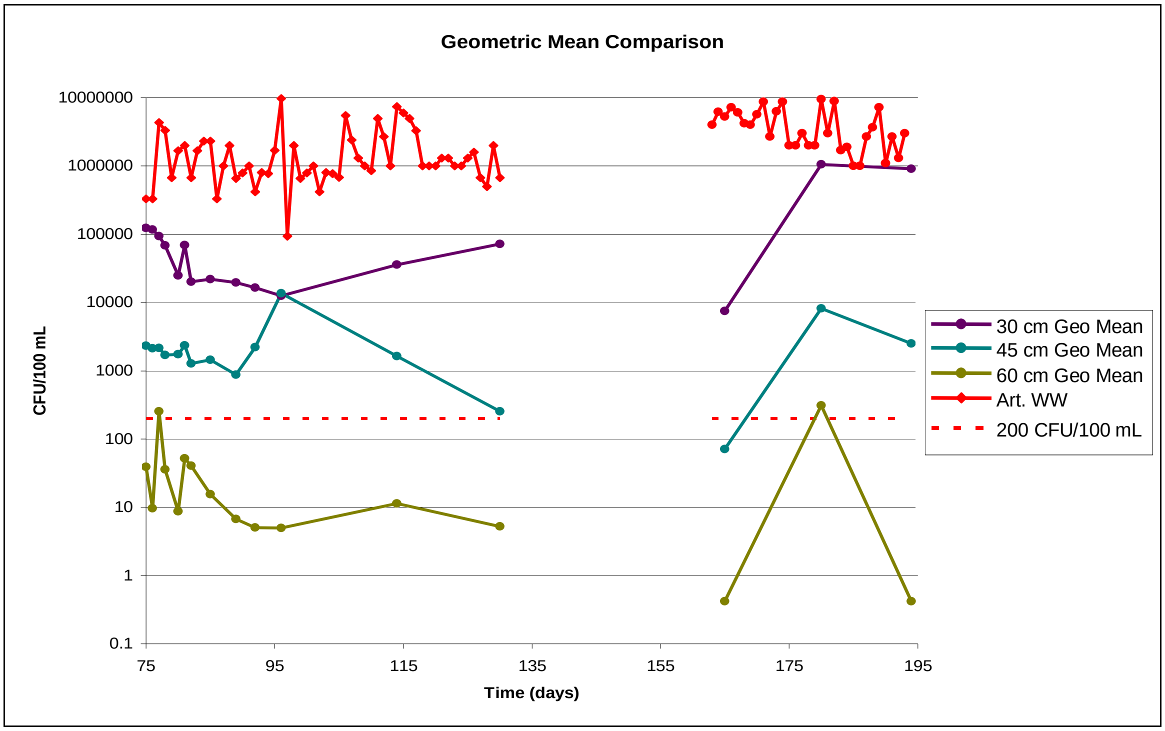


Figure 25. Geometric mean for the microbial count for all saturated trench treatments following a 30-day break.

Forty-five centimeters of saturated flow showed the same trends as 30 cm of saturated flow after a 30-day break from AWW application. After an initial decrease in CFU in the water table after the break, the concentration of *E. coli* returned to pre-break levels within 30 days of AWW application (Figure 26). Before the 30-day break, *E. coli* concentrations for four of the six replications were greater the 200 CFU/100 mL standard and two replications were below this standard. In the first sampling period after the 30-day break all six replication concentrations had decreased and four of the replications were below the 200 CFU/100 mL limit. Thirty days after the break, however, four of the replicates were above the 200 CFU/100 mL limit. Geometric means of the CFU/100 mL measurements from the top of the water table show that 45 cm of saturated flow is insufficient, even after the 30-day break (Figure 25). The geometric mean of CFU taken from the 45 cm sampling port remained above the 200 CFU/100 mL limit for the duration of application of AWW.

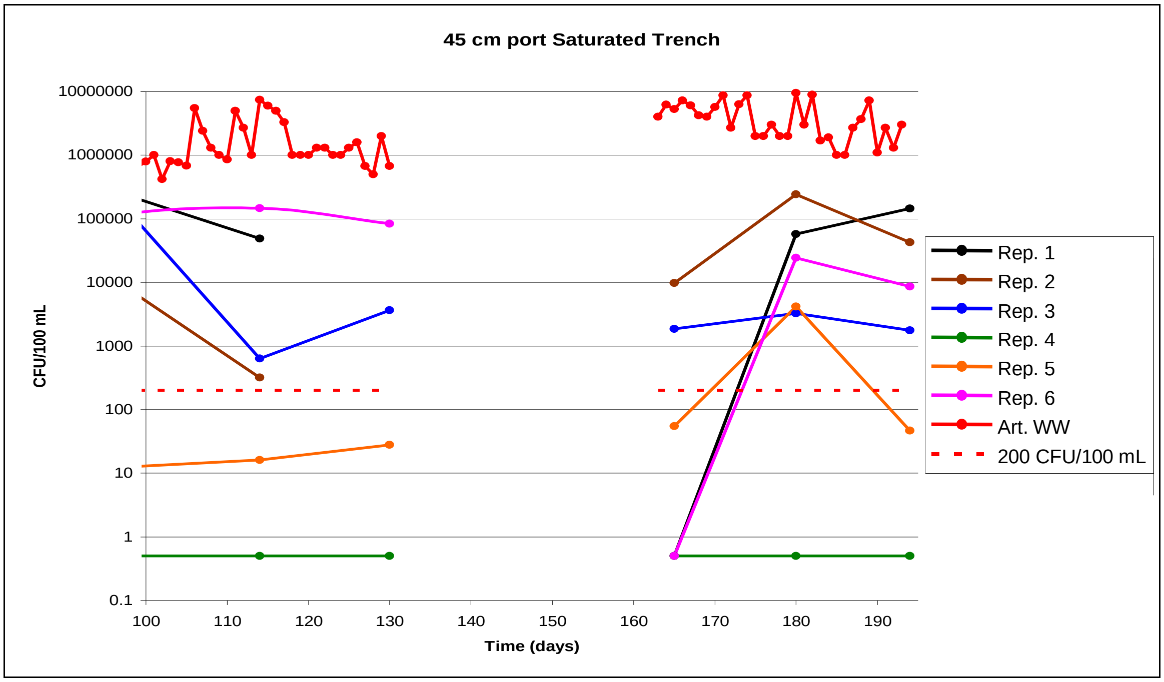


Figure 26. Concentration of *E. coli* bacteria detected at 45 cm depth in soil columns after passing AWW through 45 cm of saturated soil following a 30-day break.

Sixty centimeters of saturated flow did not show the same trends as 30 and 45 cm of saturated flow after a 30-day break from AWW application. After the 30-day break, concentrations of *E. coli* under 60 cm of saturated flow increased (Figure 27). The 30-day break had a perceived negative effect on treatment. Before the 30-day break, concentrations of *E. coli* in five of the six replications were below the 200 CFU/100 mL standard. Following the 30-day break, the first sampling period showed two of the six replicates above the standard. Within 30 days of the termination of the break, four of the six replicates exceeded the 200 CFU/100 mL standard. The geometric mean of CFU/100 mL in the samples taken after 60 cm of saturated flow show that there was adequate treatment when compared to the 200 CFU/100 mL threshold. Sampling on day 180 showed that the geometric mean exceeded the limit during this time, but returned below the cutoff point by day 195, on the last day of sampling (Figure 25).

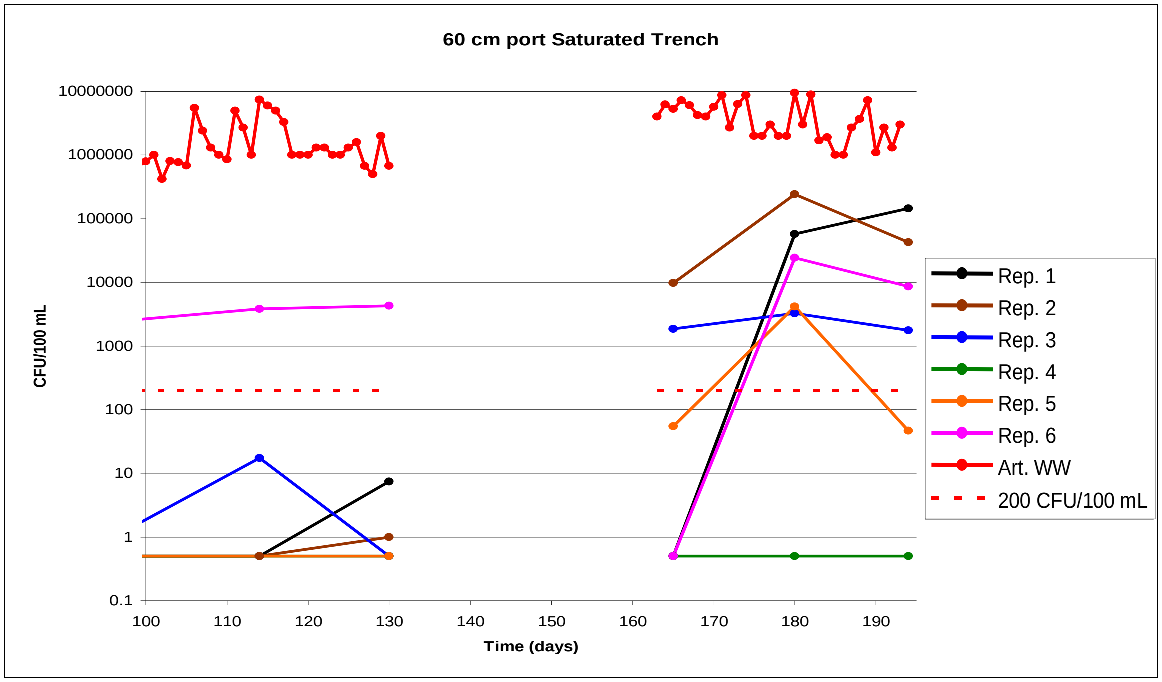


Figure 27. Concentration of *E. coli* bacteria detected at 60 cm depth in soil columns after passing AWW through 60 cm of saturated soil following a 30-day break.

Forty five cm of unsaturated flow showed the same patterns as 60 cm of saturated flow after a 30-day break. In the treatments that maintained 45 cm of unsaturated flow there were two replications above the standard of 200 CFU/100 mL before the break and three replications above the standard of 200 CFU/100 mL after the break (Figure 28). The 30-day break had a negative impact on treatment through 45 cm of unsaturated flow. The geometric mean of the data also demonstrates these results (Figure 29). Before the 30-day break, there was marginally adequate treatment (176 CFU/100 mL) through 45 cm of unsaturated soil. After the break, the geometric mean was greater than 200 CFU/100 mL and it remained above this concentration for the remainder of the experiment.

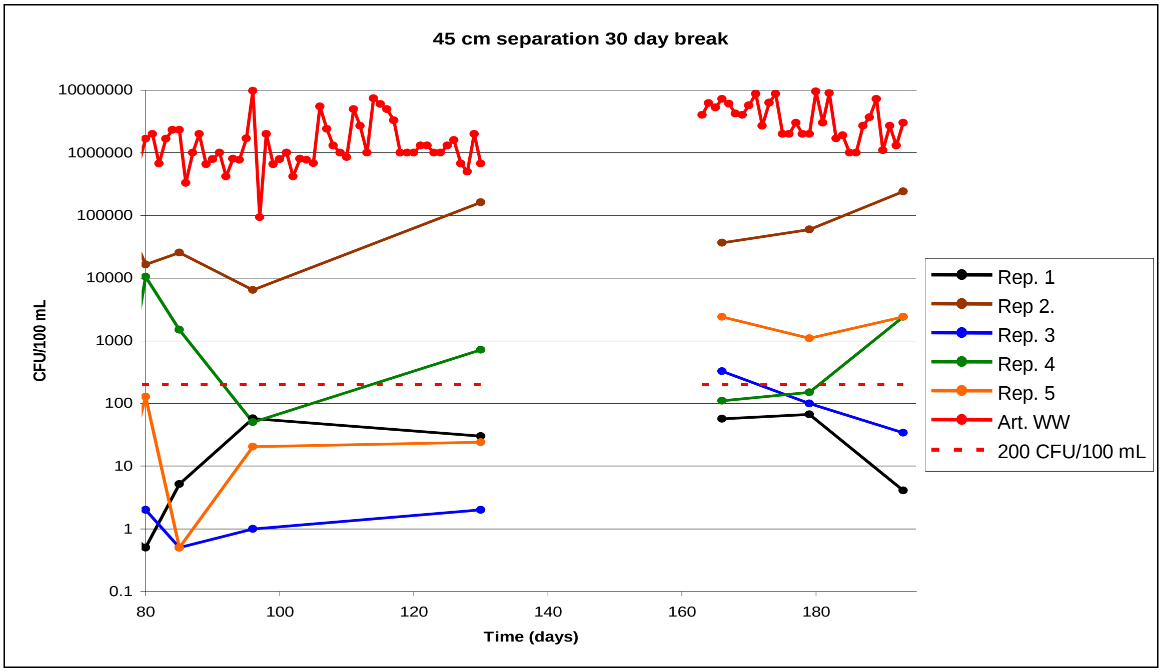


Figure 28. Concentration of *E. coli* bacteria detected at 45 cm depth in soil columns after passing AWW through 45 cm of unsaturated soil following a 30-day break.

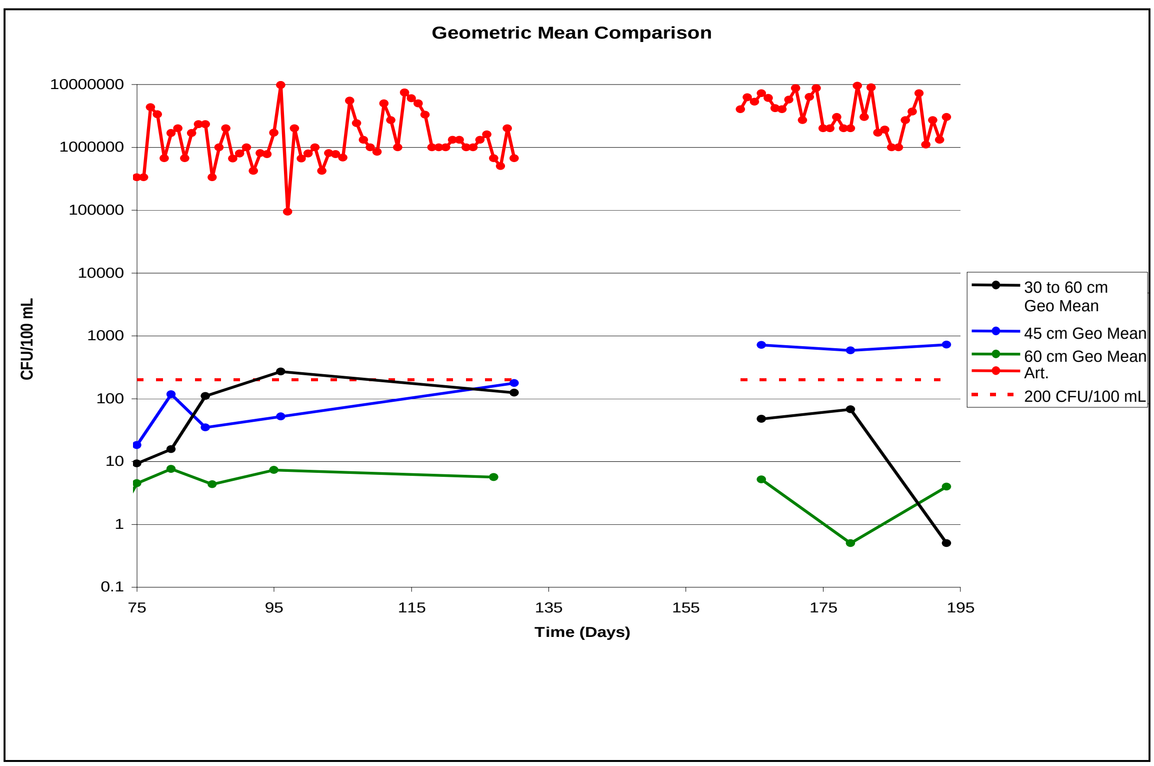


Figure 29. Geometric mean for the microbial count for all unsaturated flow treatments following a 30-day break.

Treatments that had the WT remain at 60 cm below the soil surface throughout the experiment show an overall decrease in CFU/100 mL after the 30-day break (Figure 30). Throughout the treatment, concentrations never reached the 200 CFU/100 mL limit before or after the break. The geometric mean shows that the concentrations decreased after the break, as well (Figure 29).

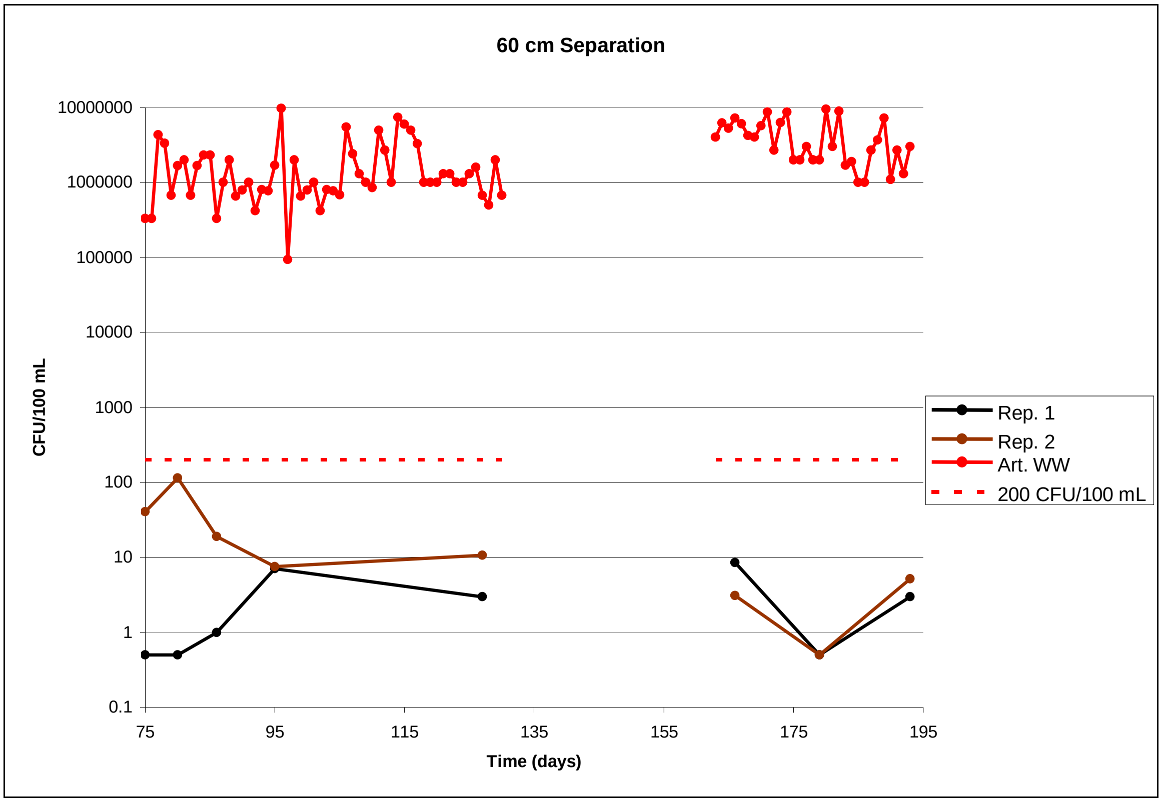


Figure 30. Concentration of *E. coli* bacteria detected at 60 cm depth in soil columns after passing AWW through 60 cm of unsaturated soil following a 30-day break.

Treatments that had the WT originally at 30 cm below the soil surface and then lowered to 60 cm below the soil surface at the beginning of the experiment showed an overall decrease in CFU/100 mL after the 30-day break (Figure 31). Before the break, one replicate was above the 200 CFU/100 mL mark and one was below. After the break, both treatments eventually dropped below the standard and stayed below this level for the last sampling period on day 195. This is the most pronounced decrease in all six treatments in this portion of the experiment. The geometric mean stays well below pre-break CFU levels in the top of the water table after the break (Figure 29).

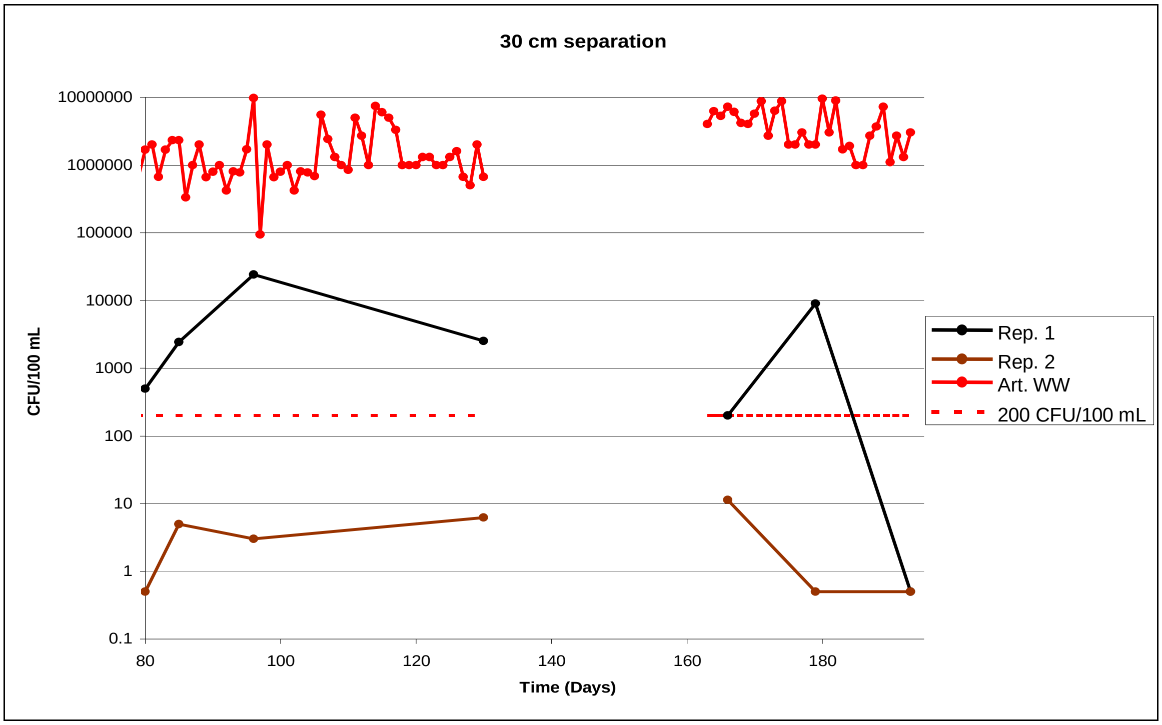


Figure 31. Concentration of *E. coli* bacteria detected at 60 cm depth in soil columns after passing AWW through 60 cm of unsaturated soil following a 30-day break.

According to the overall Kruskal-Wallis non parametric statistical test, there is statistical evidence that there was at least one treatment taken from a different population (Appendix G). Fewer pair wise statistical contrasts can be deemed significant in comparing these treatments. At $\alpha = 0.05$, the only comparison that is statistically significant is that 30 cm of saturated flow had higher concentrations of *E. coli* than 60 cm of saturated flow. The lack of statistical significance for this part of the experiment may be due to the fact that this procedure is very conservative or that there is a high variability with these data.

K_{sat} Measurement

Saturated hydraulic conductivity measured by the constant head method was relatively high and showed that this soil had a potential for rapid soil water velocity. Saturated hydraulic conductivity was approximately 0.17 cm/hour, with calculations shown in Appendix H.

Moisture Release

The moisture release curve for this repacked soil material is shown in Figure 32. From this graph, it can be approximated that the capillary fringe, where the pressure potential is negative but pores are nearly saturated (>90%), is approximately 17 cm in height. This value can be determined by the moisture release curve (Figure 32). At saturation, all pores are filled with water and this takes up approximately 44% of the bulk volume. At 17 cm of pressure head, 40% of the bulk volume (90% saturation) is still occupied by water, and is near saturation, which is assumed to be the capillary fringe.

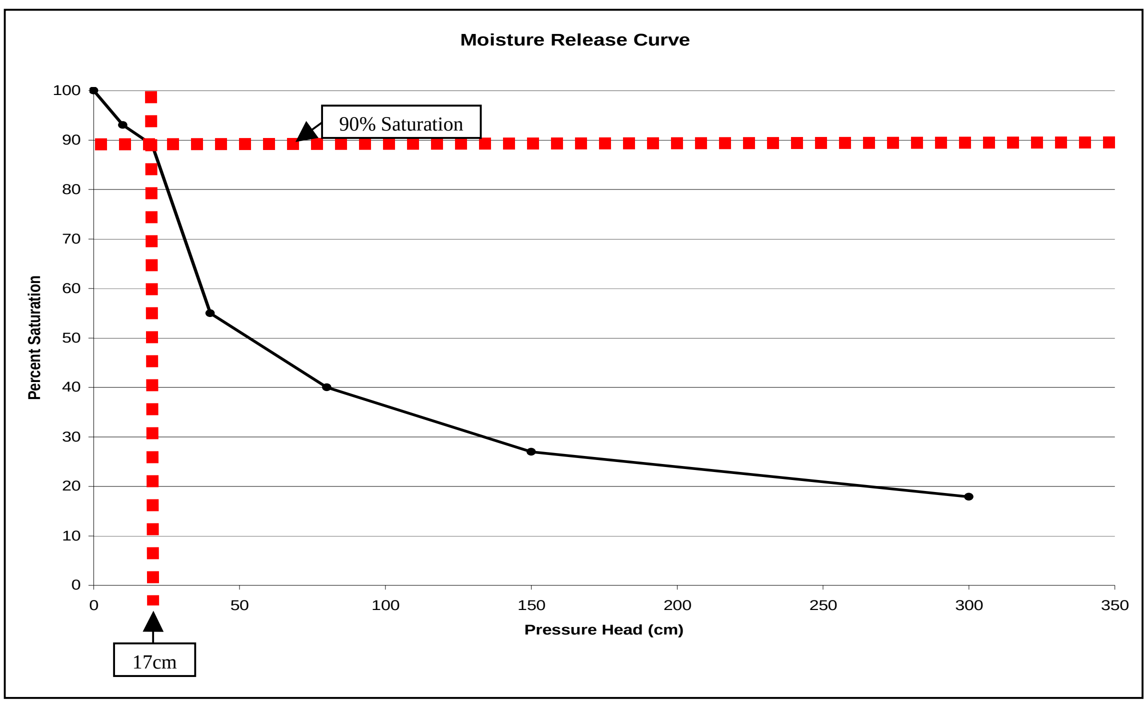


Figure 32. Soil characteristic curve for repacked Norfolk sandy loam soil material.

Redox Potential

Redox Potential

To ensure that the saturated zone in the soil columns became anaerobic, one column was selected to assess the aerobic status of the soil (i.e., availability of oxygen) using redox probes. Redox probes were installed through the side of the PVC column at depths of 10, 20, 30, 40, 50, and 60 cm below the soil surface and the reference electrode was installed approximately 10 cm below the soil surface. The baseline reading with no WT in the column taken on the first day showed that all six redox probes indicated aerobic conditions because they were all more positive than the 0.169 mV needed for anaerobic conditions (Figure 33). After saturating the columns to 30 cm depth below the surface, the soil at 50 and 60 cm below the soil surface became anaerobic quickly, in five days. Forty cm depth became anaerobic by day 13 and the top of the WT at 30 cm became anaerobic by day 20, while the 10 and 20 cm redox probes showed aerobic conditions throughout the first period of sampling (Figure 34). When AWW was applied to the column, starting on day 21, redox potentials at 40, 50, and 60 cm below the soil surface stayed anaerobic. The 30 cm redox probe measured aerobic for one day (day 28), but stayed anaerobic for the duration of measurements. Also, the redox probes at 10 and 20 cm below the soil surface stayed aerobic for the duration of measurements (Figures 33-36). When the WT was dropped from 30 cm of separation to 60 cm of separation, as was practiced for the 2nd part of the study, the 30 cm sampling probe showed aerobic conditions were reestablished within two days and the 40 cm sampling probe showed aerobic conditions were reached within six days of dropping the WT. The 50 and 60 cm probes showed that these depths never reaerated and stayed anaerobic, while the 10 and 20 cm redox probes stayed aerobic (Figure 35). When the WT was brought to the surface, the 50 and 60 cm probes showed conditions stayed anaerobic. On the 8th day after bringing the WT to the surface, the 20, 30, and 40 cm redox probes showed anaerobic conditions formed, while 10 cm below the soil surface never became anaerobic (Figure 36)

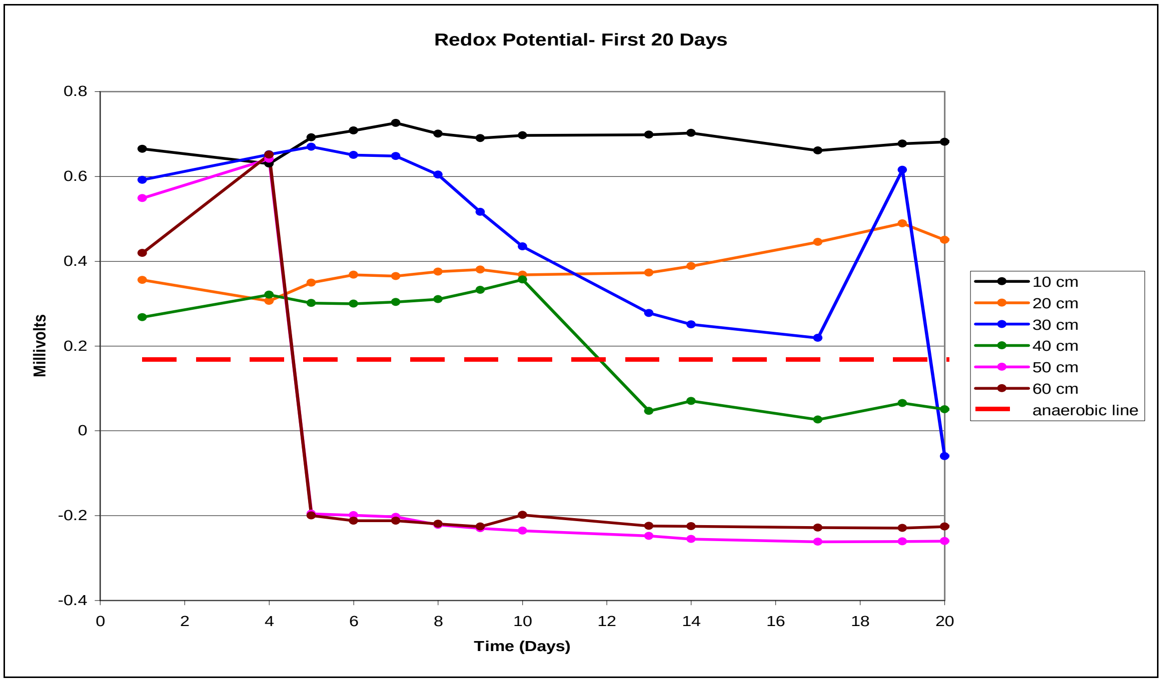


Figure 33. Redox potential at varying depths in the soil column when allowed to remain stagnant for 20 days.

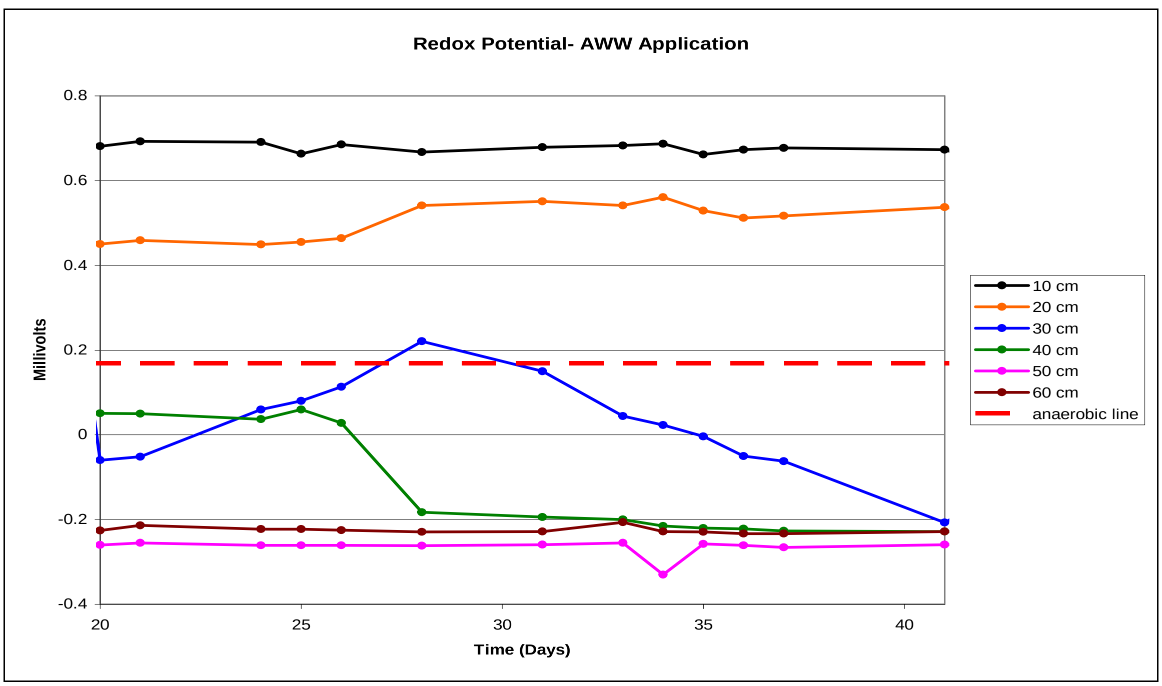


Figure 34. Redox potential at varying depths in the soil column when AWW was applied daily.

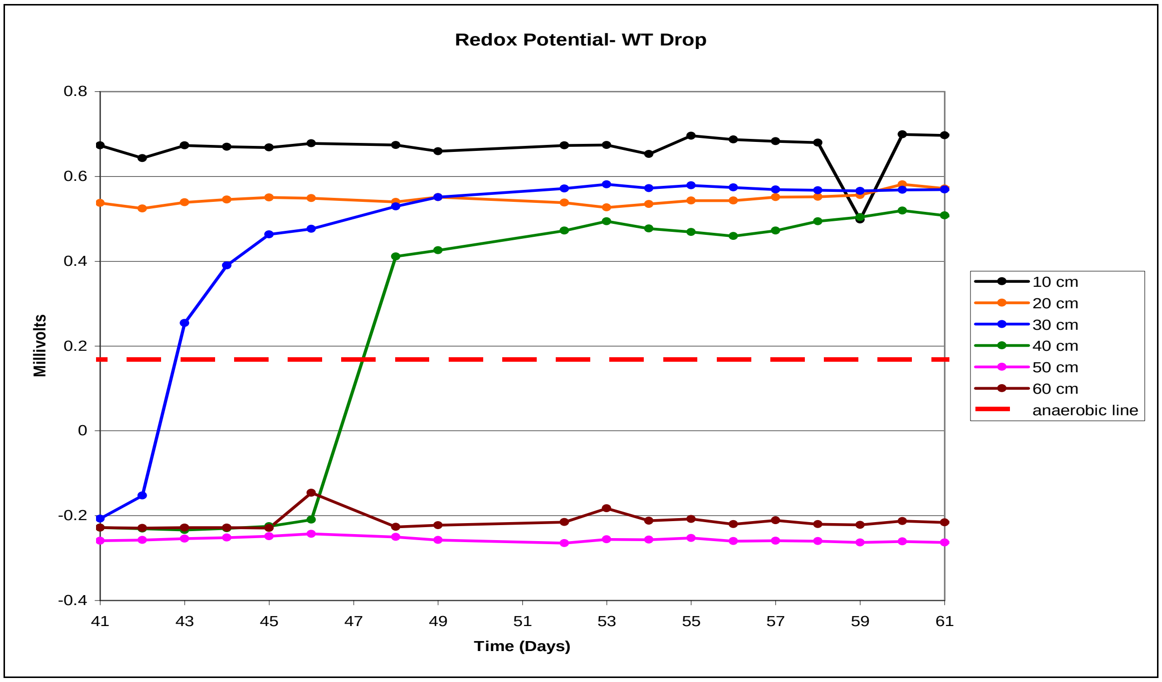


Figure 35. Redox potential at varying depths in the soil column when WT is lowered from 30 cm to 60 cm below the soil surface and AWW application continues.

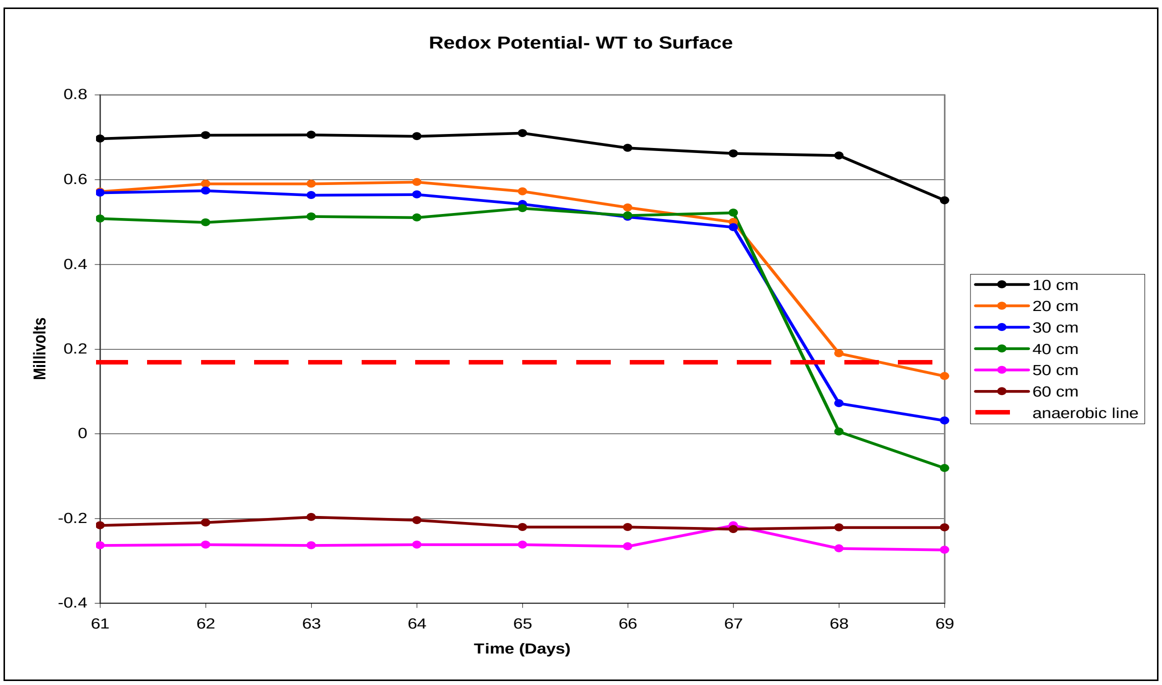


Figure 36. Redox potential at varying depths in the soil column when WT is brought to the soil surface and AWW application continues.

Moisture and Microbial Distribution in Soil Columns

Moisture content in the soil material in the columns validated assumptions made about saturation in the columns. It was assumed that the soil above the WT was unsaturated. It was also assumed that soil below the WT was saturated, where all the pore spaces were filled with water, leading to anaerobic conditions. Distribution of water in the treatment with 60 cm of separation between the top of the soil and the top of the WT shows that saturation was not reached above the WT (Figure 37). Water content increased with depth, but a measurement was not taken below the water table. Distribution of water in the 45 cm of separation treatment showed that saturation was not reached above the WT as well (Figure 38). Saturation is reached in the measurement of water content at 45 and 55 cm below the soil surface, in the saturated zone. For the treatment with the WT at the surface, the entire profile is very close to saturated or is saturated (Figure 39).

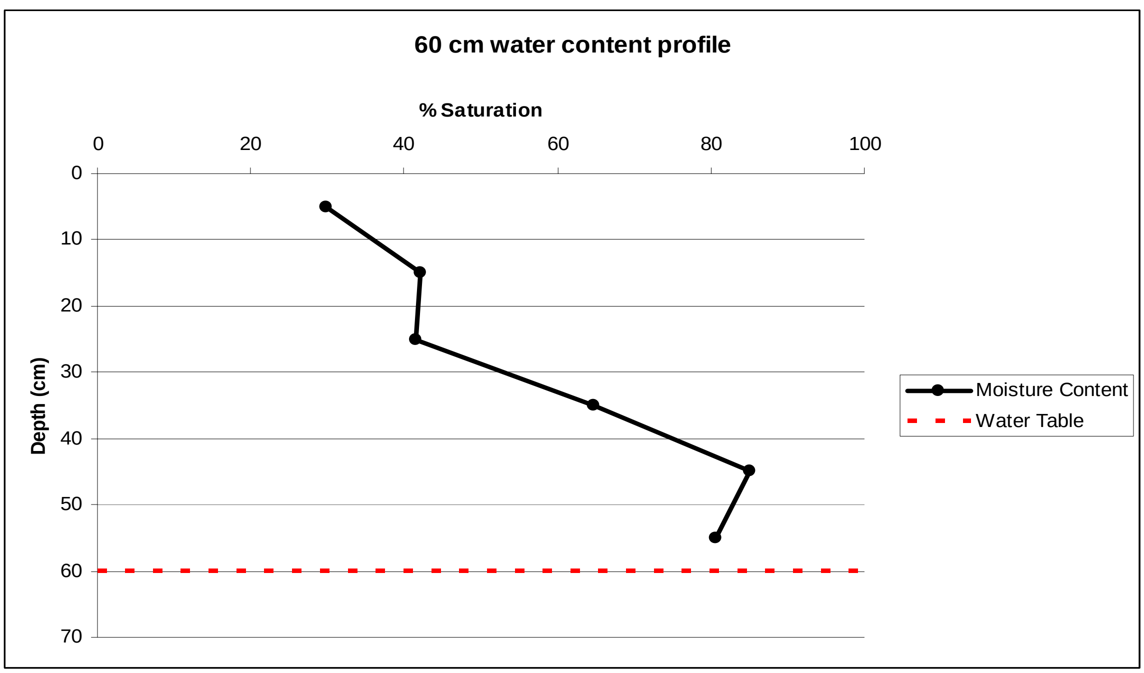


Figure 37. Moisture content profile for 60 cm of separation between the top of the water table and the top of the soil.

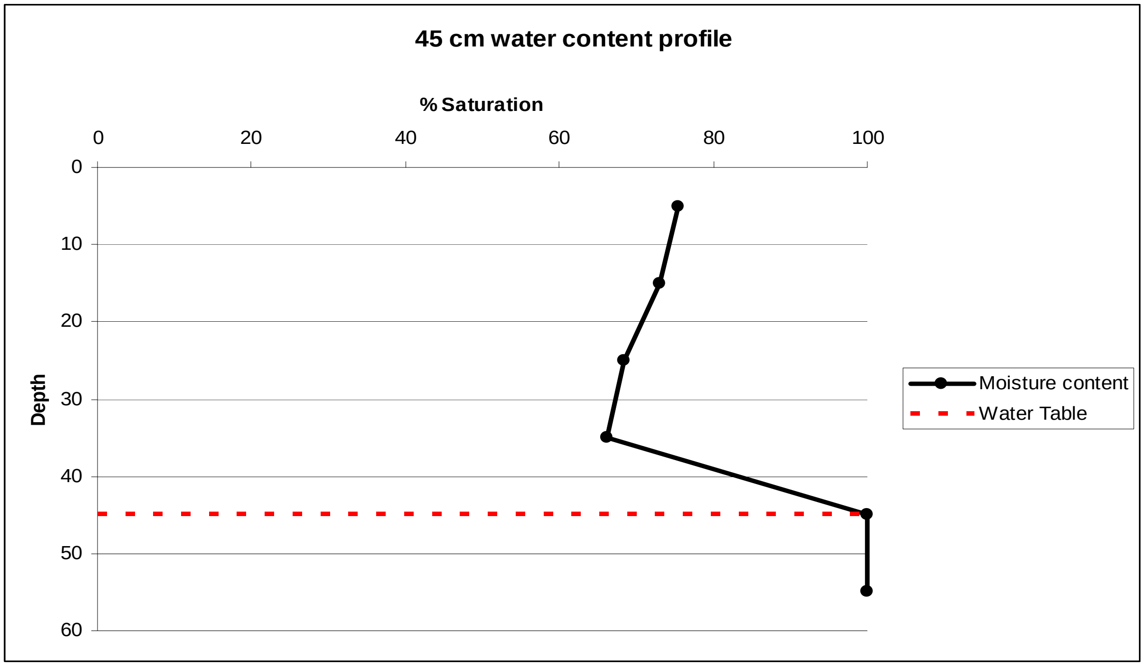


Figure 38. Moisture content profile for 45 cm of separation between the top of the water table and the top of the soil.

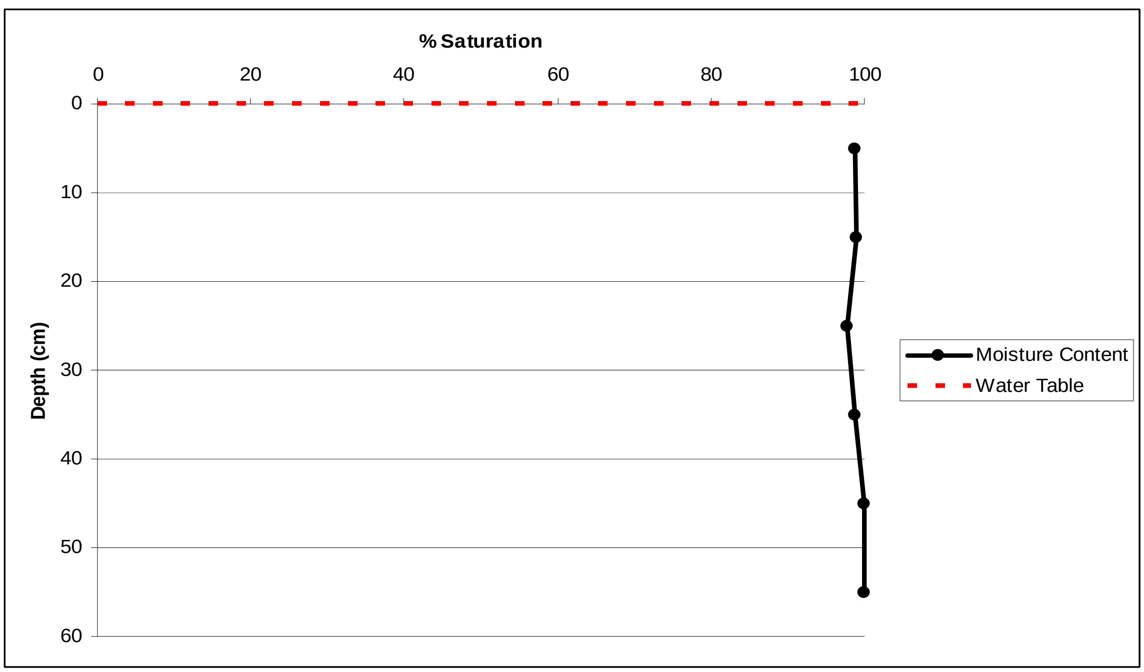


Figure 39. Moisture content profile for treatments with water table at the soil surface.

Microbial distribution concurred with results from Gerba et al. (1975) and Wollum and Cassel (1978). Both studies stated that microbial populations are highest within the first few cm of application in the soil material. Results from this study showed the same outcome where microbial populations, for the most part, were highest in the top 5 to 15 cm for most of the treatments and their replications as shown in Figure 40, Figure 41, and Figure 42.

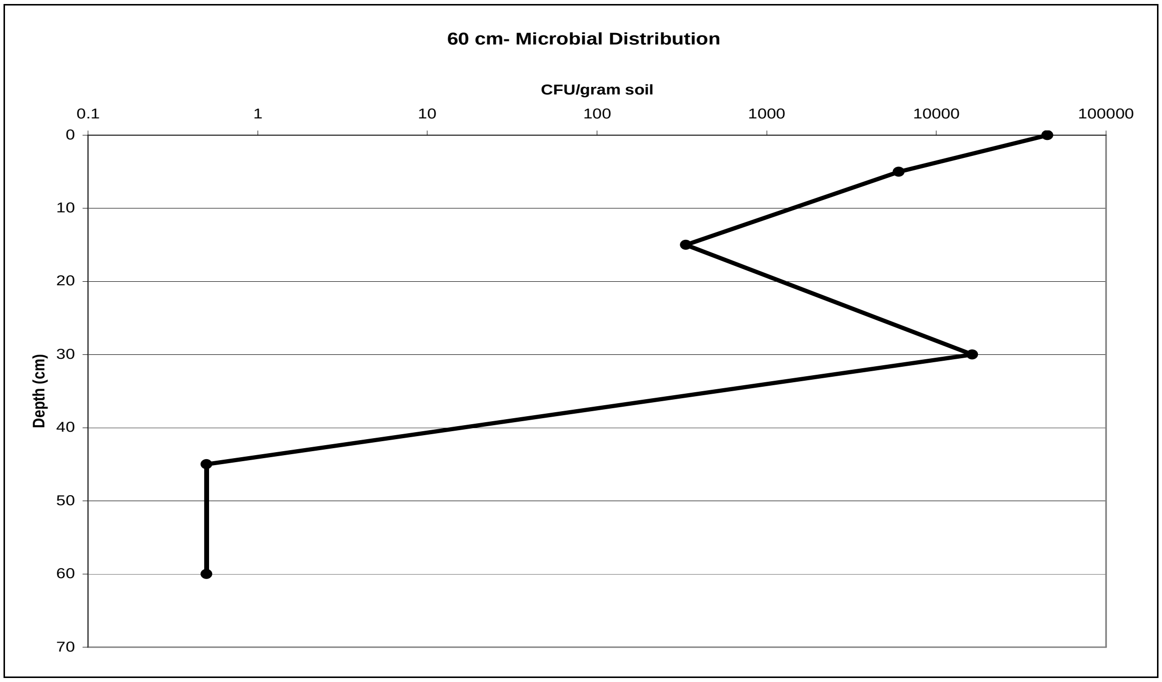


Figure 40. Microbial distribution profile for treatment with 60 cm of separation between the soil surface and the top of the WT.

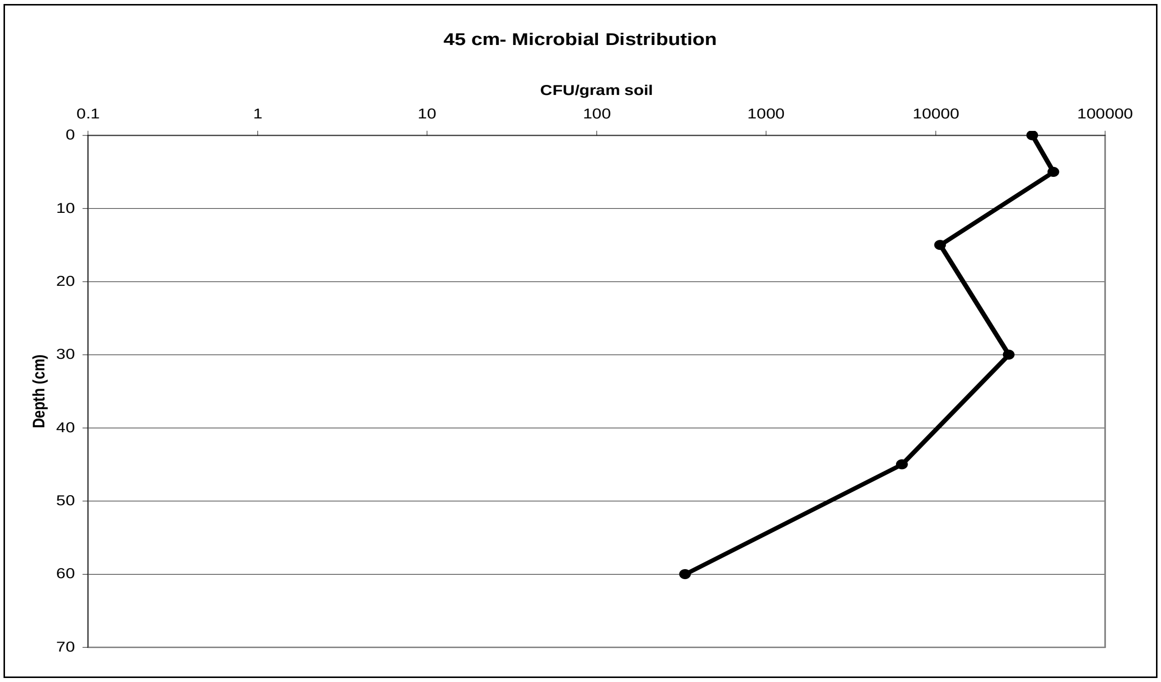


Figure 41. Microbial distribution profile for treatment with 45 cm of separation between the soil surface and the top of the WT.

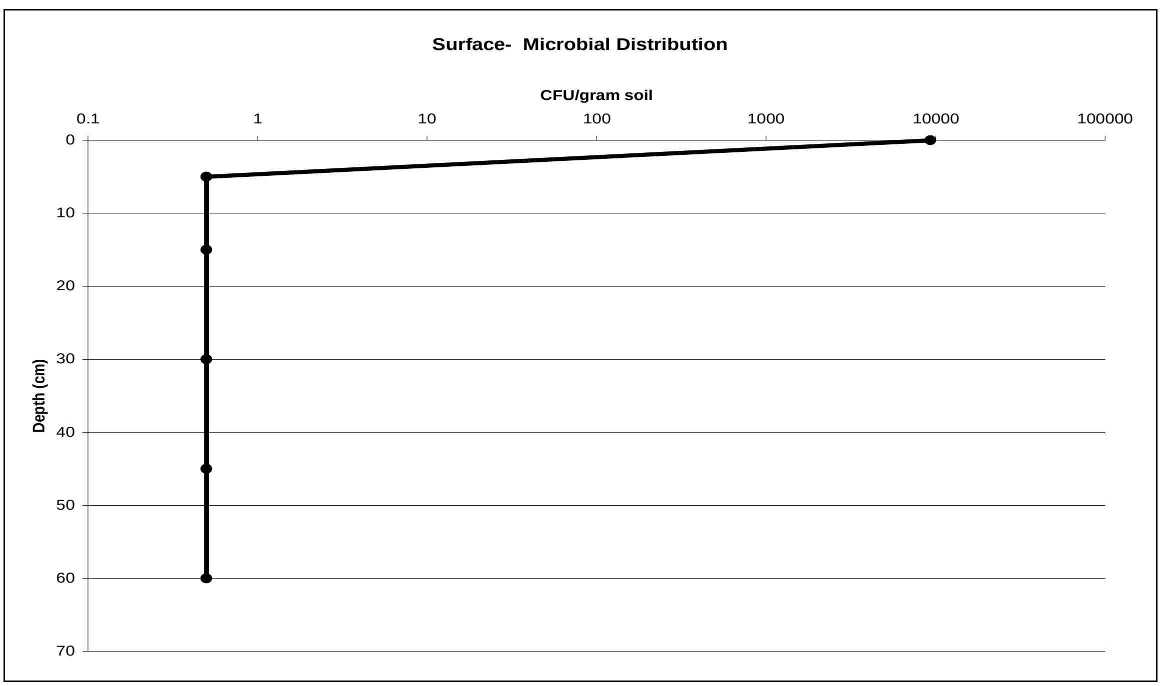


Figure 42. Microbial distribution profile for treatment with WT at the surface of the soil.

SUMMARY AND CONCLUSIONS

The purpose of this study was to ascertain the fate and transport of enteric bacteria from septic tank effluent applied to soils under varying soil moisture regimes. The study was conducted through four different experiments.

The results of the first part of this study showed that longer distances of unsaturated flow is more effective at removing anaerobic bacteria from AWW in soil columns. Sixty centimeters of unsaturated flow was more efficient at removing *E. coli* than both 30 and 45 cm of unsaturated flow, and 45 cm of unsaturated flow was more efficient at removing *E. coli* than 30 cm of unsaturated flow.

North Carolina's regulation (15 A NCAC 18A .1900, Laws and Rules for Sewage Treatment and Disposal Systems) requires 30 cm (one foot) of separation between the bottom of the trench and the seasonal high water table, but this separation distance may not be sufficient for the effective removal of enteric bacteria from AWW. In the first nine days of AWW application with 30 cm of unsaturated flow, there was a complete breakthrough of bacterial concentration indicating insufficient treatment. Concentrations of *E. coli* in samples collected from top of the saturated zone (i.e., water table) located 30 cm below the surface where wastewater was applied were on the same order of magnitude as the inflow concentration of AWW. The critical distance of unsaturated flow in this column experiment was 60 cm. In the 60 cm of unsaturated flow, *E. coli* concentrations were reduced to an acceptable level during the study period.

The second part of the study showed that microbial contamination does not travel with a receding water table. Although microbes reached the top of the saturated zone at 30 cm depth below the surface of the soil in the column (simulating the bottom of the trenches), they did not travel with the receding water table when the saturated zone was lowered from 30 to 60 cm. In dropping the water table, the concentrations of *E. coli* in the top of the WT dropped from 10^6 to 10^1 within one day.

Increasing the length of unsaturated flow path by lowering the WT increased the efficiency of the soil to treat AWW for bacterial contamination. Treatments that had less unsaturated flow for the first nine days of the experiment (30 cm of unsaturated flow) had slightly higher concentrations of *E. coli* than when the WT was never above 60 cm depth. This may be a result of the previously saturated soil acting as a source of contamination. Aerobic bacteria may have been eliminated by the saturated and anaerobic conditions and were not effectively removing introduced bacterial contamination.

In the worst case scenario when the bottom of a septic trench is saturated, there is insufficient treatment of enteric bacteria under saturated flow. Through 30 cm of saturated flow when the trench was saturated, concentration in effluent was equal to or slightly less than the concentration of *E. coli* in the AWW applied to the column throughout the duration of the experiment. At the 45 cm sampling port (i.e., after 45 cm of saturated flow), there was also inadequate treatment for the duration of the experiment. The geometric mean of the concentration of *E. coli* in effluent after 45 cm of saturated flow was above the 200 CFU/100 mL standard for the duration of this experiment. During this time, 45 cm of unsaturated flow maintained concentration of *E. coli* below this same standard. Sixty cm of saturated flow, however, appeared to be sufficient to decrease concentrations of *E. coli* in effluent below the 200 CFU/100 mL level.

It appears that a 30-day break improves treatment of bacteria in AWW for a short period of time. Under 30 and 60 cm of saturated flow and 60 cm of unsaturated flow, concentrations were decreased immediately following the 30-day stoppage of AWW application to the soil columns. However, within 30 days of application after this break, concentrations of bacteria contained in the samples were at the same order of magnitude as before the break. Under 45 cm of saturated flow and 45 cm of unsaturated flow, concentrations of *E. coli* were actually greater than pre-break concentrations of *E. coli,* following the break. The only case where it seemed to be advantageous to cease application of AWW for a period of time was the treatment that had 30 cm of unsaturated flow originally, but the water table was lowered to 60 cm. For this treatment concentrations of *E. coli* in the top of the WT were lower than pre-break levels and stayed that way throughout the 30 day application period after the break. From these data, it is difficult to distinguish whether there is a significant effect of a stoppage of AWW application to the soil.

Thirty cm of separation distance between the bottom of a septic trench and the top of the WT is not sufficient for the removal of *E. coli* contamination in AWW. Forty five cm of unsaturated flow is marginally efficient for bacterial removal. Sixty cm of separation between the trench bottom and WT may be the critical value for removal of bacteria from AWW.

The capillary fringe (CF) has a major role in the vertical transport of microbes in soil. The CF is near saturated (>90% saturated) and therefore has more of the larger pores available for transport. In these soil columns, the CF was calculated to be approximately 17 cm, so when there was 30 cm of “unsaturated conditions” in the columns, there was 13 cm of truly unsaturated flow. This remains true for 45 cm of separation only being 28 cm of unsaturated flow. As a result, 60 cm of unsaturated flow was the best in removal of microbes because the CF only extended to 43 cm below the soil surface.

Microbial contamination that reaches the WT does not travel with the descending WT. Microbial counts were elevated in the treatments with a falling WT as a result of microbial contamination only having to move 30 cm. Microbes applied to the surface of the soil must move the entire 60 cm length of unsaturated soil.

Preliminary results indicated that 30 cm of saturated flow may be marginally more effective than 30 cm of unsaturated flow. This may be a result of the saturated flow having a slower pore water velocity, allocating more time for attenuation and removal of microbes.

Resting the soil treatment area has a positive influence on treatment of bacterial contamination in the short term, but would not be a feasible way to regenerate failed soil treatment areas.

REFERENCES

Allen, M.J., and E.E. Geldreich. 1975. Bacteriological criteria for ground-water quality. Ground Water. 13: 45-52.

Amador, J.A., D.A. Potts, M.C. Savin, P. Tomlinson, J.H. Gorres, and E.L. Nicosia. 2006. Mesocosm-scale evaluation of faunal and microbial communities of aerated and unaerated leachfield soil. Journal of Environmental Quality. 35: 1160–1169.

Annan'ev, N. E., and N. S. Demin. 1971. On the spread of pollutants in sub-surface waters. Hygiene and Sanitation. 36: 292-294.

Ausland,G., T.K. Stevik, J.F. Hanssen, J.C. Kohler, and P.D. Jenssen. 2002. Intermittent filtration of wastewater-removal of fecal coliforms and fecal Streptococci. Water Resources. 36: 3507-3516.

Baars, J.V. 1957. Travel of pollution, and purification en route, in sandy soils. Bulletin World Health Organization. 16: 727.

Bitton, G., N. Lahav, and Y. Henis. 1974. Movement and retention of Klebsiella aerogenes in soil columns. Plant and Soil. 40: 373–380

Bouma, J., W.A. Ziebell, W.G. Walther, P.G. Olcott, E. McCoy, and F.D. Hole. 1972. Soil absorption of septic tank effluent. Extension Circulation no. 20. University of Wisconsin, Madison.

Bouwer, S., S.C. Lance, and M.S. Riggs. 1974. High-rate land treatment II: Water quality and economic aspects of the Flushing Meadows project. Journal of the Water Pollution Control Federation. 46: 844

Brady, N.C. 1974. The Nature and Properties of Soils (8th ed.). Macmillan, New York.

Brady, N.C., and R.R. Weil. 2002. Element of the Nature and Properties of Soils (2^{nd} Ed.). Prentice Hall, New Jersey.

Brown, K.W., H.W. Wolf, K.C. Donnelly, and J.F. Slowey. 1979. The movement of fecal coliforms and coliphages below septic lines. Journal of Environmental Quality. 8: 121–125.

Brown, K.W., S.G. Jones, and K.C. Donnelly. 1980. The influence of simulated rainfall on residual bacteria and virus on grass treated with sewage sludge. Journal of Environmental Quality. 9: 261-264.

Burton, G.A., Jr., D. Gunnison, and G.R. Lanza. 1987. Survival of pathogenic bacteria in various freshwater sediments. Applied Environmental Microbiology. 53: 633-638.

Butler, R.G., G.T. Orlob, and P.H. McGauhey. 1954. Underground movement of bacteria and chemical pollutants. Journal of American Water Works Association. 46: 97-111.

Caldwell, E.L. 1937. Pollution flow from pit latrines when an impervious stratum closely underlies the flow. Journal of Infectious Diseases. 61: 269-288.

Caldwell, E.L. and L.W. Parr. 1937. Ground water pollution and the bored-hole latrine. Journal Infectious Diseases. 61:148-183.

Canter, L.W., and R.C. Knox. 1985. Septic tank system effects on groundwater quality. Lewis Publications, Chelsea, Michigan.

Cattaneo, M.V., C. Masson, and C.W. Greer. 1997. Transport and survival of a genetically engineered Pseudomonas cepacia strain in soil columns during wet/dry cycles. Annals of the New York Academy of Sciences. 1: 280–289

Celico, F., M. Varcamonti, M. Guida, and G. Naclerio. 2004. Influence of precipitation and soil on transport of fecal enterococci in fractured limestone aquifers. Applied Environmental Microbiology. 70: 2843-2847

Clark D.L., B.B. Milner, M.H. Stewart, R.L. Wolfe, and B.H. Olson. 1991. Comparative study of commercial 4-methylumbelliferyl-beta-D-glucuronide preparations with the Standard Methods membrane filtration fecal coliform test for the detection of Escherichia coli in water samples. Applied Environmental Microbiology. 57: 1528–1534.

Cogger C. 1988. On-site septic systems: the risk of ground water contamination. Journal of Environmental Health 51: 12–16

Cogger, C.G., L.M. Hajjar, C.L. Moe, and M.D. Sobsey. 1988. Septic system performance on a costal barrier island. Journal of Environmental Quality. 17: 401-408.

Commonwealth of Massachusetts. 2006. Title 5 Alternative to percolation testing guidance for system upgrades. Department of Environmental Protection. Retrieved on 5/14/08 from http://www.mass.gov/dep/water/laws/t5sieve.doc.

Cotteral, J.A., Jr., and, D.P. Norris. 1969. Septic tank systems. American Society of Civil Engineers Journal Sanitary Engineering Division. 95: 715-746.

Crabill, C., R. Donald, J. Knelling, R. Foust, and G. Southam. 1998. The impact of sediment fecal coliform reservoirs on seasonal water quality in Oak Creek, Arizona. Water Resources. 33: 2169-2171.

Crane, S.R., P.W. Westerman, and M.R. Overcash. 1981. Die-off of fecal indicator organisms following land application of poultry manure. Journal of Environmental Quality. 9: 531–537.

Crane, S.R., and J.A. Moore. 1984. Bacterial pollution of groundwater: a review. Water Air and Soil Pollution. 22: 67–83

Craun, G. F. 1979 Waterborne outbreaks of Giardiasis. Waterborne transmission of Giardiasis. In Proceedings of a Symposium, 18–20 September 1978. USEPA/600/9-79/001. 127–147

Craun, G.F. 1984. Health Aspect of Groundwater Pollution, in G. Bitton and C.P. Gerba (Eds.) Groundwater Pollution Microbiology. John Wiley & Sons, Inc. New York.

DallaValle J.M., and R.R. Jones. 1940. Basic principles of industrial sanitation. American Journal of Public Health. 30: 369-384

Daniels, R.B., L.A. Nelson, and E.E. Gamble. 1970. A method of characterizing nearly level surfaces. Annals of Geomorphology. 14: 175-185.

Daniels, R.B., E.E. Gamble, W.H. Wheeler, and C.A. Holzhey. 1972. Some details of the surficial stratigraphy and geomorphology of the coastal plain between New Bern and Coats, North Carolina. North Carolina Geological Society and Atlantic Coastal Plain Geological Association. Field trip guidebook.

Daniels, R.B., S.W. Buol, H.J. Kleiss, and C.A. Ditzler. 1999. Soil Systems in North Carolina. Technical Bulletin 314. North Carolina State University, Soil Science Deptartment. Raleigh, NC. 27695-7619.

Department of Natural Resources. 1997. Summary of On-Site Sewage System Requirements King County, Washington. Retrieved on 4/15/08 from <ftp://dnr.metrokc.gov/wlr/dss/swdmapdx/refb.PDF>

Duda, A.M., and K.D. Cromartie. 1982. Coastal pollution from septic tank drainfields. Journal of Environmental Engineering, Division of American Society of Civil Engineering. 108: 1265-1279.

Duncan, C.S., R.B. Reneau, Jr., and C. Hagedorn. 1994. Impact of effluent quality and soil depth on domestic wastewater. Proceedings of the Seventh International. Symposium on Individual and Small Community Systems. American Society of Agricultural Engineering. St. Joseph, Michigan. 219-228.

Dunn O.J. 1964. Multiple comparisons using rank sums. Technometrics 6: 241–252.

Eckner, K.F. 1998. Comparison of membrane filtration and multiple-tube fermentation by the Colilert and Enterolert methods for detection of waterborne coliform bacteria, Escherichia coli, and Enterococci used in drinking and bathing water quality monitoring in southern Sweden. Applied and Environmental Microbiology. 64: 3079–3083.

Edberg, S.C., M.J. Allen, and D.B. Smith. 1989. National field evaluation of a defined substrate method for the simultaneous detection of total coliforms and Escherichia coli from drinking water: comparison with presence–absence techniques. Applied Environmental Microbiology. 55:1003–1008.

Edberg, S.C., M.J. Allen, D.B. Smith, and N.J. Kriz. 1990. Enumeration of total coliforms and Escherichia coli from source water by the defined substrate technology. Applied Environmental Microbiology. 56:366-369.

Edberg, S.C., M.J. Allen and D.B. Smith. 1991. Defined substrate technology method for rapid and specific simultaneous enumeration of total coliforms and Escherichia coli from water: collaborative study. Journal of the Association of Official Analytical Chemists 74:526-529.

Elsner, J.B. and A.B. Kara. 1999. Hurricanes of the North Atlantic: Climate and Society. Oxford University Press, New York.

Entry, .A., R.K. Hubbard, J.E. Theis, and J.J. Fuhrmann. 2000. The influence of vegetation in riparian filterstrips on coliform bacteria: II. Survival in soils. Journal of Environmental Quality. 29: 1215–1224.

Faust, M.A. 1982. Relationship between land use practices and fecal bacteria in soils. Journal of Environmental Quality. 11: 141–146.

Gagliardi, J.V., and J.S. Karns. 2002. Leaching of *Escherichia coli* O157:H7 in diverse soils under various agricultural management practices**.** Applied Environmental Microbiology. 66: 877–883.

Garcia, M.M., and K.A. McKay. 1970. Pathogenic microorganisms in soil: An old problem in a new perspective. Canadian Journal of Comparative Medicine. 34: 105–110

Gee, G.W., and D. Or. 2002. Particle size analysis. In: J.H. Dane and G.C. Topp, Editors, Methods of soil analysis, part 4—Physical methods, Soil Science Society of America. Book Series, SSSA, Madison, Wisconsin. 255–293.

Gerba, C.P., C. Wallis, and J.L. Melnick. 1975. Fate of wastewater bacteria and viruses in soil. Journal of Irrigation and Drainage Engineering 101: 157-174.

Gilbert, R. G., C. P. Gerba, R. C. Rice, H. Bouwer, C. Wallis, and S. L. Melnick. 1976. Virus and bacteria removal from wastewater by land treatment. Journal of Environmental Microbiology. 32:333-338.

Glotzbecker, R. A., and A. L. Novello. 1975. Poliovirus and bacterial indicators of fecal pollution in handfill leachates. News of Environmental Research in Cincinnati. January 31, 1975. United States Environmental Protection Agency National Research Center, Cincinnati, OH.

Griffin D.M., and G. Quail. 1968. Movement of bacteria in moist, particulate systems. Australian Journal of Biological Sciences. 21: 579–582.

Hagedorn, C., D.T. Hansen, and G.H. Simonson. 1978. Survival and movement of fecal indicator bacteria in soil under conditions of saturated flow. Journal of Environmental Quality. 7: 55-59.

Hagedorn, C., and E. McCoy. 1979. Soil suitability for on-site waste disposal: development of genetically marked Escherichia coli strains as tracers of subsurface water flow. Water Resources Research Institute, Oregon State University, Corvallis, Oregon. WRRI-65. 81.

Hagedorn, C., E.L. McCoy, and T.M. Rahe. 1981. The potential for ground water contamination from septic effluents. Journal of Environmental Quality. 10: 1–8.

Ho, G.O., R.A. Gibbs, and K. Mathew. 1991. Bacteria and virus removal from secondary effluent in sand and red mud columns. Water Science and Technology 23: 261-270.

Hollander, M., and D. Wolfe. 1973. Nonparametric Statistical Methods. John Wiley and Sons, New York.

Hoover, M.T. 1994. Septic systems and their maintenance-revised. Publication AG-439-13. North Carolina Cooperative Service.

Idaho DEQ. 2002. Community septic systems. DEQ-6, SSDS, 82014.

Indiana SDH. 1990. Residential sewage disposal systems. Retrieved on 5/14/08 from <http://www.state.in.us/isdh/regsvcs/saneng/pdf_s/410_IAC_6_8_1.pdf>

Karathanasis, A.D., T.G. Mueller, B. Boone, and Y.L. Thompson. 2006. Effect of soil depth and texture on fecal bacteria removal from septic effluents. Journal of Water and Health. 4: 395-404.

Kibbey, H.J., C. Hagedorn, and E.L. McCoy. 1978. Use of fecal streptococci as indicators of pollution in soil. Applied Environmental Microbiology. 35: 711–717

Kleiss H.J., F.G. Averette, and R.E. Horton. 1982. Soils of the peanut belt research station, Lewiston, North Carolina: Their technical and usability classification. N.C. Agric. Res. Serv., NCDA, and USDA, Raleigh.

Krone, R.B., G.T. Orlob, and C. Hodgkinson. 1958. Movement of coliform bacteria through porous media. Sewage Industry Wastes. 30:1-13.

Lee, B.D., D.D. Jones, and H.M. Peterson. 2005. Septic System Failure. Purdue Extension Document HENV-1-W. Department of Agronomy and Department of Agricultural and Biological Engineering, Purdue University, Indiana.

MacConnell, V., and R.O. Coburn. 2000. Microbial pathogens and on-site soil treatment systems. Retrieved on 4/24/2008 from <http://www.ces.ncsu.edu/plymouth/septic/98meschke.html>

Matson, K.C., and E.F. Fels. 1996. Approaches to automated water table mapping. Conference Proceedings for the National Center for Geographic Information and Analysis. Santa Fe, New Mexico. 21-25 January.

McCoy, E. 1969. American Society of Agricultural Engineers. Paper No. 69-430, St. Joseph, Michigan.

McCoy E.L., and C. Hagedorn. 1979. Quantitatively tracing bacterial transport in saturated soil systems. Water Air Soil Pollution. 11: 467–479.

McGauhey, P.H., and R.B. Krone. 1954. Report on the investigation of travel of pollution. Report to the California Water Pollution Control Board. Publication 11.

McGauhey, P.H., and R.B. Krone. 1967. Soil mantle as a waste water treatment system. SERL Report No. 67-11. University of California Berkeley.

McLeod, M., J. Aislabie, J. Smith, R. Fraser, A. Roberts, and M. Taylor. 2001. Viral and chemical tracer movement through contrasting soils. Journal of Environmental Quality. 30: 2134–2140.

McQuillan, D. Ground-water quality impacts from on site septic systems. 2004. Proceedings, National Onsite Wastewater Recycling Association, 13th Annual Conference, Albuquerque, New Mexico. November 7-10.

Merrell, J.C., Jr., W.F. Jopling, R.F. Bott, A. Katko, and H.E. Pintler. 1967. The Santee Recreation Project, Santee, California Final Report. Water Pollution Research Series. Publication No. WP-20-7. Federal Water Pollution Control Administration, Cincinnati, OH.

Mubiru, D.N., M.S. Coyne, and J.H. Grove. 2000. Mortality of Escherichia coli O157:H7 in two soils with different physical and chemical properties. Journal of Environmental Quality. 29: 1821–1825.

NCSS. 2005. Norfolk Series. Retrieved on 4/21/08 from <http://www2.ftw.nrcs.usda.gov/osd/dat/N/NORFOLK.html>

Newman, M. 2006. Groundwater table predition for tidal and inland conditions. Proceedings of the FICE/FDOT Design Conference. Florida Department of Transportation and the Florida Institute of Consulting Engineers. Orlando, Fl. Session 29.

North Carolina Cooperative Extension Service. 1996. Septic Systems: What you need to know. Publication number WM-1. North Carolina State University. Raleigh, NC. 27695-7619. Retrieved from http://www5.bae.ncsu.edu/programs/extension /publicat/wqwm/wm1.html on 4/22/08.

NCDENR. 2008. Laws and rules for sewage treatment and disposal systems, North Carolina General Statute 130A-333,4, 116 Rules. .1942 of Title 15A Subchapter 18A of the North Carolina Administrative Code. North Carolina Department of Environment and Natural Resources, Raleigh, North Carolina. <http://www.deh.enr.state.nc.us/ oww/Rulelaw/rules.htm>

Ontario Rural Wastewater Centre. 2006. Frequently asked questions about building regulations, standards, and codes. Retrieved 5/14/08 from <http://www.uoguelph.ca/orwc/FrequentlyAskedQuestions/FAQ_Regs.htm>

Paul, J.H., J.B. Rose, S. Jiang, C. Kellogg, and E.A. Shinn. 1995. Occurrence of fecal indicator bacteria in surface waters and the subsurface aquifer in Key Largo, Florida. Applied Environmental Microbiology. 61: 2235-2241

Potts, D.A., J.H. Gorres, E.L., Nicosia, and J.A. Amador. 2004. Effects of aeration on water quality from septic system leachfields. Journal of Environmental Quality. 33: 1828-1838.

Powelson, D.K., and A.L. Mills. 1998. Water saturation and surfactant effects on bacterial transport in sand columns. Soil Science. 163: 694–704

Powelson, D.K. and A. L. Mills. 2001. Transport of Escherichia coli in Sand Columns with Constant and Changing Water Contents. Journal of Environmental Quality. 30: 238-245.

Powelson, D. K. and C. P. Gerba. 1994. Virus removal from sewage effluents during saturated and unsaturated flow through soil columns. Water Resources. 28: 2175–2181.

Prasad, G., R. Rajput and A.K. Chopra. 2006. Sand intermittent filtration technology for safer domestic sewage treatment. Journal of Applied Sciences and Environmental Management. 10: 73-77

Purdue University. 1998. A homeowners guide to onsite system regulations. Purdue University, Agronomy and Agricultural and Biological Engineering, Purdue University, West Lafayette, IN 47907.

Pye V.I., P. Ruth, and J. Quarles. 1983. Groundwater contamination in the United States. University of Pennsylvania Press, Philadelphia, PA.

Rahe T.M., C. Hagedorn, E.L. McCoy and G.F. Kling. 1978. Transport of antibiotic-resistant Escherichia coli through western Oregon hillslope soils under conditions of saturated flow. Journal of Environmental Quality. 7: 487–494.

Reddy, K.R., R. Khaleel, and M.R. Overcash. 1981. Behavior and transport of microbial pathogens and indicator organisms in soils treated with organic wastes. Journal of Environmental Quality. 10: 255–266

Reneau, R. B., and D. E. Pettry. 1975. Movement of coliform bacteria from septic tank effluent through selected coastal plain soils in Virginia. Journal of Environmental Quality. 4: 41-44.

Reynolds, K.A. 2004. Ground water vulnerability to microbial contamination. Water Conditioning and Purification. 28-30

Romero, J.C. 1970. The movement of bacteria and viruses through porous media. Ground Water. 8: 37–48.

SAS Institute. 1989. SAS/STAT User's Guide (Version 6, 4th Ed.). SAS Inst. Inc., Cary, NC.

SAS Institute. 1994. The SAS System for Windows. Release 6.10. SAS Inst. Inc., Cary, NC.

Schaub, S.A., and C.A. Sorber. 1977. Virus and bacteria removal from wastewater by rapid infiltration through soil. 33(3): 609-619.

Sevebeck, K.P., and C.J. Kroehler. 1992. A guide to septic systems and alternatives. Virginia Water Resources Research Center. Blacksburg, VA.

Severson, E. 2005. Evaluation of several methods of predicting historic water table levels. Masters Thesis. North Carolina State University, Raleigh.

Smith, B.R., M.A. Granger, and S.W. Buol. 1976. Sand and coarse silt mineralogy of selected soils on the lower Coastal Plain of North Carolina. Soil Science Society of America Journal. 40: 928-932.

State of DE- DNREC. 2005. Minimum Isolation Distances. Exhibit C. Retreived on 5/14/08 from < http://www.dnrec.state.de.us/water2000/Sections/GroundWat/Library/Regs/regsfinal.pdf>

State of Florida. 1985. Standards for onsite sewage disposal systems. Chapter 10D-6, Florida Administrative Code. Dept. of Health and Rehabilitative Services, Tallahassee.

State of Minnesota. 2008. State Register, Rules, Executive Orders, Appointments, Commissioners' Orders, Revenue Notices, Official Notices, Grants, State Contracts & Loans, Non-State Bids, Contracts & Grants 32: 1343-1466.

State of Montana. 1991. Adopted by the Board of Environmental Review of the State of Montana. Retrieved on 5/14/08 from <http://www.deq.mt.gov/wqinfo/MPDES/RULES03/SUBCH9newRules1-10EDIT__.pdf.>

State of New York. 1990. APPENDIX 75-A to Public Health Law, 201(1)(1) New York State Wastewater Treatment Standards- Individual Household Septic Systems.

Stiles, C.W., and H.R. Crohurst. 1923. Principles underlying the movement of *E. coli* in ground water with the resulting pollution of wells. Public Health Report 38: 1350-1353.

Tate, R.L. III. 1978. Cultural and environmental factors affecting the longevity of Escherichia coli in Histosols. Applied Environmental Microbiology. 35: 925-929.

US Census Bureau. 2006. American housing survey for the United States: 2005.

UC Cooperative Extension. 2003. Septic tanks. Retrieved 10/9/2007 from <http://cecalaveras.ucdavis.edu/realp.htm>

USEPA. 1977. The report to congress. Waste disposal practices and their effect on ground water. U.S. Environmental Protection Agency. Washington, DC.

USEPA. 1980. Design manual for on-site wastewater treatment and disposal systems. USEPA Rep. 625/1-80-012. U.S. Gov. Print. Office, Washington, DC.

USEPA. 1997. Microbial Monitoring under GWDR:Analytical Methods. Retrieved on 5/20/08 from <http://www.epa.gov/OGWDW/standard/montbl2.html.

USEPA. 1999. Decentralized Systems Technology Fact Sheet. US EPA 932-F-99-075. U.S. Gov. Print. Office, Washington, DC.

USEPA. 2003. National Primary Drinking Water Regulations. USEPA 816-F-03-016. U.S. Gov. Pring. Office, Washington, DC.

USEPA. 2005a. Fecal Coliforms in sewage sludge (biosolids) by multiple-tube fermenation using lauryl tryptose broth (LTB) and EC medium. Washington, DC: Environmental Protection Agency, EPA. EPA Method. 1680.

USEPA. 2005b. A homeowner's guide to septic systems. USEPA-832-B-02-005. U.S. Gov. Pring. Office, Washington, DC.

USEPA. 2006. Monitoring and assessing water quality. Retrieved 9/27/06 from http://www.epa.gov/owow/monitoring/volunteer/stream/vms511.html.

United States Geological Survey. 2000. The Coastal Plain. Retrieved 5/12/2008 from <http://tapestry.usgs.gov/features/13coastalplain.html>.

Van Cuyk, S., R. Siegrist, A. Logan, S. Masson, E. Fischer, and L. Figueroa. 2001. Hydraulic and purification behaviours and their interactions during wastewater treatment in soil infiltration systems. Water Resources. 35: 953–964.

Viraraghavan, T., and R.G. Warnock. 1975. Current Canadian practice in using septic tank systems. Canadian Journal of Public Health. 66: 213.

Viraraghavan, T. 1976. Journal Water Pollution Control Federation. 46: 2213.

Virginia Department of Health. 1994. Personal communication with Mr. Don Alexander. Richmond, Va. From: Duncan et al., 1994.

Washington State Department of Health. 2007. Recommended standards and guidance for performance, application, design, and operation & maintenance alternating drainfields. DOH Publication #337-002.

Wan, J., J.W. Wilson, and T.L. Kieft. 1994. Influence of the gas–water interface on transport of microorganisms through unsaturated porous media. Applied Environmental Microbiology. 60: 509–516

Warrick, L.F., and J. Muegge. 1930. Safeguarding Wisconsin's water supplies. Journal of American Water Works Association. 22: 215-230.

Wesner, G.M., and D.C. Baier. 1970. Injection of reclaimed waste water into confined Aquifers. Journal of American Water Works Association. 62: 203-206.

Wollum, A.G. II, and D.K. Cassel. 1978. Transport of microorganisms in sand columns. Soil Science Society of American Journal. 42: 72–76

Wong, P.T.W., and D.M. Griffin. 1976. Bacterial movement at high matric potentials- I. in artificial and natural soils. Soil Biology & Biochemistry. 8: 215-218.

Young, R.H.F. 1973. Effects of groundwater. Journal Water Pollution Control Federation. 46: 1269-1301.

Ziebell W.A., D.H. Nero, J.F. Deininger, and E. McCoy. 1974. Use of bacteria in assessing waste treatment and soil disposal systems. Proceedings of the National Home Sewage Disposal Symposium. American Society of Agricultural Engineering. St. Joseph, Michigan.

Appendix

APPENDIX A

15A NCAC 18A .1942 SOIL WETNESS CONDITIONS

(a) Soil wetness conditions caused by seasonal high-water table, perched water table, tidal water, seasonally saturated soil or by lateral water movement shall be determined by field evaluation for soil wetness colors and field observations, and may be assessed by well monitoring, computer modeling, or a combination of monitoring and modeling as required by this Rule. All sites shall be evaluated by an Authorized Agent of the Department using Basic Field Evaluation Procedures pursuant to Paragraph (b) of this Rule.

(b) Basic Field Evaluation Procedures:

(1) A soil wetness condition shall be determined by the indication of colors of chroma 2 or less (Munsell Color Charts) at ≥2% of soil volume in mottles or matrix of a horizon or horizon subdivision. However, colors of chroma 2 or less which are relic from minerals of the parent material shall not be considered indicative of a soil wetness condition.

(2) A Soil wetness condition shall also be determined by the periodic direct observation or indication of saturated soils or a perched water table, or lateral water movement flowing into a bore hole, monitoring well, or open excavation above a less permeable horizon or horizon subdivision, that may occur without the presence of colors of chroma 2 or less. A soil wetness condition caused by saturated soils or a perched water table shall be confirmed to extend for at least three consecutive days. The shallowest depth to soil wetness condition determined by Subparagraph (b)(1) or (b)(2) of this Rule shall take precedence.

(c) Site Suitability as to Soil Wetness: Initial suitability of the site as to soil wetness shall be determined based upon the findings of the Basic Field Evaluation Procedures made pursuant to Paragraph (b) of this Rule. Sites where soil wetness conditions are greater than 48 inches below the naturally occurring soil surface shall be considered SUITABLE with respect to soil wetness. Sites where soil wetness conditions are between 36 and 48 inches below the naturally occurring soil surface shall be considered PROVISIONALLY SUITABLE with respect to soil wetness. Sites where soil wetness conditions are less than 36 inches below the naturally occurring soil surface shall be considered UNSUITABLE with respect to soil wetness. Sites where a soil wetness condition is determined based upon the observation or indication of lateral water movement within 48 inches of the naturally occurring soil surface shall be considered UNSUITABLE, except when such water can be intercepted in accordance with 15A NCAC 18A .1956(4).

APPENDIX B

NORFOLK SERIES

LOCATION NORFOLK NC+AL AR FL GA SC VA
Established Series
CMO/Rev. JAK
11/2005

MLRA(s): 133A-Southern Coastal Plain, 153A-Atlantic Coast Flatwoods, 153B-Tidewater Area
MLRA Office Responsible: Raleigh, North Carolina
Depth Class: Very deep
Drainage Class (Agricultural): Well drained
Internal Free Water Occurrence: Deep, transitory or very deep
Index Surface Runoff: Negligible to medium
Permeability: Moderate (Saturated Hydraulic Conductivity: Moderately high)
Landscape: Lower, middle, or upper coastal plain
Landform: Uplands or marine terraces
Geomorphic Component: Interfluve, side slopes
Hillslope Profile Position: Summits, shoulders, backslopes
Parent Material: Marine deposits or fluviomarine deposits
Slope: 0 to 10 percent
Elevation (type location): Unknown
Mean Annual Air Temperature (type location): 62 degrees F.
Mean Annual Precipitation (type location): 49 inches

TAXONOMIC CLASS: Fine-loamy, kaolinitic, thermic Typic Kandiudults

TYPICAL PEDON: Norfolk loamy sand--cultivated. (Colors are for moist soil unless otherwise indicated.)

Ap--0 to 9 inches; grayish brown (10YR 5/2) loamy sand; weak fine and medium granular structure; very friable; nonsticky, nonplastic; few fine and medium roots; darker-colored material in old root channels; strongly acid; clear smooth boundary. (3 to 10 inches thick)

E--9 to 14 inches; light yellowish brown (10YR 6/4) loamy sand; weak medium granular structure; very friable; nonsticky, nonplastic; few fine and medium roots; darker-colored material in old root channels; strongly acid; clear smooth boundary. (0 to 10 inches thick)

Bt1--14 to 17 inches; yellowish brown (10YR 5/6) sandy loam; weak medium subangular blocky structure; friable; slightly sticky, slightly plastic; few fine and medium roots; few faint clay films on faces of peds; strongly acid; clear wavy boundary.

Bt2--17 to 38 inches; yellowish brown (10YR 5/6) sandy clay loam; weak medium subangular blocky structure; friable; slightly sticky, slightly plastic; many fine and medium pores; few faint clay films on faces of peds; strongly acid; gradual wavy boundary.

Bt3--38 to 58 inches; yellowish brown (10YR 5/6) sandy clay loam; weak medium subangular blocky structure; friable; slightly sticky, slightly plastic; few faint clay films on faces of peds; few fine faint strong brown (7.5YR 4/6) and few prominent yellowish red (5YR 5/8) masses of oxidized iron and few fine distinct pale brown (10YR 6/3) iron depletions; strongly acid; gradual wavy boundary.

Bt4--58 to 70 inches; yellowish brown (10YR 5/6) sandy clay loam; weak medium subangular blocky structure; friable; slightly sticky, slightly plastic; few faint clay films on faces of peds; common medium distinct yellowish red (5YR 5/8) masses of oxidized iron and pale brown (10YR 6/3) and light brownish gray (10YR 6/2) iron depletions; 1 percent, firm yellowish red plinthite nodules; strongly acid; gradual wavy boundary. (Combined thickness of Bt horizon is 40 to more than 60 inches.)

BC--70 to 82 inches; variegated brownish yellow (10YR 6/6), strong brown (7.5YR 5/6), and yellowish red (5YR 5/6) sandy clay loam; weak medium subangular blocky structure; friable; slightly sticky, slightly plastic; 5 percent firm, brittle plinthite nodules; strongly acid; gradual wavy boundary. (0 to more than 15 inches thick)

C--82 to 100 inches; variegated red (2.5YR 4/8), strong brown (7.5YR 5/8), brownish yellow (10YR 6/8) and gray (10YR 5/1) sandy clay loam; massive; friable; slightly sticky, slightly plastic; strongly acid.

TYPE LOCATION**:** Robeson County, North Carolina; 1.25 miles south of Parkton; 300 feet west of State Road 1724 and 60 feet south of farm road.

RANGE IN CHARACTERISTICS:
Thickness of the sandy surface and subsurface layers: 3 to 19 inches
Depth to top of the Argillic horizon: 3 to 19 inches
Depth to the base of the Argillic horizon: 60 to more than 80 inches
Depth to top of the Kandic horizon: 3 to 19 inches
Depth to bedrock: Greater than 80 inches
Depth to Seasonal High Water Table: 40 to 72 inches, January to March
Soil Reaction: Extremely acid to strongly acid, throughout except where limed
Rock Fragment Content: 0 to 5 percent, by volume throughout; mostly quartz pebbles or ironstone nodules

Plinthite Content: 0 to 4 percent to a depth of 60 inches and 0 to 10 percent or more below 60 inches

RANGE OF INDIVIDUAL HORIZONS**:**
Ap horizon or A horizon (where present):
Color--hue of 10YR or 2.5Y, value of 4 to 7, chroma of 1 to 4
Texture--loamy sand, sandy loam, fine sandy loam, or loamy fine sand. Some pedons are fine sand or sand.

E horizon:
Color--hue of 10YR or 2.5Y, value of 4 to 7, chroma of 2 to 6
Texture--loamy sand, sandy loam, fine sandy loam, or loamy fine sand. Some pedons are fine sand or sand.

BE horizon (where present):
Color--hue of 10YR or 2.5Y, value of 4 to 6, chroma of 3 to 8
Texture--sandy loam or fine sandy loam

Bt horizon (upper):
Color--hue of 7.5YR to 2.5Y, value of 5 to 8, chroma of 3 to 8
Texture--sandy loam, fine sandy loam, sandy clay loam, or clay loam
Redoximorphic features (where present)--masses of oxidized iron in shades of red, yellow, or brown and iron depletions in shades of brown, yellow, or olive

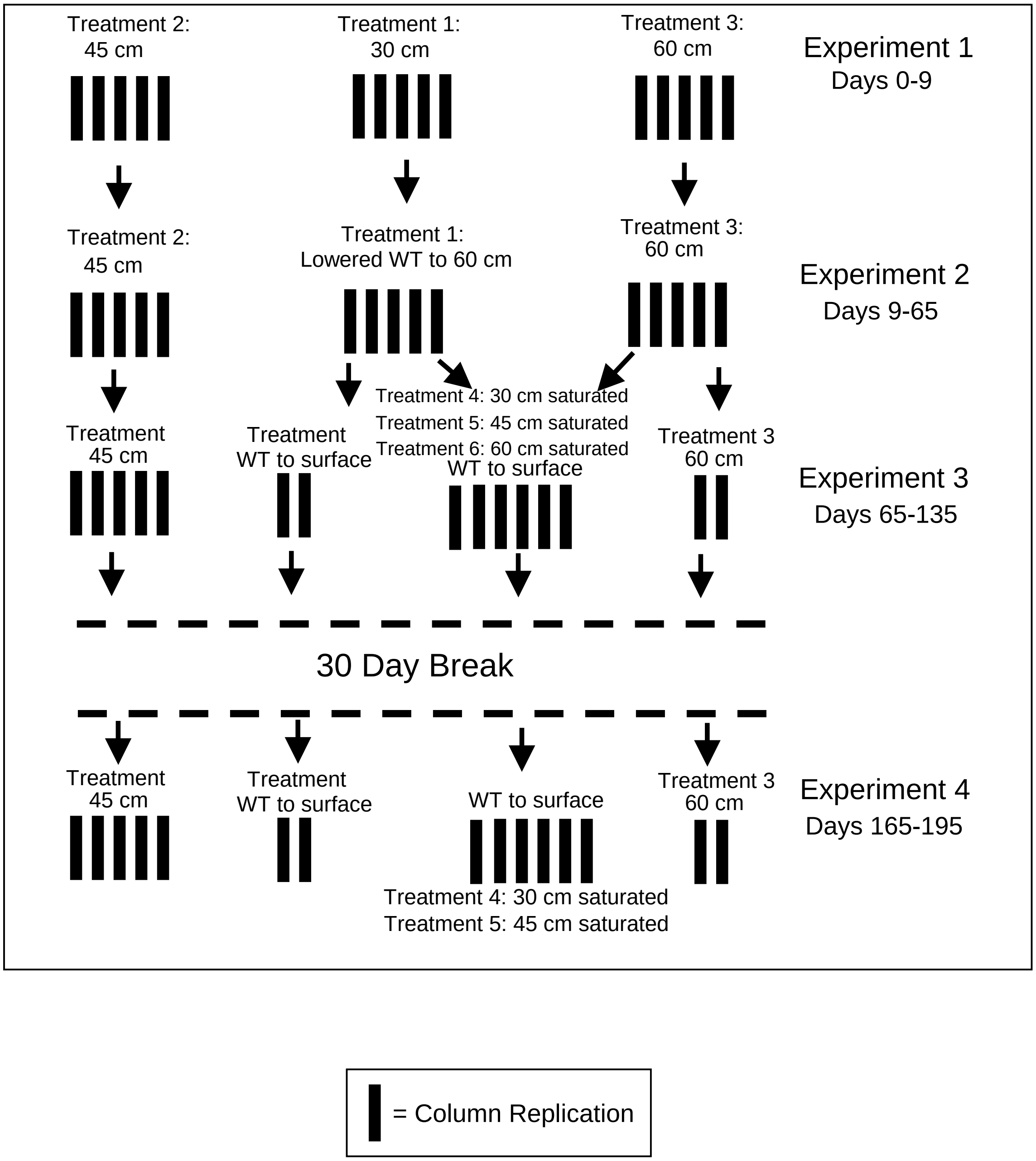
Bt horizon (lower):
Color--hue of 7.5YR to 2.5Y, value of 5 to 8, chroma of 3 to 8
Texture--sandy loam, fine sandy loam, sandy clay loam, clay loam, sandy clay, or clay
Redoximorphic features--masses of oxidized iron in shades of red, yellow, or brown and iron depletions in shades of brown, yellow, olive, or gray

BC horizon or BCt horizon (where present):
Color--hue of 5YR to 2.5Y, value of 4 to 7, chroma of 3 to 8, or variegated in shades of these colors
Texture--sandy loam, fine sandy loam, sandy clay loam, clay loam, sandy clay, or clay
Redoximorphic features--masses of oxidized iron in shades of red, yellow, or brown and iron depletions in shades of brown, yellow, olive, or gray

C horizon:
Color--hue of 2.5YR to 5Y, value of 4 to 8, chroma of 3 to 8, or is variegated in shades of these colors
Texture--loamy coarse sand, loamy sand, loamy fine sand, coarse sandy loam, sandy loam, fine sandy loam, sandy clay loam, clay loam, or sandy clay. Some pedons have layers of coarser or finer textured materials.
Redoximorphic features--masses of oxidized in shades of red, yellow, or brown and iron

APPENDIX C

Schematic Flowchart for Column Treatments



APPENDIX D

Experiment 1

The SAS System
The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable CFU
Classified by Variable treatment

treatment	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
30cm	9	199.00	126.0	18.943235	22.111111
45 cm	9	129.50	126.0	18.943235	14.388889
60 cm	9	49.50	126.0	18.943235	5.500000

Average scores were used for ties.

Kruskal-Wallis Test	
Chi-Square	20.7954
DF	2
Pr > Chi-Square	<.0001

At alpha=0.05 there is strong statistical evidence to fail to accept the null hypothesis. From these data, we can conclude that there is a significant difference between treatments

In this section, n_i, i= 1,2,3, is number of samples taken in each treatment
r_i, i= 1,2,3, is average rank of data
N is total number of samples in sample period
k is number of treatments
α is the probability level

Dunn- Experiment 1

Treatment 1- 30 cm
Treatment 2- 45 cm
Treatment 3- 60 cm

Probability level= 0.05
k(k-1)=6
2A/k(k-1)= 0.167
z-score= 2.13
N=27

n_1= 9
n_2= 9
n_3= 9
r_1= 22.111
r_2= 14.389
r_3= 5.5

Probability Level= 0.05

Treatment 1 vs. Treatment 2
Critical Value= 7.970
Calculated Statistic= 7.722

Treatment 2 vs. Treatment 3*
Critical Value= 7.970
Calculated Statistic= 8.889

Treatment 1 vs. Treatment 3*
Critical Value= 7.970
Calculated Statistic= 16.611

Probability Level= 0.075

Treatment 1 vs. Treatment 2*
Critical Value= 7.333
Calculated Statistic= 7.7222

Treatment 2 vs. Treatment 3*
Critical Value= 7.333
Calculated Statistic= 8.888

Treatment 1 vs. Treatment 3*
Calculated Value=7.333
Calculated Statistic 16.611

(Significance shown with “*”)

APPENDIX E

Experiment 2

The SAS System
The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable CFU
Classified by Variable Treatment

Treatment	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
30 to 60 cm	23	722.0	805.0	78.534119	31.391304
45 cm	23	1323.0	805.0	78.534119	57.521739
60 cm	23	370.0	805.0	78.534119	16.086957

Average scores were used for ties.

Kruskal-Wallis Test	
Chi-Square	50.2019
DF	2
Pr > Chi-Square	<.0001

At alpha= 0.05 there is strong statistical evidence to fail to accept the null hypothesis. From these data we can conclude there is a difference between treatments.

In this section, n_i, i= 1,2,3, is number of samples taken in each treatment
r_i, i= 1,2,3, is average rank of data
N is total number of samples in sample period
k is number of treatments
α is the probability level

Dunn- Experiment 2

Treatment 1- 30 cm lowered to 60 cm
Treatment 2- 45 cm
Treatment 3- 60 cm

Probability level= 0.05
k(k-1)=6
2A/k(k-1)= 0.167
z-score= 2.13
N=69

n_1= 23
n_2= 23
n_3= 23
r_1= 31.391
r_2= 57.522
r_3= 16.087

Probability Level= 0.05

Treatment 1 vs. Treatment 2*
Critical Value= 4.985
Calculated Statistic= 26.130

Treatment 1 vs. Treatment 3*
Critical Value= 4.985
Calculated Statistic= 15.304

Treatment 2 vs. Treatment 3*
Critical Value= 4.985
Calculated Statistic= 41.434

(Significance shown with "*")

APPENDIX F

Experiment 3

The SAS System
The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable CFU
Classified by Variable treatment

treatment	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
30 to 60 cm	6	164.00	255.00	57.575745	27.333333
45 cm	7	200.00	297.50	61.788980	28.571429
60 cm	6	40.50	255.00	57.575745	6.750000
30 cm s	22	1533.00	935.00	98.293280	69.681818
45 cm s	22	1213.00	935.00	98.293280	55.136364
60 cm s	21	419.50	892.50	96.804727	19.976190

Average scores were used for ties.

Kruskal-Wallis Test	
Chi-Square	68.6191
DF	5
Pr > Chi-Square	<.0001

At alpha= 0.05 there is strong statistical evidence to fail to accept the null hypothesis. From these data we can conclude there is a difference between treatments.

In this section, n_i, i= 1,2,3, is number of samples taken in each treatment
r_i, i= 1,2,3, is average rank of data
N is total number of samples in sample period
k is number of treatments
α is the probability level

Dunn- Experiment 3

Treatment 1- 30 cm lowered to 60 cm
Treatment 2- 45 cm
Treatment 3- 60 cm

Treatment 4- 30 cm saturated trench
Treatment 5- 45 cm saturated trench
Treatment 6- 60 cm saturated trench

Probability level= 0.05
k(k-1)=30
2A/k(k-1)= .003
z-score= 2.72
N=84

n_1= 6 r_1= 27.333
n_2= 7 r_2= 28.6
n_3= 6 r_3= 6.75
n_4= 22 r_4= 69.7
n_5= 22 r_5= 55.14
n_6= 21 r_6= 20

Probability Level= 0.05

Treatment 1 vs. Treatment 2
Critical Value= 36.913
Calculated Statistic= 1.238

Treatment 1 vs. Treatment 3
Critical Value= 38.306
Calculated Statistic= 20.583

Treatment 1 vs. Treatment 4*
Critical Value= 30.558
Calculated Statistic= 42.348

Treatment 1 vs. Treatment 5
Critical Value= 30.558
Calculated Statistic= 27.803

Treatment 1 vs. Treatment 6
Critical Value=30.713
Calculated Statistic=7.357

Treatment 2 vs. Treatment 3
Critical Value= 36.913
Calculated Statistic=21.821

Treatment 2 vs. Treatment 4*
Critical Value= 28.792
Calculated Statistic= 41.110

Treatment 2 vs. Treatment 5*
Critical Value= 28.792
Calculated Statistic= 26.565

Treatment 2 vs. Treatment 6
Critical Value= 28.957
Calculated Statistic= 8.595

Treatment 3 vs. Treatment 4*
Critical Value= 30.600
Calculated Statistic= 62.900

Treatment 3 vs. Treatment 5
Critical Value= 30.560
Calculated Statistic= 48.390

Treatment 3 vs. Treatment 6
Critical Value= 30.713
Calculated Statistic= 13.226

Treatment 4 vs. Treatment 5
Critical Value= 20.005
Calculated Statistic= 14.545

Treatment 4 vs. Treatment 6*
Critical Value= 20.241
Calculated Statistic= 49.706

Treatment 5 vs. Treatment 6*
Critical Value=20.2
Calculated Statistic= 35.2
(Significance shown with “*”)

APPENDIX G

Experiment 4

The SAS System
The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable CFU
Classified by Variable treatment

treatment	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
30 to 60 cm	3	18.50	28.50	8.432256	6.166667
45 cm	3	36.00	28.50	8.432256	12.000000
60 cm	3	14.50	28.50	8.432256	4.833333
30 cm s	3	50.00	28.50	8.432256	16.666667
45 cm s	3	39.00	28.50	8.432256	13.000000
60 cm s	3	13.00	28.50	8.432256	4.333333

Average scores were used for ties.

Kruskal-Wallis Test	
Chi-Square	13.6539
DF	5
Pr > Chi-Square	0.0180

At alpha=0.05 there is strong statistical evidence to fail to accept the null hypothesis. From these data, we can conclude that there is a significant difference between treatments.

In this section,
n_i, i= 1,2,3, is number of samples taken in each treatment
r_i, i= 1,2,3, is average rank of data
N is total number of samples in sample period
k is number of treatments
α is the probability level

<u>Dunn- Experiment 4</u>

Treatment 1- 30 cm lowered to 60 cm
Treatment 2- 45 cm
Treatment 3- 60 cm

Treatment 4- 30 cm saturated trench
Treatment 5- 45 cm saturated trench
Treatment 6- 60 cm saturated trench

Probability Level= 0.05
k(k-1)=30
2A/k(k-1)= .003
z-score= 2.72
N= 18

n_1= 3 r_1= 6.167
n_2= 3 r_2= 12
n_3= 3 r_3= 4.833
n_4= 3 r_4= 16.667
n_5= 3 r_5= 13
n_6= 3 r_6= 4.33

Probability Level= 0.05

<u>Treatment 1 vs. Treatment 2</u>
Critical Value= 11.856
Calculated Statistic= 5.833

<u>Treatment 1 vs. Treatment 3</u>
Critical Value= 11.856
Calculated Statistic= 1.333

<u>Treatment 1 vs. Treatment 4</u>
Critical Value= 11.856
Calculated Statistic= 10.500

<u>Treatment 1 vs. Treatment 5</u>
Critical Value= 11.856
Calculated Statistic= 6.833

<u>Treatment 1 vs. Treatment 6</u>
Critical Value= 11.856
Calculated Statistic= 1.833

<u>Treatment 2 vs. Treatment 3</u>
Critical Value= 11.856
Calculated Statistic= 7.167

<u>Treatment 2 vs. Treatment 4</u>
Critical Value= 11.856
Calculated Statistic= 4.667

<u>Treatment 2 vs. Treatment 5</u>
Critical Value= 11.856
Calculated Statistic= 1.000

<u>Treatment 2 vs. Treatment 6</u>
Critical Value= 11.856
Calculated Statistic= 7.667

<u>Treatment 3 vs. Treatment 4</u>
Critical Value= 11.856
Calculated Statistic= 11.837

<u>Treatment 3 vs. Treatment 5</u>
Critical Value= 11.856
Calculated Statistic= 8.167

<u>Treatment 3 vs. Treatment 6</u>
Critical Value= 11.856
Calculated Statistic= 0.500

<u>Treatment 4 vs. Treatment 5</u>
Critical Value= 11.856
Calculated Statistic= 3.667

<u>Treatment 4 vs. Treatment 6*</u>
Critical Value= 11.856
Calculated Statistic= 12.334

<u>Treatment 5 vs. Treatment 6</u>
Critical Value=20.2
Calculated Statistic= 8.667
(Significance shown with "*")

APPENDIX H

Calculations and data for K_{sat} measurement

	Rep. 1	**Rep. 2**	**Rep. 3**	**Rep. 4**	**Rep. 5**	**Rep. 6**	**Average**
Δx (cm)	7.60	7.60	7.60	7.60	7.60	7.60	**0.17 cm/h**
ΔH (cm)	12.70	13.25	13.35	13.50	13.15	13.60	
Vol H20 (mL)	16.46	42.77	28.00	59.60	3.25	12.72	
Time (mins)	68.00	68.00	240.00	240.00	68.00	240.00	
Q (cm/hour)	14.52	37.74	7.00	14.90	2.87	3.18	
i=ΔH /Δx	1.67	1.74	1.76	1.78	1.73	1.79	
Area (cm2)	45.36	45.36	45.36	45.36	45.36	45.36	
Ksat (cm/hour)	*0.19*	*0.48*	*0.09*	*0.18*	0.04	0.04	