Abstract

Onsite wastewater treatment systems (OWTS) have been identified as a potential source of microbial contamination of groundwater. Microbial indicators, such as *Escherichia coli* (*E.coli*) and *Enterococcus*, are transported from these systems to groundwater and may migrate in the subsurface. North Carolina Administration Code 15 A NCAC 18 A.1900 suggests that a 45 centimeter vertical separation distance should be maintained between the bottom of the drainfield and the top of the seasonal high water table (SHWT) in sandy soils and a 15 meter horizontal separation (setback distance) from the drainfield to private wells and surface water bodies is sufficient to protect water quality. The goal of this project was to examine if there was contaminant transport of *E. coli* and *Enterococcus* to surficial aquifers and surface water bodies via OWTS in eastern North Carolina. Densities of *E.coli* and *Enterococcus* were monitored in wastewater, drainfield groundwater, groundwater up-gradient and down-gradient from drainfield trenches, and drinking water samples. Septic tank effluent was sampled monthly, and groundwater was sampled bi-monthly from October 2009 through May 2010 at two residences in Washington, North Carolina. It was hypothesized that 1) the North Carolina 45 cm separation distance does not always prevent microbial contamination of groundwater, and 2) microbial contaminants from OWTS can migrate greater than the North Carolina 15 m setback distance. Results indicate that the unsaturated zone has the greatest control on microbial reduction, with nearly 99.7% reduction of *E.coli* at Site 1 and 98% reduction of *E.coli* at Site 2 occurring between the drainfield and the water table. There was 93% reduction of *Enterococcus* at Site 2; however, there was only 33% reduction of *Enterococcus* at Site 1 in the unsaturated zone. In piezometers located near the 15 m setback distance, the horizontal treatment efficiency (microbial density decline from drainfield groundwater to down-gradient piezometer) was 83%

for *E.coli* and 98.5% for *Enterococcus* at Site 1. There was no reduction of both *E.coli* and *Enterococcus* in piezometers 13 and 17 m from the drainfield at Site 2. Even though significant reduction occurred, relative to tank effluent densities, there was evidence that microorganisms could leach to the groundwater and travel greater than 15 meters down-gradient. These data suggest that more conservative separation distance and setback rules could improve water quality in sandy surficial aquifers and adjacent surface waters. Specifically, increasing North Carolina’s separation distance for sandy soils to 60 cm and setback distance to 30 m would probably reduce *E.coli* and *Enterococcus* to background groundwater levels.

Occurrence of *Escherichia coli* (*E coli)* and *Enterococcus* in shallow groundwater adjacent to onsite wastewater treatment systems in Washington, North Carolina

Thesis

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List of Abbreviations

BOD$_5$-biological oxygen demand (five Days)

C°-Celsius

cfu/100 mL-colony forming units per 100

C.v.-coefficient of variation

Cm/s-centimeters/second

Cm-centimeters

D-Box-distribution box

DO-dissolved oxygen

EPA-Environmental Protection Agency

FC-fecal coliform

FS-fecal streptococci

Ft-feet

Gpd-gallons per day

HTE-horizontal treatment efficiency

K-hydraulic conductivity

MCL-maximum contaminant level

Mg/L-milligrams per liter

M-meters

MPN L^{-1}-most probable number per liter

mS/cm-millisiemens per centimeter

NC-North Carolina

OWTS-onsite wastewater treatment system

Pfu/100 mL-plaque forming units per mL

PVC-polyvinyl chloride

SHWT-seasonal high water table

TDN-total dissolved nitrogen

VRE- vancomycin-resistant enterococci

VTE-vertical treatment efficiency

WT-water table elevation

Chapter 1: Introduction

Onsite wastewater treatment systems (OWTS) include a septic tank, drainfield, and soils. OWTS are designed to store and treat raw sewage by reducing biological oxygen demand (BOD), total suspended solids (TSS) and microorganism densities before dispersing effluent into the environment (Verma, 2008). OWTS collect, treat, and release approximately four billion gallons of effluent per day nationwide (US EPA, 2002). Approximately 25% of the United States and nearly half of North Carolina's state population use an OWTS (US EPA, 2003, 2008). In North Carolina, the potential for water quality impairment via OWTS may increase in coastal areas because nearly 60% of coastal residences use OWTS (North Carolina National Estuarine Research Reserve, 2004). *Escherichia coli* (*E. coli)* and *Enterococcus* from OWTS can cause serious illness to humans from ingestion and through skin contact (US EPA, 2006-a).

In the United States, approximately 168,000 viral illnesses and 34,000 bacterial illnesses occur each year as a result of consumption of contaminated drinking water from private wells (Verma, 2008). Onsite wastewater treatment systems have been identified as one potential source of groundwater contamination (US EPA, 2002). For example, in February 2001, a norovirus outbreak caused at least 35 people to become ill in Sheridan County, Wyoming due to an overloaded OWTS that contaminated the drinking water well at a hunting lodge (US EPA, 2006-b). A gastroenteritis outbreak occurred at a resort on Drummond Island, Michigan in 1991, in which 30 people became sick. A tracer dye injected into the septic tank appeared in the well water two days later (US EPA, 2006-b).

In eastern North Carolina, there is potential for these types of problems due to the high density of OWTS in many coastal areas and the reliance on private wells in rural areas. Approximately two million North Carolinians rely on private groundwater wells for their water supply (Kenny et al., 2009). Poorly constructed wells and OWTS that haven't been properly installed or maintained could potentially contaminate these wells (D'Amato and Devkota, 1997, O'Hara, 2006). For example, in one Indiana county, nearly a third of all OWTS constructed between 1951 and 2001, required repairs to the system. From 1990-2001, after guidelines for septic system construction and repair were established, only three percent of newly installed systems required repairs (Lee et al., 2005).

Microbial contamination of surface waters is also a major concern to public health. Elevated levels of microbes, such as *E.coli* and *Enterococcus* resulting from stormwater discharge, have caused restrictions and closures on many estuaries and lake beaches throughout the United States (Jeng et al., 2005). Agricultural runoff, primarily due to disposal of livestock waste, can also elevate pathogenic microbial densities in surface water, posing risk to human health (Sapkota et al., 2007). In eastern North Carolina surface waters may also be affected by microbial contamination. In 2010, North Carolina reported 345 beach closings in which the source of microbial contamination was attributed to stormwater runoff (Dorfman and Rosselot, 2011).

Although there have been several studies that have quantified nutrient and microbial loading to rivers via point source wastewater discharges (Meyer, 1985, Walsh et al., 2005, and Mallin et al., 2009), it is more difficult quantifying OWTS input into surface waters (Helfand and House, 1995 and Karathanasis et al., 2006). Recent studies have shown that there is increased risk of microbial contamination of groundwater from OWTS in areas where there are sandy soils and a shallow water table (Arnade, 1999 and Humphrey, 2009). Both conditions are common in coastal North Carolina (Scandura et al., 1997).

Although permitting regulations are in place for installation of new OWTS to prevent water quality impairments, OWTS are not typically monitored for compliance after installation and may not always meet the North Carolina state requirements mandated by rules 15A NCAC 18A .1900. These rules require suitable soils, compliance with setback distances, vertical separation, and appropriate tank size. For example, North Carolina Division of Environmental Health records indicated that nearly 1,500 coastal septic systems fail hydraulically (surfacing effluent and/or wastewater back-up in the home) each year (Humphrey, 2009). The wastewater plume from a non-compliant OWTS that may be loaded with harmful microbes and viruses from an OWTS that hasn't been properly treated could affect the groundwater or surface water quality of adjacent properties (Borchardt et al., 2003, Pang et al., 2003, and Lee et al., 2005). The potential for water quality impairment via OWTS grows as OWTS densities increase (Yates, 1985, Lipp et al., 2001, and Borchardt et al., 2003).

Setback distances (horizontal distance to a surface water body or private/public water well) and separation distances (vertical distance between drainfield trench and seasonal high groundwater table (SHWT)) are in place to allow for treatment of effluent. In North Carolina, setback and separation distances are generally less conservative when compared to other states (Table 1). These rules vary across the United States and in some situations may not provide adequate treatment to prevent groundwater or surface water quality impairments (Table 1). For example, the presence of *E. coli* and other bacterial and viral pathogens has led to the closure of shellfish waters numerous times since the late 1970's in Brunswick County, North Carolina, partially due to poorly performing septic systems (Cahoon et al., 2006). In Wisconsin, a link was established between increased endemic diarrheal illnesses in children and greater septic system densities (Borchardt et al., 2003). Some state OWTS setback/separation rules are based on wastewater plume models or measured relationships that have not been supported by current field data (US EPA, 2002). These studies suggest that more information is needed to verify how well water and surface water quality is protected in coastal North Carolina under the current regulations.

State	**Separation Distance (cm)**	**Source**
North Carolina	45/30 cm (sandy soils/other soil types)	NCDENR, 2008
Delaware	90 cm	State of DE-DNERC, 2005
Florida	60 cm	FL Dept. of Health, 1985
Georgia	60 cm	GA Dept. of Health, 2001
Indiana	60 cm	Indiana SDH, 2012
Kentucky	45/30 cm (sandy soils/other soil types)	Kentucky Cabinet for Health and Family Services, 2002
Massachusetts	150/120 cm (sandy soils/other soil types)	Commonwealth of Massachusetts, 2006
South Carolina	15 cm	SC DHEC, 1986
Virginia	60/45 cm	VA Dept. of Health, 2000

Table 1. Minimum separation distance between the bottom of the drainfield and SHWT for various states.

State	Setback Distances (m)	Source
North Carolina	15 m/30 m (surface water/shellfish-saltwater)	NCDENR, 2008
Florida	23 m	FL Dept. of Health, 1985
Georgia	30 m	GA Dept. of Health, 2001
Massachusetts	30 m	Commonwealth of Massachusetts, 2006
Missouri	30/15/7 m (Private Well/Permanent Stream-Lakes/Annual Stream-open ditch)	MO Dept. of Health and Senior Services, 2009
New Hampshire	30 m (Private Well-1000 gpd tank)	NH DES, 2010
South Carolina	23/30 m (less than 1500 gpd/greater than 1500 gpd)	SC DHEC, 1986
Virginia	15 m	VA Dept. of Health, 2000

Table 2. Minimum setback distances between the drainfield and private water wells and bodies of water for various states.

Systems that are in compliance with North Carolina State Rules 15A NCAC 18A .1900 can still be sources of groundwater contamination because the regulations focus on ensuring that wastewater infiltrates and does not rise to the surface, rather than on groundwater quality in the subsurface. Treatment malfunctions may occur and residents/homeowners may not be aware of the problem due to the fact that there is no clear visual evidence of it at the surface. Until 2008, North Carolina did not have a state-wide well program for permitting, inspecting, and testing private drinking water wells that were constructed, repaired, or abandoned. Therefore, wells constructed before 2008 may not have been inspected and the groundwater quality in private wells may not have been tested (Humphrey, 2009). Improper OWTS and well maintenance and installation can allow wastewater-impacted groundwater from the shallow aquifer to migrate to these wells.

There have been several studies that analyze the occurrence of *E.coli*, *Enterococcus*, and other microbial contamination of groundwater caused by OWTS in coastal settings (Arnade, 1999-Palm Springs, Florida; Lipp et al., 2001-Saratoga Bay, Florida; Cahoon et al., 2006-Brunswick County, North Carolina; Sapkota et al., 2007-the Mid-Atlantic region; and Habteselassie et al., 2011-coastal North Carolina). These studies have provided links between OWTS contamination and surface water contamination in these coastal areas. However, there have not been many field-based studies to determine the overall reduction and elimination of *E.coli* and *Enterococcus* from OWTS before discharge to groundwater and adjacent surface waters. Specifically, if microbes from these OWTS can affect surface water, like the Pamlico River, and drinking water wells, this could potentially become a human health hazard. Based on past studies mentioned above in sandy surficial aquifers it was hypothesized that 1) the North Carolina 45 cm separation distance does not always prevent microbial contamination of groundwater (15 A NCAC 18. 1900) and 2) microbial contamination from OWTS can migrate greater than the North Carolina 15 m setback distance (15 A NCAC 18A .1950). The study results will provide guidance to help determine if current North Carolina regulations adequately protect shallow groundwater and surface water resources in the Coastal Plain of North Carolina.

Background

Onsite Wastewater Treatment Systems (OWTS)

Onsite wastewater treatment systems typically consist of a septic tank, distribution box, drainfield, and the underlying soils (Fig 1). Septic tanks are typically made of concrete. They can have multiple compartments, but most have two (Fig 1). Septic tanks function by the process of gravity separation. Effluent enters the tank via a pipe connected to the home/property main drain. Heavier solids settle to the bottom of the tank while lighter grease and solids create a layer on the top of the tank. Anaerobic bacteria in the tank digest a large quantity of solids. After several days (residence time depends on the size of tank and the residential water use), the liquid effluent is discharged into the drainfield trench via a distribution box (D-box) (Hoover, 2004). The purpose of the trenches is to store and deliver effluent to the soil below the drainfield. Below established trenches is a 2-16 cm zone called the biomat, which is a tar-like zone composed of organic matter, suspended solids, microorganisms, and fine particles (Finch, 2006). The biomat thickens over time and slows the infiltration rate of wastewater into the soil. Within the biomat, there are living anaerobic bacteria that feed on organic matter but also contribute to the mat, upon their death (Kaplan, 1991). This is an area in which significant reduction of microbial and chemical pollutants occur (Hoover, 2004).

Theoretically, biological, physical, and chemical processes occurring within the vadose zone (aerated area between bottom of trench and water table) break down residual waste matter.

These natural treatment processes should reduce the likelihood of negative water quality impacts to the groundwater.

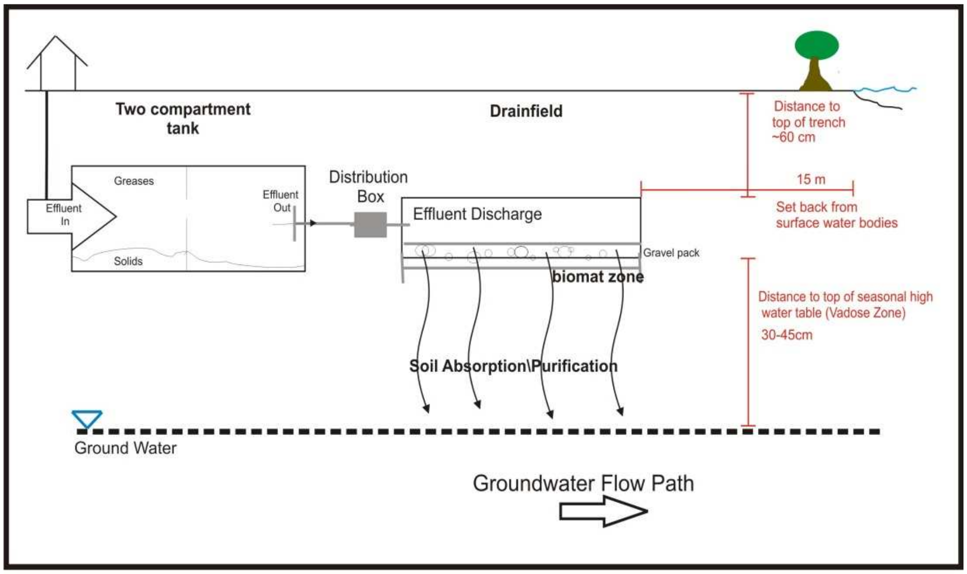

Figure 1. Diagram of an OWTS. Raw effluent enters the two compartment septic system then drains into the drainfield. Effluent is further treated in the vadose zone until the liquid percolates to the underlying groundwater system. Red text refers to North Carolina 15 A NCAC 18. 1900. Prepared with assistance from Shawn Thieme. Not drawn to scale.

The efficiency of OWTS treatment depends on several factors, such as effluent composition, application rate, groundwater depth, flow rates, water chemistry, temperature, climate, and soil properties (Yates et al., 1989; Van Cuyk and Siegrist, 2007; and Verma, 2008). North Carolina regulations state that separation between the bottom of the drainfield and SHWT is 30 cm for groups II (coarse loamy soils), III (fine loamy soils), and IV soils (clayey soils) and 45 cm for group I (sandy soils) (NCDENR, 2008) (Table 1). Previous studies in laboratory or controlled settings have shown that the thickness of soil between OWTS drainfield trenches and the water table affects virus and bacteria removal, with larger separations providing better removal or treatment (Nicosia et al., 2001; Stall, 2008; and Soupir and Mostaghimi, 2011). In general, field studies have also supported the idea that greater separation distances improve virus and bacteria removal. For example, Cogger et al (1988), Scandura and Sobsey (1997), Humphrey and O'Driscoll (2011), and Humphrey et al (2011), have shown that groundwater viruses and bacteria concentrations in groundwater increase as the water table is either close to or breaches OWTS drainfield trenches.

Microbial Indicators of Water Quality

E. coli are anaerobic, gram-negative [layer of phospholipids and lipoproteins outside a thinner peptidoglycan layer that does not retain Gram stain when washed with ethyl alcohol] rod-shaped microorganisms that live in the intestinal tracts of both healthy and diseased animals (Chappelle, 1993 and Health Protection Agency, 2007). *E. coli* is a useful microbial water quality indicator because it can suggest the presence of wastewater contamination in water supplies (Arnade, 1999). Inadequate bacteria removal during wastewater treatment can cause *E.coli* colonies to thrive and persist in the environment for extended periods of time, ranging from 50 to 132 days (Banning et al., 2002). Not only does the presence of *E. coli* indicate potential contamination of the harmful strains, such as O157:H7, its presence in surface and groundwater can indicate that other harmful bacteria, viruses, or parasites are present (US EPA, 2006). In one case, seven people died and 2300 people became ill from ingesting *E. coli* [*0157:H7*] and *Campylobacter jejuni* contaminated water in Walkerton, Ontario during an outbreak that occurred in May, 2000 (Hrudley et al, 2003). Because of the risks associated with elevated *E.coli* levels in surface water and groundwater, the US EPA has developed maximum contaminant levels (MCL) (Table 3). The contact standard for *E.coli* in freshwater bodies is 126 ($10^{2.10}$) cfu/100 mL based on a statistically sufficient number of samples (generally not less than five samples equally spaced over a 30-day period (US EPA, 2003). The contact standard for

E.coli in freshwater/marine waters designated for swimming is 235 ($10^{2.37}$) cfu/100 mL, if only one sample is taken (Giddings and Oblinger, 2004).

Contaminate	**Type of Standard**	**Maximum Contaminant Level (MCL)**
Fecal coliform and *E.coli* (US EPA, 2009)	Drinking Water	Zero
E.coli	Freshwater/Marine Water	126 cfu/100 mL (generally not less than 5 samples equally spaced over a 30-day period)
E.coli	Freshwater/Marine	235 cfu/100 mL (single sample)
E.coli	Freshwater/Marine	576 cfu/100 mL (single sample designated for water body that is infrequently used for full-body contact recreation)
Enterococcus	Freshwater	33 cfu/100 mL (generally not less than 5 samples equally spaced over a 30-day period)
Enterococcus	Marine Water	35 cfu/100 mL (generally not less than 5 samples equally spaced over a 30-day period)
Enterococcus	Marine Water	104 cfu/100 mL (single sample maximum at Designated Bathing Beaches)

Table 3. Various rules governing the maximum contaminant level (MCL) in drinking, freshwater, and marine water (US EPA, 2003; Giddings and Oblinger, 2004; and US EPA, 2009).

Enterococcus is another commonly used indicator of microbial water quality. *Enterococcus* is a gram-positive [inner membrane with a relatively thick layer of peptidoglycan covering it that retains the crystal violet pigment in Gram stain due to its thick peptidoglycan layer] facultative anaerobic coccus (spherical-shape) (Chappelle, 1993 and Talaro et al., 2009). They are naturally found in the intestinal tract of humans and other animals. The two strains of most significance to human health are *E. faecalis* and *E. faecium*. *E. faecalis* occurs in 80% to 90% of cases of enterococcal infections, and *E. faecium* occurs in 5%-10% of cases of enterococcal infections (Cermak et al, 2009). Contact with *Enterococcus* can cause urinary tract infections, intra-abdominal or pelvic wounds, and Enterococcal meningitis (Moellering Jr., 1992). Also, *Enterococcus* has emerged as a greater threat to public health due to the rise of vancomycin-resistant enterococci (VRE) (Talaro et al., 2009). Vancomycin is an antibiotic used to treat enterococcal infections.

The presence of *Enterococcus* is used as an indicator of fecal pollution and the possible presence of enteric pathogens, which are bacteria that originally resided in the intestines of animals (Talaro et al., 2009). The US EPA MCL contact standard for *Enterococcus* is 33 ($10^{1.52}$) cfu/100mL in freshwater and 35 ($10^{1.54}$) cfu/100 in saltwater (US EPA, 2003) (Table 3). The significance of finding Enterococci in recreational water samples is that there is a direct relationship between the density of Enterococci in the water and the occurrence of swimming-associated gastroenteritis at marine and fresh water bathing beaches (US EPA, 2002). *Enterococcus* species can tolerate increased concentrations of sodium chloride (NaCl) (up to 6.5%), and bile salts (up to 40%), as well as higher substrate pH values (up to pH 9.6) (Cermak et al, 2009). This is the primary reason why these microbes are a better indicator of fecal contamination in brackish waters than *E.coli.*

Although the common practice for evaluating microbial water quality is to use indicator bacteria such as *E. coli* and Enterococci, there are drawbacks to using microbial indicators as a proxy to predict the concentration and transport of viruses. Microbial indicators can predict the probable presence of viruses in water, but cannot precisely predict the level of occurrence (Payment and Locas, 2010). One shortcoming of using microbial indicators as a primary indicator of water quality is that viruses tend to be more resistant to disinfection; therefore densities of microbial indicators in water may not always correspond with the viral concentrations. Information derived from microbiological analysis takes time due to incubation periods and generally samples are not obtained in a continuous manner and the survivability differences between microbes and viruses could misrepresent the true concentrations (Figueras and Borrego, 2010). Also, *E.coli* have been consistently found in pristine tropical rain forest aquatic and plant systems, as well as soils (Hazen et al., 1990 and Lasalde et al., 2005). Microbes and viruses can thrive in certain environments and employing multiple testing of these indicators will provide a more robust synopsis of water quality (Verstraeten et al., 2005 and Conn et al., 2012). In this study, geochemical indicators, such as nitrogen species, specific conductivity, dissolved oxygen, temperature, pH, were collected and used to help verify the presence and migration of wastewater. In a parallel study, *C. perfringens* (bacteria), F+ phage (MS2) and somatic phage (ΦX174) (viruses) were also collected by the CDC and will be used to help confirm conclusions.

Chapter 2: Site Descriptions-Methods and Materials

Regional Setting and Climate

The North Carolina Coastal Plain is underlain by an eastward dipping and thickening wedge of sediments and sedimentary rocks ranging from Late-Cretaceous to recent (Richards, 1950). Beaufort County sits on the edge of the Tidewater/Inner Coastal Plain boundary. The Tidewater region is extremely flat, averages less than 6.1 m above sea level and contains large swamps and lakes indicative of poor drainage conditions (Orr et al., 2000).

In Beaufort County, July is typically the wettest month and November is typically the driest month. However, rainfall does vary yearly due to unpredictable phenomena such as tropical systems (Climate Office of North Carolina, 2010). Annual daily mean temperatures for the area ranged from 15.6°-18.3°C. From 1971-2000, the annual precipitation was approximately 132 cm. The area received 71.3 cm of precipitation from November 1st, 2009-May 31st, 2010 during the groundwater sampling period of this current study (Climate Office of North Carolina, 2010). Rainfall during the study period was higher than average. Historical data from 1971-2000 indicated that Beaufort County has received an average of 65 cm of precipitation from November through May (Climate Office of North Carolina, 2011).

Site Selection and Characterization

The study was conducted in Washington, Beaufort County, North Carolina. According to the 1990 census data, approximately 70% of all residences in Beaufort County used OWTS as their primary method of wastewater disposal (US Census, 1990). There is evidence that microbial contaminants in surface waters may present an environmental health risk in the coastal regions of North Carolina, which include Beaufort County (Dorfman et al., 2010 and Humphrey et al., 2011). In 2009, it was reported that Beaufort County had the highest exceedance rate (7%) of the state's daily maximum bacterial standards for North Carolina's coastal waters (Dorfman et al., 2010).

The residential OWTS of 115 Goose Creek Drive, Washington, North Carolina (Site 1), (0.27 hectares) and 109 Fairway Drive, Washington, North Carolina (Site 2) (0.23 hectares), (Fig 2, 3, and 4) were chosen based on > 45 cm separation distance (during initial site selection surveys) between bottom of drainfield trench and SHWT indicators. These sites also have the appropriate setback distances outlined by 15 A NCAC 18A .1950 (Humphrey et al., 2010). Site 1 was chosen because of the high occurrence of OWTS in the county and the proximity to the Pamlico River.

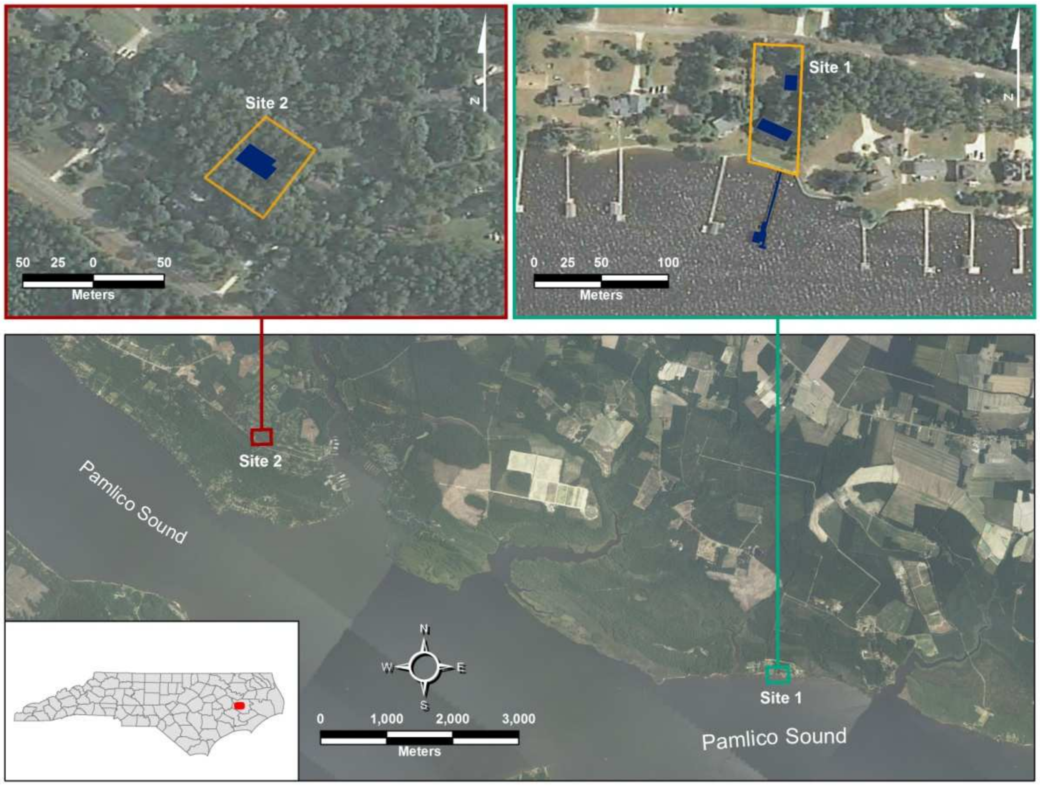

Figure 2. Aerial view of Site 1 and Site 2 in relation to the Pamlico River. Prepared with assistance from Robert Howard.

During initial site visits, detailed descriptions of soil morphology, such as soil texture, structure, consistence, and color with depth (Appendix A), were obtained via auger borings using methods described by Deal et al (2007). Soil descriptions were provided by N. Deal, previously at North Carolina State University. Soil colors were quantified using a Munsell Soil Color book (Munsell Soil Color Charts, 2000). Estimated depth to SHWT, or theoretical SHWT, was determined on the basis of two or one chroma colors in accordance with North Carolina Rules for Sewage Treatment and Disposal Systems (NCDENR, 2008). Measured SHWT was determined using continuous water level (WL) data collected from the HOBO Dataloggers. There was an 18 cm separation distance between the bottom of the drainfield and measured SHWT (1/30/2010 to 2/13/2010) at Site 1 and a 40 cm separation distance between the bottom of the drainfield and measured SHWT (2/4/2012-2/18/2012) at Site 2 (Appendix A).

The soil profile for Site 1 as described by N. Deal indicates predominantly sandy soils. The soil profile for Site 2 was similar in structure and texture to Site 1 with the exception of a layer of sandy loam at the depths between 90 and 125 cm. No chroma 2 or 3 mottles were initially documented within 150 cm of the surface on either site, though there was spatial variability of soil morphology near the OWTS (Humphrey, 2010) (Fig 3).

Figure 3. Soil profile of soil taken from drainfield at Site 2. The first 90 cm consist of predominantly sandy soils. A layer of sandy loam is present at depths between 90 and 125 cm. (Humphrey, 2010).

A soil test analyzing pH, nutrients, and other parameters was performed on soils at the top of the chroma 2 layer for each site (Appendix B). The test was performed by North Carolina Department of Agricultural and Consumer Services Agronomic Division. Although the soil properties were similar at both sites; one notable difference between Site 1 and Site 2 soils was that the soil was more acidic at Site 2 (5.1) than Site 1 (6.9).

Geoprobe sediment cores were collected at each site (Appendix C and D). Based on one geoprobe core to a depth of 4 m, surficial aquifer at the Site 1 is predominantly sandy sediments. The surficial aquifer at Site 2 is also comprised predominantly of sandy sediments, however approximately 3-4 m below land surface, clayey sand lenses are present. At Site 2, a low permeability organic-rich clay and woody debris layer were observed between 4.8 and 5 m. Twenty-five slug tests were performed to determine the hydraulic conductivity (K) of the surficial aquifer at each site. The mean K at Site 1 was 2.08×10^{-3} cm/s (Table 4) (Shawn Thieme, personal communication). The mean K at Site 2 was 6.24×10^{-4} cm/s (Table 5) (Shawn Thieme, personal communication). Both values are within K values predicted for sandy/sandy loam environments (Heath, 2004).

Initially, three groundwater wells were installed at each site to determine the direction of groundwater flow (Humphrey et al., 2010). The results showed that at Site 1, groundwater flowed in a south-southeast direction towards the estuary (Appendix E). At Site 2, the initial survey indicated that groundwater was flowing in a southwest direction. However, seasonal water table elevations suggested that flow direction was seasonally variable (Appendix F).

Septic Tank Sampling and Piezometer Installation

Septic tank and drainfield locations were determined by tile-drain probing and a permit sketch (Humphrey, 2010). Samples were collected directly from inlet and outlet compartments of the OWTS. Manhole covers on both OWTS were removed and replaced with concrete lids fitted with PVC cleanouts. A rigid plastic sampling tube was installed in each cleanout (Fig 4). The pump could then be easily connected to the sampling locations at the inlet and outlet of both tanks to collect samples (Humphrey, 2010). Tubing used for sampling was sterilized with a chlorine bleach solution before and after each sample.

Figure 4. Installation of the PVC Cleanout at Site 2. Flex tubing was attached to the rigid tubing and fitted with a nipple used for connection to the peristaltic pump, which was used to retrieve effluent samples from the inner and outer section of the OWTS.

Twenty-one and fourteen PVC piezometers were installed at Site 1 and Site 2, respectively, based on location of the plume, groundwater level, and direction of groundwater flow. Approximate locations of wastewater plumes at each site were determined using Electrical Resistivity Surveys (Humphrey et al., 2010). Many of the piezometers were placed in clusters installed at different depths (Table 4 and 5). Piezometer screens were 61 cm long. Piezometers ranged in depth from 1.4 m to 3.7 m at Site 1 and 1.9 m to 3.7 m at Site 2. A Topcon laser theodolite was used to survey piezometer elevations at the site.

Figure 5. Site map of Site 1. Piezometers/Well clusters are indicated by the red dot. Drainfield area is represented by a box north of the OWTS (1sto/1sti). Red circle represents the approximate 15 meter (50 ft) setback radius from D-box location in accordance with North Carolina State rules 15 A NCAC 18A .1950.

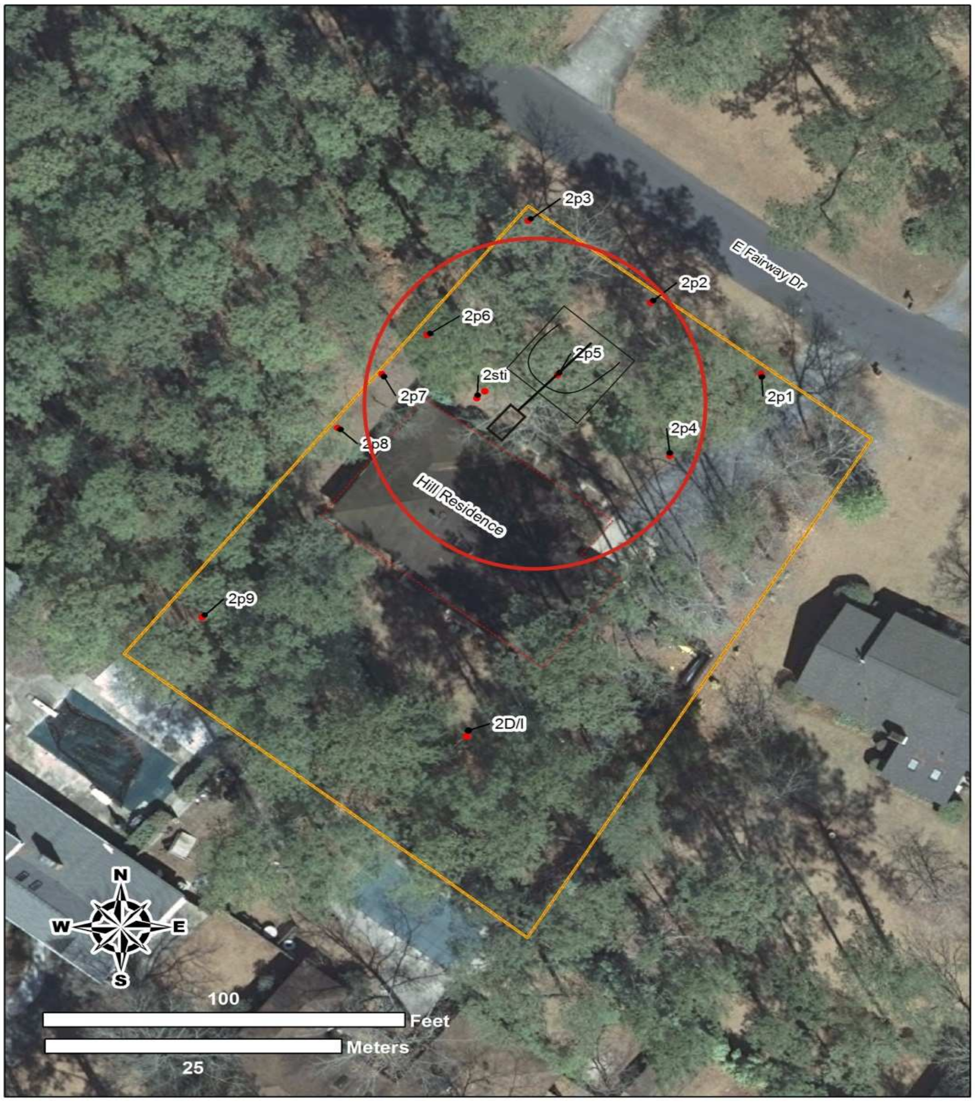

Figure 6. Site map of Site 2. Piezometers/Well clusters are indicated by the red dot. Drainfield area is represented by a box north of the OWTS (2sto/2sti). Red circle approximates the 15 meter (50 ft) setback radius from D-box location in accordance with North Carolina State rules 15 A NCAC 18A .1950.

Well ID	Top of casing elevation relative to sea level (m)	Depth of well (m)	Length of screen (cm)	Radius of Screen (cm)	Survey (z value) (m)	Latitude	Longitude	Hydraulic conductivity (cm/s)	Category Groupings
1p1	2.1	3.7	60.96	1.4	0.520	35°27.675	67°53.163	3.16E-05	GW>15 m
1p2	1.8	1.7	60.96	1.4	0.238	35°27.658	67°53.170		BG
1psonde2	1.8	1.8	60.96	4.2	0.267	35°27.672	67°53.170	3.16E-05	BG
1p3	1.7	1.7	60.96	1.4	0.146	35°27.658	67°53.167	1.79E-03	GW<15 m
1p4s	1.7	1.8	60.96	2.2	0.186	35°27.652	67°53.163	4.17E-03	DF
1p4d	1.7	2.3	60.96	2.2	0.175	35°27.652	67°53.163		DF
1p5s	1.7	1.9	60.96	2.2	0.174	35°27.646	67°53.161	1.58E-03	GW<15 m
1p5d	1.7	2.4	60.96	2.2	0.148	35°27.646	67°53.161	2.28E-03	GW<15 m
1psonde5	1.7	2.1	60.96	4.2	0.153	35°27.646	67°53.161		GW<15 m
1p6s	1.6	1.4	60.96	2.2	0.031	35°27.642	67°53.163		GW<15 m
1p6d	1.6	2.2	60.96	2.2	0.023	35°27.642	67°53.163	5.90E-03	GW<15 m
1p7s	1.5	1.6	60.96	2.2	-0.030	35°27.637	67°53.161	1.48E-03	GW>15 m
1p7d	1.5	2.0	60.96	2.2	-0.020	35°27.637	67°53.161	1.86E-03	GW>15 m
1p8s	1.4	1.7	60.96	2.2	-0.160	35°27.634	67°53.161	3.13E-03	GW>15 m
1p8d	1.4	2.0	60.96	2.2	-0.172	35°27.634	67°53.161	3.99E-03	GW>15 m
1p9	1.4	1.5	60.96	2.2	-0.125	35°27.637	67°53.163	3.58E-03	GW>15m
1p10	1.3	1.9	60.96	1.4	-0.235	35°27.633	67°53.161	1.34E-03	GW>15 m
1p16	0.9	1.6	60.96	1.4	-0.650	35°27.652	67°53.163	1.90E-06	Estuary
Septic Tank						35°27.658	67°53.167		Tank

Table 4. Site 1 well depth, elevation relative to sea level, screen length, well diameter, survey value, latitude, longitude, and category groupings. Well depth is the vertical length of the well. To approximate elevation relative to sea level, Site 1 datum was set at the lowest water level reading in the piezometer adjacent to the estuary (1p16), which was recorded on 4/14/2010. TOC =Top of casing

Well ID	Top of casing elevation relative to sea level (m)	Vertical Length of well (m)	Length of screen cm	Radius cm	Survey (z value) (m)	Latitude	Longitude	Hydraulic conductivity (cm/s)	Category Groupings
2p1	2.4	4.0	60.96	1.4	0.378	35^{o}29.666	76^{o}58.258	1.74E-05	GW<15 m
2p2	3.7	3.9	60.96	1.4	0.340	35^{o}29.667	76^{o}58.626	3.38E-05	GW>15 m
2p3	2.4	3.8	60.96	1.4	0.266	35^{o}29.667	76^{o}58.271		BG
2psonde4	2.4	4.0	60.96	4.2	0.387	35^{o}29.611	76^{o}58.263	6.60E-03	GW>15 m
2p5s	2.3	3.9	60.96	1.4	0.342	35^{o}29.669	76^{o}58.268	1.46E-05	DF
2psonde5	2.6	3.9	60.96	4.2	0.351	35^{o}29.669	76^{o}58.268	4.45E-05	DF
2p5d	3.5	3.9	60.96	1.4	0.343	35^{o}29.669	76^{o}58.268		DF
2p6s	1.9	3.8	60.96	1.4	0.214	35^{o}29.672	76^{o}58.278	3.88E-05	GW>15 m
2p6d	2.7	3.8	60.96	1.4	0.227	35^{o}29.672	76^{o}58.278		GW>15 m
2p7s	2.2	3.8	60.96	1.4	0.236	35^{o}29.672	76^{o}58.281	2.20E-05	GW>15 m
2p7d	2.8	3.8	60.96	1.4	0.226	35^{o}29.672	76^{o}58.281	1.45E-05	GW>15 m
2p8s	2.2	3.8	60.96	1.4	0.219	35^{o}29.669	76^{o}58.283	1.37E-05	GW<15 m
2p8d	2.5	3.8	60.96	1.4	0.215	35^{o}29.669	76^{o}58.283	2.55E-05	GW<15 m
2p9	3.0	4.2	60.96	1.4	0.662	35^{o}29.658	76^{o}58.294	3.50E-05	GW<15 m
Septic Tank						35^{o}29.661	76^{o}58.277		Tank

Table 5. Site 2 well depth, elevation relative to sea level, screen length, well diameter, survey value, latitude, longitude, and category groupings. Well depth is the vertical length of the well. Site 2 datum was set off the road adjacent to the site. The road's elevation was approximated as 396 cm obtained from the USGS topographic quadrangle (Blounts Bay, USGS 1993).

Water Quality, Precipitation, and Groundwater Level Monitoring

Water quality was monitored seasonally for *E.coli* and *Enterococcus* from November 2009- May 2010. Groundwater samples were collected on November 16th, 2009, January 25th, 2010, March 15th, 2010 and May 24th, 2010 (Appendix G). Each sample was collected using a new bailer assigned to each well to avoid cross contamination. In addition, bailers were only used once and disposed of immediately after sampling. Nitrile gloves were worn at all times to avoid contamination during sampling bottles. Once filled with 1000 mL of sample, the samples were immediately capped and placed in a cooler filled with ice. The cooler was then sealed and shipped overnight to the Centers for Disease Control (CDC) and Prevention National Center for Emerging and Zoonotic Infectious Diseases/Waterborne Disease Prevention Branch in Atlanta, Georgia for further analysis.

During field sampling events, specific conductivity, dissolved oxygen, pH, and water temperature were determined using a YSI 556 MPS meter. The YSI 556 MPS was calibrated prior to sampling by N. Deal. In addition, nutrient (dissolved nitrogen) and chloride concentrations were analyzed at the ECU Central Environmental Lab for each sampling date (Appendix H and I). Groundwater levels were collected prior to sampling with a Solinst TLC water level meter. Between sampling events, groundwater levels and specific conductance and dissolved oxygen were monitored in select piezometers by Onset HOBO pressure dataloggers and YSI 6920 v2 sondes, respectively (Appendix J).

Membrane Filtration

The *E. coli* densities were determined using the process of membrane filtration using the Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC) method by CDC lab personnel at the CDC in Atlanta, Georgia. Membrane filtration provides a direct count of *E.*

coli in water, based on the development of colonies that grow on the surface of a membrane filter. Samples are filtered through Modified mTEC membranes, which were incubated at 35 ± 0.5°C for 2 hours to resuscitate the injured or stressed bacteria, and then incubated at 44.5 ± 0.2°C for 22 hours after contact with modified mTEC agar. If the filters from the modified mTEC agar were red or magenta, *E. coli* colonies were present and counted (US EPA, 2002).

Enterococcus densities were determined using the process of membrane filtration using membrane-*Enterococcus* Indoxyl-b-D-Glucoside Agar (mEI) method. *E. faecalis* and *E. faecium* are the two strains that the method can detect, but others strains of enterococci can grow on the mEI agar (Chandra Schneeberger, personal communications). The water samples were filtered, using 0.45μm pore size of mixed cellulose ester medium, through the membrane which retains the bacteria. Following filtration, the membrane containing the bacterial cells was placed on the mEI agar medium, and incubated for 24 hours at 41°C. All colonies with a blue halo were recorded as Enterococci colonies. A stereoscopic microscope and a small fluorescent lamp were used for counting to give maximum visibility of colonies (US EPA, 2002).

Statistical Analysis of Microbial Populations

Microbial densities used for analysis were based off the highest measurement observed in each piezometer cluster (when nested piezometers existed, i.e. one shallow and one deep) for each sampling date. Microbial densities that were below detection limits (Appendix K, L, M, and N) were indicated by <, these data provide an approximation, but could not be verified. Standard industry practice is to reduce the detection limit of microbial densities by half and use that number for statistical analysis (Humphrey, 2011). Water samples that had microbial densities that were less than one were rounded up to one, to allow for statistical analysis.

Boxplots, mathematical equations, and other forms of statistical analysis were performed using EXCEL and Minitab statistical software.

Microbial measurements were grouped into seven categories at Site 1 and six categories at Site 2 (Table 4 and 5). They are: Tank (1sto, 2sto), background groundwater (BG) (1p2, 2p3), drainfield groundwater (DF) (1p4, 2p5), piezometers in which horizontal setback distance was within 15 m of drainfield (GW<15 m) (1p5, 1p6, 2p2, 2p4, 2p5, 2p6, 2p7), piezometers in which horizontal setback distance was 15 m or greater of drainfield (GW>15 m) (1p7, 1p8, 1p9, 1p10, 2p1, 2p8, 2p9), estuary groundwater (Est GW) (1p16), and drinking/irrigation water samples (1 D/I, 2 D/I). Distances between the drainfield and piezometers were calculated from each sites drainfield distribution box.

A Mann-Whitney test is a non-parametric test used to compare two independent groups of sampled data (Freund and Wilson, 2003). Mann–Whitney tests were used to determine if significant differences existed between median *Enterococcus* and *E.coli* densities at each site and its relationship with distance from each site's drainfield. Tank microbial densities were compared to drainfield groundwater microbial densities to determine whether the biomat and soils adequately reduced the microbial densities. Microbial densities of groundwater within 15 m from drainfields were compared to microbial densities of groundwater greater than 15 m from drainfields (greater than the NC setback distance) to determine if surficial aquifer treatment processes were effective at reducing microbial concentrations. Microbial densities in drainfield groundwater were compared to microbial densities in background groundwater and Site 1/Site 2 drinking/irrigation water to help assess the impacts of OWTS on groundwater in the surficial aquifer.

E. coli and Enterococci reduction, or treatment, efficiency in the biomat and vadose zone was determined using the following equation:

Equation 1:

$$\mathrm{VTE} = \{[\mathrm{ST} - \mathrm{DF}) / \mathrm{ST}] * 100\}$$

Where: VTE = Vertical (Unsaturated) Treatment Efficiency (% cfu 100 mL), ST=median septic effluent in tank (cfu/100 mL), and DF= median drainfield groundwater (cfu/100 mL) (Humphrey et al., 2010).

The saturated (horizontal) treatment efficiency was also calculated to determine the percentage of microbial reduction that has occurred from the drainfield groundwater to piezometers at various distances by using the following equation (Equation 2) and the percentage of microbial reduction that occurred from the septic tank to piezometers at various distances (Equation 3).

Equation 2:

$$\mathrm{HTE} = \{[(\mathrm{DF} - (\text{Well ID}) / \mathrm{DF}\} * 100\}$$

Where: HTE= horizontal (saturated) treatment efficiency (% cfu/100 mL), DF= median drainfield groundwater (cfu/100 mL) and Well ID=median microbial densities of specified piezometer (cfu/100 mL) (Humphrey et al., 2010).

Equation 3:

$$\mathrm{TE} = \{[(\mathrm{ST} - (\text{Well ID}) / \mathrm{ST}\} * 100\}$$

Where: TE= overall treatment efficiency (% cfu/100 mL), ST= median septic tank effluent in tank (cfu/100 mL), and Well ID=median microbial densities of specified piezometer (cfu/100 mL) (Humphrey et al., 2010).

Chapter 3: Results

Vertical (Unsaturated Zone) Treatment Efficiency: Does the North Carolina 45 cm Separation Distance Prevent Microbial Contamination of Groundwater?

Microbial Densities and Separation Distance in the Drainfield

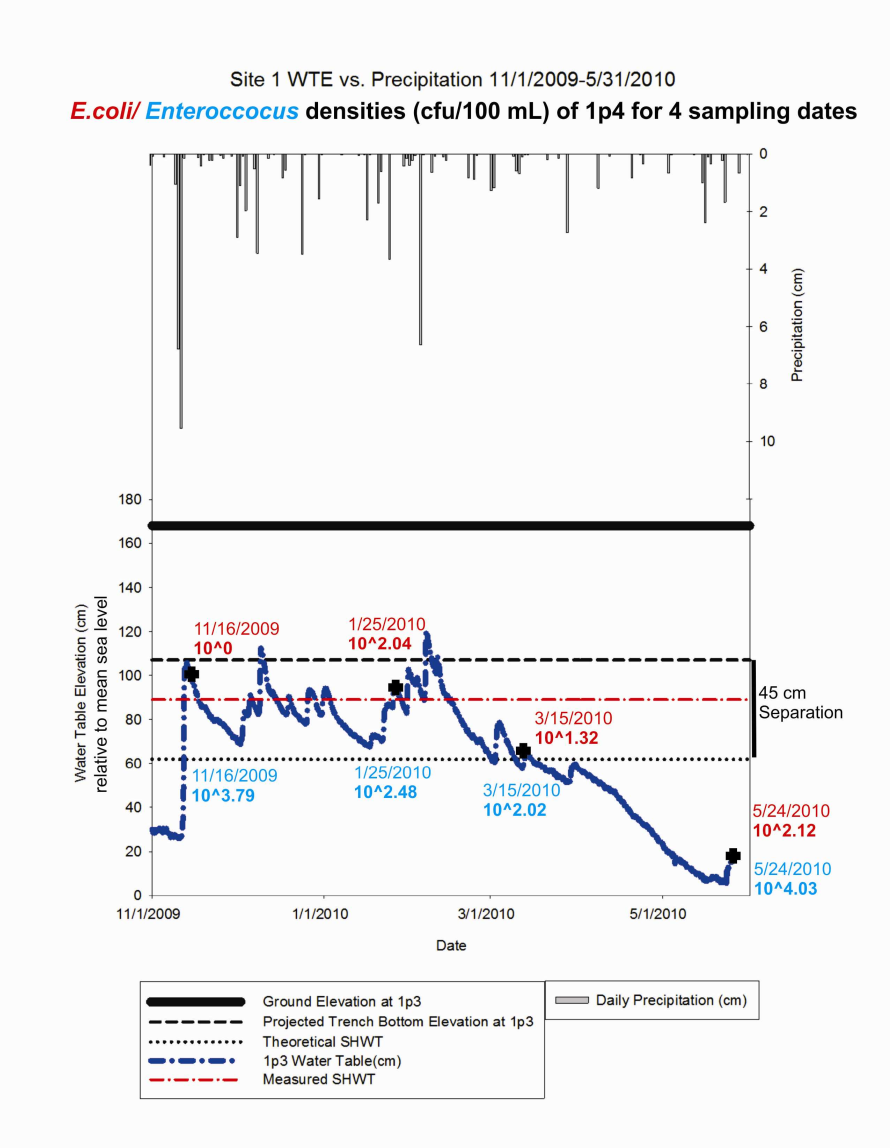

Figure 7. Groundwater hydrograph for Site 1 with *E.coli* and *Enterococcus* drainfield densities (1p4) sampling date data. Representation of the groundwater table in relation to precipitation events and *E.coli* and *Enterococcus* densities in drainfield collected on four sampling dates. Cross symbol represents sampling date.

The water table (WT) at Site 1 breached the 45 cm separation zone for a total of 122 days (Fig 7). At Site 1, the WT was the deepest during the 5/24/2010 sampling date. *E.coli* density in drainfield groundwater was variable throughout the study. The greatest *E.coli* density in drainfield groundwater occurred on 5/24/2010 ($10^{2.12}$ cfu/100 mL). In contrast, *E.coli* was below the detection limit in drainfield groundwater on the 11/16/2009 sampling date. Median tank *E.coli* was more variable, with a coefficient of variation (c.v.) of 88% (median= $10^{4.34}$cfu/100 mL), than drainfield groundwater *E.coli* which had a c.v. of 72% (median =$10^{1.82}$ cfu/100 mL).

At Site 1, on the 1/16/2009 sampling date, the WT was within the 45 cm separation zone and the drainfield groundwater had a $10^{3.79}$ cfu/100 mL *Enterococcus* density (Fig 7). *Enterococcus* densities on the two following sampling dates (1/25/2010 and 3/15/2010) were relatively consistent, with drainfield groundwater densities of $10^{2.48}$ cfu/100 mL and $10^{2.02}$ cfu/100 mL. The highest density in drainfield groundwater at Site 1 was observed on the 5/24/2010 sampling date, when the drainfield groundwater yielded a $10^{4.03}$ cfu/100 mL sample. Median tank *Enterococcus* was more variable with a c.v. of 209% (median=$10^{3.51}$cfu/100 mL) when compared to drainfield groundwater *Enterococcus* with a c.v. of 32% (median=$10^{3.69}$ cfu/100 mL).

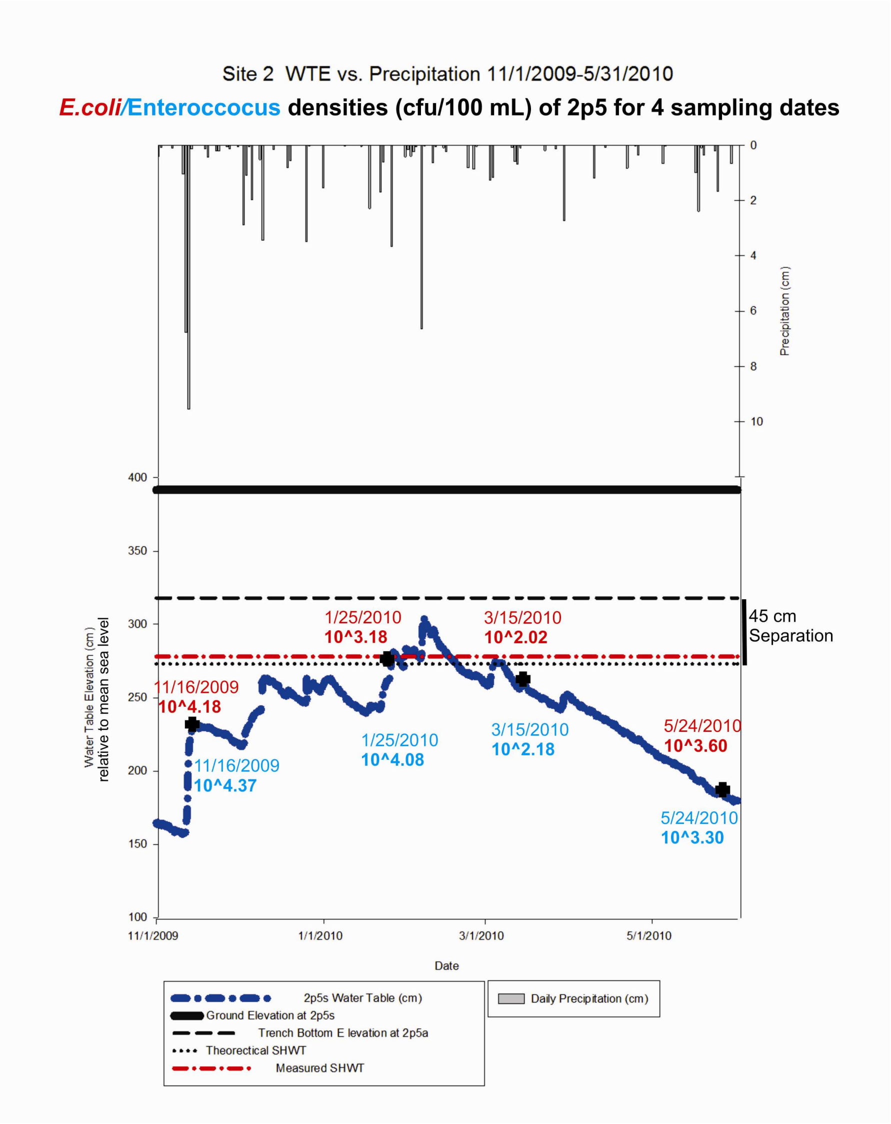

Figure 8. Hydrograph for Site 2 with *E.coli* and *Enterococcus* drainfield densities (2p5) sampling date data. Representation of the groundwater table in relation to precipitation events and *E.coli* and *Enterococcus* densities in DF collected on four sampling dates. Cross symbol represents sampling date.

At Site 2, the WT breached the 45 cm separation zone for a total of 20 days (Fig 8) (Appendix O). Groundwater *E.coli* densities beneath drainfield trenches on 1/25/2010 and 3/15/2010 were $10^{3.18}$ cfu/100 mL and $10^{2.02}$ cfu/100 mL, respectively. The WT was at its highest and closest to the 45 cm separation distance during these dates (Fig 8). Median tank

E.coli was more variable with a c.v. of 98% (median=$10^{5.21}$cfu/100 mL) when compared to drainfield groundwater *E.coli* with a c.v. of 28% (median=$10^{3.44}$ cfu/100 mL).

There was a two orders of magnitude reduction of *Enterococcus* between the sampling events on 1/25/2010 ($10^{4.08}$cfu/100 mL) and 3/15/2010 ($10^{2.18}$ cfu/100 mL) at Site 2 (Fig 6). *Enterococcus* density was $10^{3.30}$ cfu/100 mL, when WT was at the lowest. Median tank *Enterococcus* was more variable with a c.v. of 198% (median=$10^{4.97}$ cfu/100 mL) when compared to drainfield groundwater *Enterococcus* with a c.v. of 28% (median= $10^{3.85}$ cfu/100 mL).

At both sites, the WT rose within the zones between the measured SHWT and theoretical SHWT (Fig 7 and 8). The water table was deeper at Site 2 and groundwater had a greater separation distance from the drainfield for the duration of the study at Site 2, relative to Site 1. There were times at Site 1 in which the WT rose above the bottom of the trench (Fig 7).

Vertical Treatment Efficiency

Site 2 median tank effluent *E.coli* ($10^{5.21}$ cfu/100 mL) and *Enterococcus* ($10^{4.97}$ cfu/100 mL) were an order of magnitude greater than Site 1's *E.coli* ($10^{4.34}$ cfu/100 mL) and *Enterococcus* ($10^{3.69}$ cfu/100 mL) median tank effluent. Site 2 median *E.coli* ($10^{3.44}$ cfu/100 mL) and *Enterococcus* ($10^{3.85}$ cfu/100 mL) drainfield groundwater densities were greater than Site 1's *E.coli* ($10^{1.82}$ cfu/100 mL) and *Enterococcus* ($10^{3.51}$ cfu/100 mL) drainfield groundwater densities. Both sites were more efficient at reducing *E.coli* densities in the unsaturated zone than *Enterococcus* densities prior to groundwater recharge (Fig 9 and 11).

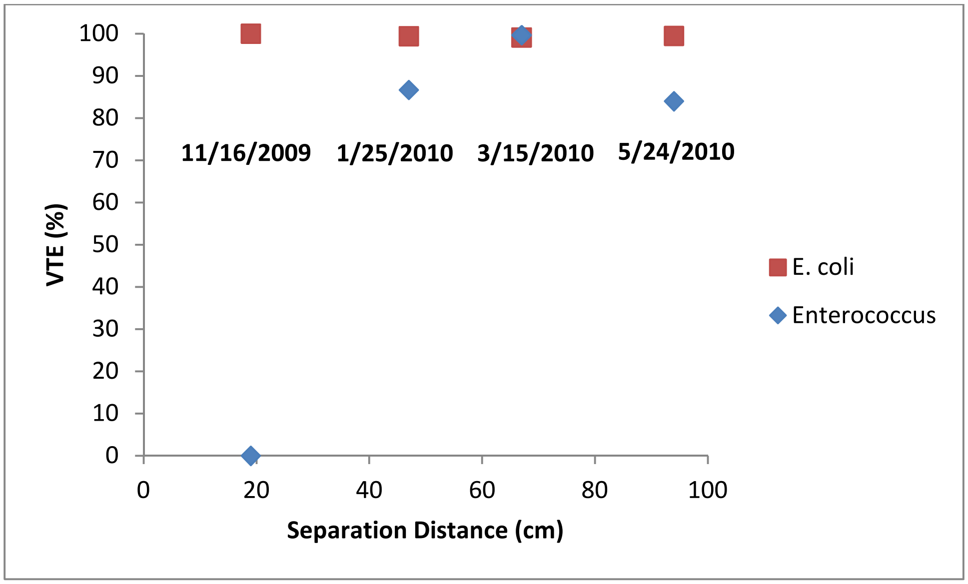

Figure 9. Vertical Treatment Efficiency (VTE) between the septic tank and drainfield (1p4) of *E.coli* and *Enterococcus*. Separation distance represents the unsaturated area between the bottom of the drainline and the WT on each sampling date.

The VTE from septic tank effluent to drainfield groundwater at Site 1 yielded various results. *E.coli* densities were reduced by 99% and greater on all four sampling dates (Fig 9), with a median VTE of 99.7%. The median VTE of *Enterococcus* was 33% (Equation 1). The median VTE of *Enterococcus* not including the 11/16/2009 sampling date was 94%. *Enterococcus* densities on 11/16/2009 were greater in drainfield groundwater ($10^{3.79}$ cfu/100 mL) than in the tank ($10^{3.42}$ cfu/100 mL). This occurred when groundwater depth was the shallowest, 19 cm from the bottom of the drainfield (Fig 7). The area received 17.7 cm of precipitation two weeks prior to sampling (Fig 10). Conductivity and chloride concentrations of the drainfield groundwater (1p4) during this date were 1.044 mS/cm and 93 mg/L, respectively, which was the highest concentration, observed of all four sampling dates. The septic tank conductivity and chloride concentrations were 1.236 mS/cm and 87 mg/L, respectively, on the 11/16/2009 sampling date.

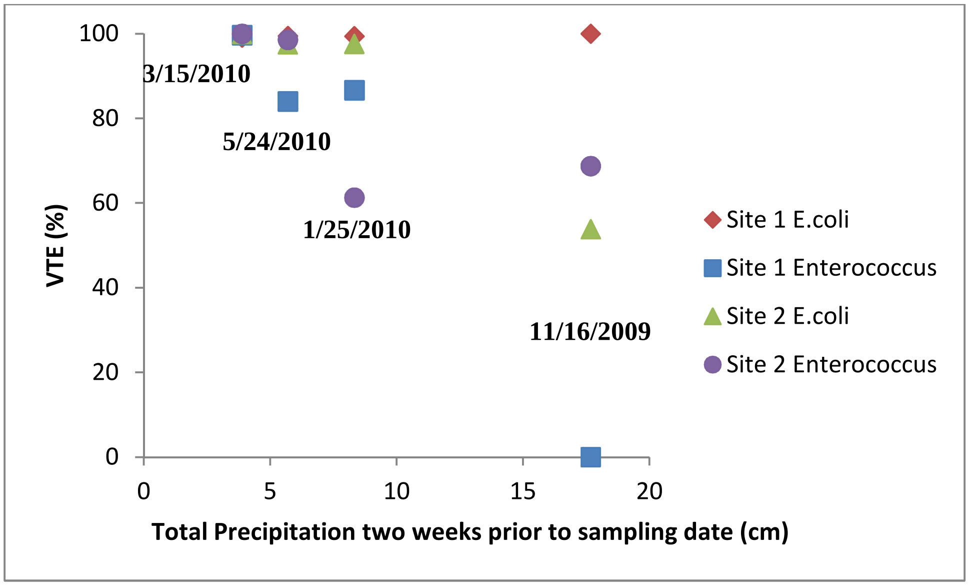

Figure 10. VTE vs. total precipitation two weeks prior to four sampling dates. Generally, as precipitation totals increased, VTE decrease.

The VTE of *Enterococcus* at Site 1 was 87% on 1/25/2010 and 84% on 5/24/2010, when the WT was 47 and 94 cm from the bottom of the drainfield, respectively (Fig 11). The only date in which there was near complete reduction of *Enterococcus* was the 3/15/2010 sampling date, in which *Enterococcus* densities were reduced by 99.7%. This corresponds to the lowest conductivity concentration measured in drainfield groundwater at 0.553 mS/cm. Precipitation two weeks prior to sampling was 3.9 cm (Fig 10).

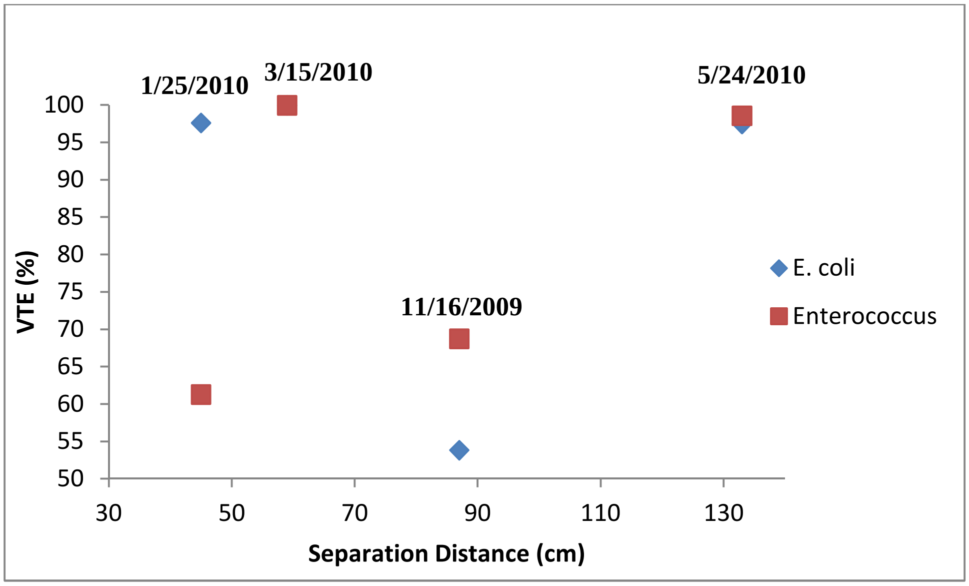

Figure 11. Vertical Treatment Efficiency (VTE) between the septic tank and drainfield (2p5) of *E.coli* and *Enterococcus*. Separation Distance represents the unsaturated area between the bottom of the drainline and the WT on each sampling date.

There were several instances at Site 2 when low levels of treatment occurred, even with separation distances greater than 45 cm. For example, the 11/16/2009 sampling date indicated that *E.coli* and *Enterococcus* VTE were only 54% and 69%, respectively (Fig 11). The WT was 87 cm below the bottom of the drainfield on this date. The area received 17.7 cm of precipitation two weeks prior to the 11/16/2009 sampling date (Fig 10). The mean conductivity of drainfield groundwater was 0.823 mS/cm, which was seven times greater than background conductivity (2p3) levels (Appendix P and Appendix Q). There was an *E.coli* VTE of 98%, but an *Enterococcus* VTE of only 64% during the 1/25/2010 sampling date. The mean conductivity of drainfield groundwater was 0.810 mS/cm, which was nearly eight times greater than background conductivity levels. The area received 8.83 cm of precipitation two weeks prior to the 1/25/2010 sampling date.

The median *E.coli* VTE was 98% and median *Enterococcus* VTE was 93% (Equation 1). On the 3/15/2010 sampling dates, both *E.coli* and *Enterococcus* VTE was greater than 99.9%.

The 5/24/2010 sampling dates, *E.coli* VTE was 98% and *Enterococcus* VTE was 99%. The WT was at its deepest during this time (Fig 8). On the 3/15/2010 and 5/24/2010 sampling dates, conductivity levels were 0.522 mS/cm in the drainfield at Site 2.

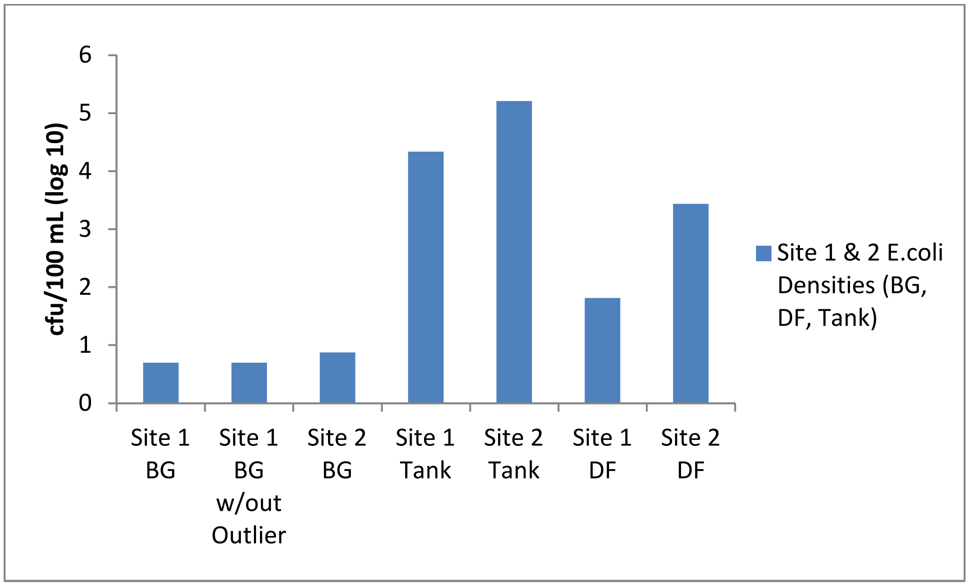

Figure 12. VTE of *E.coli* at Site 1 and Site 2 in relation to background levels. Both sites reduced *E.coli* densities between the tank and the drainfield significantly, with a three orders of magnitude reduction of *E.coli* at Site 1 and a two orders of magnitude reduction of *E.coli* at Site 2. However, reduction never reached background levels. BG=Background groundwater, BG w/o O=Background groundwater without outlier (1p2 on 5/24/2010), DF=drainfield groundwater.

The median septic tank densities of *E.coli* at Site 1 and Site 2 were $10^{4.34}$ cfu/100 mL and $10^{5.21}$ cfu/100 mL, respectively. The median drainfield groundwater densities were $10^{1.82}$ cfu/100 mL (1p4) and $10^{3.44}$ cfu/100 mL (2p5), respectively. The median VTE was 99.7% at Site 1 and 98% at Site 2 (Fig 12). *E.coli* densities were elevated in drainfield groundwater in contrast to background groundwater densities at both sites. Median *E.coli* densities in drainfield groundwater at Site 1 were 13 times greater than median background groundwater densities. Median *E.coli* densities in drainfield groundwater at Site 2 were 367 times greater than median background groundwater densities.

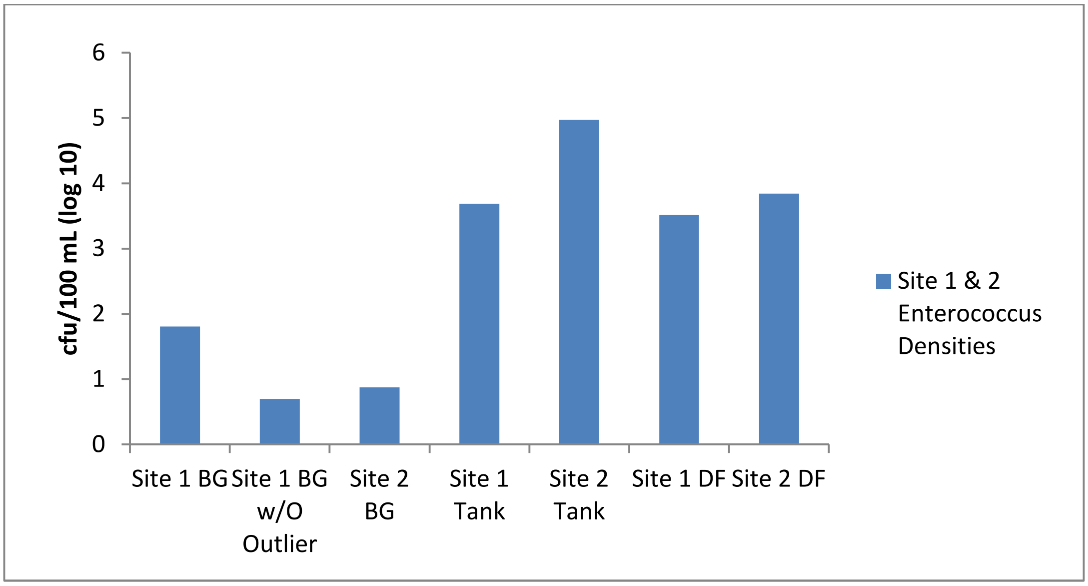

Figure 13. VTE of *Enterococcus* at Site 1 and Site 2 in relation to background levels. Site 1 reduction of *Enterococcus* between the tank and drainfield was (33%) (Equation 1). In contrast, the *Enterococcus* reduction between the tank and drainfield at Site 2 was 93%. However, reduction never reached background levels. BG=Background groundwater, BG w/o O=Background groundwater without outlier (1p2 on 5/24/2010), DF=drainfield groundwater.

The median septic tank densities of *Enterococcus* at Site 1 and Site 2 were $10^{3.69}$ cfu/100 mL and $10^{4.97}$ cfu/100 mL, respectively. The median drainfield groundwater densities were $10^{3.51}$ cfu/100 mL (1p4) and $10^{3.85}$ cfu/100 mL (2p5). The median VTE of *Enterococcus* was 33% at Site 1 and 93% at Site 2 (Fig 13). Neither site reduced *Enterococcus* densities below background levels. Median *Enterococcus* densities in drainfield groundwater at Site 1 were 51 times greater than median background groundwater densities. Median *Enterococcus* densities in drainfield groundwater at Site 2 were 933 times greater than median background groundwater densities.

Horizontal (Saturated) Treatment Efficiency: Can microbial contamination from OWTS migrate greater than the North Carolina 15 m setback distance?

Pooled E.coli and Enterococcus in Relation to Location and Distance

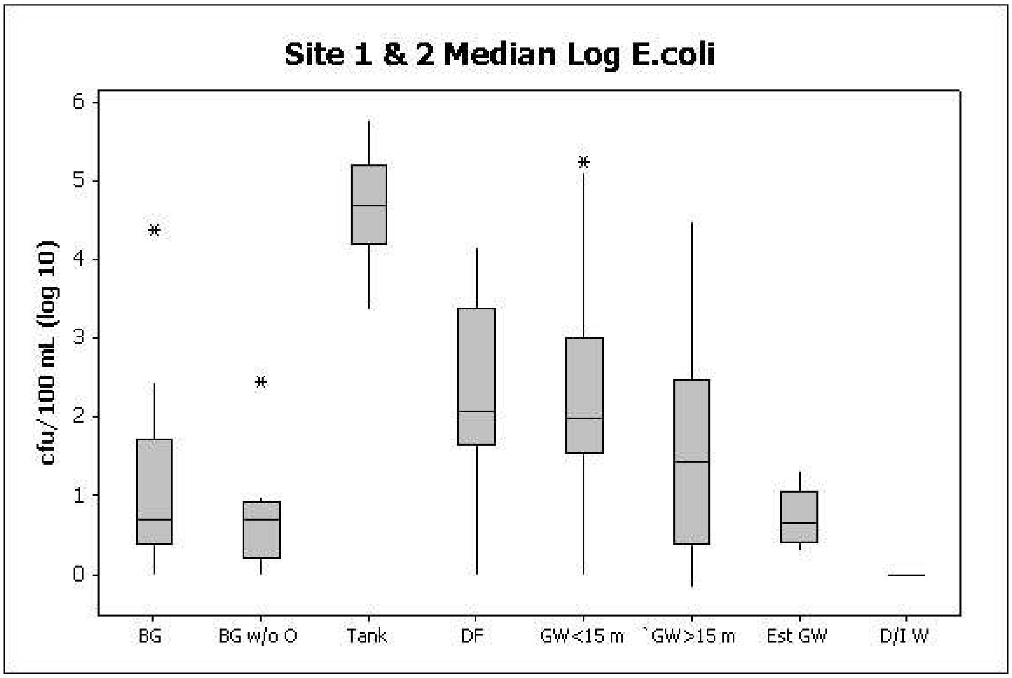

Figure 14. *E.coli* densities for Site 1 and Site 2. Colonies were grouped into areas based on location and distance. BG=Background groundwater, BG w/o O=Background groundwater without outlier (1p2 on 5/24/2010), DF=Drainfield groundwater, Tank= Septic tank effluent, GW<15 meters=groundwater in piezometers less than 15 m of OWTS, GW>15 m =groundwater in piezometers greater than 15 m of OWTS, Est GW=Estuary groundwater (1p16), D/I W-drinking/irrigation water.

The pooled data for Sites 1 and 2 showed elevated *E. coli* densities in septic tanks relative to all the other sampling points at a median of $10^{4.69}$ cfu/100 mL. Median concentrations decreased in the following pattern: drainfield groundwaters, groundwater within 15 m of the drainfields, groundwater greater than 15 of the drainfields, background groundwaters with 5/24/2010 outlier, estuary groundwater, and background groundwaters without the 5/24/2010 outlier having the lowest median *E.coli* densities at 10^{054} cfu/100 mL. Median tank *E.coli* densities indicated that populations are greater than all other groupings at $p \leq 0.05$ (Fig 14)

(Appendix R). No *E.coli* was found in the drinking/irrigation piezometers. Drainfield groundwaters had a greater median *E.coli* density than the background piezometer at p≥0.10. If the outlier that occurred on 5/24/2010 at Site 1 is not included, the p-value is less than 0.05 (Appendix R).

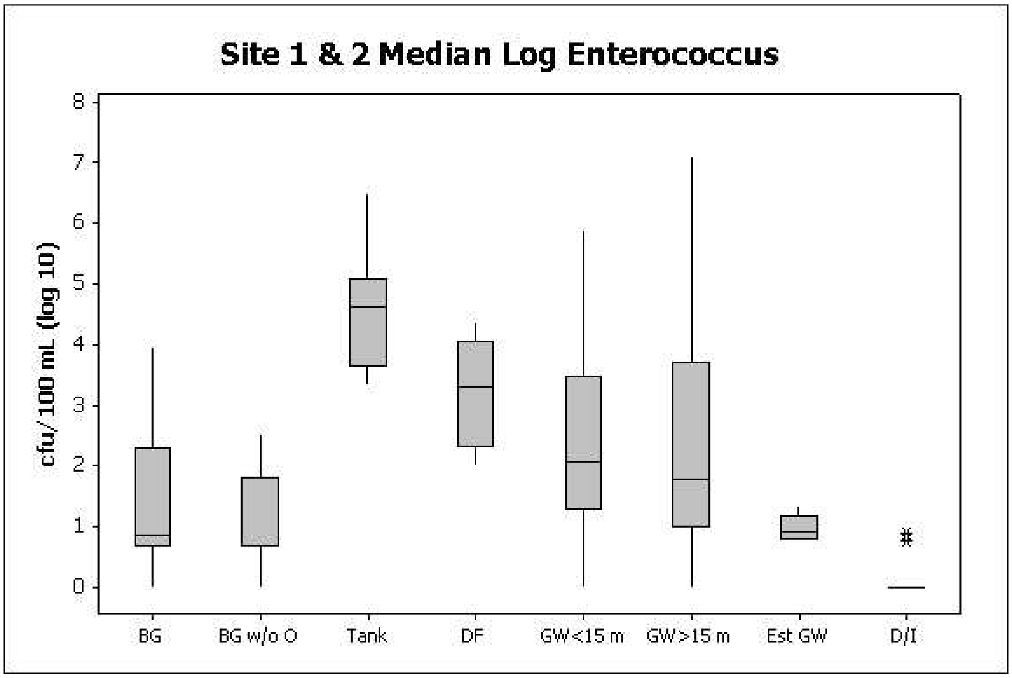

Figure 15. *Enterococcus* densities for Site 1 and Site 2. Water quality data were grouped based on location and distance from the drainfield. BG=Background groundwater, BG w/o O=Background groundwater without outlier (1p2 on 5/24/2010), DF=Drainfield groundwater, Tank= Septic tank effluent, GW<15 m=groundwater in piezometers less than 15 m of OWTS, GW<15 m=groundwater in piezometers greater than 15 m of OWTS, Est GW=Estuary groundwater (1p16), D/I W drinking/irrigation water.

Pooling the Enterococci data for Sites 1 and 2, median concentrations resulted in being the highest for the septic tank at $10^{4.65}$ cfu/100 mL. Median concentrations decreased in the following pattern: drainfield groundwaters, groundwater within 15 m of the drainfields, groundwater greater than 15 m downgradient from the drainfields, background groundwaters with and without 5/24/2010 outlier, and estuary groundwater having the lowest median *Enterococcus* densities at $10^{0.92}$ cfu/100 mL. Drinking/irrigation well water had a median of

$10^{0.00}$ cfu/100 mL; however *Enterococcus* was discovered on two sampling dates (Fig 15). Groundwater within 15 m of the drainfields did not have significantly different *Enterococcus* densities from groundwater sampled at distances greater than 15 m of the drainfields (Appendix R). All other groundwater category comparisons (Tank vs. Drainfield, etc.) had a significant difference of p≤0.05, indicating that densities originated from similar populations (Appendix R).

Horizontal Treatment Efficiency

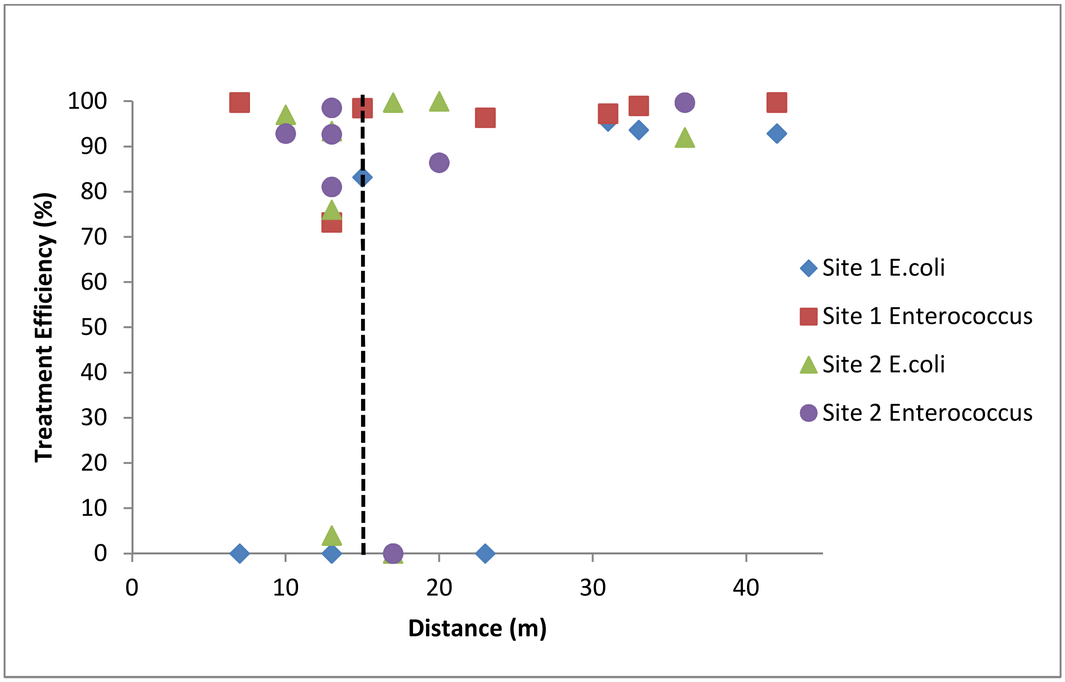

Figure 16. Site 1 and Site 2 *E.coli* and *Enterococcus* horizontal treatment efficiencies (HTE) from drainfield to various distances. All piezometers except for drainfield and background HTE were plotted in relation to distance from the drainfield. The dashed line represents the setback distance requirement stated by North Carolina State rules 15 A NCAC 18A.1950.

Generally, as distance increased, HTE increased. At Site 1, the only piezometers that did not reach 90% *E.coli* reduction after 15 meters were 1p9 (Equation 2). The piezometer closest to the setback distance requirement minimum at Site 1 was 1p7, which was slightly greater than 15 m from the drainfield. The HTE indicates that there was an 83% reduction from drainfield to 1p7. 1p8 was 31 m from the drainfield and the HTE was 96%. *E.coli* HTE that occurred between the drainfield and estuary groundwater (1p16) was 93%, which was 42 m from the drainfield (Fig 16).

At Site 1, there was a 99% *Enterococcus* reduction from drainfield to 1p7, which was slightly greater than 15 m from the drainfield. The HTE between the drainfield and 1p8 was 97%, 31 m from the drainfield. The HTE between the drainfield and the estuary groundwater (1p16) was 99.7%, which was 42 m from the drainfield (Fig. 16).

At Site 2, the piezometers closest to the setback distance requirement minimum were 2p7 (13 m) and 2p8 (17 m) (Equation 2). The HTE between the drainfield and 2p7 was 4% (*E.coli*) and 81% (*Enterococcus*). The median *E.coli* and median *Enterococcus* groundwater densities at 2p8 were greater than drainfield groundwater *E.coli* and *Enterococcus* densities. The groundwater at 2p9, which was 36 m away from the drainfield, had an *E.coli* HTE of 92% and an *Enterococcus* HTE of 99.7% (Fig 16).

Comparison of VTE and HTE

The comparision between the *E.coli* and *Enterococcus* at Site 1 indicated that there was a three orders of magnitude reduction of *E.coli* in the unsaturated zone (VTE) (Table 6). The remainder was reduced to background levels approximately 30 m from the drainfield. Reduction of *Enterococcus* in the unsaturated zone at Site 1 was very limited. Site 1 was not as effective in treating/eliminating *Enterococcus* in the trench, biomat, and unsaturated zone between the septic tank and the drainfield, whereas *E.coli* treatment at Site 1 was very effective (Table 6).

Site 1 E.coli	Well ID	Median	Median (log 10)	Treatment Efficiency (%) From DF to Piezometer	Treatment Efficiency (%) From Tank to Piezometer	Distance from Drainfield (m)
VTE	Tank	21750	4.34			
	1p4	65.5	1.82	**99.70**	**99.70**	
HTE	1p5	114.7	2.06	**0**	**99.50**	7
	1p6	280.0	2.45	**0**	**98.70**	13
	1p7	11.0	1.04	**83**	**99.90**	15
	1p8	2.9	0.46	**96**	**99.99**	31
	1p9	84.2	1.93	**0**	**99.60**	23
	1p10	4.2	0.62	**94**	**99.98**	33
	1p16	4.7	0.67	**92.90**	**99.98**	42
Site 1 *Enterococcus*						
VTE	Tank	4875	3.69			
	1p4	3250	3.51	**33**	**33**	
	1p4 w/out O	300	2.48	**94**	**94**	
HTE	1p5	8.5	0.93	**99.70**	**99.80**	7
	1p6	870.0	2.94	**73**	**82**	13
	1p7	48.8	1.69	**99**	**99.00**	15
	1p8	89.0	1.95	**97**	**98**	31
	1p9	118.4	2.07	**96**	**98**	23
	1p10	33.0	1.52	**99**	**99.30**	33
	1p16	8.3	0.92	**99.70**	**99.80**	42

Table 6. Site 1 VTE and HTE for *E.coli* and *Enterococcus*

At Site 2, there was a two orders of magnitude reduction of *E.coli* in the unsaturated zone. There were also a two orders of magnitude reduction in the saturated zone from drainfield groundwater to 2p9, but there was never a reduction to background groundwater levels (2p3). Site 2 *Enterococcus*, was reduced by an order of magnitude in the unsaturated zone. There was a two orders of magnitude reduction in the saturated zone form drainfield to 2p9; almost reaching background groundwater (2p3) levels (Table 7).

Site 2 E.coli	Well ID	Median	Median (log 10)	Treatment Efficiency (%) From DF to Piezometer	Treatment Efficiency (%)From Tank to Piezometer	Distance from Drainfield (m)
VTE	Tank	162500	5.21			
	2p5 (DF)	2750	3.44	**98**	**98**	
HTE	2p1	90	1.95	**97**	**99.90**	20
	2p2	181	2.26	**93**	**99.90**	13
	2p3	7.5	0.88	**99.70**	**99.99**	17
	2p4	659	2.82	**76**	**99.60**	13
	2p6	81	1.91	**97.10**	**99.95**	10
	2p7	2640	3.42	**4**	**98**	13
	2p8	7500	3.88	**0**	**95**	17
	2p9	220	2.34	**92**	**99.90**	36
Site 2 *Enterococcus*						
VTE	Tank	93750	4.97			
	2p5 (DF)	7000	3.85	**93**	**93**	
HTE	2p1	950	2.98	**86**	**99.00**	20
	2p2	101	2.00	**99.00**	**99.90**	13
	2p3	7.5	0.88	**99.90**	**99.99**	17
	2p4	511	2.71	**93**	**99.50**	13
	2p6	500	2.70	**93**	**99.50**	10
	2p7	1325	3.12	**81**	**99.00**	13
	2p8	13150	4.12	**0**	**86**	17
	2p9	20	1.30	**99.70**	**99.98**	36

Table 7. Site 2 VTE and HTE for *E.coli* and *Enterococcus*

EPA Surface Water Contact Standard

Number of Samplings Dates GW Exceeded EPA Single Sample Contact Standard			
	E. coli	***Enterococcus***	**Distance from drainfield (m)**
1p2	1	2	40
1p4	0	4	0
1p5	1	1	7
1p6	2	3	13
1p7	0	1	15
1p8	1	2	31
1p9	1	2	23
1p10	0	0	33
1p16	0	0	42
1 D/W	0	0	27

Table 8. Site 1 EPA Surface Water Contact Standard Exceedence Rate. The number of sampling dates microbial densities exceeded the single sample surface water contact standards of $10^{2.37}$ (235) cfu/100 mL (*E.coli*) and $10^{2.02}$ (104) cfu/100 mL (*Enterococcus*).

The EPA Surface Water Contact Standard was used to determine if groundwater that upwelled into surface water bodies would have the potential to cause adverse health effects (Table 3 & 8). At Site 1, piezometers 1p2, 1p5, 1p6, 1p8 and 1p9 exceeded the *E.coli* Single Sample Contact Standard of $10^{2.37}$cfu/100 mL at least once during the duration of the study. Every piezometer except for 1p10 and 1p16 exceeded the *Enterococcus* Single Sample Contact Standard of $10^{2.02}$ cfu/100 mL (Table 8) at least once during the study. On the 10/2/2009 and 12/9/2009 sampling dates, *Enterococcus* was also detected in the drinking/irrigation well (Appendix L).

Number of Samplings Dates GW Exceeded EPA Single Sample Contact Standard			
	E. coli	***Enterococcus***	**Distance from drainfield (m)**
2p1	1	3	20
2p2	2	1	13
2p3	1	1	17
2p4	2	2	13
2p5	3	4	0
2p6	1	2	10
2p7	2	2	13
2p8	3	3	17
2p9	2	1	36
2 D/W	0	0	37

Table 9. Site 2 EPA Surface Water Contact Standard Exceedence Rate. The number of sampling dates microbial densities exceeded the single sample surface water contact standards of $10^{2.37}$ (235) cfu/100 mL (*E.coli*) and $10^{2.02}$ (104) cfu/100 mL(*Enterococcus*) at Site 2.

Every piezometer at Site 2 exceeded the EPA *E.coli* contact standard of $10^{2.37}$cfu/100 mL and *Enterococcus* contact standard of $10^{2.02}$ cfu/100 mL (Table 9) at least once during the study. No *E.coli* and *Enterococcus* was detected in the drinking/irrigation water.

Chapter 4: Discussion

Treatment Efficiency vs. Separation Distance (Hypothesis 1): the NC 45 cm separation distance does not always prevent microbial contamination of groundwater

The North Carolina 45 cm separation distance did not always prevent microbial contamination of groundwater. There was usually at least an order of magnitude reduction in microbial densities between the tank and groundwater beneath drainfields. There were significant reductions in microbial densities between the tanks and drainfield groundwaters at Sites 1 and 2. However, microbial densities were not reduced to background levels in the drainfields, where microbial densities ranged from 13 to 933 times greater than background groundwater densities (Fig 12 and 13). Also, *Enterococcus* densities at both Site 1 and *E.coli* and *Enterococcus* densities at Site 2 exceeded the EPA Single Sample Contact Standard in groundwater beneath the drainfield (Tables 3, 8, and 9). This indicates that the groundwater adjacent to both sites' drainfields is affected by wastewater disposal, especially in sandy soils with a small separation to the water table.

Case Studies

Source	Soil Type	Distance	VTE (if applicable)	Type of Study
O'Luanaigh et al., 2012	Sandy gravel, Sandy gravely silt	90 cm		Field
Humphrey & O'Driscoll, 2011	Sandy soils, sandy loams, sandy clay loams	45 cm-sandy loams, sandy clay loams; 60 cm-sandy soils		Field
Gill et al., 2007	Gravel, sand, sandy clay	100 cm		Field
Karathanasis et al., 2006	All soils types (Group I-IV)	60 cm	Mean: FC=91.8%, FS=88.6% (for Group I soils)	Laboratory
Van Cuyk et al., 2001	Sand	30 cm	90%+	Laboratory
Duncan et al., 1994	N/A	15 cm		Laboratory
Cogger et al., 1988	Fine Sand	60 cm		Field
Tyler et al., 1977	Sandy Soils	60 cm		Laboratory

Table 10. A summary of studies and their suggestions of appropriate separation distance and the vertical treatment efficiency between the septic tank and drainfield groundwater. FC-fecal coliform and FS-fecal streptococci.

Numerous studies in sandy surficial aquifers have shown that the 45 cm separation distances may not eliminate microbial indicators (Table 10). Van-Cuyk et al (2001) conducted a laboratory experiment where four three-dimensional lysimeters were installed with the same medium sand and either an aggregate-laden (AL) or aggregate-free (AF) infiltration surface with 60- or 90-cm of soil between surface and depth to groundwater. Each lysimeter was dosed four times a day with septic tank effluent for 48 weeks. From week 20 on, there was a 96-99% reduction of the dosed fecal coliform bacteria that percolated through the lysimeters. After the 48 week testing period, they analyzed the core samples and concluded that the densities of fecal coliform bacteria decreased with increased depth and none were detected in sand samples at 30 cm or deeper.

At Site 1, the *E.coli* VTE was within ranges that Van-Cuyk et al (2001) suggested. Site 1 *Enterococcus* densities and Site 2 *E.coli* and *Enterococcus* densities varied throughout the year, and microbial reduction was less than 96%. Elevated microbial densities were also observed in groundwater at depths greater than 30 cm beneath the bottom of the drainfield. It is reasonable that treatment is slightly worse in field settings when compared to lab columns, because macropores (such as root cavities, animal burrows, earthworm voids, etc.) would be more common in a field setting and they can act as conduits for infiltrating effluent to penetrate the subsurface and migrate at a more rapid rate in contrast to packed column studies (Pang et al., 2003 and Morari et al., 2010).

Karathanasis et al (2006) conducted a laboratory study in Kentucky to evaluate the effects of soil texture and thickness on the treatment of fecal bacteria. Soil monoliths were excavated at 30, 45, and 60 cm from ten sites where new septic systems were to be installed. Domestic wastewater was leached into the monoliths. In Group I soils, which are similar to the soils at Site 1 and Site 2, the mean VTE of fecal coliform was 91.8±15 % at 30 cm, 96.8±7.5% at 45 cm and 86.6±20.6% at 60 cm. The overall fecal coliform VTE means for Group I soils were 91.8±15.7. The mean VTE of fecal streptococci was 91.1±19.5% at 30 cm, 90.9±20.9% at 45 cm, and 23.6±25.1% at 60 cm. The overall fecal streptococci VTE mean was 88.6±22.0%. They concluded that fecal bacteria treatment efficiency increased with increasing clay content. The study also provided evidence that relying solely on treatment efficiencies as the only criterion for assessing treatment differences between soil groups can be misleading. Even though the mean fecal coliform treatment efficiency was greater than 90.5%, only 20% of soil monoliths were in compliance with EPA maximum discharge limit of 10^3 cfu/100 mL. Also, the authors suggested that Kentucky's 30-45 cm separation distance regulation, which is similar to North Carolina's, is insufficient to properly treat fecal bacteria. They suggest that increasing the separation distance to 60 cm for all soil types (Group I-IV) would improve treatment efficiencies.

The variable relationship established between VTE and separation distance in the Karathanasis et al (2006) study was similar to what was observed at Site 1 and Site 2. When the vadose zone was 19 cm thick at Site 1, *E.coli* VTE was 99.997%, which is in agreement with what Karathanasis et al (2006) observed at 30 cm for fecal coliform. However there was no decline for *Enterococcus* between tank and drainfield on the same date. When the vadose zone at Site 1 was 47 cm thick, *E.coli* VTE was 99.4% and *Enterococcus* VTE was 87%, which were within ranges of what Karathanasis et al (2006) observed at similar depth (45 cm) for fecal coliform and fecal Streptococci.   At Site 2 when the vadose zone was 45 cm thick, *E.coli* VTE was 98%, which is in agreement with what Karathanasis et al (2006) observed at similar depth (45 cm) for fecal coliform. *Enterococcus* VTE was only 61%, which was below what Karathanasis et al (2006) observed at similar depth (45 cm) for fecal streptococci. When the vadose zone was 67 cm at Site 1, both *E.coli* and *Enterococcus* VTE was above 99%, which is in agreement with Karathanasis et al (2006) at 60 cm for both fecal coliform and fecal streptococci. Similarly, when the vadose zone was 59 cm at Site 2, both *E.coli* and *Enterococcus* VTE was above 99%, this is in agreement with Karathanasis et al (2006) for both fecal coliform and fecal streptococci.

Site 1 *E.coli* and Site 2 *E.coli* and *Enterococcus* densities were reduced in the drainfield by at least 93% or greater throughout the study. However, the VTE on the four sampling dates varied. Site 1 median *Enterococcus* reduction was only 33%, but that was heavily influenced by the 11/16/2010 sampling date, in which densities in the drainfield groundwater were greater than they were in the tank. It appears this is related to high levels of recent rainfall associated with a nor'easter (Fig 10). The 3/15/2010 sampling date was the only date in which microbial indicators were reduced by 99%+ on both sites. The separation distance at Site 1 was 67 cm and 59 cm at Site 2. If the goal is to reduce microbial densities to background levels, then the data suggest that 45 cm is not enough. However, if the 99.9% elimination of pathogenic microorganism in groundwater is the measure to determine the effectiveness of elimination, as stated by Pekdeger and Mattness, (1983), then a separation distance of approximately 60 cm would be required to achieve that level. This would be in agreement with Karathanasis et al (2006) mean value of Group I soils within one standard deviation.

Wastewater Strength /Concentration

Wastewater strength is based on internal and external factors, such as number and quality of water-using fixtures and appliances, the number of occupants in a residence, the age of the residence, eating habits, pharmaceutical, personal care products, cleaning products, water-use habits of the residences, and design and maintenance of the OWTS (US EPA, 2002). Wastewater strength is very important in evaluating the risk of microbial contamination of groundwater. Site 2 median tank effluent *E.coli* ($10^{5.21}$ cfu/100 mL) and *Enterococcus* ($10^{4.97}$ cfu/100 mL) were an order of magnitude greater than Site 1's median tank effluent *E.coli* ($10^{4.34}$

cfu/100 mL) and *Enterococcus* ($10^{3.69}$ cfu/100 mL) densities. Site 1 and Site 2 *E.coli* (10^4-10^6 cfu/100 mL) and *Enterococcus* (10^4-10^5) densities were within range of domestic wastewater observed by other studies (Lowe et al., 2007 and Humphrey et al., 2010). Site 2 *E.coli* and *Enterococcus* median VTE was 98% and 93%, however the median *E.coli* and *Enterococcus* densities that remained in the groundwater beneath the drainfield were higher at Site 2 than at Site 1. If wastewater strength at Site 1 was similar to Site 2, it is likely that the levels of microbial indicators in groundwater would have been as elevated at Site 1, particularly for *Enterococcus* since the VTE was low (33%). Even though there was significant reduction of microbial indicators between the tank and groundwater below the drainfield, the elevated levels of microbial indicators in groundwater underlying the drainfields could also be a hazard to public health (Lipp et al., 2001). For example, there were two dates in which low levels of *Enterococcus* were detected in the irrigation well at Site 1. This could be a potential health hazard because the residence at Site 1 used the water from that well to hydrate their vegetation. Groundwater with elevated levels of microbes can discharge into the Pamlico River and other surrounding surface waters, such as streams, estuaries, wetlands, lakes, and ditches, which could potentially harm humans and animals that come into contact with that water.

Differences in tank and drainfield groundwater microbial abundance also may be due to several factors, such as nitrogen and chlorides, among others (Appendix S). The mean total dissolved nitrogen was higher in both the tank (84.8 mg/L) and the drainfield groundwater (23.2 mg/L) at Site 1 than the tank (57.6 mg/L) and drainfield groundwater (8.9 mg/L) at Site 2. The abundance of nitrogen is an indicator of groundwater contamination and elevated levels of dissolved nitrogen can correlate to elevated levels of microbes (Humphrey, 2009). Also, the residents at Site 1 are older than the residents of Site 2. It is possible that if these residents are taking medications, such as antibiotics, their residue can affect microbial populations in the tanks and drainfields (US EPA 2002). Further analysis would need to be conducted to test whether or not this could affect microbial densities within OWTS.

Pulsation/Flushing Events

At Site 2, the water table went above the bottom of the 45 cm separation during late January through February and briefly in mid-March (Fig 8). Comparable to Site 1, Site 2 allowed for more vadose zone residence time to allow natural processes to reduce the microbes. The VTE at Site 2 varied seasonally, with *E.coli* and *Enterococcus* VTE of >98% on the 3/15/2010 and 5/24/2010 sampling dates. However, the VTE during the 11/16/2009 and 1/25/2010 sampling dates were not as efficient, even though separation distances between the bottom of the drainfield and WT were 87 and 45 cm, respectfully. The 11/16/2009 and 1/25/2010 sampling dates were dates in which the most precipitation fell two weeks prior (Fig 10). Conductivity concentrations were elevated in piezometers 2p5 on 11/16/2009 and 1/25/2010 sampling dates, compared to the 3/15/2010 and 5/24/2010 sampling dates, indicating that wastewater is the source of the contamination (Appendix Q).

The following situation is an example of how recent rains that occurred prior to sampling may have influenced the VTE of microbial densities on the sampling dates in the vadose zone (Appendix O). In environments where there is a variable flux of nutrients, such as a shallow groundwater system underlying an OWTS, microbial population shifts between exponential growth, stationary, and death phases are common (Chappelle, 1993). Dry soil conditions and a deeper water table beneath the drainfield can allow more time for natural processes, such as filtration and predation, to reduce bacteria (Davis, 2010). However, if there is a sudden increase of precipitation, bacteria from the surface as well as bacteria in the soil may move through the soil at a quicker pace and enter the groundwater system. For example, infiltration of precipitation over loamy sand can transport as much as 100 times more fecal coliforms to groundwater following rainfall events than during dry periods (Gagliardi and Karns, 2000).

Bouwer et al (1974) observed this phenomenon in a study of the Flushing Meadows Project in Phoenix, Arizona. Bouwer et al (1974) analyzed groundwater fed by secondary sewage effluent that was discharged into rapid infiltration basins. They observed that groundwater microbial colonies increased in response to a release of wastewater, which occurred after an extended dry period. This could indicate that more microbes are entering groundwater without being properly treated.

The scenario mentioned above could have occurred at least three out of the four sampling dates. Two weeks prior to sampling on the 11/16/2009 sampling date, the area received 17.7 cm of precipitation. Two weeks prior to the 5/24/2010 sampling date, the area received 5.7 cm of precipitation the week two weeks prior to sampling date. The area received 3.15 cm of rain from 4/10/2010-5/9/2010. More specifically, 1.88 cm of rain fell on the day before and on the morning of the sampling date (5/24/2010). Also during the week of increased rainfall, a spike in DO was observed at both Site 1 and Site 2 (Appendix T & U), suggesting groundwater recharge. In conclusion, it is possible that the influx observed at both sites on 11/16/2009, 1/25/2010, and 5/24/2010 sampling dates was an observation of increased microbes in the groundwater due to recharge following a sudden increase in precipitation (Fig 10).

E. coli and Enterococcus Transport with Distance from Drainfield (Hypothesis 2): microbial contamination from OWTS can migrate greater than the NC 15 m setback

The primary goal of establishing setback distances is to prevent effluent from the drainfield area from entering a well or surface water as well as reducing the probability of the effluent plume intersecting with other plumes (Froese et al., 2009). However, there are physical, chemical, and biological constituents in groundwater that can provide a hostile environment for microbes and can eliminate these microbes further, especially given time and distance (Pekdeger and Matthess, 1983). Significant reduction in the unsaturated zone can prevent elevated levels of microbes from entering the groundwater, therefore increasing the overall effectiveness of source density reduction occurring laterally.

In the current study, both sites were in compliance with the North Carolina State Regulation 15A NCAC 18A .1950 with respect to setback distances from surface waters (>15 m). North Carolina regulations dictate that residential systems discharging less than 3000 gallons/day should be placed no closer than 15 m from surface water bodies (30 m from shellfish waters) so that wastewater will not adversely impact surface waters. In the U.S., states have implemented setback distances ranging from 15-91 m, with typical values ranging from 15-30 m (Yates and Yates, 1989) (Table 2). As groundwater moved further away from the drainfield, both *E.coli* and *Enterococcus* showed significant density reductions (Fig 14, 15, and 16). However, the groundwater data at both sites suggested that the migration of *E.coli* and *Enterococcus* from OWTS through the surficial aquifer extended greater than 15 m. The degree of reduction varied temporally and spatially.

The setback distance minimum of 15 m yielded various results. At Site 1, there was an *E.coli* HTE of 83% and an *Enterococcus* HTE of 98.5%, slightly greater than 15 m away from the drainfield (1p7). Groundwater specific conductance within 15 m of the drainfield averaged 0.469 mS/cm, which is nearly eight times greater than background groundwater levels. This indicates that groundwater 15 m from OWTS may have been affected by wastewater. Electrical resistivity survey data from Site 1 indicated that wastewater-affected groundwater extended from the OWTS to the Pamlico River (Appendix V). Therefore, it is likely that the OWTS plume with elevated dissolved ions and microbes is discharging into the estuary.

At Site 2, there was no reduction of *E.coli* and *Enterococcus* in 2p8 which was 17 m from the drainfield. Median values of E.coli ($10^{3.88}$ cfu/100 mL) and *Enterococcus* ($10^{4.12}$ cfu/100 mL) were higher in piezometer 2p8 than in the drainfield. There was no significant difference between the medians of the two areas (Appendix W and X). At 2p7, which is 13 m from the drainfield, there was an *E.coli* HTE of 4% and an *Enterococcus* HTE of 81%. The mean specific conductance at 2p7 and 2p8 were 0.121 mS/cm and 0.245 mS/cm. These values were equal and two times greater than background levels. The mean chloride concentrations for 2p7 and 2p8 were 14 mg/L and 12 mg/L, which were less than mean background groundwater concentrations of 24 mg/L. The median TDN concentration at 2p7 and 2p8 was 0.6 mg/L and 1.0 mg/L, respectfully, which was lower for background TDN concentrations (1.1 mg/L). Conductivity, chloride, and TDN indicated that 2p7 and 2p8 did not appear to be heavily affected by wastewater, especially when compared to background, tank, and drainfield tracer data (Appendix I, Q, and Z).

Case Studies

Source	Soil Type	Setback required to reduce/eliminate microbes/viruses	Type of Study
Habteselassie et al., 2011	Sandy Loam	10 meters	Field
Pang et al., 2005	Coastal sand aquifers	37-44 meters	Laboratory/Field
Pang et al., 2003	Pumice Sand	16 meters-*E.coli*, 48 meters-viruses	Laboratory
DeBorde et al., 1998	Sand/Gravel	45.5 meters	Field
Yates and Yates., 1989	N/A	80 meters	Laboratory
Yates et al. 1988	Silty clay loam-sandy gravel	1.5-125 meters	
Bouwer et al., 1974	Sand/Gravel	91 meters	Field
Young, 1973	Sand	6.1 meters	Field

Table 11. A summary of studies and their suggestions of appropriate setback distance to reduce or eliminate microbes and viruses.

Several scientific studies have addressed what is an appropriate setback distance to protect water quality (Table 11). Yates and Yates (1989) using disjunctive kriging, created a model to calculate the probability of eliminating viruses from groundwater in the city of Tucson, Arizona. Their objective was to determine an adequate setback distance to reduce virus densities. They concluded that to eliminate virus contamination by seven orders of magnitude, there is a 70% probability that a 15 m setback distance would eliminate viruses, an 85% probability that a 30 m setback distance would eliminate viruses, and to reduce viruses with 99% probability, a setback distance of 80 m would be necessary.

Pang et al (2005) conducted a field/ laboratory combination experiment to compare two methods (advection-dispersion model vs. filtration theory) of determining reductions of microbial densities in different aquifers. The objective was to derive parameter values that can be used to describe the filtration of microbes in coarse alluvial gravel aquifers on a field scale and to provide recommendations on setback distances in alluvial gravel aquifers. *Bacillus subtilis* spores and the F-RNA phage (MS2) were used as the tracer to determine bacterial and viral reduction. Their results indicated that a seven orders of magnitude reduction would require 125–280 m travel in clean coarse gravel aquifers, 1.7–3.9 km travel in contaminated coarse gravel aquifers, 33–61 m travel in clean sandy fine gravel aquifers, 33–129 m travel in contaminated sandy fine gravel aquifers, and 37–44 m travel in contaminated river and coastal sand aquifers (Pang et al., 2005).

Habteselassie et al (2011) conducted a study in eastern North Carolina to examine the effects of microbial transport of four OWTS (two that were properly functioning and two that were failing) on surrounding water quality.   Water samples were collected from monitoring wells located near drainfields, as well as nearby ditches. *Enterococcus*, *E.coli*, Rhodamine WT (RWT) and coliphage MS2 were used as tracers to determine fate and transport. For the two properly functioning OWTS, there was over 99% reduction of *E.coli* and *Enterococcus* that occurred within 10 m from the drainfield. The two failing OWTS had groundwater *Enterococcus* and *E.coli* densities that exceeded the EPA threshold for both indicators 15 m away from the OWTS. They concluded that properly functioning OWTS in eastern North Carolina are effective in treating wastewater. However OWTS that are/have failed can negatively affect groundwater quality, especially after a precipitation event.

Habteselassie et al (2011) results of HTE within 10 m of the D-box were similar to this study HTE of *Enterococcus* (99.7%) at Site 1 and the *E.coli* (93%) and *Enterococcus* (99%) at Site 2. However there was no reduction of *E.coli* within 15 m of the drainfield at Site 2. *E.coli* and *Enterococcus* densities exceeded EPA Single Sample Contact Standard at both sites at the same distance (15 m), similar to the two failing OWTS of Habteselassie et al (2011).

Comparisons of VTE and HTE

The comparison between VTE and HTE for both Site 1 and Site 2 suggests that VTE is responsible for greater reduction of microbial abundance. This is in agreement with other studies such as Pang et al (2003) and Froese et al (2009). There were significant reduction of *E.coli* at Site 1 and *E.coli* and *Enterococcus* at Site 2 that occurred between the drainfield trenches and groundwater. Once microbes reached the groundwater, and if conditions are favorable, microbes can survive and not be reduced for 100's to 1000's of meters (Froese et al., 2009).

Site 1 is located on the Pamlico River and the brackish and saline waters may have a direct impact on the groundwater quality of the area (US EPA, 2003). Mean groundwater chloride concentrations were much higher at Site 1 (77 mg/L) that Site 2 (22 mg/L). Mean tank chloride concentrations were higher at Site 1 (80 mg/L) than Site 2 (55 mg/L). Site 1 *E.coli* VTE was 99%+ for all four sampling dates, whereas *Enterococcus* VTE achieved 99%+ reduction only once. One possible reason is that *Enterococcus* can survive in saline conditions better than *E.coli*. The mean chloride concentration in the drainfield was 64 mg/L. The mean septic tank chloride concentration was 80 mg/L. The elevated salt concentrations in the tank and groundwater at Site 1could have created a much more favorable environment for *Enterococcus* to thrive in, therefore creating an environment that was more hostile to *E.coli.*

EPA Compliance 15 meters and further from the drainfield

Even though there were significant reductions of *E.coli* and *Enterococcus* densities from the tank, many of the piezometers from both sites exceeded the EPA Single Sample Contact Standard contained at least once during the study (Table 8 and 9). For example, at Site 1, 1p7 exceeded the *E.coli* Single Sample Contact Standard. Piezometers 1p8 and 1p9, which were 31 m and 23 m from the drainfield, exceeded both the *E.coli* and *Enterococcus* Single Sample Contact Standard. Every piezometer exceeded the *E.coli* and *Enterococcus* Single Sample Contact Standard at Site 2. Piezometers 2p1, 2p8, and 2p9, are 20 m, 17 m, and 36 m from the drainfield. This is a concern because all of these piezometers are greater than 15 m from the drainfield. Theoretically, if there was surface water in the areas these piezometers are located, the surface water would be impaired for recreation, bathing, or consuming purposes. So when considering the effectiveness of a setback distance, microbial reduction to below EPA contact standards must also be considered (Karathanasis et al., 2006).

The HTE and EPA contact standards indicate that a setback distance of 30 m would provide a greater likelihood of reducing microbes to background levels and below EPA contact standards. Increasing the setback distances would help reduce the impact from new OWTS. At Site 1, the average distance between the OWTS and the estuary in the residence subdivision is 40 m. However, there were some OWTS that were closer than 30 m to the estuary (O'Driscoll et al., 2012).

The OWTS at Site 2 was not as effective in reducing *E.coli* and *Enterococcus* as Site 1 OWTS. There are various potential reasons for the differences in treatment across the sites. One possible explanation is wastewater at Site 2 was more concentrated than at Site 1. There were times, such as on the 3/15/2010 and the 5/24/2010 sampling dates, which the outermost piezometers (2p1, 2p3, and 2p9) indicated that there was no lateral movement of groundwater, suggesting that wastewater may have been concentrated. There is a clay lens approximately 5 m below land surface that could possibly promote lateral movement of groundwater in multiple directions instead of one general direction (Appendix D). It is possible that groundwater is more stagnant at Site 2; the hydraulic conductivity was an order of magnitude lower at Site 2 (6.24×10^{-4} cm/s) than Site 1 (2.08×10^{-3} cm/s), indicating that the surficial aquifer may transmit less groundwater than Site 1 (Thieme, personal communication).

At Site 1 and Site 2, microbial densities could have been influenced by sources other than the septic tank. Habteselassie et al (2011) suggested that both domestic and wild animals can contribute to microbial contamination to water resources. The homeowners at Site 2 had a pet dog that roamed the property. Site 1 was not fenced off and sits on the Pamlico River, meaning that animals, such as dogs, cats, and birds, can potentially contribute to microbial contamination.

OWTS construction and maintenance

Most septic tanks are prefabricated, and may have cracks and leaks that could potentially contaminate ground and surface waters and lead to structural failure of the OWTS (D'Amato & Devkota, 1997). Regulatory inspections for installed tanks have historically been inconsistent, infrequent, and in the long term, ineffective (D'Amato & Devkota, 1997). OWTS typically become less effective over time unless they are properly maintained (O'Hara, 2006). At Site 1, the septic tank wasn't pumped for at least 15 years. The tank was pumped a month prior to the first sampling date on 10/2/2009. Also, rainfall that occurred prior to the 11/16/2009 sampling date could have affected tank chemistry due to groundwater seeping into the tank. In summary, assuring that an individual's OWTS is properly maintained can reduce the potential harmful effects the system can have on the environment. Also, the construction, installation, and maintenance of these units needs to be properly regulated by all parties involved (individual, county, and state).

Chapter 5: Conclusions and Management Implications

The 15 m setback distance was not always sufficient in reducing microbial densities to below EPA standards at Site 1 and Site 2. There were areas in which the groundwater greater than 15 m down-gradient from the OWTS exceeded the EPA Single Sample Contact Standard at both sites. Site 2 patterns were less clear, in part because of variations in groundwater flow from the drainfield. Ideally, a recommendation of 30 m or greater setback would be required based on this study to reduce *E.coli* and *Enterococcus* to background groundwater levels. High densities of OWTS in sandy soils with shallow water tables can increase the risk of these microbes contaminating water resources and precautions must be taken to reduce the risk.

The data suggest that the unsaturated zone between the bottom of the drainfield and the water table had a greater influence on reducing *E.coli* and *Enterococcus* densities in wastewater effluent than the surficial aquifer. Generally, *E.coli* and *Enterococcus* in wastewater were treated more effectively at Site 1 than at Site 2 throughout the four sampling dates (Fig 7 & 8). However at both sites, elevated microbial densities were detected when the water table was 87 cm or deeper than the bottom of the drainfield trenches. Minimum separation distance required by the state of North Carolina is 45 cm in sandy soils. As discussed earlier, the greater separation distances between the drainfield and the water table, the greater the chance that microbes and viruses are filtered or become inactive before entering groundwater. Pekdeger and Mattness (1983) suggested that 99.9% reduction of pathogenic microorganism in groundwater should be the standard to determine the effectiveness of treatment. Based on that standard, in sandy/silty surficial aquifers, a minimum 60 cm separation between the bottom of the drainfield and the SHWT would be appropriate. This would correspond to what is described in current regulations in states such as Florida and Virginia (Stall, 2008) (Table 2). This would also be in agreement with recent recommendations based on systems in eastern North Carolina by Humphrey et al (2011).

Horizontal treatment efficiency is also important in reducing *E.coli* and *Enterococcus* densities. The 15 m setback distance minimum achieved significant reduction at Site 1 and HTE of 90%+ as distance increased from 15 m at both Site 1 and Site 2. Even though there was significant reduction, the remaining microbes could still be a source of water contamination.

North Carolina's population is expected to grow to 12 million people by 2030. Beaufort County's population is expected to increase by 14% between 2010 and 2020 and by 12% between 2020 and 2030. Eastern North Carolina is expected to grow by 15% by 2020 and by 12% between 2020 and 2030 (North Carolina Office of State Budget and Management, 2011). In rural areas, such as eastern North Carolina, the increase in population could potentially increase septic tank density. Yates (1985) suggested that septic tank density within an area is the single most important indicator to reduce the effect that these systems have on groundwater. The US EPA has designated areas with septic tank densities greater than 40 OWTS per square mile as regions of potential groundwater contamination (Yates, 1985). At Site 1, the neighborhood contained 27 OWTS and one community OWTS, in a 0.07 square mile region. The increase in population will increase the OWTS densities in these areas if municipal wastewater treatment does not occur, thus intensifying the potential threat of microbial contamination.

Sea level rise in response to climate change could have a tremendous impact on the effectiveness of OWTS. Climate models have indicated that North Carolina's average temperature and rainfall will increase (North Carolina Climate Office, 2011). Some "best case" climate models predict that the increased rainfall amounts will be higher in intensity and frequency (US EPA, 2010). This could have an effect on SHWT, as WT could increase due to sea level rise, decreasing the separation distances between the bottom of the drainfield and SHWT on existing systems. Also, pulsation/recharge events would be more common. This could increase the possibility of water-borne outbreaks due to the degradation of water quality (Howard et al, 2010).

When properly constructed and maintained, OWTS can be a safe and practical alternative to municipal wastewater treatment, especially in rural areas in which large sewage treatment facilities are not feasible. As noted earlier, if these systems are not properly implemented, degradation of water quality can occur, which can become hazardous to human health. Improved wastewater regulations are needed to help to reduce the risk of water quality impairment.

Future Work

Sampling before, during, and after storms and more frequently could help improve understanding of temporal variability and the fate and transport of these microbes. To evaluate the effects of recharge events a similar study with greater sampling frequency and storm-specific sampling could help to better explain the temporal variability of on-site wastewater treatment in sandy coastal soils. This type of study would be important to advance the understanding of groundwater-OWTS dynamics in storm-prone coastal areas and how it relates to climate change. Similarly, projects similar to this one would need to be conducted in other soil types (Group II-IV).

Further work is needed to quantify the potential sources of microbial water quality impairment in rivers, estuaries, and shallow groundwater in eastern North Carolina. An ongoing parallel study by the CDC will aim to determine the source of *E.coli* and *Enterococcus* by analyzing microbial DNA, to determine whether origin is from human, dog, cat, etc. This type of work may help to improve the understanding of the various sources of microbial water quality impairments in the estuary and other surface water bodies.

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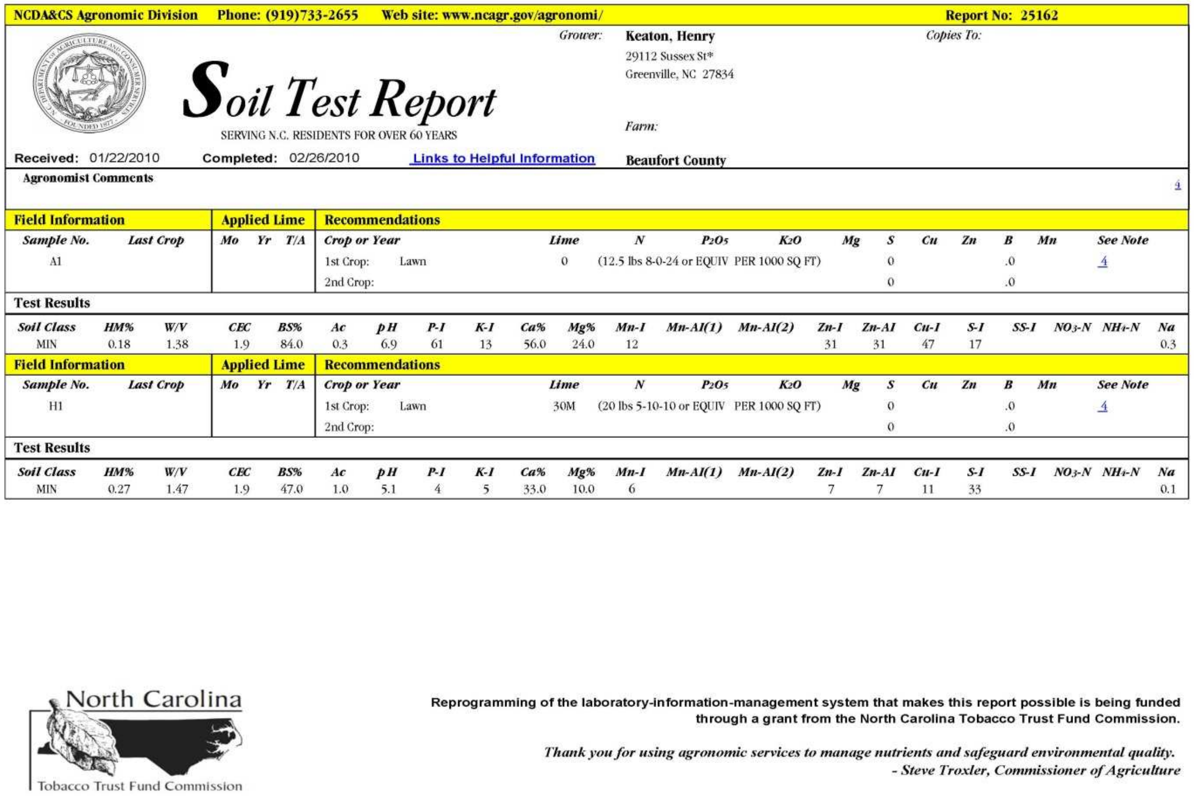
Appendix

Appendix A. Soil morphology of Site 1 and Site 2. Modified from Humphrey et al., 2010.

Site	**Morphological Parameter**			**Horizon**			**Boring Summary**	
		1	**2**	**3**	**4**	**5**	Soil group*	1
1	Depth (cm)	0-38	38-58	58-109	109-150+		Depth (cm) to SHWT (Chroma 2)	>150
	Texture	s	s	s	s		Depth (cm) to Chroma 3 (cm)	>150
	Structure	SG	SG	SG	SG		Depth (cm) to Concentrations	109
	Consistence	l	l	l	l		Depth (cm) to saturation today	132
	Soil color matrix	10YR 2/1	10YR 4/4	10YR 5/6	10YR 4/6		Depth (cm) to top of trench	30-50
	Soil color mottle	none	none	none	7.5YR 5/8 5%		Projected trench bottom depth (cm)	61-91
	Soil color mottle	none	none	none	10YR 5/6 5%		Projected separation to SHWT (cm)	59-89
							Elevation of Land Surface above Sea Level (cm)	168
							Elevation of Trench Bottom (cm)	107
							Measured SHWT: 1/30/2010-2/13/2012 (cm)	89
							Measured Separation to SHWT (cm)	18
2	Depth (cm)	0-23	23-56	56-91	91-125	125-150+	Soil group	1
	Texture	ls	s	s	sl	ls	Depth to SHWT (Chroma 2)	>150 cm
	Structure	SG	SG	SG	wf SBK	SG	Depth to Chroma 3	>150 cm
	Consistence	fr	fr	fr	fr	fr	Depth to Concentrations	91
	Soil color matrix	2.5Y 3/2	2.5Y 5/4	2.5Y 6/4	10YR 4/6	10YR 5/6	Depth to saturation today	>150
	Soil color mottle	none	none	none	none	2.5Y 7/3	Projected trench bottom depth (cm)	74
	Soil color mottle	none	none	2.5Y 6/6	none	30%	Depth to top of trench	43
							Projected separation to SHWT (cm)	76
							Elevation of Land Surface above Sea Level (cm)	392
							Elevation of Trench Bottom (cm)	318
							Measured SHWT: 2/4/2010-2/18/2010 (cm)	278
							Measured Separation to SHWT (cm)	40

Key									
S	Ls	sl	Wf	SBK	SG	ls	fr	SHWT	Soil Group
Sand	Loamy sand	Sandy loam	Weak, fine, subangular	Blocky	Single grained	Loose	Friable	Seasonal high water table	per (Title 15A NCAC 18A.1900)

Appendix B. Soil Test Report Conducted by North Carolina Department of Agricultural and Consumer Services Agronomic Division.



NCDA&CS Agronomic Division **Phone: (919)733-2655** **Web site: www.ncagr.gov/agronomi/** **Report No: 25162**

Soil Test Report

SERVING N.C. RESIDENTS FOR OVER 60 YEARS

Grower: **Keaton, Henry**
29112 Sussex St*
Greenville, NC 27834

Copies To:

Farm:

Beaufort County

Received: 01/22/2010 **Completed:** 02/26/2010 Links to Helpful Information

Agronomist Comments 4

Field Information		Applied Lime			Recommendations												
Sample No.	*Last Crop*	*Mo*	*Yr*	*T/A*	*Crop or Year*		*Lime*	*N*	*P₂O₅*	*K₂O*	*Mg*	*S*	*Cu*	*Zn*	*B*	*Mn*	*See Note*
A1					1st Crop:	Lawn	0	(12.5 lbs 8-0-24 or EQUIV PER 1000 SQ FT)				0			.0		4
					2nd Crop:							0			.0		

Test Results

Soil Class	*HM%*	*W/V*	*CEC*	*BS%*	*Ac*	*pH*	*P-I*	*K-I*	*Ca%*	*Mg%*	*Mn-I*	*Mn-AI(1)*	*Mn-AI(2)*	*Zn-I*	*Zn-AI*	*Cu-I*	*S-I*	*SS-I*	*NO_3-N*	*NH_4-N*	*Na*
MIN	0.18	1.38	1.9	84.0	0.3	6.9	61	13	56.0	24.0	12			31	31	47	17				0.3

Field Information		Applied Lime			Recommendations												
Sample No.	*Last Crop*	*Mo*	*Yr*	*T/A*	*Crop or Year*		*Lime*	*N*	*P₂O₅*	*K₂O*	*Mg*	*S*	*Cu*	*Zn*	*B*	*Mn*	*See Note*
H1					1st Crop:	Lawn	30M	(20 lbs 5-10-10 or EQUIV PER 1000 SQ FT)				0			.0		4
					2nd Crop:							0			.0		

Test Results

Soil Class	*HM%*	*W/V*	*CEC*	*BS%*	*Ac*	*pH*	*P-I*	*K-I*	*Ca%*	*Mg%*	*Mn-I*	*Mn-AI(1)*	*Mn-AI(2)*	*Zn-I*	*Zn-AI*	*Cu-I*	*S-I*	*SS-I*	*NO_3-N*	*NH_4-N*	*Na*
MIN	0.27	1.47	1.9	47.0	1.0	5.1	4	5	33.0	10.0	6			7	7	11	33				0.1

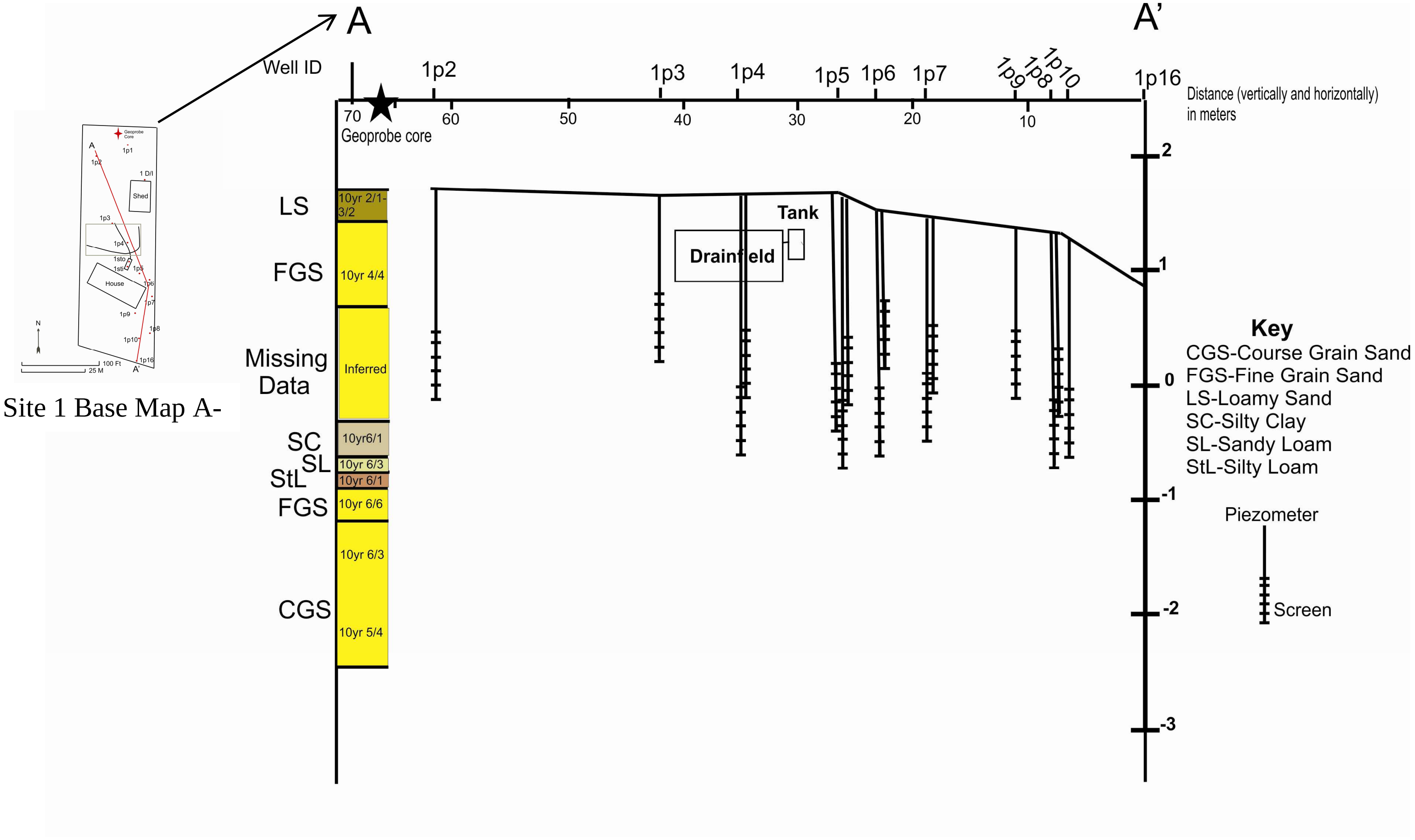
North Carolina
Tobacco Trust Fund Commission

Reprogramming of the laboratory-information-management system that makes this report possible is being funded through a grant from the North Carolina Tobacco Trust Fund Commission.

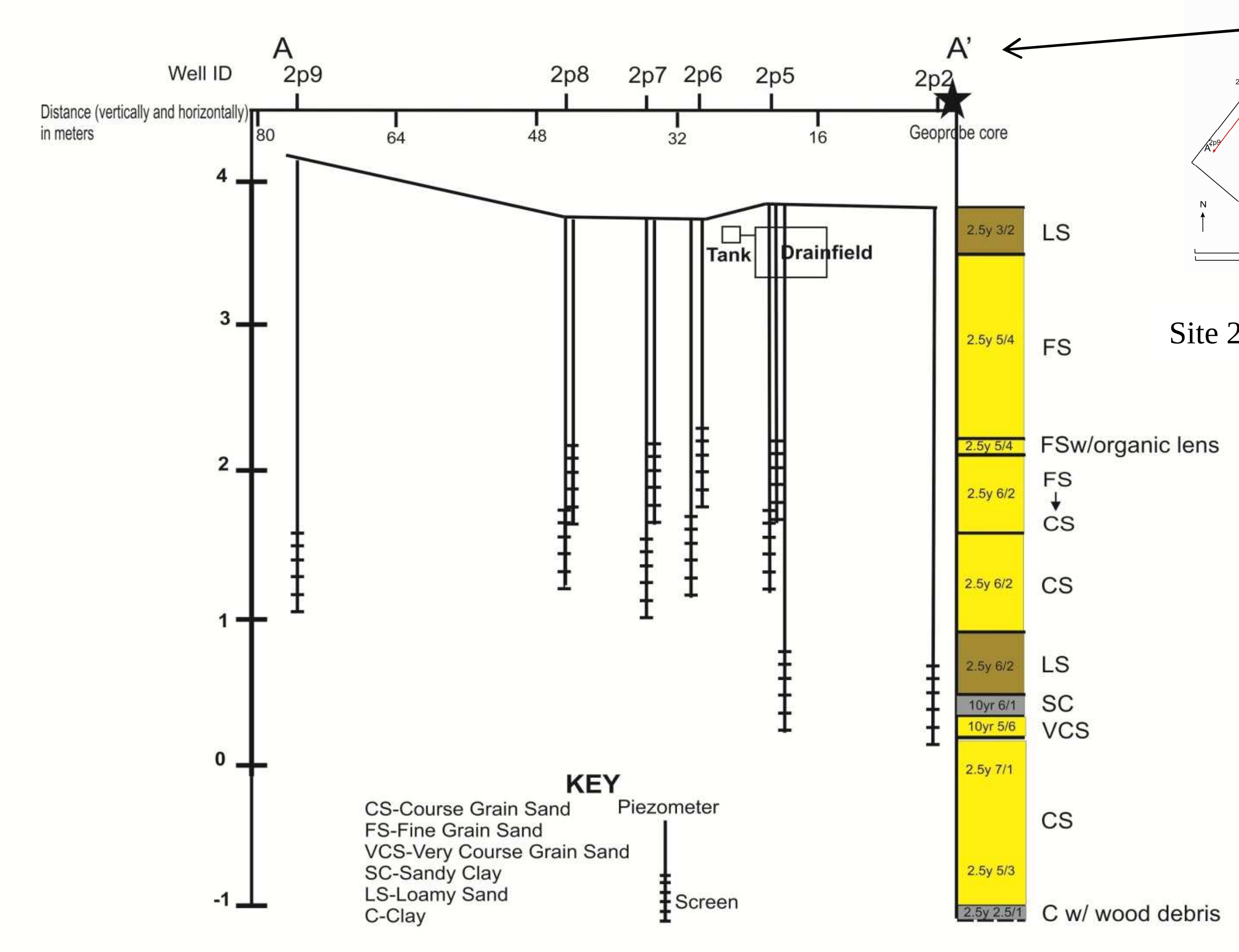
Thank you for using agronomic services to manage nutrients and safeguard environmental quality.
- Steve Troxler, Commissioner of Agriculture

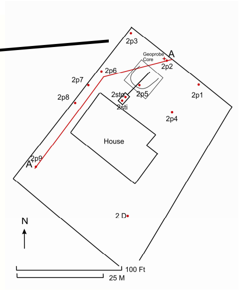
*Note. A-1 represents Site 1 and H-1 represents Site 2.

Appendix C. Soil profile of Site 1. (Prepared with assistance from Shawn Thieme).



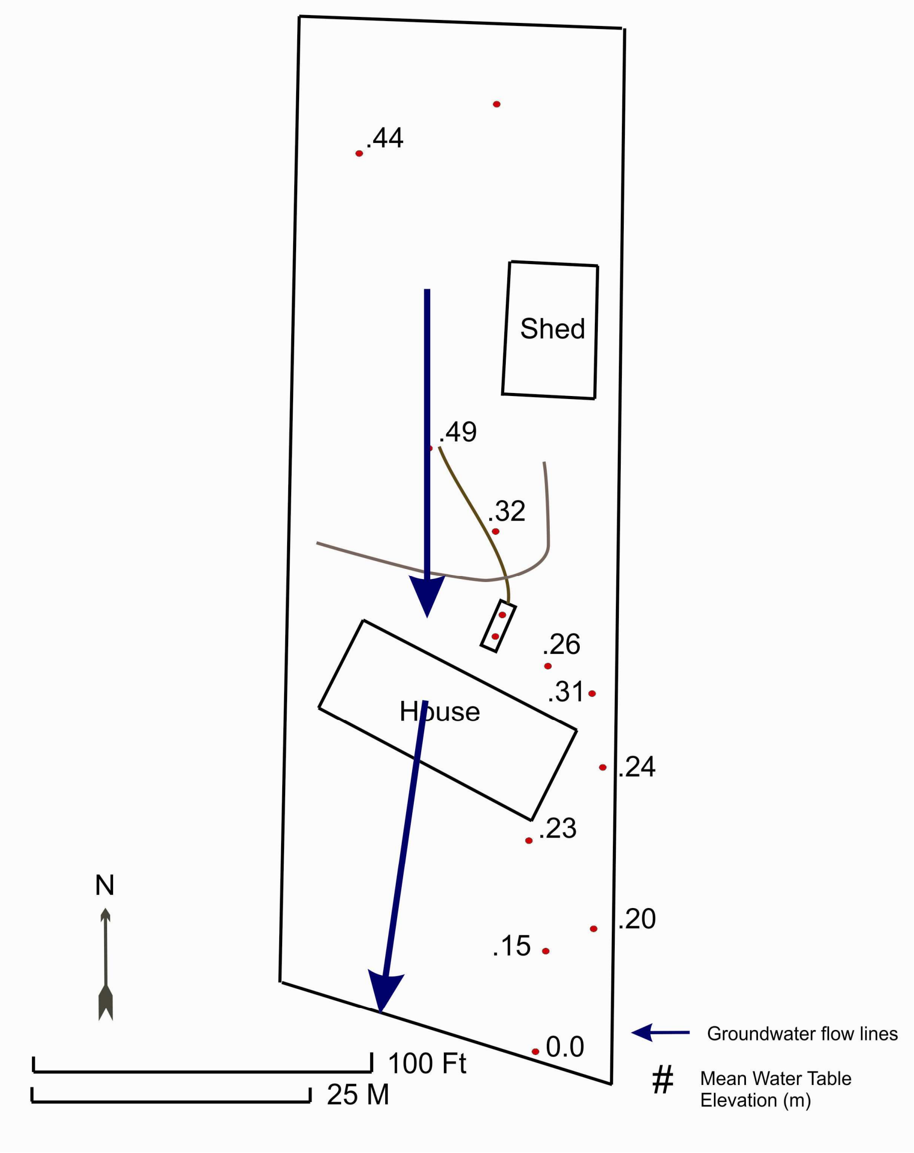
Appendix D. Soil profile of Site 2. (Prepared with assistance from Shawn Thieme).



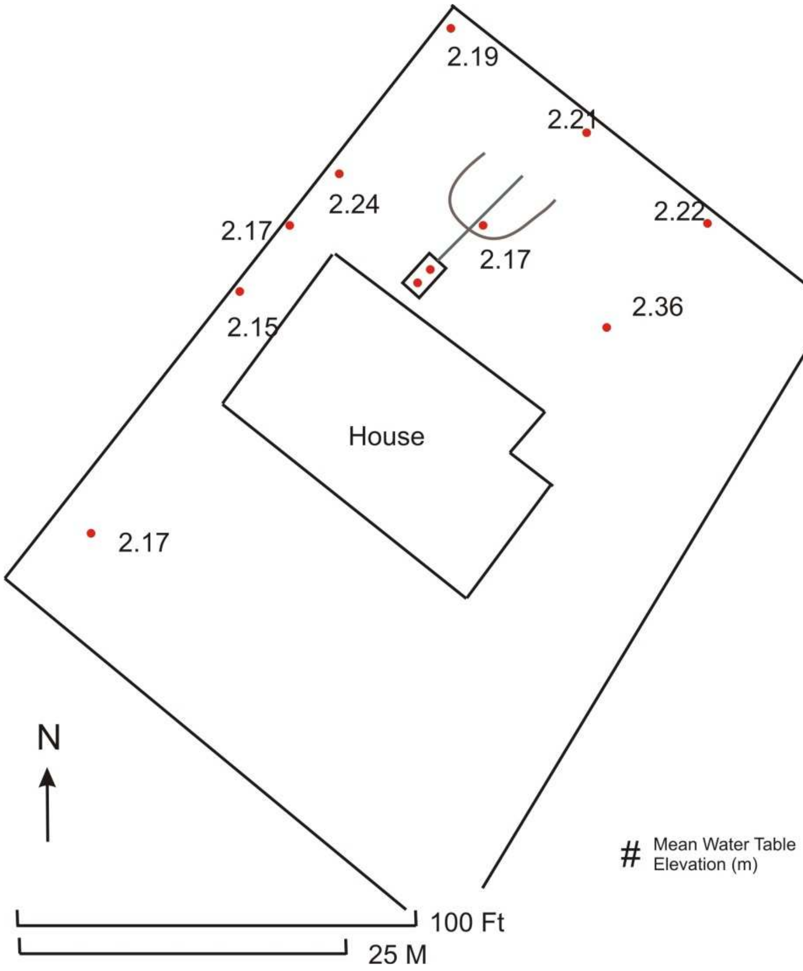


Site 2 Base Map A-

Appendix E. Groundwater Direction at Site 1



Appendix F Average Groundwater Direction (m) at Site 2.



Appendix G. Sampling dates and the total number of water/effluent samples taken from both sites.

Date	Drinking/Irrigation Samples	Tank Samples	Groundwater Piezometer Wells Samples	Total Samples
10/1/2009	2	4	0	6
11/16/2009	2	4	28	34
12/7/2009	2	4	0	6
1/25/2010	2	4	30	36
2/15/2010	2	4	0	6
3/15/2010	2	4	30	36
4/19/2010	2	4	0	6
5/24/2010	2	4	27	33

Appendix H. Site 1 Total Dissolved Nitrogen (TDN) for four sampling dates.

Well ID	11/16/2009	1/25/2010	3/15/2010	5/24/2010
1sto	56.0	93.1	95.5	94.6
1sti	56.3	92.0	89.7	94.7
1psonde2	0.9	0.4	0.3	1.2
1p4s	9.8	17.5	10.0	45.5
1p4d	9.6	39.0	43.4	14.9
1p5d	4.7	3.5	1.1	10.8
1psonde5	2.0	0.9	0.7	2.4
1p6s	3.0	2.1	1.8	
1p6d	5.8	0.8	0.7	1.3
1p7s	2.9	1.8	1.5	
1p7d	10.6	0.9	1.0	0.7
1p8s	2.4	2.2	1.4	2.3
1p8d	1.9	1.9	1.2	4.1
1p9	1.4	0.8	0.4	2.1
1p10	11.0	6.2	1.3	2.1
1p16	1.0	12.3	2.4	0.9

Appendix I. Site 2 Total Dissolved Nitrogen (TDN) for four sampling dates.

Well ID	**11/16/2009**	**1/25/2010**	**3/15/2010**	**5/24/2010**
2sto1	55.1	64.8	44.5	66.2
2sti1	52.8	66.2	44.1	65.0
2p1	7.5	1.2	1.1	8.8
2p2	7.2	10.5	9.4	10.0
2p3	1.8	0.4	0.3	2.9
2p4	7.0	1.8	1.6	24.0
2p5s	9.7	12.1	10.7	13.4
2psonde5	9.7	3.4	9.7	9.7
2p5d	9.3	1.6	9.6	8.4
2p6s	3.6	2.0	1.8	
2p6d	0.4	0.4	0.3	0.6
2p7s	6.0	1.2	0.6	16.6
2p7d	3.0	0.2	0.4	0.9
2p8s	3.3	1.6	1.6	1.2
2p8d	0.5	0.3	0.9	0.3
2p9	1.7	4.6	2.7	2.1

Appendix J. Instrument location for Site 1 and Site 2.

	Hobo Datalogger	YSI 6920 v2 Sonde
Site 1	1psonde2, 1p3, 1psonde5, 1p16, Atmospheric pressure logger	1psonde2, 1psonde5
Site 2	2p4, 2psonde5, 2p5s, 2p5d, 2p6d, 2p7d, 2p8s, 2p8d, 2p9	2p4, 2psonde5

Appendix K. Site 1 E.coli densities (cfu/100 mL)

Well ID	10/1/2009	11/16/2009	12/7/2009	1/25/2010	2/15/2010	3/15/2010	4/19/2010	5/24/2010
E. coli								
1 STI	N/D	48000	49950	20500	5250	9500	9000	28500
1 STO	58700	39500	63500	18500	6800	2350	4500	25000
1psonde2		5		1		5		25000
1psonde2 (rep)		5		1.3		2		11000
1p2		5		1.3		5		25000
1p4s		1		74		2.67		40
1p4d		0.5		110		21		133
1p4		1		110		21		133
1psonde5		220		2		0.25		79.5
1p5d		N/A		4		9.33		176000
1p5		220		4		9.3		176000
1p6s		460		100		4		N/S
1p6d		366		20		25		800
1p6		460		100		25		800
1p7s		20		1		1		N/S
1p7d		8		2		0.5		30
1p7		20		2		1		30
1p8s		1.5		0.65		2		31000
1p8d		3.33		0.65		2.5		64
1p8		3.3		0.7		2.5		31000
1p9		163.33		5		1		446
1p10		3.33		2		5		30
1p16		21.33		2		3.33		6
1 D/I W	0.5	0.25	0.215	0.25		0.25	0.25	0.25

Appendix L. Site 1 Enterococcus densities (cfu/100 mL)

Well ID	10/1/2009	11/16/2009	12/7/2009	1/25/2010	2/15/2010	3/15/2010	4/19/2010	5/24/2010
1 STI	280000	3500	2650	1650	1800	27000	3700	63500
1 STO	370000	2650	2870	2250	2900	31500	6850	67500
1psonde2		5		12		5		9200
1psonde2 (rep)		5		122.7		2		8800
1p2		5		122.7		5		9200
1p4s		6200		300		104		3600
1p4d		1.33		2		3		10800
1p4		6200		300		104		10800
1psonde5		5		0.5		1.5		760
1p5d		N/S		12		0.67		770000
1p5		5		12		1.5		770000
1p6s		180		340		8		N/S
1p6d		1400		10		1		59000
1p6		1400		340		8		59000
1p7s		40		36		1		N/S
1p7d		46		2800		0.5		51.5
1p7		46		2800		1		51.5
1p8s		1.65		13		2		41000
1p8d		1.5		4.7		165		82000
1p8		1.7		13		165		82000
1p9		16.7		220		1		13000000
1p10		1.65		80		5		61
1p16		23.33		10		6.67		6
1 D/I W	6	0.125	8	0.125		0.125	0.085	0.07

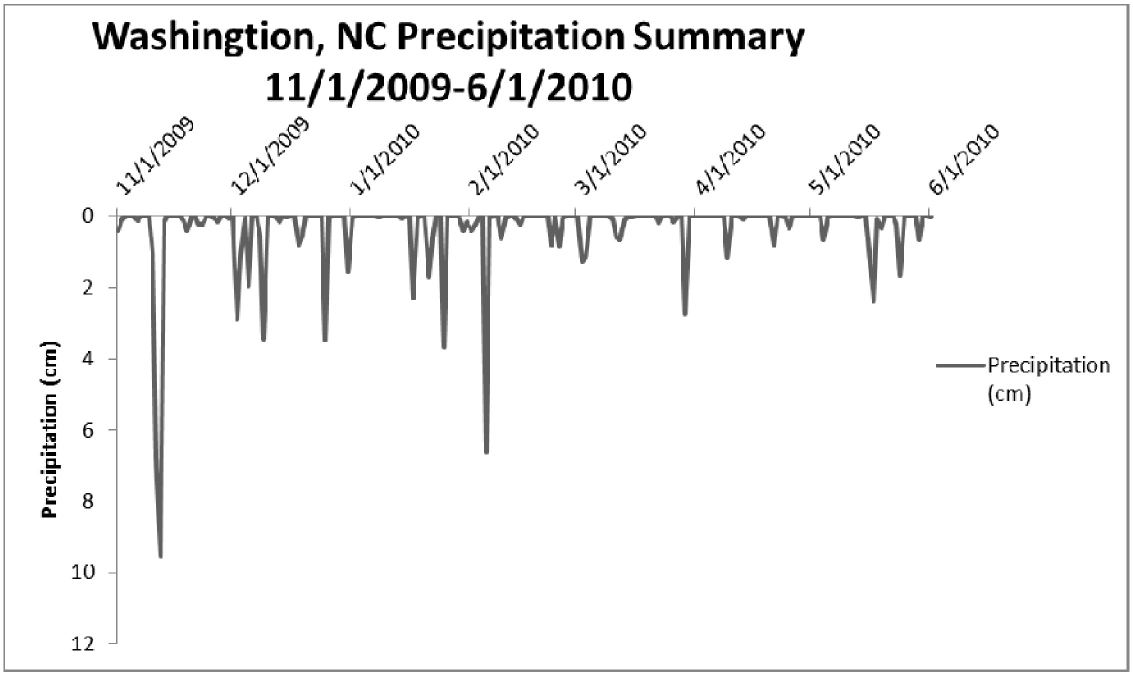
Appendix M. Site 2 E.coli densities (cfu/100 mL)

Well ID	**10/1/2009**	**11/16/2009**	**12/7/2009**	**1/25/2010**	**2/15/2010**	**3/15/2010**	**4/19/2010**	**5/24/2010**
2 STI	165000	21000	305000	46000	23000	280000	680000	160000
2 STO	610000	32500	165000	63000	14000	290000	545000	160000
2p1		100		80		1		7200
2p1 (rep)				2		0.5		465
2p1		100		80		1		7200
2p2		62		600		2		300
2p3		10		287		1		5
2psonde4		18		1300		1		130000
2p5s		300		1100		50		316
2p5d		220		1500		8		300
2psonde5		15000		600		105		4000
2p5		15000		1500		105		4000
2p6s		50		5600		50		
2p6d		20		160		12		112
2p6		50		5600		50		112
2p7s		50		5200		80		5300
2p7d		10		3800		8		200
2p7		50		5200		80		5300
2p8s		2900		15500		104		6300
2p8d		9000		6000		200		11800
2p8		9000		6000		200		11800
2P9		340		100		2.5		311000
2 D/I W	0.5	0.25	0.145			0.25	0.25	0.25

Appendix N. Site 2 Enterococcus Densities (cfu/100 mL)

Well ID	**10/1/2009**	**11/16/2009**	**12/7/2009**	**1/25/2010**	**2/15/2010**	**3/15/2010**	**4/19/2010**	**5/24/2010**
Enterococcus								
2 STI	80000	57000	46000	44500	3050000	515000	31500	63500
2 STO	112000	75500	47000	31000	3100000	735000	41500	140000
2p1		500		1400		2		17200
2p1 (rep)				520		10		18400
2p1		500		1400		10		18400
2p2		112		13200		0.5		90
2p3		10		343		1		5
2psonde4		122		900		1		203000
2p5s		2900		12000		150		400
2p5d		23600		3800		8		900
2psonde5		3000		1200		36		2000
2p5		23600		12000		150		2000
2p6s		50		6800		50		
2p6d		800		480		12		200
2p6		800		6800		50		200
2p7s		50		3600		32		100
2p7d		10		3100		8		2600
2p7		50		3600		32		2600
2p8s		840		15000		56		1700
2p8d		17000		30000		124		9300
2p8		17000		30000		124		9300
2P9		10		30		2		460000
2 D/I W	0.5	0.25	0.07			0.125	0.085	0.07

Appendix O. Monthly Rainfall Total for Washington, North Carolina. Precipitation was recorded at Warren Field and Tranters Creek stations (Climate Office of North Carolina, 2010)



	Nov-09	Dec-09	Jan-10	Feb-10	Mar-10	Apr-10	May-10
Precipitation (cm)	19.32	16.54	8.94	10.06	6.95	2.45	7.05

Appendix P. Site 1 conductivity (mS/cm) taken on various sampling days using the YSI 556 MPS meter.

Well ID	10/1/2009	11/16/2009	12/7/2009	1/25/2010	2/15/2010	3/15/2010	4/19/2010	5/24/2010
1sto	1.252	1.238	1.198	1.107	1.018	0.978	1.221	1.347
1sti	1.280	1.234	1.189	1.106	1.021	0.990	1.167	1.337
1 D/I W	0.063	0.073	0.058	0.068	0.059	0.061	0.070	0.081
1psonde2		0.095		0.064		0.055		0.028
1p4s		1.060		0.757		0.373		1.189
1p4d		1.027		0.948		0.732		0.737
1p5d		1.320		0.342		0.490		0.916
1psonde5		1.022		0.775		0.433		0.795
1p6s		0.030		0.289		0.192		
1p6d		1.100		0.449		0.292		0.552
1p7s		0.360		0.311		0.181		
1p7d		1.393		0.306		0.300		0.432
1p8s		1.053		0.342		0.249		0.289
1p8d		1.154		0.420		0.220		0.302
1p9		0.400		0.397		0.192		0.278
1p10		1.403		0.684		0.263		0.835
1p16		15.920		0.770		0.375		0.962

Appendix Q. Site 2 conductivity (mS/cm) taken on various sampling days using the YSI 556 MPS meter.

Well ID	10/1/2009	11/16/2009	12/7/2009	1/25/2010	2/15/2010	3/15/2010	4/19/2010	5/24/2010
2sto	1.038	0.914	0.930	0.985	0.888	0.922	1.020	1.013
2sti	1.040	0.918	0.936	1.000	0.867	0.920	1.013	1.000
2 D/I W		0.361	0.330			0.263	0.339	0.358
2p1		0.589		0.077		0.048		0.124
2p2		0.237		0.493		0.458		0.355
2p3		0.119		0.104		0.067		0.121
2p4		0.113		0.288		0.065		0.319
2p5s		1.010		1.054		0.548		0.473
2psonde5		0.681		0.821		0.562		0.841
2p5d		0.779		0.556		0.457		0.343
2p6s		0.057		0.079		0.177		
2p6d		0.057		0.079		0.056		0.098
2p7s		0.161		0.111		0.081		0.145
2p7d		0.131		0.088		0.055		0.202
2p8s		0.277		0.176		0.112		0.325
2p8d		0.385		0.194		0.122		0.369
2p9		0.500		0.523		0.283		0.320

Appendix R. Pooled E.coli and Enterococcus Mann-Whitney results

Significant Differences: Pooled *E.coli*			**Testing parameters**	**Legend**
$p\leq0.05$	$p\leq0.10$	$p\geq0.10$		
Tank≠All sampling points GW<15 m ≠GW>15 m DF≠BG w/o outlier		DF≠BG	Tank≠ All sampling points DF≠BG DF≠BG w/o O GW< 15 m ≠GW >15 m	Tank=Septic Tank Effluent DF=Groundwater beneath drainfield BG=Background groundwater BG w/o O=Background groundwater without outlier GW<15 m= Groundwater within 15 meters of OWTS GW>15 m= Groundwater greater than 15 meters of OWTS Est GW=Estuary groundwater D/IW=Drinking/Irrigation water

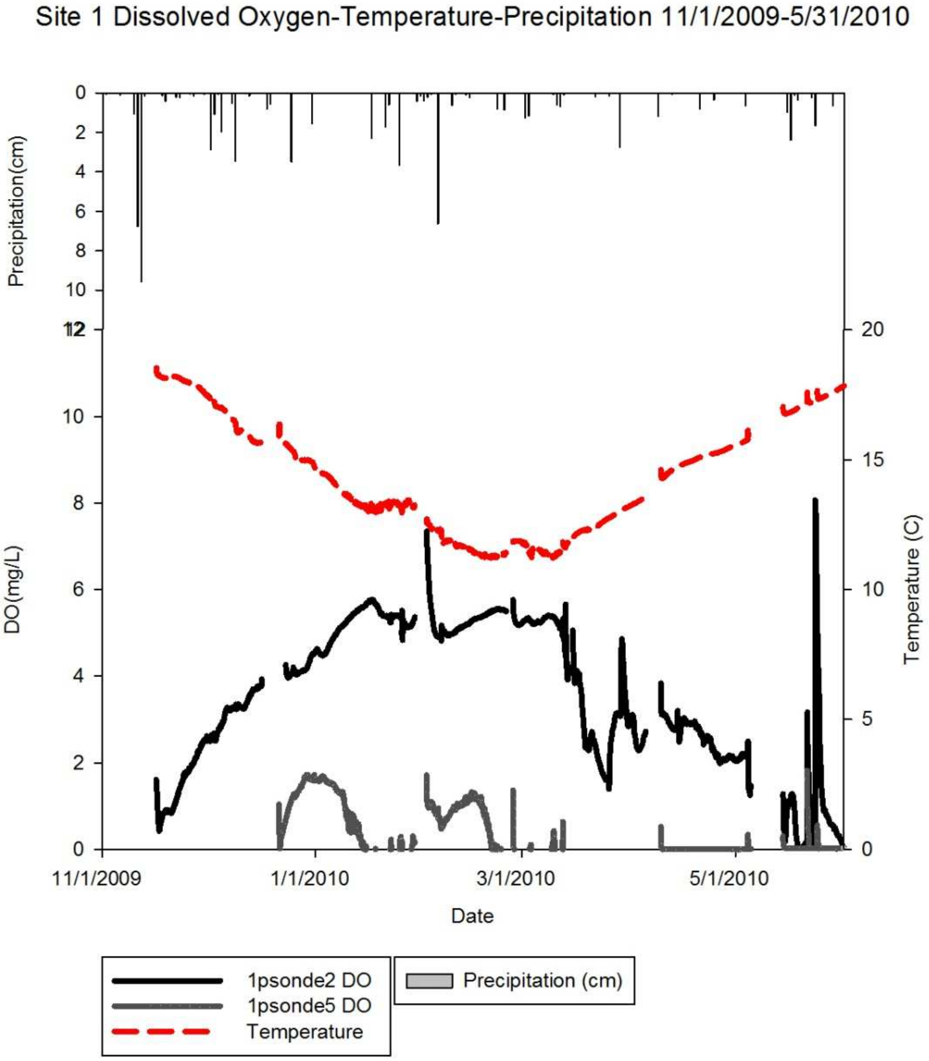
Significant Differences: Pooled *Enterococcus*			**Testing parameters**	**Legend**
$p\leq0.05$	$p\leq0.10$	$p\geq0.10$		
Tank≠All sampling points DF≠BG BG≠D/I W DF≠BG w/o outlier		GW<15 m ≠GW>15 m	Tank≠ All sampling points DF≠BG GW< 15 m ≠GW >15 m BG≠ I/D W DF≠BG w/o O	Tank=Septic Tank Effluent DF=Groundwater beneath drainfield BG=Background groundwater BG w/o O=Background groundwater without outlier GW<15 m= Groundwater within 15 meters of OWTS GW>15 m= Groundwater greater than 15 meters of OWTS Est GW=Estuary groundwater D/IW=Drinking/Irrigation water

Appendix S. Constituent mass loadings and concentrations in typical residential wastewater. Adapted from (US EPA, 2002).

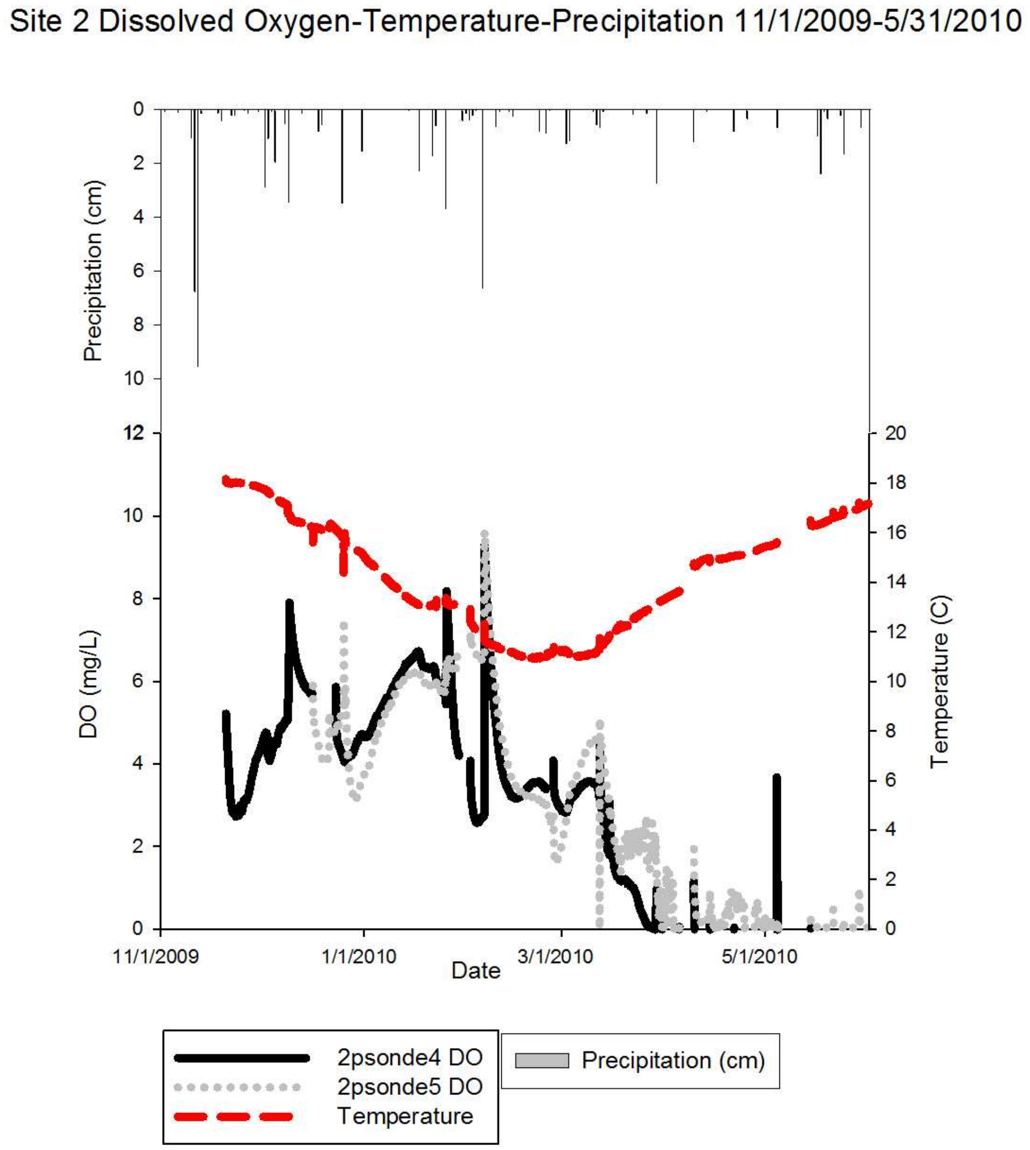
Constituent mass loadings and concentrations in typical residential wastewater

Constituents	Mass Loading (grams/person/day)	Concentration (mg/L)
Total Solids	115-200	500-880
Volatile Solids	65-85	280-375
Total Suspended solids	35-75	155-330
Volatile suspended solids	25-60	110-265
5-day biochemical oxygen demand (BOD_5)	35-65	155-286
Chemical Oxygen Demand (COD)	115-150	550-660
Total Nitrogen (TN)	6-17	26-75
Ammonia (NH_4)	1-3	4-13
Nitrites and Nitrates (NO_2-N; NO_3-N)	<1	<1
Total Phosphorus	1-2	6-12
Fats, oils and grease	12-18	70-105
Volatile organic compounds (VOC)	0.02-0.07	0.1-0.3
Surfactants	2-4	9-18
Total Coliform (TC)		10^8-10^{10}
Fecal Coliform (FC)		10^6-10^8

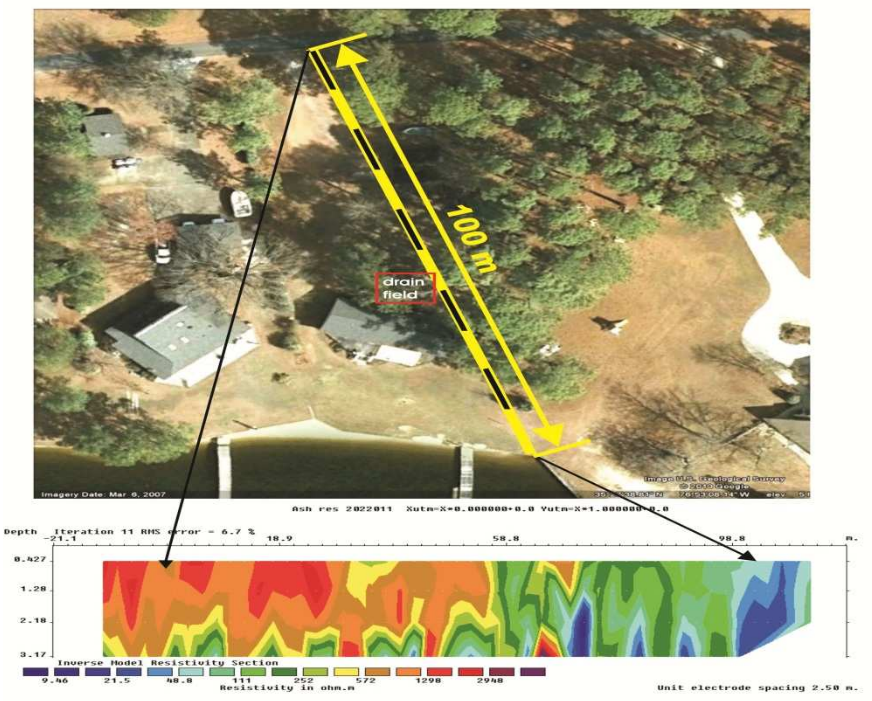
Appendix T. Site 1 Dissolved Oxygen and Temperature recorded from YSI 6920 v2 sonde. Comparison of DO of 1psonde2 (BG) and 1psonde5 (DF) in relation to temperature and precipitation.



Appendix U. Site 2 Dissolved Oxygen and Temperature recorded from YSI 6920 v2 sonde. Comparison of DO of 2p4 and 2psonde5 (DF) in relation to temperature and precipitation.



Appendix V. Electrical resistivity survey conducted at Site 1 on 2/2/2011. Courtesy of O'Driscoll et al., 2012



An initial electrical resistivity survey (OhmMapper, Geometrics, Inc.) was conducted at each site on September 2009 (Humphrey et al., 2010). During February 2011, a two-dimensional file was collected, spanning from the edge of the road to the Pamlico River. Resistivity declined approximately 50 meters from the road, in the vicinity of the drainfield. Low resistivity data after 50 meters from the road suggests that wastewater affects the conductivity of the groundwater and that the wastewater plume extends to the estuary.

Appendix W. Site 1 and Site 2 E.coli Mann-Whitney results.

Significant Differences: Site 1 *E.coli*			**Testing parameters**	**Legend**
p≤0.05	p≤0.10	p≥0.10		
Tank≠All sampling points Except for BG GW<15 m ≠GW>15 m	Tank ≠ BG	DF≠BG DF≠BG w/o outlier	Tank≠ All sampling points DF≠BG DF≠BG w/o O GW< 15 m ≠GW >15 m	Tank=Septic Tank Effluent DF=Groundwater beneath drainfield BG=Background well groundwater BG w/o O=Background without outlier GW<15 m= Groundwater within 15 meters of OWTS GW>15 m= Groundwater greater than 15 m of OWTS Est GW=Estuary groundwater D/IW=Drinking/Irrigation water

Significant Differences: Site 2 *E.coli*			**Testing parameters**	**Legend**
p≤0.05	p≤0.10	p≥0.10		
Tank≠All sampling points	DF≠BG	GW<15 m ≠ GW>15 m DF≠GW>15 m DF≠GW<15 m	Tank≠ All sampling points DF≠BG GW< 15 m ≠GW >15 m DF≠GW>15 m DF≠GW<15 m	Tank=Septic Tank Effluent DF=Groundwater beneath drainfield BG=Background well groundwater GW<15 m= Groundwater within 15 meters of OWTS GW>15 m= Groundwater greater than 15 m of OWTS D/IW=Drinking/Irrigation water

Appendix X. Site 1 and Site 2 Enterococcus Mann-Whitney results.

Significant Differences: Site 1 *Enterococcus*			Testing parameters	Legend
p≤0.05	p≤0.10	p≥0.10		
Tank≠All sampling points	DF≠BG	GW<15 m ≠ GW>15	Tank≠ All sampling points DF≠BG GW< 15 m ≠GW >15 m	Tank=Septic Tank Effluent DF=Groundwater beneath drainfield BG=Background well groundwater GW<15 m= Groundwater within 15 meters of OWTS GW>15 m= Groundwater greater than 15 m of OWTS D/IW=Drinking/Irrigation water

Significant Differences: Site 2 *Enterococcus*			Testing parameters	Legend
p≤0.05	p≤0.10	p≥0.10		
Tank≠All sampling points	DF≠BG	GW<15 m ≠GW>15 m DF≠GW>15 m DF≠GW<15 m	Tank≠All sampling points DF≠BG GW< 15 m ≠GW >15 m DF≠GW>15 m DF≠GW<15 m	Tank=Septic Tank Effluent DF=Groundwater beneath drainfield BG=Background well groundwater GW<15 m= Groundwater within 15 meters of OWTS GW>15 m= Groundwater greater than 15 m of OWTS D/I W=Drinking/Irrigation water

Appendix Y. Site 1 Chloride (mg/L) taken on sampling days.

Well ID	**10/1/2009**	**11/16/2009**	**12/7/2009**	**1/25/2010**	**2/15/2010**	**3/15/2010**	**4/19/2010**	**5/24/2010**
1sto	77	87		88	74	74	81	78
1sti	78	87		89	72	73	81	80
1sti (rep)							80	
1 D/I W	11	14	12	16	9	12	13	12
1 D/I W (rep)	11		12				13	
1psonde2		16		12		11		2
1psonde2 (rep)				12		10		2
1p4s		95		43		42		70
1p4d		92		60		62		45
1p5d		142		43		43		61
1psonde5		114		46		26		44
1p6s		0		39		23		
1p6d		93		34		19		33
1p7s		81		70		39		
1p7d		120		40		22		42
1p8s		247		55		43		44
1p8d		201		78		43		32
1p9		57		47		25		28
1p10		134		109		22		57
1p16		565		475		99		122

Appendix Z. Site 2 Chloride (mg/L) taken on sampling days.

Well ID	**10/1/2009**	**11/16/2009**	**12/7/2009**	**1/25/2010**	**2/15/2010**	**3/15/2010**	**4/19/2010**	**5/24/2010**
2sto	41		55	57	60	58	57	52
2sto (rep)							58	
2sti	40		57	58	57	57	56	50
2sti (rep)							55	
2 D/I Water	7	15	7			7	7	8
2 D/I Water (rep)	8	15	7				9	
2p1		15		8		8		10
2p1 (rep)				7		8		10
2p2		19		14		42		30
2p3		26		15		12		16
2p4		19		5		7		15
2p5s		56		20		45		27
2psonde5		39		6		39		62
2p5d		33		2		35		19
2p6s		45		8		15		
2p6d		21		11		10		14
2p7s		22		15		7		13
2p7d		16		4		7		25
2p8s		13		22		15		13
2p8d		8		3		10		8
2p9		32		85		45		24