Stream channel properties and their effect on the release of *Escherichia coli* from bottom sediment

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Abstract

Escherichia coli (E. coli) is the standard fecal indicator bacteria used to monitor water quality in rivers, particularly around recreational areas. Recent research indicates that while agricultural and urban wastewater runoff remains the dominant source of *E. coli* to rivers, there is growing evidence to suggest that the disturbance-induced resuspension of *E. coli* from bottom sediments contributes significantly to microbial contaminant levels in the water column. This research was focused on the question, "How does the resuspension of *E. coli* from bottom sediments contribute to the overall microbiological impairment of the Rivanna River and its tributaries?" Sediment cores were collected from 14 sites throughout the Rivanna watershed in Central Virginia and analyzed for their *E. coli* concentration, organic matter content, and particle size. Linear regressions were used to identify any significant relationship between *E. coli* concentrations in the water column, derived from existing data gathered by the Rivanna Conservation Alliance between 2018 and 2022, and sediment *E. coli* concentrations. Relationships between stream channel properties and subwatershed land use with the observed water column and sediment *E. coli* concentrations were also explored. A significant finding of this study was the relationship between sediment *E. coli* concentrations and water column concentrations at a turbidity level of 5 NTU ($p=0.025$), indicating a potential link between *E. coli* supply in sediment and *E. coli* transport during periods of high flow. Sediment *E. coli* concentrations were not significantly related to any of the factors considered, although it was concluded that texture is more predictive of microbial levels than subwatershed land use practices. This study offers insight into the physical mechanisms affecting the persistence and prevalence of *E. coli* in recreational areas, thereby improving public health initiatives aimed at identifying sources of water quality impairment.

Acknowledgments

First and foremost, I wish to express my deepest gratitude to my thesis advisors, Todd Scanlon and Aaron Mills. Their generosity, patience, and invaluable guidance made this research possible. As a student with a background in social sciences, their mentorship not only enabled me to navigate the complexities of scientific research but also instilled in me a newfound passion for microbiology. The lessons and insights they imparted will continue to inspire my interest in this field indefinitely.

Secondly, I would like to thank the additional collaborators who supported this project. Meg Miller, your generosity in providing access to your lab space for processing is sincerely appreciated. Thank you to the Rivanna Conservation Alliance (RCA), specifically Lisa Wittenborn and Claire Sanderson. Their support went beyond providing access to materials and space; they taught me crucial lessons on the importance of safeguarding our local watershed. Through their dedication to the Rivanna, I gained insight into the broader implications of my research.

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Table of Contents

1: Introduction

Research surrounding water quality and the presence of pathogenic microbes in water sources has been stimulated by the widespread occurrence of fecal contamination in water bodies across the globe (Peterson & Hubbart, 2020). According to the World Health Organization, microbiologically contaminated water is estimated to cause some 1 million diarrheal deaths each year, making it a leading cause of death in the developing world (WHO, 2023). Within the United States, waterborne pathogens are largely a recreational public health concern as rivers and lakes are utilized for recreational purposes throughout the year. The CDC approximates that, annually, 1 in 44 individuals in the United States falls ill due to waterborne diseases, either by ingesting, inhaling, or coming into contact with contaminated water on their skin, eyes, ears, or other mucous membranes (CDC, 2023).

The Rivanna River, located in central Virginia, serves as both a predominant drinking water source and as a recreational area for the City of Charlottesville as well as Albemarle and Fluvanna counties. The federal Clean Water Act of 1972 and the Virginia State Water Control Act mandate timely water quality testing to protect the public users of the water body in the event of high levels of bacteria (“Bacteria monitoring”, 2023). Microbiological contamination of drinking, irrigation, and recreational water is commonly monitored using fecal indicator bacteria (FIB) (Cho et al., 2010). When evaluating microbiological water quality in recreational bodies, *Escherichia coli* (*E. coli*) is the standard FIB used - as mandated by the EPA - because it is a strong indicator of fecal waste and suggests other pathogenic organisms may also be present (Herrig et al., 2015).

In 2021, the Virginia Department of Environmental Quality set the water quality standard for *E. coli* in recreational areas at 410 colony forming units (CFU) per 100mL. The Virginia

Department of Health has historically recommended 235 CFU per 100mL for the threshold at which a person's risk exposure to pathogenic organisms increases (VADEQ, 2023). The Rivanna Conservation Alliance (RCA) - a nonprofit organization located in Charlottesville, Virginia - established a volunteer-based bacteria monitoring program in 2018 to keep a detailed record of *E. coli* concentrations at locations within the Rivanna River Watershed. The Virginia DEQ has certified RCA's bacteria monitoring program at level III - the highest level attainable in Virginia - indicating that RCA's collected data are of the same quality as that of the agency itself. In 2021, 11 out of the 19 sites within the Rivanna River Watershed failed to meet the DEQ's recreational water quality standard ("Bacteria monitoring", 2023). Using the Most Probable Number (MPN) method to count the levels of *E. coli* in water samples, eight sites failed to meet the standard because they had two or more samples above 410 MPN per 100mL within 90 days of each other. The other three sites failed to meet the standard because greater than 10% of samples exceeded 410 MPN per 100mL in a 90-day period. The overall impairment of the Rivanna River Watershed underscores the urgent need for a better understanding of the sources and proliferation of bacterial contaminants.

The most dominant source of *E. coli* to rivers is agricultural and urban wastewater, originating from either point or diffuse sources such as sewer overflows, damaged sewer pipes, or manure runoff from cropland and pastures (Herrig et al., 2015). The RCA has historically only monitored *E. coli* within the water column, though it has been amply demonstrated that freshwater streambed sediments can harbor substantial amounts of these indicator organisms (Cho et al., 2010; Shelton et al., 2014). Without accounting for the resuspension of *E. coli* from bottom sediments, previous studies have failed to explain the bacterial flux observed in the rising limb of storm hydrographs (Shelton et al., 2014). However, it's important to note that dynamic

fluctuations in *E. coli* concentrations are influenced by more than just heavy rainfall events (Chen & Chang, 2014). Shelton et al (2014) asserts that sediment microbiological reservoirs can also affect water quality during less intense interactions between the sediment and water column during low flow periods.

Minimal research has been conducted on the magnitude and sources of variability in the release of *E. coli* from streambeds within a mixed-use watershed such as those in the Charlottesville/Albemarle area. Moreover, on a broader scale, there are numerous knowledge gaps concerning the factors that influence resuspension of bacteria stored in sediment. Advancing the understanding of the presence of *E. coli* in freshwater sediments will, therefore, better inform policy makers and aid in reducing the health consequences linked to unsafe water conditions. By better characterizing the physical mechanisms that control *E. coli* concentrations in streams and rivers, predictive models can be developed to better assess the origination of bacterial hotspots in widely utilized recreational areas such as those found throughout the Rivanna River Watershed.

2: Prior Research

Fecal contamination from humans and animals, along with a variety of contaminants such as metals, a variety of halogenated hydrocarbons, pesticides, and various other organics, can enter water bodies through storm runoff, especially in industrial or urban areas (Makepeace et al., 1995; Eriksson et al., 2005; Baun et al., 2006; Huber et al., 2016). Bacteria, including those of fecal origin, can reach the sediment in large numbers as particulate matter settles into the bed (Liao, et al., 2015). The large numbers of fecal bacteria surviving in sediment motivates a question of the importance of the stream sediment as a source of bacterial contamination to the water column, especially during storms that induce resuspension of sediment and attached

bacteria.

Various meteorological, hydrologic, physiochemical, and land use variables influence the persistence of fecal indicator bacteria (Herrig et al., 2015). In a stretch of the lower Lahn River, Germany, NH_4^+-N concentrations, turbidity, and shortwave solar irradiance were identified as the three most predictive variables for bacterial indicators (Herrig et al., 2015). Rainfall amounts, pH, chlorophyll a, and oxygen content were supplementary indicators that, when used to optimize regression models developed from a 12-month monitoring period, could explain 69% of the observed spatial variance in *E. coli* concentrations (Herrig et al., 2015). Stream sediment characteristics behave as additional predictor variables as they provide conditions that are relevant for *E. coli* survival. *E. coli* can adhere to both inorganic and organic substances within the sediment through either physical, electrostatic, or chemical binding. *E. coli* thrive off the increased nutrient and organic matter content, optimal light exposure, and protection from abiotic and biotic stressors such as protozoa that is provided by the sediment (Peterson & Hubbart, 2020).

Shelton et al. (2014) examined how coliform populations in streambed sediment respond to variations in water nutrient concentrations, aiming to enhance understanding of how nutrient-rich sediment foster bacterial growth and colonization. They observed a half-order magnitude increase in total coliform concentrations in sandy sediment across all three nutrient level spikes (1x, 0.5x, 0.1x). In contrast, there were no significant changes observed in total coliform levels in the water column at lower nutrient concentrations. This finding confirms the anticipated benefits of phosphorus, carbon, organic nitrogen, ammonium, and nitrate.

Prior research indicates that survival of *E. coli* ranges from days to weeks in the water column but can be from weeks to months in the sediment, leading to higher cell concentrations

being found within the sediment, as opposed to the water column (Garzio-Hadzick et al., 2010; USDA, 2011). Van Donsel and Geldreich (1971) found fecal coliform concentrations were up to 1000 times greater in the sediment than in the water column. Sediment disturbances such as recreational activity, heavy rain events, animal crossings, and commercial activity contribute to the resuspension of particulate matter and *E. coli* into the water column (Salamet et al., 2021), with 98% of the annual *E. coli* load associated with heavy rain events (McKergow & Davies‐Colley, 2010). Concentrations within the water column can increase one to two orders of magnitude due to resuspension following a sediment disturbance (Abia et al., 2017; Pandey & Soupir, 2014). Accurate predictions of *E. coli* levels within riverine systems should include resuspension following heavy rain events, making turbidity a highly predictive variable in this regard.

Matson et al. (1978) were the first to study sediment and water column interactions. Subsequently, multiple studies have replicated their methods for quantifying the proportion of bacteria released from the sediment. In the majority of these studies, the number of bacteria released from the sediment is quantified by the number of bacteria in the sediment multiplied by the mass of resuspended sediment. Muirhead et al. (2004) conducted artificial high flow events and restricted point sources of fecal contamination to isolate resuspension as a source. These events caused a two-order of magnitude increase in *E. coli* concentrations with no substantial input from land sources (Muirhead et al., 2004). It is common for bacterial resuspension to be mistaken as new contamination, which may explain why land surface runoff has often been implicated as the source of bacterial contamination during storm events.

Prior studies have modeled E. coli release and transport using a single set of parameters for the whole stream or reservoir. Cho et al. (2010) proposed that modeling streambed *E. coli*

resuspension with reach-specific parameters can provide substantially better accuracy, as sediment is subject to continual transformation downstream due to the complex interplay of erosion, deposition, other geomorphological processes, as well as subwatershed land use practices. Cho et al. (2010) analyzed bacterial resuspension in stream reaches with significantly different bottom textures, defined by their particle size. Bacteria are generally associated with fine-grained particles; *E. coli* concentrations per unit mass of clay and silt particles are on average two to six times higher than *E. coli* concentrations per unit of total sediment (Cho et al., 2010; USDA, 2011). Peterson and Hubbart (2020) used hydrophilic, nylon net filters to yield concentrations of *E. coli* in different soil particle size classifications and found that more than 90% of *E. coli* was in <5 μm size class across all four sampling locations. Finer particles both protect bacteria from predators and have a greater attachment capacity as a result of the larger surface area per unit mass (Wu et al., 2019). However, Cinotto (2005) reported that larger particles, between 125 μm and 500 μm, contained the highest median concentration of *E. coli* - suggesting that the competitive advantage of fine-grained particles is not always universal.

It is understood that subwatershed land use practices within stream reaches influence both the composition of sediment as well as the presence of FIB. Several papers have studied the prevalence of *E. coli* in isolated single-use land areas, though reports on highly mixed-use areas remain sparse. Using a georeferenced collection of *E. coli* isolates from almost 1,500 soil samples across a semi-rural landscape, Dusek et al (2018) found an elevated presence and abundance of *E. coli* in wooded areas and pastures relative to cropland. The per sample probability of *E. coli* isolation from surface soil in croplands was almost 20-fold lower than in forested areas (Dusek et al., 2018). Moreover, the proximity of a sample to impervious surfaces was a stronger indicator of *E. coli* presence compared to physical and biological soil attributes

like moisture, temperature, and pH. The impact of land use practices on the prevalence of *E. coli* in soils and adjacent waterways is evident.

Sampling in a manner that segments a stream into multiple reaches also works to address spatial variability. Byappanahalli et al. (2010) found no notable difference in *E. coli* concentrations in sediment between areas close to the stream bank and those in the middle of the stream. However, concentrations did vary significantly between site locations upstream and downstream. Other findings suggest that it is not unusual for two samples taken at the same site or within the same watershed to be two to five orders of magnitude different between maximum and minimum concentrations (Berry et al., 2007), highlighting the high degree of spatial variability that can sometimes exist. High concentrations in localized areas are referred to as hot spots, and they are frequently missed when using grab sampling techniques (Cho et al., 2010). Therefore, to obtain accurate measures of *E. coli* contamination at a site, multiple samples are required to account for the spatial variation in *E. coli* levels in bottom sediment.

Within-stream variability can partially be attributed to the processes and factors affecting the solar inactivation of *E. coli.* Whitman et al (2004) studied the diurnal patterns of *E. coli* in recreational freshwater and found cell concentrations in water decline exponentially as solar insolation increases throughout the day, with a mean decrease of over 900 CFU per 100mL during the first sampling hour. Because it causes photobiological damage to *E. coli,* solar radiation was the most influential factor affecting *E. coli* counts in their study. Water temperature was the second most predictive parameter. Hence, any obstruction of light caused by the stream's banks, such as tree cover resulting in shaded areas, or variations in water temperature due to solar exposure, may result in differences in *E. coli* concentrations across different sections of the stream.

This research was focused on the question, "How does the resuspension of *E. coli* from bottom sediments contribute to the overall microbiological impairment of the Rivanna River and its tributaries?" The objectives of this work are: i) to examine the correlation between *E. coli* concentrations in the water column and those in the bottom sediment; ii) to determine the relationship between the concentrations of *E. coli* in the sediment and in the water column and various sediment and stream channel properties such as particle size, nutrient composition, and exposure to light; iii) to investigate the impact of land use on the spatial variation of *E. coli* sediment levels and water column concentrations within the Rivanna River and its tributaries. Meeting these objectives will result in improved predictions and understanding of hotspot regions containing *E. coli* and other fecal bacteria within the Rivanna River Watershed. This information can then establish a foundation for future analyses and public health initiatives dedicated to safeguarding individuals from exposure to harmful levels of pathogens.

3: Methods

3.1 Site Description and Existing Data

The study area is located throughout the Rivanna River Watershed in Central Virginia, USA, spanning approximately 1984 square kilometers. The Rivanna River is a 67.8-km long tributary of the James River, and a part of the greater Chesapeake Bay Watershed. Preliminary research using the RCA's 2018 to 2022 Bacterial Monitoring Program data was carried out to first analyze the relationship between *E. coli* levels in the water column and turbidity and to determine which RCA sites were to be used for the remainder of the study. From March to November, water samples are collected and analyzed monthly at each of their 19 identified locations. From Memorial Day to Labor Day, additional samples are collected weekly at three sites with high recreational use, including Darden Towe and Riverview parks and Pollocks

Branch in Jordan Park. Regression models of *E. coli* versus turbidity were created through MATLAB, with variables log-transformed to realize a more normal distribution and stabilize the variance. The slope of the regressed relationships (*b*) was recorded as a measure of *E. coli* enrichment (enrichment factor) with respect to turbidity, and the *E. coli* concentration at a turbidity level of 5 NTU (Nephelometric Turbidity Unit) (C_5) were determined to normalize for the effects of turbidity (Figure 1). Due to inconsistencies in the timing of when the sites were sampled, these metrics were selected to reduce the impact of sampling bias in the water column *E. coli* concentrations. This approach contrasts with merely averaging turbidity and *E. coli* measurements across the 2018 to 2022 sampling period. Variable numbers of paired measurements of *E. coli* (MPN/100mL) and turbidity were collected at each site (Appendix A). While some sites amassed over 100 paired measurements during the span of four years, others had fewer than 5 due to the inconsistency of turbidity readings. Consequently, a threshold of 40 paired measurements was chosen to exclude sites with insufficient data on the relationship between *E. coli* and turbidity, while ensuring a sufficient number of sites for the remainder of the study. The 14 sites that met this threshold were included in this study (Figure 2).

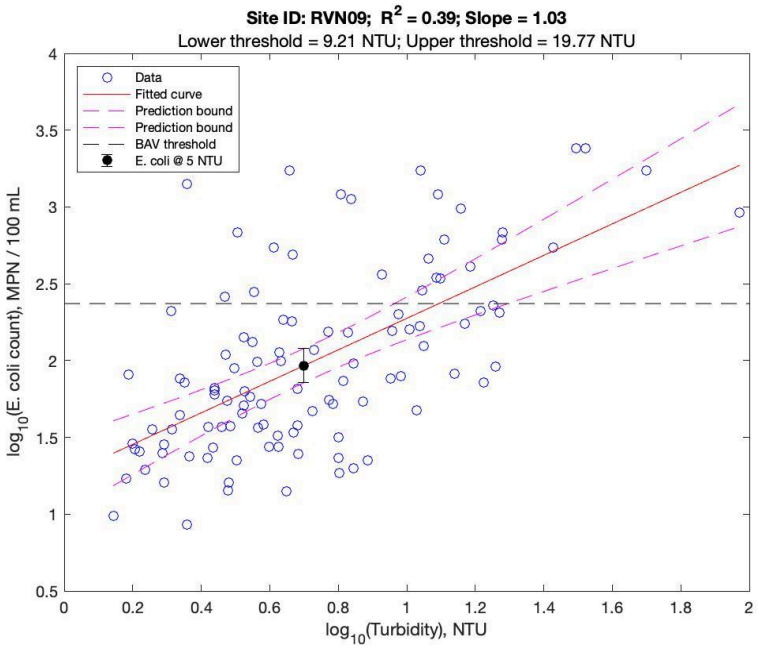

Figure 1: *Scatter plot indicating how to calculate the enrichment factor (b) and C_5 concentration for site RVN09. Enrichment factor is calculated by determining the slope of the line of best fit. C_5 concentration is at the point $log_{10}(5)$, denoted by the black dot.*

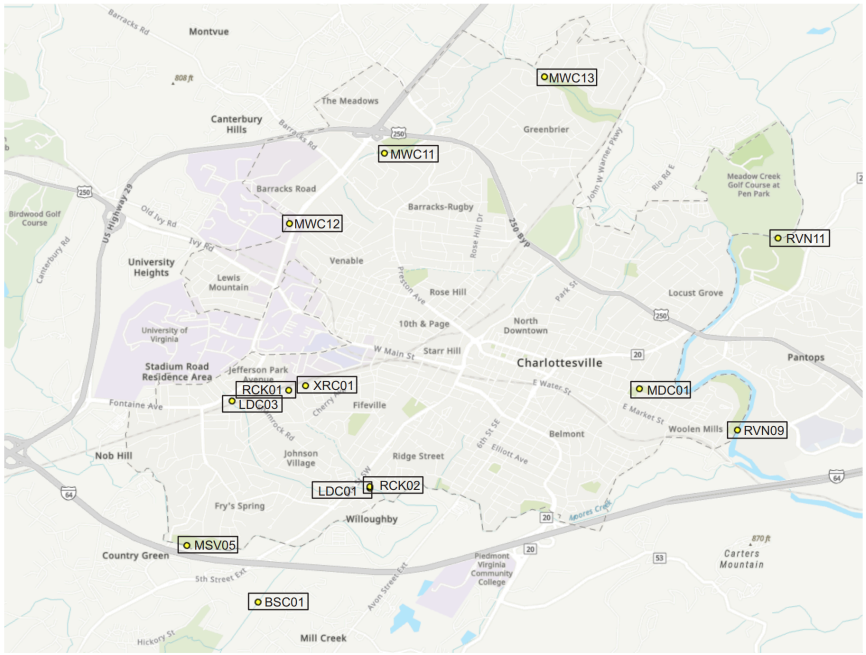

Figure 2: *13 site locations where samples were conducted throughout the Rivanna River Watershed. An additional site was located along the Rivanna River (RVN12) outside of Charlottesville in Palmyra, Virginia at the Palmyra boat launch (37.857763, -78.26679).*

3.2 Sediment Collection

Several hydrological and geochemical methods were employed to characterize the relationship between *E. coli* levels in the sediment and the associated physical properties of the sediment. Sampling took place over a month-long span throughout November and December 2023. Sampling was conducted during base flow conditions on days with no precipitation within the three days preceding the sampling event. This limitation was implemented to isolate the effects of stream channel entrainment at base flow from those induced by runoff following a rain event. Site coordinates and descriptions of where to conduct each sample were provided by the RCA's Bacteria Monitoring Program site guide ("Bacteria monitoring", 2023). At each site, climatic conditions were recorded including air temperature, water temperature, and cloud cover. Due to the potential for spatial variability, each stream site was divided into thirds. Depending on the accessibility of the river or tributary, this division was implemented as either the middle of the stream and either bank or three different points along the most accessible bank. Before extracting a sample, a measuring tape was used to determine the depth of the water column and the water temperature within each sector was measured using a thermometer.

A 60mL, detipped plastic syringe (Herlihy & Mills, 1985) was used to take cores of the bottom sediment. The syringe barrel was inserted into the bottom sediment while keeping the plunger stationary at the sediment surface, creating a vacuum in the process. The syringe was inserted upstream of any disturbance that was caused from entering the water. Because each site exhibited different levels of bottom sediment roughness, the depth to which the syringe was inserted into the soil varied and lacked consistency, ranging from an average of 5cm to 10cm. After removing the syringe from the bottom sediment, a 3mL detipped syringe was used to take a subsample of 1mL from the original core, which was then transferred to individual 100mL

IDEXX bottles for analysis and placed in an ice chest for preservation. The remaining sample in the 100mL syringe was ejected and transferred into a plastic bag labeled with the site ID before being stored in the ice chest for preservation. This procedure was replicated for each of the three samples taken at every site.

3.3 Bacteria Processing

Samples were transported to the RCA laboratory on ice within 24 hours of extraction and analyzed for *E. coli* concentrations using the Colilert® process. The Colilert® technique is a water test, approved by the EPA, used to quantify coliforms. This advanced technique can be performed by untrained lab techs and eliminates the subjective interpretation found in traditional methods.

The workspace was cleaned with alcohol wipes to eliminate any coliforms that could interfere with the analysis. 100mL of distilled water was added to the IDEXX® bottles to ensure the solution was diluted appropriately, as the Colilert® technique only records *E. coli* concentrations up to 2419 MPN. The bottles were shaken by hand for three minutes to detach and disperse the bacteria from the sediment. One packet of Colilert® reagent, containing two nutrient indicators, was then added to each bottle and thoroughly shaken until dissolved. The final solution was poured into an IDEXX Quanti-Tray® with 97 wells and sealed using a Colilert®-grade sealer (Figure 3). The trays were then incubated at 35°C for 24 hours. To determine the Most Probable Number (MPN) (Appendix B) of *E. coli,* wells that fluoresced under UV lighting were tallied (Figure 4a). The MPN of total coliforms was determined in room light (visible) by the number of yellow wells within each Quanti-tray® (Figure 4b). Whereas a typical Colilert® test reports results as MPN/100mL, the sediment *E. coli* counts were reported as MPN/1mL.

Figure 3: *IDEXX® bottles filled with 100mL of distilled water alongside Colilert® reagent and IDEXX Quanti-Tray® in preparation for bacteria processing*

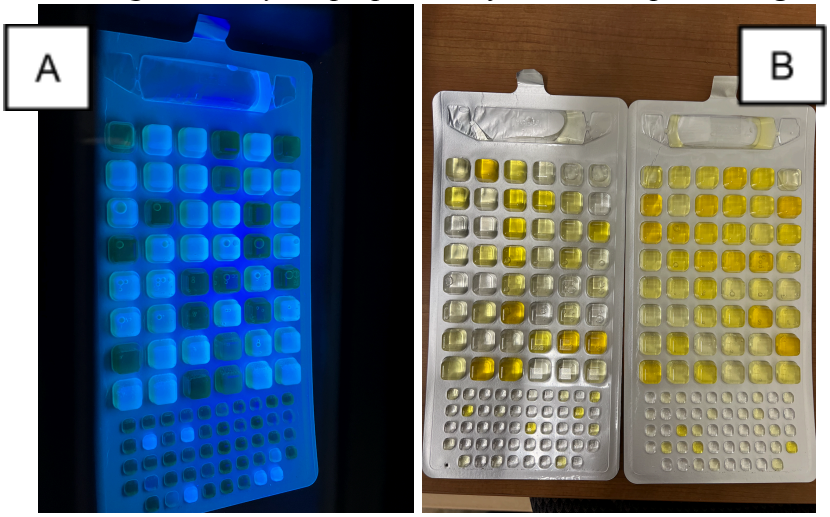

Figure 4 (a,b): *A: IDEXX Quanti-Tray® under UV lighting. Fluorescent wells indicate E. coli presence. B: IDEXX Quanti-Tray® in natural lighting. Yellow wells indicate coliform presence.*

3.4 Sediment Size Processing

The sediment not used for bacterial analysis was kept frozen prior to particle-size analysis using the hydrometer method (Liu & Evett, 1984). Oven-dried sediment was sifted using a 2mm sieve, and a 40g portion was combined with 50mL of Clorox® for 24 hours to

remove organic matter from the sample. One hundred milliliters of sodium hexametaphosphate was added to the mixture as a dispersing agent, and the sample was blended for 5 minutes to ensure complete dispersal. The sample was then transferred to a hydrometer jar and filled with distilled water up to 1000mL (Figure 5). After inverting the cylinder to suspend all the particles, hydrometer readings were recorded at 30 seconds, and subsequently at 3, 10, 30, 60, 90, and 120 minutes.

Figure 5: *Hydrometer jars used for particle-size analysis through the hydrometer method*

The hydrometer (151H) was calibrated in a solution of 50mL Clorox® and 100mL dispersant solution diluted to one liter in a separate cylinder. An additional hydrometer reading was obtained in a column of distilled water. Before processing, the ambient air temperature was recorded as hydrometer readings must be corrected for the variation in temperature due to the viscosity of the water.

The percentage of soil remaining in the suspension was calculated using:

$$P = [(100000/W)G](R_c - G_L)/(G - G_L),$$

where *W* is the oven dry weight of the sample (g), *G* is the specific gravity of soil particles (=2.65), G_L is the specific gravity of liquid (=1), and R_C is the hydrometer reading corrected by the composite correction factor related to temperature. The following correction factor was used:

$$R_C = R - R_L,$$

where *R* is the reading taken from the soil suspension and R_L is the difference between the hydrometer reading in distilled water at the same temperature as the settling column, and the hydrometer reading in the column of the dispersing agent plus hypochlorite (Liu & Evett, 1984).

The diameter of the soil particle corresponding to the percentage of soil remaining in the suspension was computed as:

$$D = K\sqrt{\frac{L}{T}},$$

where *D* is the diameter (mm) of the particle, *K* is a constant that depends on the temperature of the suspension and the specific gravity of the soil particles (Appendix C), and *L* is the distance in cm from the surface of the suspension to the level at which the density is being measured (Appendix C).

The percentage of sand particles in the sample was determined as the percentage passing after 30 seconds. The percentage of clay within the sample was determined as the percentage still suspended in the column after 120 minutes. The percentage of silt within the sample was calculated as the remaining percentage of the total (i.e., (100 - % Sand) - %Silt) (Liu & Evett, 1984).

3.5 Carbon and Nitrogen Analysis

Portions of 20 to 40 mg of the oven-dried sediment were used to determine carbon and nitrogen content. Each sample was first ground with a mortar and pestle. The samples were then

folded into tin capsules, weighed, and processed in a Carlo Erba elemental analyzer to be oxidized by flash combustion. The resulting combustion gasses were swept in a helium stream into a molecular sieve chromatographic column, where a thermal conductivity detector measured the concentration of the individual components proportional to a reference stream of pure helium gas. The percentage of carbon and nitrogen per sediment sample was determined from the thermal conductivity results.

3.6 Land Cover Modeling

The contributing area for each of the sample sites was determined using the U.S.G.S. StreamStats tool (U.S. Geological Survey, 2019). By locating each sample site on the map-based user interface, the tool proceeded to delineate the site's contributing area. The following basin characteristics were retrieved following the delineation of each subwatershed using the classification system from NLCD2011: The drainage area (DRNAREA), the percentage of the watershed that was cropped land including hay (CRPHAY, classes 81-82), the percentage of developed land (DEV, classes 21-24), the percentage of land covered by forest and shrubs (FORSHB, classes 41-52), the percentage of land covered by grass (GRASS), the percentage of land classified as wetland (WETLND, classes 90-95), the percentage of open water (WATER, class 11), and the percentage of impervious surface (IMP). The downloaded data was sourced from 2011, representing the most recent update available in StreamStats (Appendix D).

4: Results

4.1 Objective A

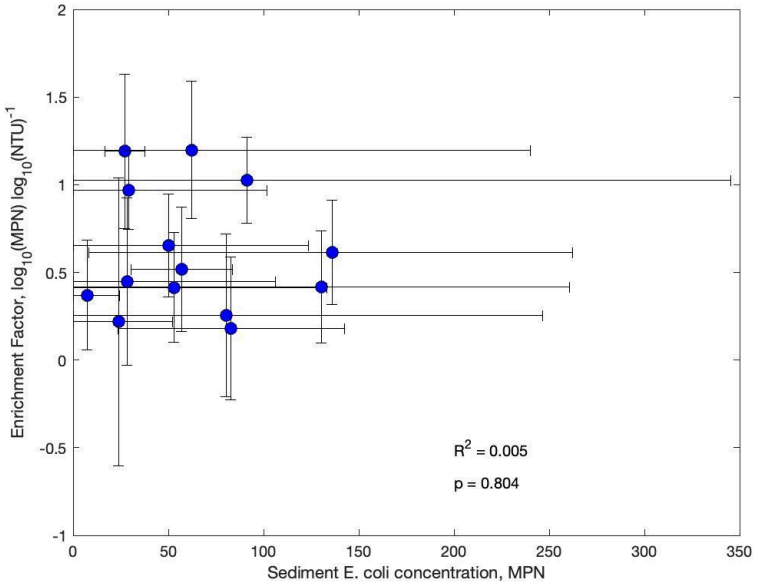

Figure 6: *Scatter plot displaying the relationship between sediment E. coli concentrations and enrichment factor (b). Sediment E. coli concentrations (MPN per 1 mL) represent the average of the three samples taken at each site. 95% confidence bounds are included for both the enrichment factor and E. coli concentrations.*

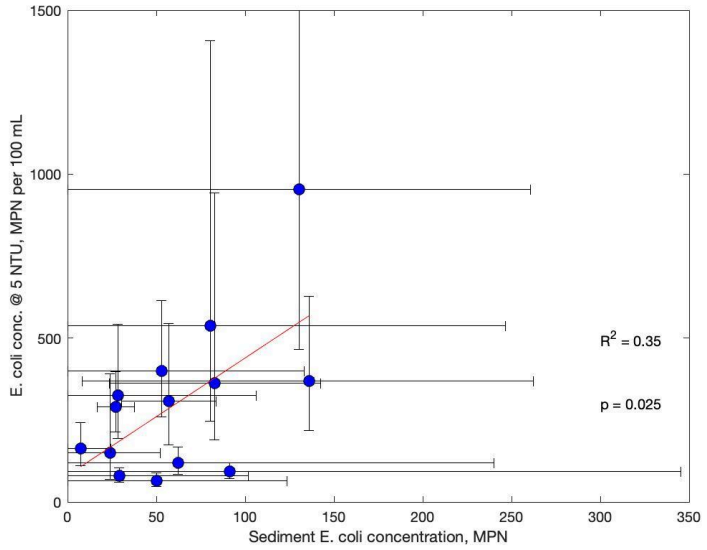

Figure 7: *Scatter plot displaying the relationship between sediment E. coli concentrations and water column C_5 concentrations. Sediment concentrations (MPN per 1mL) represent the average of the three samples taken at each site. 95% confidence intervals are included for both C_5 and sediment E. coli concentrations.*

There was no clear relationship between sediment *E. coli* concentrations and enrichment factors (Figure 6). There was a significant, positive relationship (p=0.025) between *E. coli* levels in the sediment and C_5 concentrations (Figure 7).

4.2 Objective B

Sediment texture (i.e., percent sand, silt, clay), nutrient concentrations (i.e., percent nitrogen and carbon), and depth to the bottom sediment had no influence on sediment *E. coli* concentrations (Appendix E). Similarly, these sediment and stream channel properties had no relationship to water column *E. coli* levels, including both the enrichment factor (b) and the C_5 concentrations.

4.3 Objective C

Land use practices within each subwatershed did not have a significant effect on sediment *E. coli* concentrations (Appendix E). However, various basin characteristics were significantly correlated with *E. coli* levels in the water column. Both the enrichment factor (b) and C_5 concentrations exhibited correlations with the percentage of forested land within each subwatershed, albeit in opposite directions. Specifically, the percentage of forested land was positively correlated with the enrichment factor (p=0.0314), while negatively correlated with the C_5 values (p=0.0127) (Figure 8a, 8b).

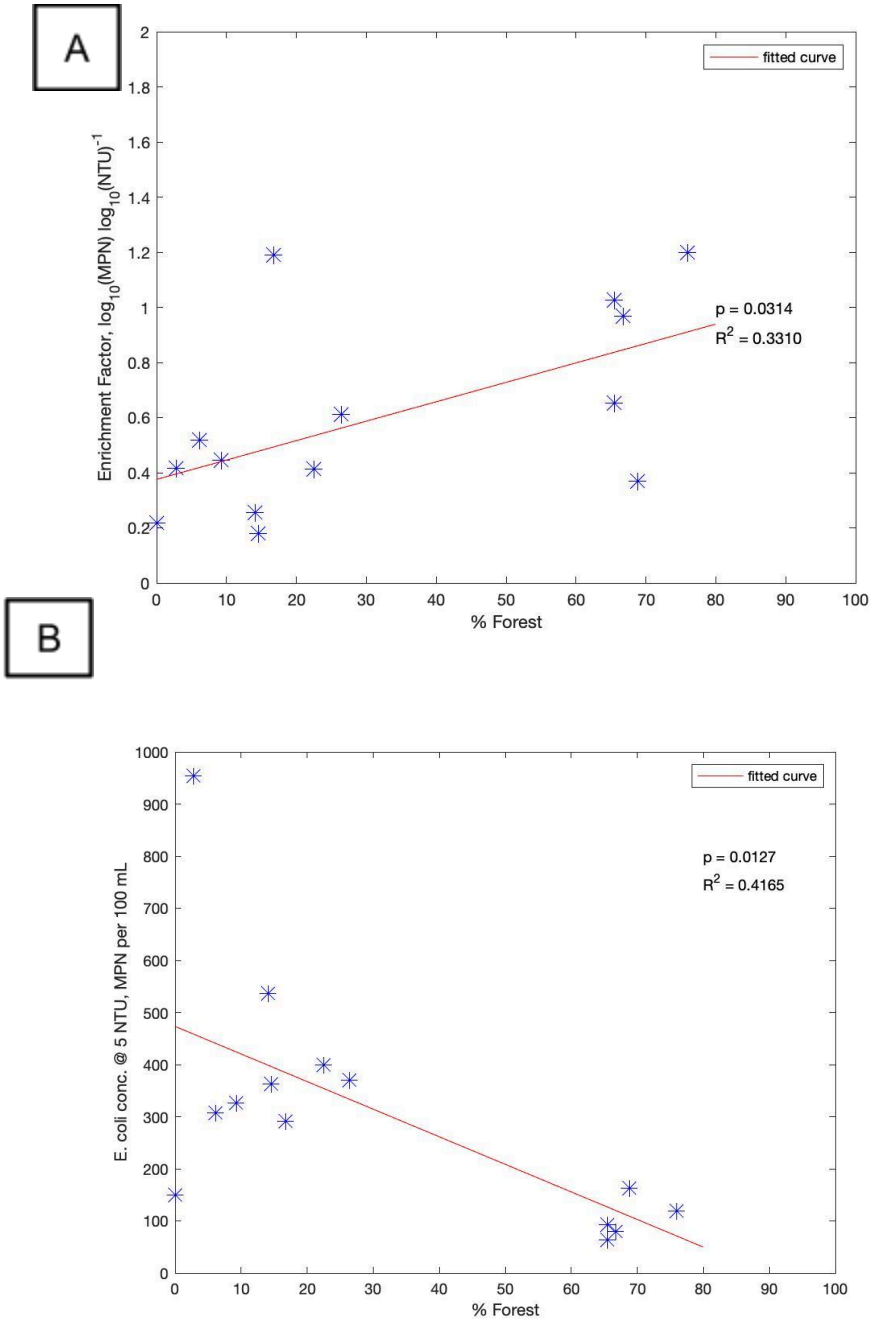

Figure 8 (a,b): *Impact of forested land on E. coli concentrations in the water column. Graph A depicts the impact of forested land on the enrichment factor. Graph B depicts the impact of forested land on C_5 concentrations.*

The enrichment factor was negatively correlated with the percentage of developed land within the subwatershed (p=0.0268) (Figure 9a); however, C_5 concentrations were positively

correlated with the percentage of developed land within each subwatershed (p=0.0105) (Figure 9b).

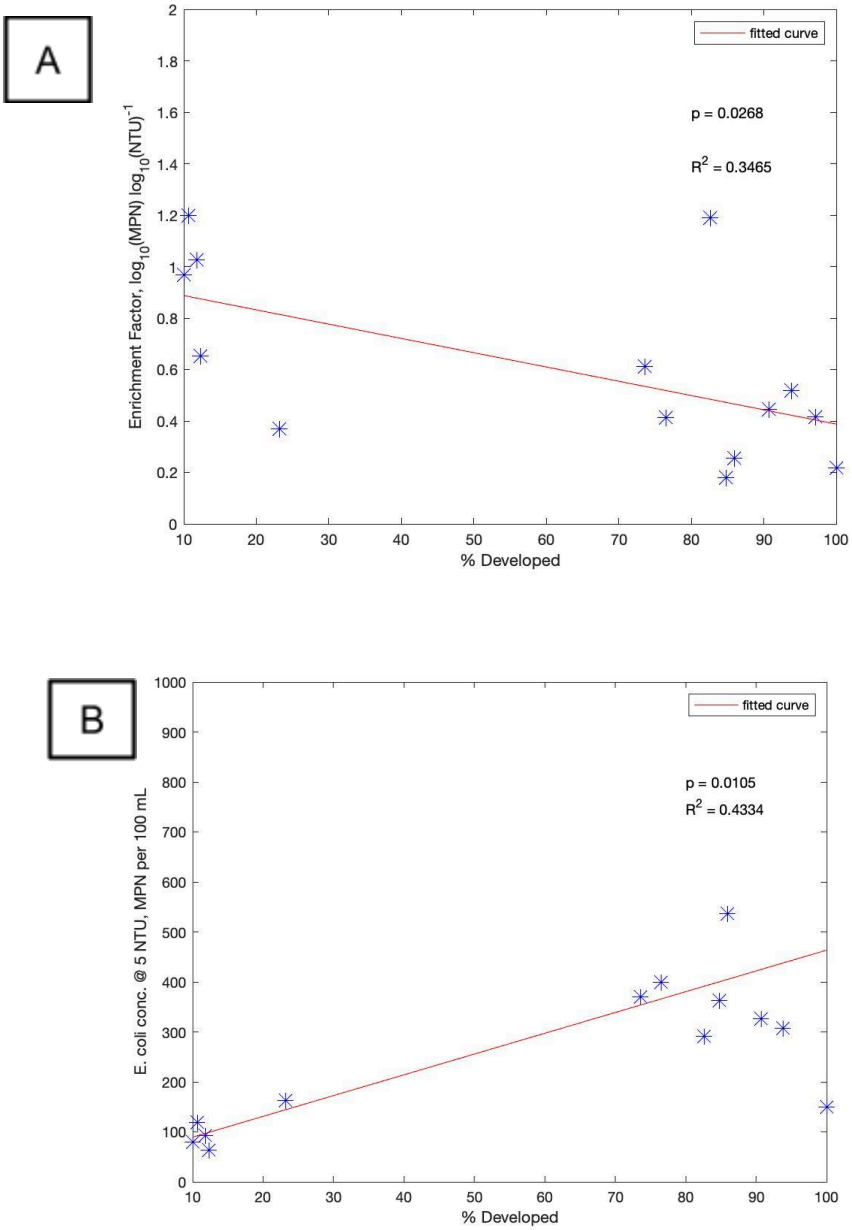

Figure 9 (a,b): *Impact of developed land on E. coli concentrations in the water column. Graph A depicts the impact of developed land on the enrichment factor. Graph B depicts the impact of developed land on C_5 concentrations.*

Furthermore, whereas the enrichment factor was positively correlated with the combined

percentage of grass, cropland, and hay within the subwatershed (p=0.0304) (Figure 10a), C_5 concentrations were negatively correlated (p=0.0166) (Figure 10b).

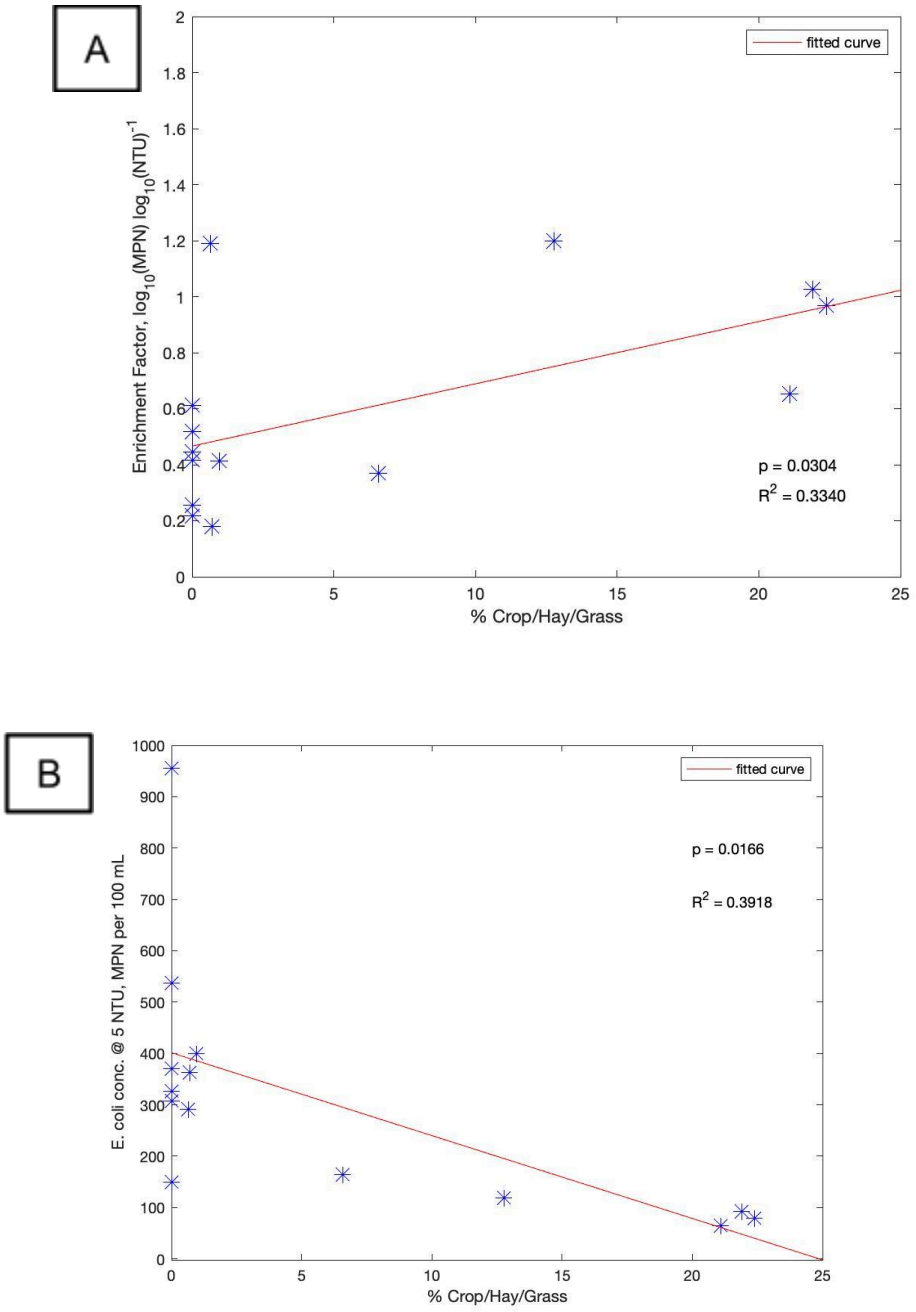

Figure 10 (a,b): *Impact of cropland/hay/grass on E. coli concentrations in the water column. The percentage of land covered by grass was aggregated with the percentage of cropland and hay (classes 81 and 82). Graph A depicts the impact of cropland/hay/ and grassland on the enrichment factor. Graph B depicts the impact of cropland/hay/ and grassland on C_5 concentrations.*

4.4 Principal Component Analysis (PCA)

A principal component analysis was conducted using IBM SPSS Statistics to attempt to reduce the number of independent variables influencing sediment *E. coli* levels because of the insignificant results from objective B. Three components were extracted, explaining 80% of the variance among the independent variables (Table 1).

Component	% of Variance	Cumulative %
1	54.869	54.869
2	15.214	70.083
3	10.014	80.097
4	5.591	85.687
5	4.701	90.389
6	4.128	94.517
7	3.303	97.821
8	0.997	98.817
9	0.917	99.734
10	0.213	99.947
11	0.052	99.999
12	0.001	100.000
13	1.607×10^{-15}	100.000
14	-5.105×10^{-15}	100.000
15	-5.938×10^{-15}	100.000

Table 1: *Percentage of variance explained by each of the extracted variables from the Principal Component Analysis. The variance explained pertains to the independent variables included in the PCA and should not be confused with the variance among sediment MPN concentrations.*

Variable Entered	Principal Component (3 Components extracted)		
	PC1	PC2	PC3
BARE	**0.626**	0.413	0.548
CRPHAY	**0.935**	0.183	-0.008
DEV	**-0.963**	-0.094	0.130
FORSHB	**0.936**	0.060	-0.169
GRASS	**0.820**	0.424	-0.029
IMP	**-0.922**	0.025	0.119
WATER	**0.876**	-0.044	0.025
WETLND	**0.791**	0.282	0.427
WATER_COLI	**-0.776**	-0.279	0.046
SLOPE	0.546	0.291	-0.585
N	-0.588	0.550	-0.262
C	-0.564	0.550	-0.234
SAND	0.576	**-0.717**	-0.124
SILT	-0.563	**0.722**	0.038
CLAY	-0.169	0.038	0.687

Table 2: *Component matrix showing the variable weightings (factor scores) in each of the three extracted components. Factor scores represent the contribution of the individual variable to the principal component, analogous to Beta weightings in a multivariate regression. A negative weighting indicates a negative relationship with sediment E. coli concentrations.*

Agreed upon by the general scientific community, a variable with greater than 0.6 weighting within the component matrix is considered material (Table 2). PC1 grouped together land use related variables, along with C_5 *E. coli* concentrations (bolded). With positive factor scores, cropland/hay, barren land, forest, grassland, water, and wetland exhibited positive relationships with sediment *E. coli* levels. PC2 grouped together sediment texture, specifically sand and silt (bolded). With a positive factor score, silt exhibited a positive relationship with sediment *E. coli* levels. Aside from the variable clay, PC3 did not have a variable that met the predetermined threshold. When converted to PC scores (Appendix G) - which refers to the value

of the principal component for each sampling location - and plotted against sediment *E. coli* concentrations, the relationships were at best weak. Though there was an absence of correlation between land use variables and sediment *E. coli* (PC1) (Figure 13), texture (PC2) exhibited a positive correlation with sediment *E. coli* (Figure 14). The R^2 value for PC1 was 0.037 with a p-value of 0.5285, and the R^2 value for PC2 was 0.072 with a p-value of 0.3741.

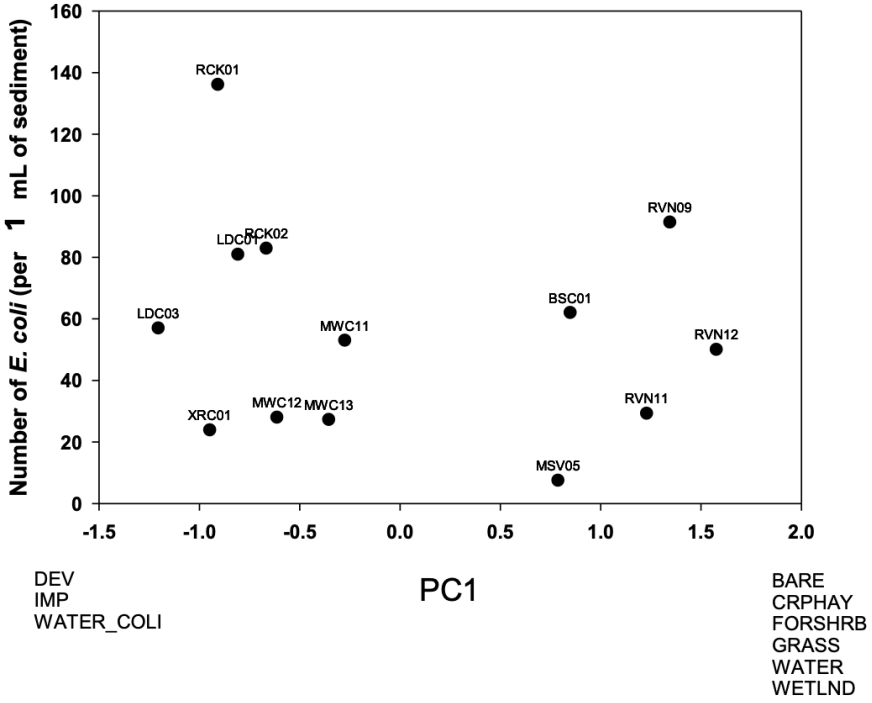

Figure 13: *Scatter plot illustrating the relationship between factor scores from PC1 and sediment E. coli concentrations, with each data point representing a sample site. The graph depicts the absence of a correlation between land cover variables and sediment E. coli concentrations.*

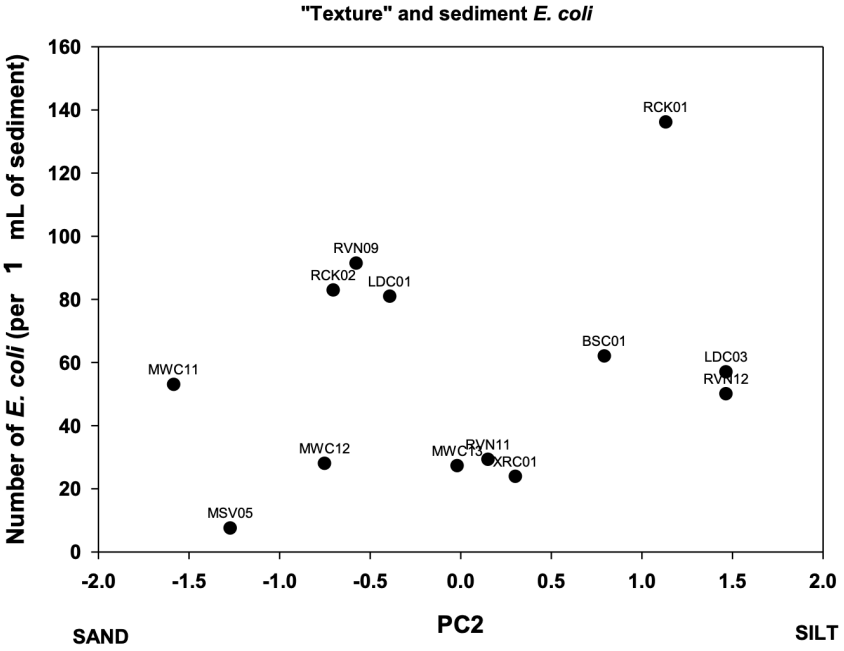

Figure 14: *Scatter plot illustrating the relationship between factor scores from PC2 and sediment E. coli concentrations, with each data point representing a sample site. The graph illustrates an overall positive correlation between sediment texture and sediment E. coli concentrations.*

5: Discussion

A notable finding of this study was the significant relationship between sediment *E. coli* concentrations and C_5 concentrations ($p=0.025$), with 35% of the variability in C_5 concentrations explained by sediment concentrations. This result suggests a mutual influence between water column *E. coli* levels and the quantity of *E. coli* within bottom sediments. This validates earlier findings suggesting that the interplay between suspended particulate matter and the water column may result in increased microbiological contamination. However, the specific direction of this interaction remains uncertain. It is unclear whether resuspended bottom sediment contributed to *E. coli* counts within the water column or if high concentrations within the water column led to more *E. coli*-laden sediment settling out.

The absence of significant relationships between sediment *E. coli* concentrations and various stream channel and sediment properties was somewhat unanticipated, considering the insights gleaned from existing literature. This includes the expectation that fecal indicator bacteria (FIB) adhere to fine-grained particles and thrive in nutrient-rich sediment environments. Whereas the percentage of sand and silt per sample had relative variation, the percentage of clay fell between 22% and 26% for every sample, indicating the overall coarseness of sediment within the Rivanna River and its tributaries. The degree to which the sampling method impacted the hydrometer testing remains unclear. Although the detipped syringe was expected to create a vacuum, clay particles could have washed away when removing the syringe from the water. It is also likely that fine sediment continued to wash downstream rather than settling during autumn streamflow, and it may subsequently be replenished during periods of lower flow. In the 2021 water year, November 2020 reported higher than average stream flow (USGS, 2022), suggesting that clay may not settle out during this time.

Nutrient levels within the sediment were also unexpectedly low, with only 19% of samples registering any detectable nitrogen content and carbon levels remaining, on average, below 2%. The observed C:N ratios fell within the typical range for riverbed sediments, aligning with findings from studies examining C:N ratios that have reported an order of magnitude variability (Venkatesh & Anshumali, 2020). However, both sediment and water column *E. coli* levels showed no dependence on the ratios. There were no obvious relationships between C:N ratios and land cover data, which could have been evident if adjoining stream vegetation and litter biomass contributed to organic carbon content. Similarly, depth to the sediment as a measure of light exposure had no significant influence on sediment *E. coli* concentrations. Depth serves as just one factor influencing light accessibility to bottom sediments. Other factors that

could affect the amount of solar radiation reaching the sediment include total suspended solids, turbidity, and the level of shade provided by vegetation.

The results from objective B imply subwatershed land use practices had a greater impact on water column *E. coli* concentrations compared to sediment *E. coli* concentrations. C_5 concentrations exhibited negative relationships with percent forested land and percent cropland/hay/grass. This aligns with prior research findings and indicates that waterbodies adjacent to less developed land exhibit lower bacterial levels, likely due to natural filtration and reduced exposure to contaminated runoff. The negative correlation with the percentage of cropland/hay suggests that agricultural fields susceptible to manure runoff did not result in heightened *E. coli* contamination in the water. Complementing these findings, C_5 concentrations were positively correlated with the percentage of developed land. As the proportion of developed land in the watershed increases, characterized by an increase in impervious surfaces, the rate of stormwater runoff carrying pollutants from adjacent industrial and mechanical sources also increases. Site MDC01 (Meade Creek at Meade Park) was located at the apex of Figure 6, indicating both high sediment and water column *E. coli* concentrations, while also being situated in an area with only 2.85% forested land (Appendix F).

The enrichment factor exhibited opposite relationships with these variables, which was unanticipated given the enrichment factor is a measure of *E. coli* concentrations in the water column as well. The negative relationship between the enrichment factor and both forested land and cropland/hay/grass infers that less developed sites experience a more significant rise in *E. coli* following a disturbance event. Likewise, sites with higher levels of development experienced a smaller rise in *E. coli* levels after a disturbance event. This is possibly due to the fact that baseflow water column *E. coli* levels are already elevated in more developed

environments. Consequently, after a heavy flow event, *E. coli* levels may not increase as proportionally as they would in undeveloped areas. It is important to note that for the graphs depicting the percentage of forested and developed land, there were few data points in the middle, with a greater concentration of data points at either end. Although the p-values fall below the significance level, it is important to consider how this may affect the degree of confidence regarding the strength of these observed relationships.

The PCA results also suggest that subwatershed land use practices have an insignificant effect on sediment *E. coli* levels. The higher R^2 value (0.072) and lower p-value (0.3741) from PC2 suggest that sediment texture and local in-stream conditions had more of an effect on sediment *E. coli* concentrations. The strong, positive factor score associated with silt specifically indicates that those sites with a higher proportion of silt particles also exhibited elevated sediment *E. coli* levels. Clay did not have a factor score high enough (0.038) to be considered influential in PC2. This is likely attributed to the fact that clay is not settling out from the water column, either due to its small size and colloidal nature or because of the relatively high stream velocity, as mentioned earlier. Moreover, there was little variation in the proportion of clay within the sediment across different sites, indicating it had no discernable impact on *E. coli* levels. In the Rivanna River and its tributaries, it's probable that bacteria are adhering to finer silt particles as opposed to clay, as silt makes up a greater portion of the sediment samples and is able to settle out more readily.

6: Conclusion and Recommendations for Future Work

This study's limitations lie primarily in the variable nature of *E. coli* detection, along with inadequate sediment sampling. Three samples were taken at each site for consistency and additional samples were not collected due to the duration of the study. Although this sampling

approach likely provided sufficient representation of smaller tributaries based on their stream width and area, it may not have been adequate to cover the larger sampling sites, such as the Rivanna River locations. Additionally, in the time between sampling and processing, sediment samples collected from site MDC01 (Meade Creek at Meade Park) were inadvertently misplaced. This explains why any analysis concerning sediment texture or nutrient concentrations reflected data from 13 sites rather than 14.

Although some of the results of this study deviated from what was anticipated, the data set produced can serve as a baseline for future studies, specifically those looking to compare *E. coli* levels within the Rivanna River Watershed before and after the wastewater spillage event that took place in January 2024. Charlottesville's largest wastewater facility (Rivanna Pump Station) failed during a heavy rain event, resulting in 6 million gallons of wastewater overflowing into the Rivanna (Armesto, 2024). Since these samples were taken in the months preceding the spillage event, the dataset can serve as a reference point for research aiming to assess both the longevity of the introduced *E. coli* and the duration required for bacterial levels to return to safe recreational levels. In future studies, it is advisable to increase the number of samples taken per site and ensure that sampling is conducted at a specific depth within the sediment. This approach will enhance understanding of the depth at which *E. coli* are present. Furthermore, there are an additional 5 sites identified by the RCA that were not sampled but could be included to encompass the remainder of the Charlottesville area, as well as other high-use recreational areas not sampled by the RCA such as Totier Park located near Scottsville and the South Fork Rivanna River Reservoir. The broader significance of this study offers valuable insights for designing more inclusive *E. coli* monitoring initiatives and broadening scientific consensus about sediment as an under-recognized source of water quality impairment.

Based on this study's results, the following conclusions can be drawn:

I. Those sites with elevated *E. coli* levels in the water column, specifically MDC01, LDC01, and RCK01, also showed higher quantities of *E. coli* in bottom sediment, due to the observable mutual influence between the water column and suspended particulate matter.

II. Land use practices have a significant impact on *E. coli* levels in the water column. However, in areas where concentrations are already high, any disturbance in the water column does not lead to proportionally high increases in *E. coli* concentrations.

III. Sediment texture is more predictive of sediment *E. coli* concentrations compared to land use practices. Within the Rivanna River and its tributaries, it is likely that *E. coli* adhere to finer silt particles, rather than clay.

The new findings indicate that in the scientific community's efforts to mitigate microbial contamination in rivers in the interest of public health, the class of undeveloped land - be it agricultural, forested, or grassland - is less significant for predictive modeling than the presence of impervious surfaces within the subwatershed. Similarly crucial is the need to caution communities against using recreational areas after a period of high flow given the relationship between *E. coli* supply in sediment and *E. coli* transport. Furthermore, rather than narrowing the scope of a study to focus solely on *E. coli* interaction with clay particles, analyses concerning bacteria monitoring should now give precedence to understanding the properties of silt particles settling out of the water column.

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Appendix A

Paired measurements of water column *E. coli* concentrations and turbidity data used in pre-analysis to determine which sites would be used in the remainder of the study. The samples were collected by the Rivanna Conservation Alliance's volunteer-based Bacteria Monitoring program from 2018 to 2022.

ID	total_meas	paired_meas
{'RVN09' }	125	109
{'RVN11' }	121	106
{'MDC01' }	73	57
{'MWC11' }	58	50
{'BSC01' }	55	49
{'MWC13' }	60	49
{'MSV05' }	55	47
{'RVN12' }	50	45
{'LDC01' }	59	42
{'LDC03' }	45	42
{'RCK01' }	50	42
{'RCK02' }	56	41
{'MWC12' }	55	40
{'XRC01' }	51	40
{'RVN10' }	42	37
{'SCK01' }	50	35
{'XLC01' }	34	26
{'XLD01' }	36	25
{'XLC01A' }	19	19
{'BSC03' }	18	18
{'TWN02' }	20	18
{'XLD01A' }	18	17
{'PBR01' }	14	13
{'PLK02' }	19	13
{'PLK01' }	5	5
{'RVN11_BoatRamp'}	5	5
{'MDU01' }	2	2
{'MDU02' }	2	2
{'TWN01' }	2	2
{'BVE01' }	1	1
{'MDC02' }	1	1
{'MDU03' }	1	1
{'MDU04' }	1	1
{'MSC04' }	1	1
{'QTR03' }	1	1
{'RVN11_Original'}	5	1

Appendix B

IDEXX Quanti-Tray® MPN table used for the Colilert® method to determine the Most Probable Number of both total coliforms and *E. coli* based on the number of small and large wells that turn yellow or fluorescent depending on the variable of interest.

IDEXX Quanti-Tray®/2000 MPN Table *(per 100ml)*

# Large Wells Positive	# Small Wells Positive																							
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6	21.6	22.7	23.7	24.8	25.8	26.9
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8	33.9
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3	35.4
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4	38.6
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.5	40.7	41.9
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.2	42.4	43.6
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.9	44.2	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
17	20.3	21.6	22.8	24.1	25.3	26.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.9	47.2	48.5	49.8	51.2
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.5	51.9	53.2
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	39.0	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0	55.4
20	24.9	26.2	27.5	28.8	30.1	31.5	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.6
21	26.5	27.9	29.2	30.5	31.8	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.9
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.8	62.3
23	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8	67.3
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4	70.0
26	35.5	36.9	38.4	39.9	41.4	42.8	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2	75.9
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.9	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.5	82.4
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.8
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0	107.3
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2	112.7
37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	115.9	118.6
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2	125.0
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.9	93.4	95.9	98.4	101.0	103.6	106.3	109.0	111.8	114.6	117.4	120.3	123.2	126.1	129.2	132.2
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.8	93.3	95.9	98.5	101.2	103.9	106.7	109.5	112.4	115.3	118.2	121.2	124.3	127.4	130.5	133.7	137.0	140.3
41	78.0	80.5	83.0	85.5	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.4	138.8	142.3	145.9	149.5
42	82.6	85.2	87.8	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.9	120.1	123.4	126.7	130.1	133.6	137.2	140.8	144.5	148.3	152.2	156.1	160.2
43	87.6	90.4	93.2	96.0	99.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.2	159.4	163.8	168.2	172.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	125.9	129.6	133.4	137.4	141.4	145.5	149.7	154.1	158.5	163.1	167.9	172.7	177.7	182.9	188.2
45	99.3	102.5	105.8	109.2	112.6	116.2	119.8	123.6	127.4	131.4	135.4	139.6	143.9	148.3	152.9	157.6	162.4	167.4	172.6	178.0	183.5	189.2	195.1	201.2	207.5
46	106.3	109.8	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	146.7	151.5	156.5	161.6	167.0	172.5	178.2	184.2	190.4	196.8	203.5	210.5	217.8	225.4	233.3
47	114.3	118.3	122.4	126.6	130.9	135.4	140.1	145.0	150.0	155.3	160.7	166.4	172.3	178.5	185.0	191.8	198.9	206.4	214.2	222.4	231.0	240.0	249.5	259.5	270.0
48	123.9	128.4	133.1	137.9	143.0	148.3	153.9	159.7	165.8	172.2	178.9	186.0	193.5	201.4	209.8	218.7	228.2	238.2	248.9	260.3	272.3	285.1	298.7	313.0	328.2
49	135.5	140.8	146.4	152.3	158.5	165.0	172.0	179.3	187.2	195.6	204.6	214.3	224.7	235.9	248.1	261.3	275.5	290.9	307.6	325.5	344.8	365.4	387.3	410.6	435.2

09-63235-01

Appendix C

Tables used for hydrometer analysis calculations. The first table displays values for the constant, *K,* that is used in computing the diameter of particles. The second table displays values for the constant, L, which is the distance in cm from the surface of the suspension to the level at which the density is being measured. Both tables are taken from Liu and Evett, 1984.

Temperature	*Specific Gravity of Soil Particles*								
(°C)	2.45	2.50	2.55	2.60	2.65	2.70	2.75	2.80	2.85
16	0.01510	0.01505	0.01481	0.01547	0.01435	0.01414	0.01394	0.01374	0.01356
17	0.01511	0.01486	0.01462	0.01439	0.01417	0.01396	0.01376	0.01356	0.01338
18	0.01492	0.01467	0.01443	0.01421	0.01399	0.01378	0.01359	0.01339	0.01321
19	0.01474	0.01445	0.01425	0.01403	0.01382	0.01361	0.01342	0.01323	0.01305
20	0.01456	0.01431	0.01408	0.01386	0.01365	0.01344	0.01325	0.01307	0.01289
21	0.01438	0.01414	0.01391	0.01369	0.01348	0.01328	0.01309	0.01291	0.01273
22	0.01421	0.01397	0.01374	0.01353	0.01332	0.01312	0.01294	0.01376	0.01258
23	0.01404	0.01381	0.01358	0.01337	0.01317	0.01297	0.01297	0.01279	0.01243
24	0.01388	0.01365	0.01342	0.01321	0.01301	0.01282	0.01264	0.01246	0.01229
25	0.01372	0.01349	0.01327	0.01306	0.01286	0.01267	0.01249	0.01232	0.01215
26	0.01357	0.01334	0.01312	0.01291	0.01272	0.01253	0.01235	0.01218	0.01201
27	0.01342	0.01319	0.01297	0.01277	0.01258	0.01239	0.01221	0.01204	0.01188
28	0.01327	0.01304	0.01283	0.01264	0.01244	0.01225	0.01208	0.01191	0.01175
29	0.01312	0.01290	0.01269	0.01249	0.01230	0.01212	0.01195	0.01178	0.01162
30	0.01298	0.01276	0.01256	0.01236	0.01217	0.01199	0.01182	0.01165	0.01149

(Taken from Liu and Evett, 1984)

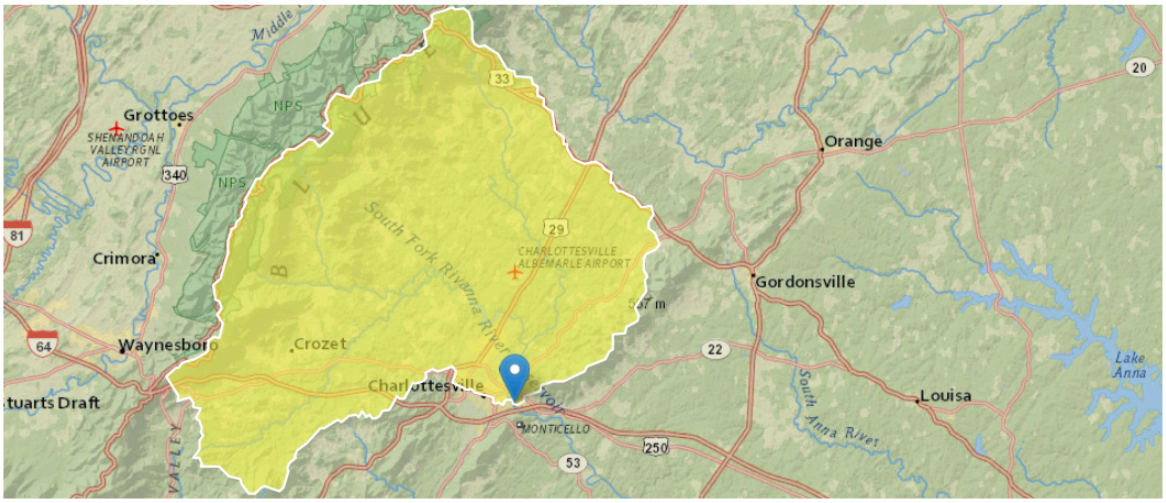
Actual Hydrometer Reading	*Effective Depth L(cm)*	*Actual Hydrometer Reading*	*Effective Depth L(cm)*	*Actual Hydrometer Reading*
1.000	13.3	1.013	12.9	1.026
1.001	16.0	1.014	12.6	1.027
1.002	15.8	1.015	12.3	1.028
1.003	15.5	1.016	12.1	1.029
1.004	15.2	1.017	11.8	1.030
1.005	15.0	1.018	11.5	1.031
1.006	14.7	1.019	11.3	1.032
1.007	14.4	1.020	11.0	1.033
1.008	14.2	1.021	10.7	1.034
1.009	13.9	1.022	10.5	1.035
1.010	13.7	1.023	10.2	1.036
1.011	13.4	1.024	10.0	1.037
1.012	13.1	1.025	9.7	1.038

Appendix D

Basin characteristics for site RVN09 retrieved from StreamStats online delineation tool. Note, percentages do not add up to 100. Rather, the percentage of impervious area is determined from a separate dataset - NLCD 2011 impervious data set. Impervious surface is a subcategory of developed land.

RVN09 - Rivanna River at Riverview Park

Region ID: VA
Workspace ID: VA20240312212556590000
Clicked Point (Latitude, Longitude): 38.02335, -78.45386
Time: 2024-03-12 17:26:21 -0400



Collapse All

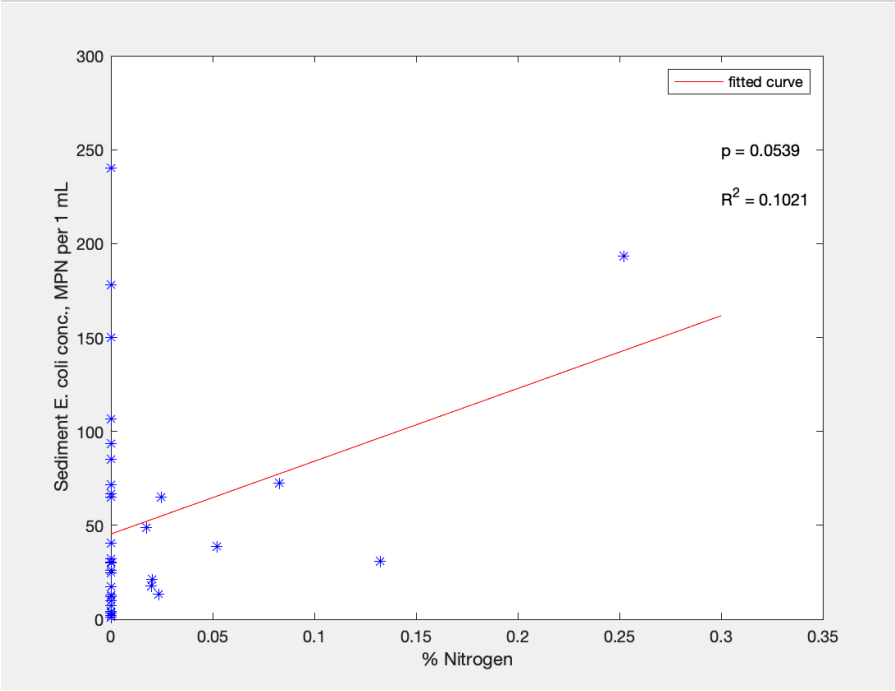
Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	468	square miles
LC11BARE	Percentage of barren from NLCD 2011 class 31	0.01	percent
LC11CRPHAY	Percentage of cultivated crops and hay, classes 81 and 82, from NLCD 2011	21.56	percent
LC11DEV	Percentage of developed (urban) land from NLCD 2011 classes 21-24	11.8	percent
LC11FORSHB	Percentage of forests and shrub lands, classes 41 to 52, from NLCD 2011	65.5	percent
LC11GRASS	Percent of area covered by grassland/herbaceous using 2011 NLCD	0.33	percent
LC11IMP	Average percentage of impervious area determined from NLCD 2011 impervious dataset	2.33	percent
LC11WATER	Percent of open water, class 11, from NLCD 2011	0.72	percent
LC11WETLND	Percentage of wetlands, classes 90 and 95, from NLCD 2011	0.11	percent

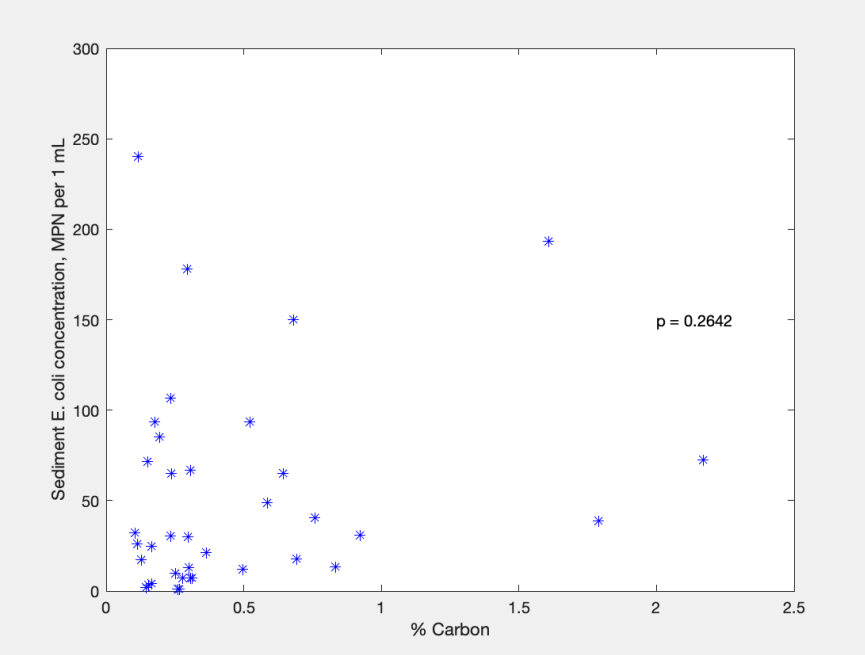
Appendix E

Additional scatter plots addressing objectives a, b, and c. These graphs do not produce any significant relationships between various independent variables (including sediment texture, nutrient composition, and land use practices) and sediment *E. coli* concentrations along with water column concentrations.

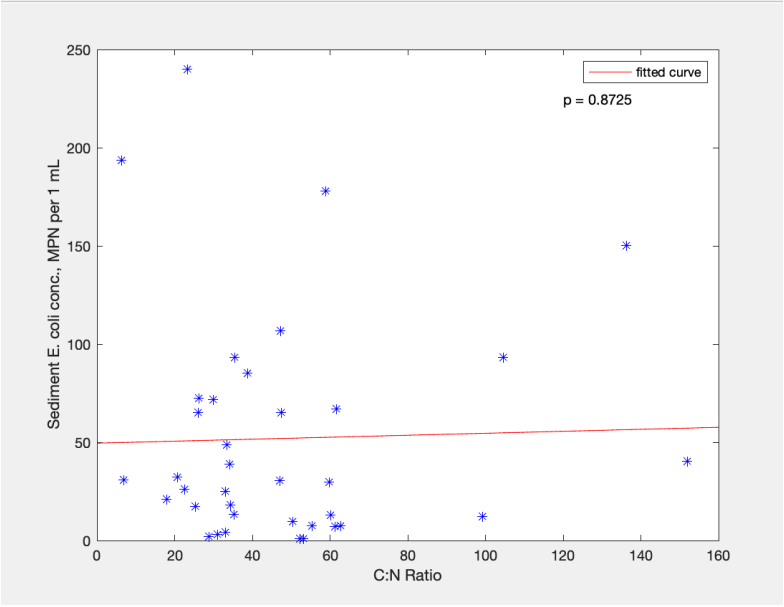
Nutrient Concentrations vs. Sediment and Water Column E. Coli Concentrations



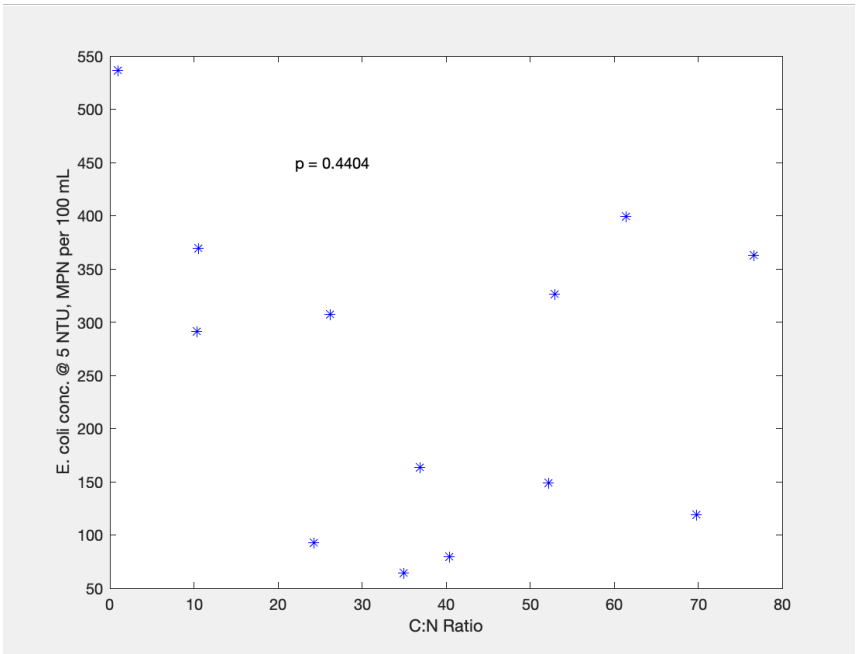
Graph 1: Percentage of nitrogen within sediment sample versus *E. coli* concentration of sediment sample. Each point represents a single sample, rather than an average. Nitrogen within the bottom sediment had an almost significant impact on the concentration of *E. coli* within the sediment. Though, only 19% of samples recorded any percent nitrogen, suggesting that the significance level may be impacted by the small sample size.



Graph 2: Percentage of nitrogen within sediment sample versus *E. coli* concentration of sediment sample. Each point represents a single sample, rather than an average.

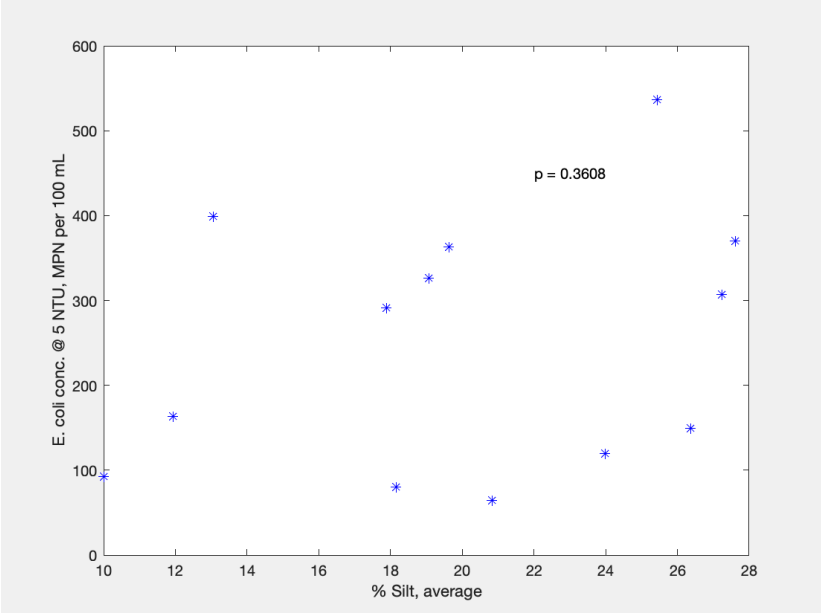


Graph 3: C:N ratio versus sediment *E. coli* concentration. Each point represents a single sample, rather than an average. C:N ratio was calculated by dividing the percentage of carbon per sample by the percentage of nitrogen. If a sample did not contain any nitrogen, to prevent a division by zero error, the data was adjusted so that the sample registered 0.005%, which is half of the minimum detection limit (0.01%).

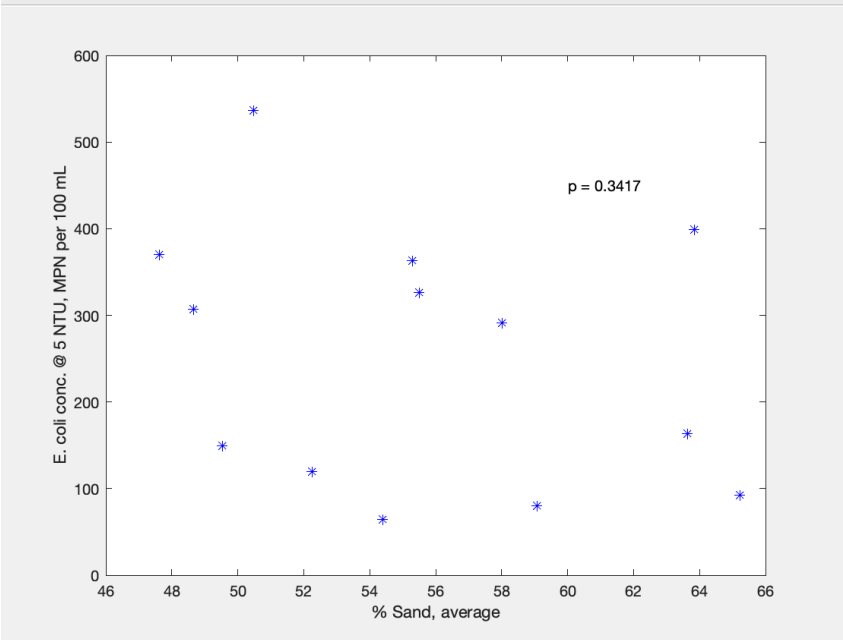


Graph 4: C:N ratio versus C_5 concentrations. Each data point reflects the mean C:N ratio observed at each site. Similarly, if a sample did not contain any nitrogen, to prevent a division by zero error, the data was adjusted so that the sample registered 0.005%, which is half of the minimum detection limit (0.01%).

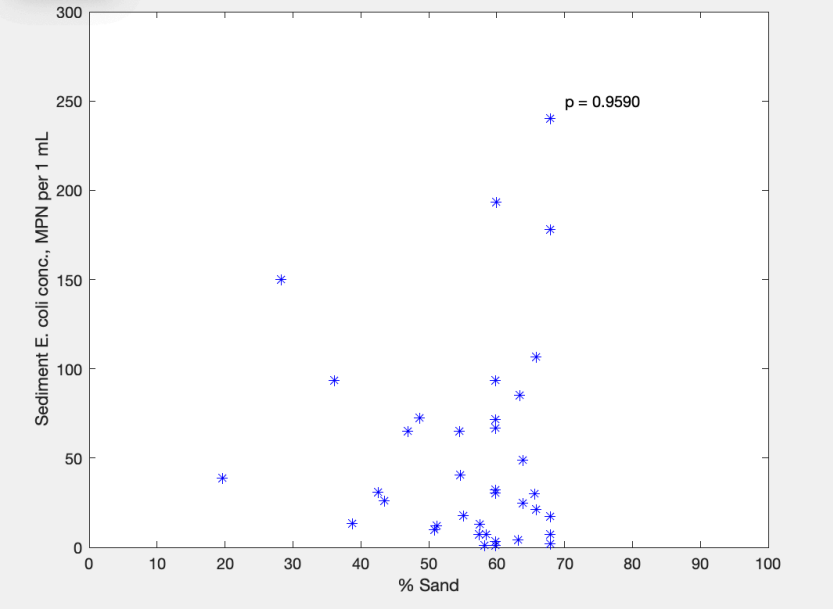
Texture vs. Sediment and Water Column E. Coli Concentrations



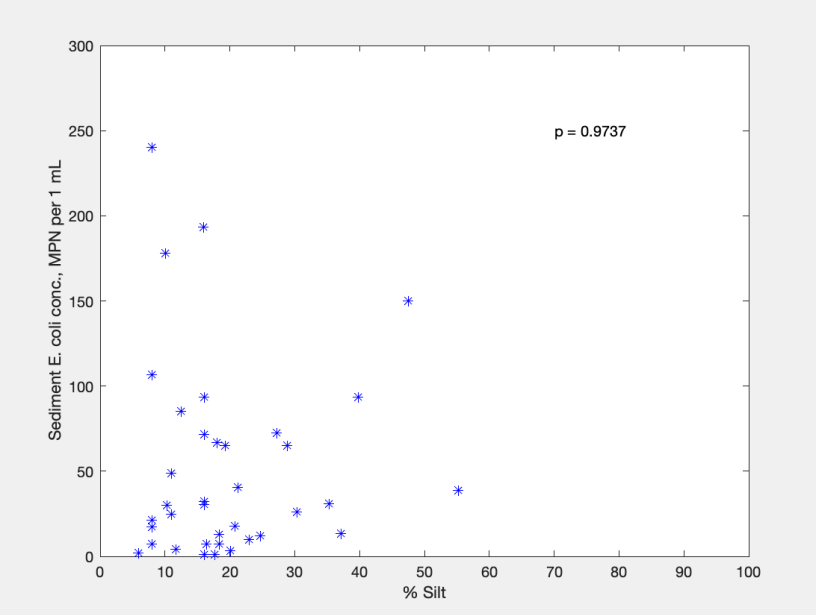
Graph 5: Percent silt versus C_5 concentrations. Each data point reflects the mean percent silt observed at each site.



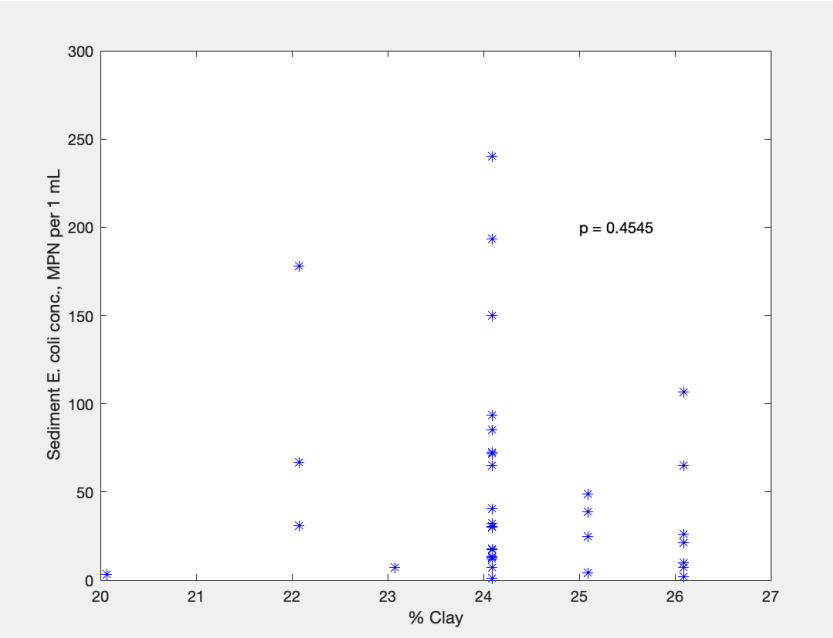
Graph 6: Percent sand versus C_5 concentrations. Each data point reflects the mean percent sand observed at each site.



Graph 7: Percent sand versus sediment *E. Coli* concentrations. Each data point reflects an individual sample.

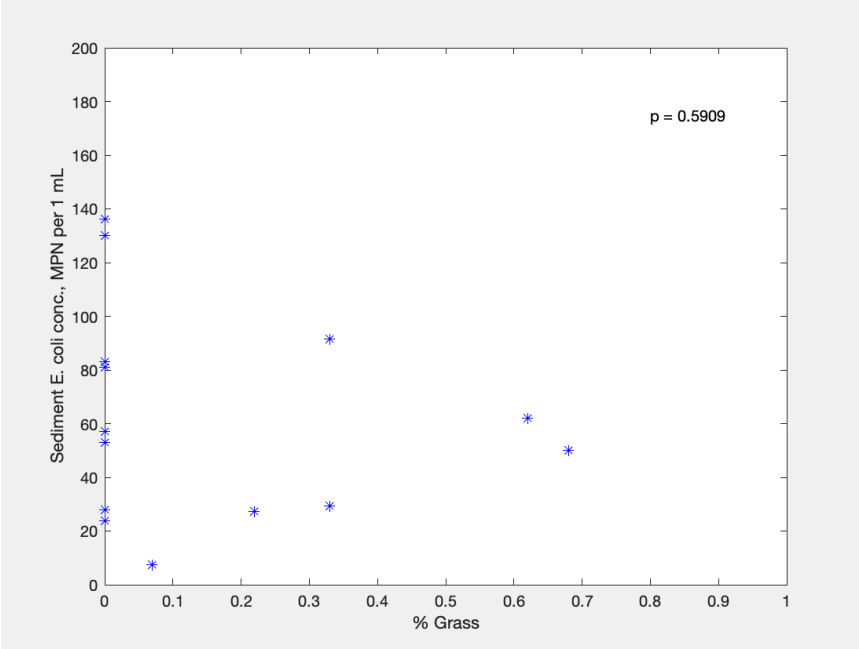


Graph 8: Percent silt versus sediment *E. Coli* concentrations. Each data point reflects an individual sample.

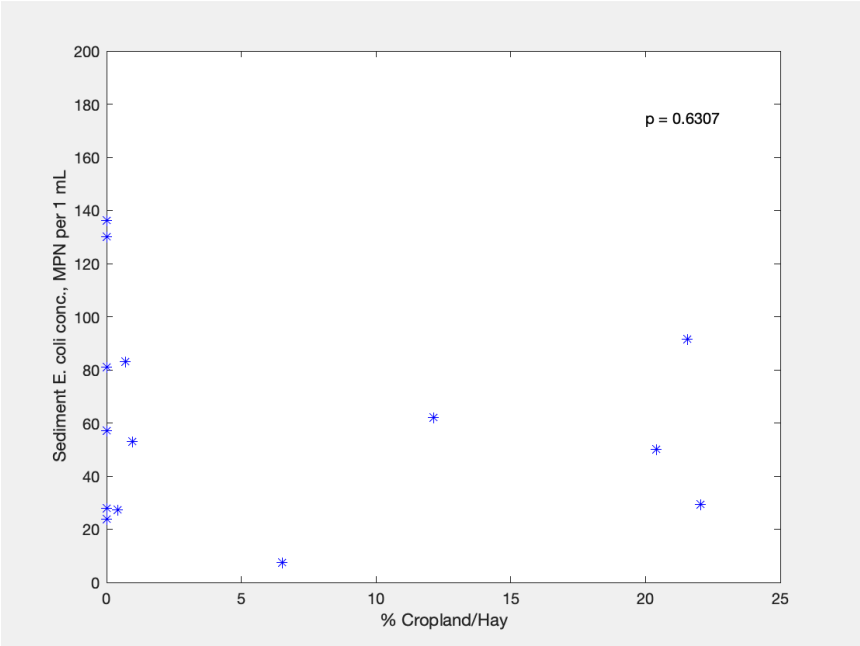


Graph 9: Percent clay versus sediment *E. Coli* concentrations. Each data point reflects an individual sample.

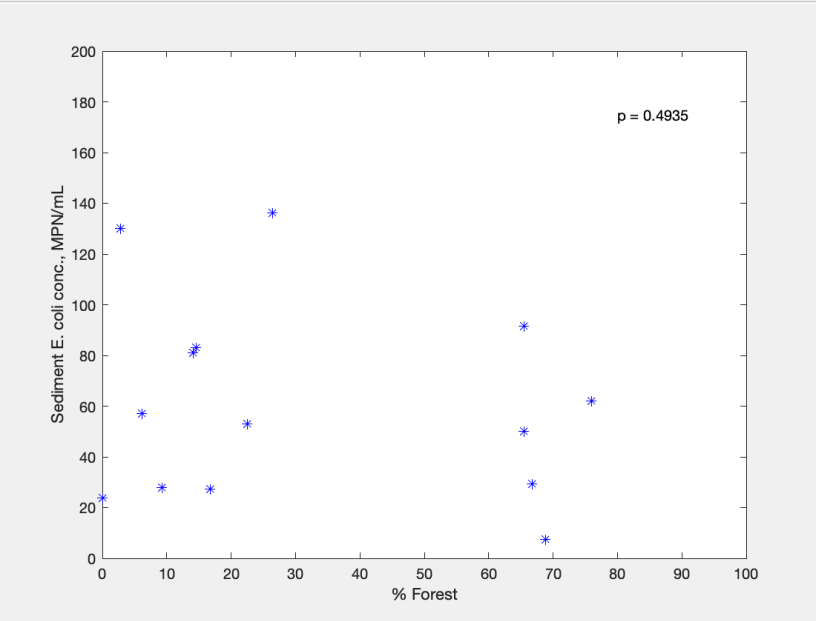
Land Use Characteristics vs. Sediment E. Coli Concentrations



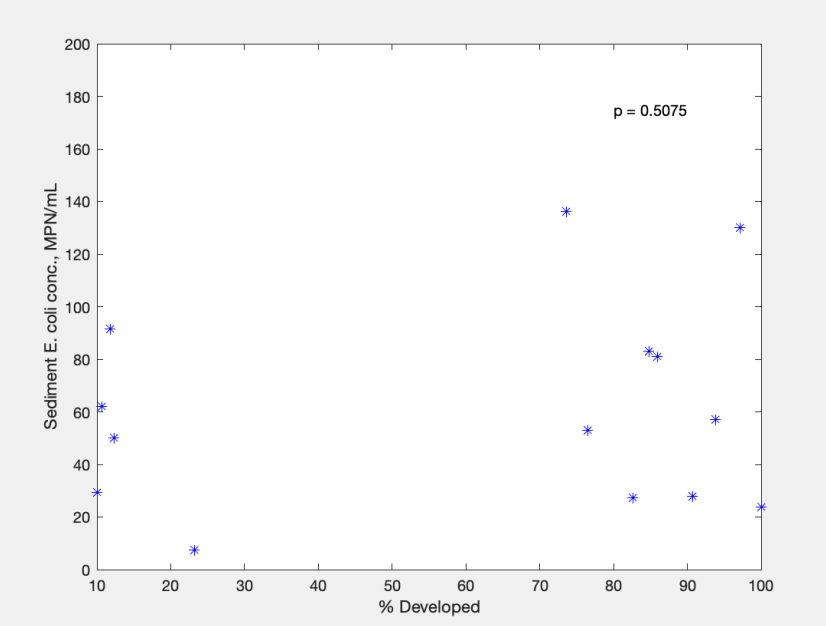
Graph 10: Percent grass within each subwatershed versus sediment *E. Coli* concentrations. Each data point represents the average *E. coli* concentration per site.



Graph 11: Percent cropland/hay within each subwatershed versus sediment *E. Coli* concentrations. Each data point represents the average *E. coli* concentration per site.

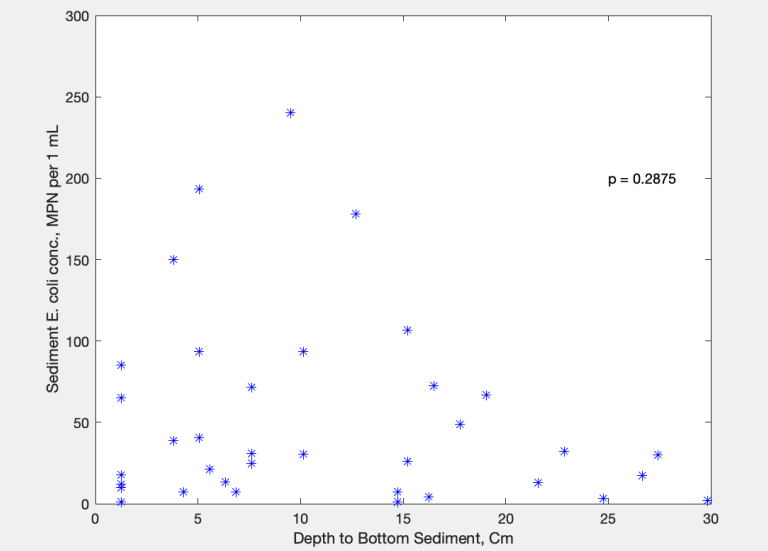


Graph 12: Percent forested land within each subwatershed versus sediment *E. Coli* concentrations. Each data point represents the average *E. coli* concentration per site.



Graph 13: Percent developed land within each subwatershed versus sediment *E. Coli* concentrations. Impervious surface is a sub-category of developed land. Each data point represents the average *E. coli* concentration per site.

Depth to Sediment vs. Sediment E. Coli Concentrations



Graph 14: Depth to bottom sediment as a measure of light availability versus sediment *E. coli* concentrations. Each data point reflects an individual sample.

Appendix F

Full data table, encompassing every collected variable for all 14 sample sites. Note missing values for site MDC01. In the time between sampling and processing, sediment samples, aside from bacteria samples, for site MDC01 were misplaced.

Site ID	BARE (%)	CRPHAY (%)	CRPHAY GR(%)	DEV (%)	FORSHB (%)	GRASS (%)	IMP (%)	WATER (%)	WETLND (%)	Total Coliforms (MPN/1mL)*	SedimentE. coli (MPN/1mL)*
RCK01	0	0	0	73.6	26.42	0	30.3	0	0	1011.20	136.17
MDC01	0	0	0	97.2	2.85	0	56.6	0	0	1107.00	130.00
RVN09	0.01	21.56	21.89	11.8	65.5	0.33	2.33	0.72	0.11	1018.13	91.43
RCK02	0	0.71	0.71	84.8	14.52	0	36	0	0	1555.30	82.93
LDC01	0	0	0	85.9	14.08	0	29.7	0	0	1560.00	81.00
BSC01	0	12.15	12.77	10.7	76	0.62	1.66	0.55	0	734.00	62.00
LDC03	0	0	0	93.8	6.18	0	45.4	0	0	1211.00	57.00
MWC11	0	0.96	0.96	76.5	22.56	0	27.5	0	0	796.00	53.00
RVN12	0.05	20.41	21.09	12.3	65.46	0.68	2.39	0.85	0.23	1384.17	50.03
RVN11	0.01	22.05	22.38	10	66.8	0.33	1.56	0.72	0.1	290.50	29.30
MWC12	0	0	0	90.7	9.33	0	37.2	0	0	95.00	28.00
MWC13	0	0.43	0.65	82.6	16.72	0.22	36.5	0.04	0	1053.33	27.27
XRC01	0	0	0	100	0	0	59.5	0	0	1067.67	23.90
MSV05	0	6.51	6.58	23.2	68.87	0.07	5.63	1.26	0.04	439.00	7.50

Site ID	Sediment E. Coli 95% Lower Bound (MPN/1mL)	Sediment E. Coli 95% Upper Bound (MPN/1mL)	Water Column E.Coli (MPN/100mL) @ 5 NTU	Water E.Coli (MPN/100mL) @ 5 NTU Lower Bound	Water E. Coli (MPN/100mL) @ 5 NTU Upper Bound	Slope of Water Column E.Coli (MPN/100mL) vs. Turbidity	Slope Lower Bound	Slope Upper Bound	% N
RCK01	62.208	210.1254717	369.693	217.689	627.835	0.613	0.316	0.910	0.092
MDC01	55.384	205.683	954.705	464.624	1961.720	0.416	0.096	0.736	
RVN09	0.000	238.035	92.509	71.594	119.533	1.025	0.779	1.271	0.000
RCK02	48.634	117.232	363.101	190.197	943.273	0.179	-0.227	0.586	0.006
LDC01	0.000	176.375	536.508	246.207	1406.209	0.255	-0.209	0.719	0.017
BSC01	0.000	164.938	119.340	84.639	168.267	1.197	0.805	1.589	0.000
LDC03	41.556	72.311	307.262	173.229	545.001	0.518	0.163	0.872	0.083
MWC11	6.933	99.467	398.992	259.249	614.061	0.414	0.101	0.727	0.000
RVN12	7.464	92.602	64.162	46.069	89.360	0.654	0.359	0.948	0.000
RVN11	0.000	71.235	79.719	61.293	103.685	0.968	0.744	1.192	0.000
MWC12	0.000	73.354	325.914	193.500	541.561	0.447	-0.030	0.924	0.000
MWC13	21.191	33.342	291.182	213.587	396.966	1.191	0.750	1.631	0.051
XRC01	7.531	40.269	149.203	69.560	390.119	0.219	-0.603	1.040	0.015
MSV05	0.000	17.262	163.450	110.399	241.995	0.370	0.056	0.684	0.000

Site ID	% C	% Sand	% Silt	% Clay	Nlim	C:N Ratio
RCK01	0.978	47.617	27.627	24.757	0.092	10.594
MDC01						
RVN09	0.122	65.227	10.017	24.757	0.005	24.321
RCK02	0.449	55.287	19.623	25.090	0.006	76.609
LDC01	0.017	50.473	25.440	24.087	0.017	0.975
BSC01	0.349	52.253	23.993	23.753	0.005	69.837
LDC03	2.171	48.670	27.240	24.090	0.083	26.200
MWC11	0.307	63.855	13.060	23.085	0.005	61.465
RVN12	0.175	54.400	20.843	24.757	0.005	34.980
RVN11	0.202	59.080	18.170	22.750	0.005	40.378
MWC12	0.265	55.513	19.063	25.423	0.005	52.968
MWC13	0.529	58.020	17.893	24.087	0.051	10.379
XRC01	0.762	49.543	26.367	24.090	0.015	52.233
MSV05	0.185	63.643	11.933	24.423	0.005	36.911

Appendix G

Principal component scores taken from the principal component analysis. PC scores refer to the value of the principal component for each sampling location

Station	PC1	PC2	PC3
RCK01	-0.90883	1.13214	-0.21557
RVN09	1.34456	-0.57829	-0.02682
RCK02	-0.66709	-0.70405	1.0052
LDC01	-0.8089	-0.39259	0.48248
BSC01	0.84842	0.79315	-1.41677
LDC03	-1.20557	1.46468	-0.63574
MWC11	-0.27622	-1.58544	-0.61672
RVN12	1.57639	1.4642	2.06486
RVN11	1.22864	0.1507	-1.13982
MWC12	-0.61409	-0.75248	1.02718
MWC13	-0.35594	-0.02022	-1.03568
XRC01	-0.94844	0.30169	0.4757
MSV05	0.78707	-1.27348	0.03169