2019

Variability of E. coli in Streambed Sediments and its Implication for Water Quality

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VARIABILITY OF *E. COLI* IN STREAMBED SEDIMENTS AND ITS IMPLICATION FOR WATER QUALITY

BY

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A dissertation submitted in partial fulfillment of the requirements for the

Doctor of Philosophy

Major in Agricultural, Biosystems and Mechanical Engineering

South Dakota State University

2019

DISSERTATION ACCEPTANCE PAGE

Sadia Salam

This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ACKNOWLEDGEMENTS

I would like to express my gratitude to all those who have contributed their support one way or, other to the success of my Ph.D. dissertation.

I would like to thank my major advisor, Dr. Rachel McDaniel, for her continuous support, time, understanding and guidance she gave throughout my time at South Dakota State. I want to extend my sincere thanks to my committee member Dr. Bruce Bleakley, Dr. Todd Trooien and Dr. Jayarama Gunaje for their support and cooperation.

I would like to acknowledge the funding agencies, USEPA, SD DENR and USDA Hatch projects SD00H604-15 and SD00H452-14 courtesy of the SDSU Agricultural Experiment Station for providing monetary support for this research work.

I also want to thank my husband, my parents and my family for their continuous support and encouragement throughout my Ph.D. program.

Finally, I would like to extend my gratitude to my colleague Louis, Sara, Pavan, Abhinav, Suraiya and undergraduate assistant Miranda, Brian, Zeb, Moriah of Dr. McDaniel’s research group for their help and support both in the field and laboratory.

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ABBREVIATONS

Abbreviation	Full Name
BMP	Best Management Practice
CFU	Colony Forming Unit
GI	Gastrointestinal
MPN	Most Probable Number
MST	Microbial Source Tracking
RAM	Riparian Area Management
SD DENR	South Dakota Department of Environmental and Natural Resources
SRAM	Seasonal Riparian Area Management
TMDL	Total Maximum Daily Load
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
WWTP	Wastewater Treatment Plant

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ABSTRACT

VARIABILITY OF *E. COLI* IN STREAMBED SEDIMENTS AND ITS IMPLICATION FOR WATER QUALITY

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Fecal indicator bacteria (FIB), including *E. coli*, are the number one cause of water quality impairments in the United States according to the USEPA. FIB are used as a predictor to identify the possible presence of pathogens in waterbodies. *E. coli* is a useful indicator of gastrointestinal (GI) related illnesses from contact with fresh water. While surface water is routinely monitored for water quality, streambed sediments are rarely considered as a source of FIB to the overlying water column. This study focuses on understanding the variation of *E. coli* concentrations in streambed sediments and the potential impact of sediment sources on microbial water quality impairments. A total of five sites were monitored, including four sites located on Skunk Creek and one site located on Six Mile Creek, both of which are tributaries to the Big Sioux River in eastern South Dakota. The Skunk Creek monitoring sites are abbreviated as Sk1, Sk2, Sk3 and Sk4 and cover approximately three miles along a stream reach. Sk1 is the site farthest upstream and has direct cattle access, while the other three sites are under seasonal riparian area management which is a cattle exclusion-based management practice. The monitoring site at Six Miles Creek is abbreviated as SM and a swine facility located near the site is a possible source of microbial contamination in the creek.

A total of 25 sediment samples were collected from each of the five sites by creating a 5×5 grid. The detailed spatial grid provided insight into the variation of *E. coli* in sediment throughout the stream cross section. Samples were processed two times, within 8-hour and 24-hour of sample collection, to understand temporal stability of *E. coli* in sediment samples and the uncertainty related to sediment storage. Samples were also analyzed for sediment texture. The results showed high spatial variation of *E. coli* in bed sediment, ranging from 4 to 997 CFU g^{-1} (8.9×10^{2} to 2.1×10^{5} CFU 100 mL^{-1}). The highest and lowest *E. coli* concentration and variability was observed at Sk1 and Sk4, respectively. Pockets of high *E. coli* concentrations were measured at all sites, and were typically located at the edge of the stream with the exception of Sk1 where the high pockets of *E. coli* were located in the middle of the stream, likely due to direct deposition of fecal matter by cattle. Due to the high variability of *E. coli* in stream sediment, large sample sizes of 4 to 65 samples were required to appropriately represent *E. coli* in sediment with a moderate margin of error (E= 60 CFU g^{-1}) at a 95% confidence interval. Sediment holding times up to 24-hour after collection will not result in a statistically significant change in a majority of cases (80%). There was a strong correlation between the quantity of fine particles and *E. coli* concentration in sediment, though the direction was inconsistent.

The four monitoring sites in Skunk Creek were also monitored over the course of two recreation seasons and surrounding a series of storm events. Three to five sediment samples were collected from May to October to analyze seasonal variation for a two-year period (2017-2018). Sk2 and Sk4 were selected for storm event monitoring which included nine samples collected in a 3×3 grid from both sites prior to, after, and five to

seven days after the storm events. A significant reduction of *E. coli* concentrations in sediment were observed in 2018 compared to 2017 at the cattle crossing site. All sites showed higher *E. coli* in the late season (August to October) compared to the early season (May to July). Among the four storm events monitored, only one resulted in a noticeable hydrological response and a corresponding significant increase in *E. coli* concentrations in both water and sediment samples.

The highest median *E. coli* concentration was observed at Sk3 (85 CFU g^{-1}) followed by Sk1 (53 CFU g^{-1}), while Sk2 and Sk4 showed significantly lower *E. coli* concentrations in the sediment. Higher attachment rates of *E. coli* were observed in the sediment as compared to the water column. The sediment attachment rate to settleable particles (> 0.004 mm) ranged from 37% to 78% and was highest at Sk2 and Sk3. Phenotypic antibiotic resistance was measured at Sk1 and Sk2, and Sk2 demonstrated a significantly higher proportion of *E. coli* isolates resistant to ampicillin, tetracycline and sulfisoxazole, while erythromycin resulted in no significant change. In addition, the average organic content ranged from 4.2% to 8.2% and, overall, the organic content had little correlation with *E. coli* concentrations in sediments. However, there was a significant positive relationship between sediment organic matter and sediment *E. coli* concentrations taken from the middle of the stream at Sk1 and Sk2, possibly due to direct fecal deposit from cattle.

The findings from this study will expand the knowledge regarding sediment sources of water pollution which can be useful in developing water quality monitoring projects and strategies. Additionally, the information can be incorporated into the

development of microbial fate and transport models to better predict the contribution of streambed sediment on poor water quality.

CHAPTER 1: INTRODUCTION

1.1 Background

Fecal indicator bacteria (FIB) are natural inhabitants of the gastrointestinal (GI) system of humans and warm-blooded animals. It is not unusual to detect FIB in the natural environment. They enter the environment through feces of warm-blooded animals. Most FIB are harmless and usually not disease-causing bacteria (Haack, 2017; Myers et al., 2014), but they are associated with fecal contamination and typically used as an indicator for the possible presence of pathogens (Myers et al., 2014). Generally, FIB are used to assess the microbiological water quality as it is easy to test, can be used as a surrogate for many other pathogens, and is effective for both fresh and marine aqueous environments (Haack, 2017; Myers et al., 2014). *Escherichia coli* (*E. coli*) has been identified as a useful predictor for GI illness in fresh water environments, while enterococci are useful predictors for marine environments (Wade et al., 2003).

Historically it was thought that FIB do not live long in the natural environment, as their primary environment is the GI tract of warm-blooded animals (Haack, 2017). Thus, their presence in the environment is thought to indicate fresh fecal contamination (Garzio-Hadzick et al., 2010; Wade et al., 2003). But recent research shows that FIB can adapt to the natural environment given suitable conditions (Pachepsky and Shelton, 2011 ; Ishii et al., 2006), including the availability of nutrients, temperature, predation, UV radiation and oxygen concentrations (Craig et al., 2004; Hughes, 2003; Thomas et al., 1999; Davies et al., 1995). The most common sources of FIB released to the environment are livestock, wildlife, wastewater treatment plants, leaking septic systems, storm drains, and pets.

According to the United States Environmental Protection Agency (USEPA), fecal indicator bacteria, including *E. coli*, are the leading cause of water quality impairments in rivers and streams within the United States (USEPA, 2019). A waterbody is impaired when it cannot meet its designated beneficial uses (East Dakota Water Development District, 2005). Beneficial uses can vary from drinking water supply, irrigation, recreational, limited contact recreational, fish and wildlife propagation and industrial (USEPA, 2018a). Under the Clean Water Act, the USEPA has set water standards for waterbodies and implements pollution control programs to reach its standard (USEPA, 2009).

While many common sources of microbial pollution have been monitored to understand their influence on water quality, streambed sediments are often overlooked as potential sources of FIB to the water column. The FIB can survive in the water for a few hours to several days but can survive longer in sediment, from days to months (Haack, 2017; Garzio-Hadzick et al. 2010; Czajkowska et al. 2005). FIB decay rates depend on their adaptation to a specific environment (Pachepsky and Shelton, 2011 ; Ishii et al., 2006). Streambed sediment is one such environment which can nurture and harbor bacteria (Jamieson et al., 2005b; McElhany and Pillai, 2011). Protection from predators (Jamieson et al., 2005a;b), organic matter and nutrient availability (Jamieson et al., 2005a;b), and protection from sunlight (Koirala et al., 2008) makes sediment more favorable to bacterial survival and growth (Garzio-Hadzick et al., 2010). In addition to surviving for long periods in the sediment, some strains of *E. coli* have adapted to become indigenous to the environment (Ishii et al., 2006). Sediment concentrations of FIB can range from 100 to 1000 times higher than the water column (Karbasdehi et al.,

2017; Norman et al., 2013; Van Donsel and Geldreich, 1971). Thus, streambed sediment is a reservoir for FIB (Pachepsky and Shelton, 2011).

When the streambed sediments are disturbed, the sediments and associated FIB can resuspend into the water column, thus contributing to poor water quality (Pandey and Soupir, 2014; Bai and Lung, 2005). Streambed sediment can be disturbed through storm events (Pandey and Soupir, 2014; Fries et al., 2006; Nagels et al., 2002), recreational activities such as swimming (Roslev et al., 2008; An et al., 2002), animals crossing the stream (Abia et al., 2017; Sherer et al., 1988), shipping (Pettibone et al., 1996), or other disturbances such as raking (Abia et al., 2017). The resuspension of FIB to water column may appear as recent fecal contamination, while it is actually re-entry of FIB from sediment-bed storage. This may provide inaccurate information on water quality assessments (Haller et al., 2009 a,b).

Although the resuspension of sediment can significantly impact microbial water quality, bacterial association with particles in stream sediment have not been well studied. Bacteria can be attached to particles or remain unattached (Pachepsky et al., 2006; Thurston-Enriquez et al., 2005; Jamieson et al., 2004; Fiener and Auerswald, 2003). Attached bacteria can settle quickly and can be removed by sedimentation, but by associating with bacteria, sediment increases bacterial survival and persistence in the environment (Haack, 2017; Characklis et al., 2005). On the other hand, unattached bacteria are buoyant and can travel farther from their origin, but FIB cannot survive as long as free cells in the environment (Haack, 2017; Characklis et al., 2005). Thus, knowledge about the bacterial association with particles is important to understand

bacterial fate and transport (Jamieson et al., 2005a; Pachepsky and Shelton, 2011), and determine potential removal mechanisms (Kunkel et al., 2013; Characklis et al., 2005).

In addition to resuspension, runoff, direct fecal deposits, and point source pollution are other ways of microbial transport to natural water. There are many management practices that have been used to reduce fecal contamination in waterbodies. Riparian area management (Parkyn, 2004), vegetative treatment systems (Harmel et al., 2018a), cattle exclusion (Bragina et al., 2017; Smolders et al., 2015), and controlled tile drainage (Sunohara et al., 2016; Wilkes et al., 2014; Sunohara et al., 2014) are some of the best management practices (BMP) commonly used for reducing FIB from waterways.

1.2 Goals and Objectives

The overall goal of this study is to understand the variability of *E. coli* in streambed sediment and its potential impact on microbial water quality impairments. The objectives of the study are to:

i. Assess the variability of *E. coli* in streambed sediment across the stream cross-section and determine the implications for sediment sampling;
ii. Monitor *E. coli* variability in stream sediment during a range of temperature and flow conditions;
iii. Examine the variation of *E. coli* along a stream reach to understand reach-specific differences in *E. coli* concentrations; and
iv. Evaluate the impact of direct cattle access on *E. coli* concentrations in streambed sediments.

1.3 Hypotheses

The hypotheses for this study are:

i. The spatial variation of *E. coli* in stream sediment is high;

ii. Seasonal change has a significant impact on the *E. coli* concentration in streambed sediment; and

iii. Limiting direct access of cattle can reduce *E. coli* from bed sediment.

1.4 Thesis Organization

To achieve the project goal, both field and laboratory studies were performed. Chapter 2 contains the literature review on the impact of streambed sediment as a source of microbial water quality impairment. Chapter 3, Chapter 4 and Chapter 5 are papers containing detailed information about the results of *E. coli* in stream sediment and its potential impact on water quality. Chapter 6 contains the project's conclusions including the implications and recommendations for future work to expand knowledge with further research.

CHAPTER 2: LITERATURE REVIEW

2.1 Fecal Indicator Bacteria (FIB) and their Impact on Water Quality

2.1.1 FIB

The USEPA reported FIB are one of the major causes of water quality impairments in the United States (USEPA, 2000) making it a major focus in the environmental research (Pachepsky and Shelton, 2011). The FIB are bacteria which live in the gut of warm-blooded animals and enter the environment via fecal matter. Though they are generally not disease causing, they are associated with fecal contamination and the possible presence of pathogens (i.e. disease-causing bacteria) which also live in the human and animal intestinal systems. Thus, the presence of FIB in streams indicates the possible presence of pathogenic microorganisms (Myers et al., 2014). It is difficult, expensive, and time-consuming to detect all varieties of pathogens in the environment. The FIB are easier to isolate and detect, are present in greater numbers than pathogens, and are safer to work with than pathogens (Mubiru et al., 2000; Tate et al., 2000). Hence, FIB are generally used as a surrogate for measuring pathogens in environmental samples, such as water and soil (Elmund et al., 1999; Rochelle-Newall et al., 2015). An ideal fecal indicator is able to predict illness accurately and consistently within a variety of environments (Wade et al., 2003). Common FIB include *E. coli* and enterococci which are useful predictors of gastrointestinal (GI) illness in freshwater and marine environments, respectively (Wade et al., 2003).

2.1.2 FIB and Human Health

Human exposure to contaminated waters poses a serious threat to human health because of the possible presence of human enteric pathogens (Borade et al., 2014, Scott et al., 2003). An outbreak analysis by Yoder et al., (2008) reported 19 out of 20 outbreaks of waterborne diseases in recreational waters occurred from untreated waters from 2005 to 2006. Past studies have also observed an association between FIB and illness of beach swimmers (Colford et al., 2007; Wade et al., 2006; Wade et al., 2003). For example, Marion et al. (2010) performed a study on identifying the GI illness risk of inland recreational water users. They found 48 individuals out of 965 were affected by GI-related illness (Marion et al., 2010), and of these affected individuals, the exposed and unexposed individuals to recreational waters were 45 out of 806 and 3 out of 159, respectively. Similar results were observed by Dufour (1984) in a freshwater bathing beach study where they found GI illness incidence of 38 to 61 cases per 1000 swimmers and 19 to 53 cases per 1000 non-swimmers (Dufour, 1984). Another study compared the proportion of swimmer to non-swimmer symptoms of GI illness and concluded that even exposure in a lightly contaminated water increased the risk for GI illness (Cabelli et al., 1982). In short, swimmers reported more GI-related illness symptoms than non-swimmers.

Furthermore, FIB, such as *E. coli,* are used as a predictor of fecal contamination in the freshwater environment by the USEPA (USEPA, 1986; Dufour, 1984) as direct contact with waters containing high FIB, like *E. coli,* has been linked to an increased risk of GI illness (Abhirosh et al., 2010; Marion et al., 2010; Cabelli et al., 1982). For example, Wade et al. (2003) comprehensively analyzed 27 epidemiological studies which

included 247 to 26,686 participants that linked specific microbial indicators with health outcomes. In the case of GI illness, there was a high correlation (0.86) with *E. coli,* indicating GI illness is a function of *E. coli* density (Wade et al., 2003). These results supported the USEPA's position on identifying the risk of fecal contamination by using *E. coli* to reduce GI illness risk.

2.1.3 Water quality impairment due to FIB

The presence of FIB in waterbodies can cause water quality impairments, depending on the designated use. These impaired waterbodies are a concern for both human health and aquatic life and cannot fulfill their designated beneficial use. Beneficial use is the benefits to be gained from a waterbody. FIB alone threaten over 100,000 miles of rivers and streams in the United States (USEPA, 2018b) and are the leading cause of water quality impairments in the assessed rivers and streams across the nation (USEPA, 2000b, USEPA, 2002, USEPA, 2004).

2.1.4 Water Quality Standards

Since direct contact with water contaminated with fecal material is harmful to human health, it is important to have a standard value for FIB in waterbodies, which indicates fecal contamination (USEPA, 2012). The water quality standard for FIB is used to determine the ability of the waterbody to fulfill its designated or assigned uses, such as recreational activities, public water supply, and/or aquatic life (USEPA, 2018a). To protect human health and aquatic life, FIB standards for recreational waterbodies have been developed. The water standards are set by states, territories, local or federal law, and must be approved by USEPA. The water quality standards provide a legal basis to control

pollutants introduced to the waterbodies (USEPA, 2018a). Usually states, territories, local and federal authorities follow similar steps as described:

To meet USEPA approval, the states, local authority, or federal authority must include four basic elements in their proposed water quality standard (USEPA, 2018a) as follows:

i. Designated uses of the waterbody;

ii. Water quality criteria to protect designated uses;

iii. An antidegradation policy to maintain and protect existing uses and high-quality water; and

iv. General policies addressing implementation issues.

The designated beneficial uses can vary from public drinking water supply, recreational, protection and propagation of fish, shellfish, wildlife, agricultural, navigational, industrial and other purposes (SDDENR, 2004). The USEPA develops water quality criteria for ambient waterbodies so the quality of water can reflect the up-to-date scientific knowledge on the effects of contaminants on human health and the environment (USEPA, 2018a). These criteria include aquatic life, biological, human health, recreational/ microbial, suspended and bedded sediment criteria (USEPA, 2018a). The USEPA has set water quality standards for FIB, including *E. coli*. The primary contact recreation standard for *E. coli* is a geometric mean of 126 CFU 100 mL^{-1} (USEPA, 1986).

2.2. Microbial Pollution Sources

The transmission of pollutants to the natural environment is classified in two ways: (i) point source pollution and (ii) non-point source pollution. Point sources are any identifiable single pollution source which discharges pollutants, while non-point source pollution is spread out and known as “diffused source” (USEPA, 2018b).

2.2.1 Point Sources

Point source pollution is defined as one single identifiable pollution discharge that can be traced back to a pipe. Examples of point sources include wastewater treatment plant outfalls (Templar et al., 2016, Cho et al., 2010a, Haller et al., 2009a, Garcia-Armisen and Servais, 2009 and Petersen et al., 2005), industrial and municipal discharge (Karbasdehi et al., 2017; Borade et al., 2014; Ouattara et al., 2011; Cho et al., 2010a and Haller et al., 2009a), sewer outflows (Petersen et al., 2005 and Kay et al., 2008), failed or leaking sewer systems (Sercu et al., 2011; McLellan et al., 2007; Weiskel et al., 1996), and storm drains (Haack et al., 2003).

The FIB within point sources are transported through pipes or conduits which makes them concentrated potentially leading to high concentration (Petersen et al., 2005). Literature has observed point source pollution dominates the contamination to waterbody during dry weather periods (Petersen et al., 2005; Stein and Tiefenthaler, 2005). Such as, Petersen et al., (2005) observed both wastewater treatment plant (WWTP) and storm sewer discharge dominated during dry weather conditions, while non-point source pollution was relatively consistent throughout the year. The highest point source FIB load in reviewed literature was observed by Petersen et al. (2005) for a WWTP and storm sewer that had a measured average *E. coli* load of 1.4×10^{12} MPN and 6.6×10^{10} MPN per

day, respectively. Overall, past literature observed higher FIB concentration from pollution sources of WWTP followed by sewer outflow and storm drains. The observed range of FIB concentration for WWTP, raw sewage and storm drain or storm outfall were 10^6 to 10^7 per 100 mL (Garcia-Armisen and Servais, 2009), 10^5 to 10^6 per 100 mL (Hyer, 2007), 10^3 to 10^5 per 100 mL (Ellis and Butler, 2015; Sauvé et al., 2012; Irvine et al., 2011; Lewis et al., 2005; Stein and Tiefenthaler, 2005; Schiff and Kinney, 2001; Schillinger and Gannon, 1985), respectively.

Regardless of the type of point sources, the observed average FIB concentration in the literature was at least 10^2 per 100 mL. For example, Lewis et al. (2005) reported an average total coliform concentration in storm drains and gutters of 2.4×10^4 and 7.8×10^2 CFU 100 mL^{-1}, respectively. Another study on sewer misconnection by Ellis and Butler (2015) found *E. coli* concentration was 44×10^4 MPN 100 mL^{-1} from storm water outfall. In addition, the range of *E. coli*, fecal coliforms, total coliforms and enterococci concentrations in the reviewed literature were 10^2 to 10^7 per 100 mL, 10^0 to 10^6 per 100 mL, 10^2 to 10^5 per 100 mL and 10^1 to 10^6 per 100 mL, respectively (Sauvé et al., 2012; Irvine et al., 2011; Garcia-Armisen and Servais, 2009; Hyer, 2007; Reeves et al., 2004; Stein and Tiefenthaler, 2005; Schiff and Kinney, 2001; Marino and Gannon, 1991; Schillinger and Gannon, 1985).

2.2.2 Non-point Sources

In the United States, FIB contamination in waterbodies is a national concern (USEPA, 2018a; Petersen et al., 2005). Generally, it is difficult to trace the contribution of non-point sources of pollution to water quality impairments due to the diffused and diverse sources of bacteria sources in the environment. The National Pollutant Discharge

Elimination System (NPDES) and National Research Council (NRC) control point source pollution and assess the total maximum daily load (TMDL) program, respectively. However, non-point sources are left unchecked. Also, it is challenging to identify the origin of non-point sources of pollution as it is widespread and highly variable both spatially and temporally (Bradford et al., 2013).

Examples of non-point sources include agricultural runoff (Garzio-Hadzick et al., 2010; Oun et al., 2014), stormwater runoff (Curtis and Trapp, 2014); livestock (Sherer et al., 1988; An et al., 2002; Davies-Colley et al., 2004; Oun et al., 2014; Smolders et al., 2015; Abia et al., 2017); manure (Davies-Colley et al., 2004; Oun et al., 2014); wildlife (Wilson et al., 2016; Whitlock et al., 2002; Schiff and Kinney, 2001; Weiskel, et al., 1996); and recreation (Orear and Dalman, 2011; Wilson et al., 2016; Roslev et al., 2008 and Stumpf et al., 2010).

2.2.2.1 Agriculture

Agriculture is the second most probable source of pollution for impaired waterbodies in the United States (USEPA, 2018b). This includes microbial pollution in streams which has a strong correlation with agricultural development (Goss and Richards, 2008 and Roser and Nicholas, 2005). Vant (2001) found that 70-80% of fecal bacteria load in the Waikato River, New Zealand was from agriculture, mostly resulting from runoff from intensive pastural farms.

In agricultural watersheds, runoff from pastureland, manure applied land, and wildlife areas carry fecal contamination to surface water which can subsequently be deposited into bed sediments (Garzio-Hadzick et al., 2010). The pollution sources in agricultural settings consist of livestock grazing or crossing the stream, animal feeding

operations, animal housing, manure application in the field, manure storage units, and manure applied to fields (USEPA, 2018; Garzio-Hadzick et al., 2010; Oun et al., 2014).

2.2.2.1.1 Livestock

Livestock is a major concern for microbial water quality and can be a source of FIB to the stream (Webster et al., 2004), as demonstrated by the strong correlation between *E. coli* concentrations and the presence of cattle (Valcour et al., 2002 and Michel, 1998). Livestock waste can carry many pathogenic organisms (Becher et al., 2004 and Coklin et al., 2007) and direct defecation from livestock as well as runoff containing livestock-derived fecal material (Davies-Colley et al., 2004 and Wilcock et al., 2007) can result in human health concerns (Becher et al., 2004 and Coklin et al., 2007).

Unrestricted or direct contact of livestock with waterbodies increases FIB both in the water column and sediment (Davies-Colley et al., 2004; Byers et al., 2005; Smolders et al., 2015; Bragina et al., 2017). For example, Davies-Colley et al. (2004) examined a cattle herd crossing a stream and found cows defecated 50 times more per unit length in the stream than elsewhere on the raceway. The *E. coli* concentration measured in the water column was 166-fold higher while the cattle had access to the stream when compared to the background *E. coli* concentration. Similarly, Vidon et al., (2008) reported *E. coli* concentrations that were 36-fold higher in the water column during the summer when cattle had direct access to the stream as compared to spring when cattle are rarely near the stream. Not only does cattle access impact microbial contamination, but the density of livestock that have access to the stream also influences the FIB concentrations. Aitken (2003) assessed risk of 117 farms in two river catchments in

Scotland and reported a 4 to 8-fold higher *E. coli* concentration in a stream with high livestock density than a stream that had low livestock density.

Livestock are not only associated with high concentrations of *E. coli* in the water column; higher *E. coli* concentrations have also been observed in stream-bed sediment in the presence of livestock. Bragina et al. (2017) performed a study that monitored streambed sediment with and without cattle access to the stream. They observed fencing is a useful mitigation technique for reducing fecal contamination from bed sediment. Alternatively, any evidence of recent cattle presence or application of animal waste in the agricultural field elevated the *E. coli* concentration in the sediment, even in the fenced sites (i.e. without cattle access) (Bragina et al., 2017). Another study reported adding fresh fecal matter to the stream resulted in FIB deposition to the streambed sediment (Sherer et al., 1988). Thus, any disturbances of the bed sediment, such as livestock crossing the stream or high flow can contribute to impairments via resuspension of sediment particles and the associated FIB to the water column, increasing FIB concentration in the water by over one order of magnitude (Abia et al., 2017 and Sherer et al., 1988). Furthermore, the presence of FIB in sediment can have a long-term effect on water quality impairments because sediment provides a favorable environment for FIB survival (Garzio-Hadzick et al., 2010). For example, Haack (2017) reported that FIB in water can survive for a few hours to days; while in sediment, survival increased to days or months.

2.2.2.1.2 Manure

Though animal manure is a good source of nutrients for row crop agriculture and a natural way of reusing waste, if manure is stored and handled improperly it can

contribute to microbiological water quality impairments. Livestock manure contains high concentrations of FIB (McDaniel et al., 2013; Davies-Colley et al., 2004; Thelin and Gifford, 1983) with mean *E. coli* concentrations in one cow pat ranging from 12×10^{6} CFU g^{-1} wet weight (Davies-Colley et al., 2004) to 1.1×10^{9} CFU g^{-1} (McDaniel et al., 2013). Witzel et al. (1966) measured FIB in bovine manure and observed total coliform concentrations ranging from 3.4 to 5.6×10^{5} MPN g^{-1}, fecal coliform concentrations ranging from 3.2 to 5.6×10^{5} MPN g^{-1}, and fecal streptococci concentrations ranging from 3.5 to 5.6×10^{5} MPN g^{-1}. High fecal coliform and fecal streptococci concentrations were also found by Maki and Picard (1965) who measured 6×10^{5} MPN g^{-1} and 6×10^{5} MPN g^{-1} for fecal coliform and fecal streptococci, respectively.

There are several pathways for pollution from animal waste to reach waterbodies, including runoff and leaching from manure applied land, manure storage facilities, feedlots and animal housing (Bragina et al., 2017; Oun et al., 2014; Sherer et al., 1988). When manure is applied to agricultural fields, runoff can transport the manure to nearby waterbodies which, in turn, results in elevated FIB concentrations in the water column. This is supported by Jenkins et al. (2005), who assessed the microbiological runoff from a manure-applied agricultural land and reported the average *E. coli* concentration was 2.9 $\log_{10}$ MPN 100 mL^{-1}, the average total coliform concentration was 5.2 $\log_{10}$ MPN 100 mL^{-1}, and the average fecal streptococci concentration was 1.1 $\log_{10}$ MPN 100 mL^{-1} in runoff water. Another study by Thurston-Enriquez et al. (2005) examined three different types of manure, including fresh cattle manure, aged cattle manure, and swine manure slurry, and observed FIB loads ranging from 10^{5} to 10^{9} CFU in fresh manure, 10^{4} to 10^{8}

CFU in aged manure, and 10^5 to 10^8 CFU in swine manure slurry during rainfall events (Thurston-Enriquez et al., 2005).

Like the water column, stream-bed sediment can also be affected by runoff from manure-applied agricultural land. Bragina et al. (2017) monitored *E. coli* in bed sediment in catchments with intensive cattle production. Monitoring was conducted for both fenced (i.e. no cattle access) and unfenced streams (i.e. direct cattle access) and compared to the result of a low-density agricultural catchment. The study showed significantly higher *E. coli* in sediment in high density agricultural catchments, even in the fenced stream, as compared to the low density agricultural catchment, likely due to the runoff from the manure-applied agricultural fields (Bragina et al., 2017).

Elevated FIB concentrations are also found in tile drainage water from manure-applied agricultural lands (Ball Coelho et al., 2007 and Geohring et al., 1998). Pappas et al., (2008) assessed the impact of manure application on FIB concentration in subsurface tile drain water for a three-year period and compared the results to subsurface drainage water without manure treatment in the overlying land. Significantly higher *E. coli* and enterococcus concentrations in tile drainage water were reported where manure had been applied when compared to the no manure treatment. In addition, the timing of manure application also impacts FIB concentrations observed in tile drainage water, with the highest concentrations occurring immediately following manure application to the field (Geohring et al., 1998).

2.2.2.2 Wildlife and Pets

Wildlife, such as waterfowl and raccoons, and pets, such as dogs, can substantially contribute to FIB in waterways (Whitlock et al., 2002; Schiff and Kinney,

2001 and Weiskel et al., 1996). For instance, in a study on a recreational lake, researchers observed higher *E. coli* concentrations in samples from areas near the boat dock due to the presence of wildlife, such as ducks, who were living around the boat-launching facilities (An et al., 2002). In another study, Whitlock et al. (2002) studied the identification of fecal contamination sources in Stevenson Creek throughout a season from June to December. They observed higher wildlife contribution during the wet season (June and July) as compared to the dry season (December). The study reported the range of fecal coliform counts contributed by wildlife at five locations were 1600 to 20,000 CFU 100 mL^{-1}, 1320 to 10,620 CFU 100 mL^{-1}, 0 to 1 CFU 100 mL^{-1}, 0 to 115 CFU 100 mL^{-1}, 0 CFU 100 mL^{-1} in June, July, August, September, October and December, respectively (Whitlock et al., 2002).

Wildlife can provide substantial fecal contamination in different aquatic environments. For example, Woodruff et al. (2009) performed a microbial source tracking study and observed a higher contribution of wild mammals (e.g. deer, rabbit, raccoon, elk, otter, beaver, rodents and marine mammals) to *E. coli* contamination in freshwater than marine water. The study found 189 (26.2%) out of 719 and 60 (22.6%) out of 265 *E. coli* isolates were contributed by wild mammals in freshwater and marine water, respectively. In addition, Renter et al. (2001) analyzed fecal samples and identified *E. coli* O157:H7 in 0.25% of the collected samples (7 out of 1426) from free-ranging deer in Nebraska.

The contribution of pets to fecal contamination can be elevated in urban areas. For example, Whitlock et al. (2002) observed elevated fecal coliforms from dogs near a park with concentrations ranging from 0 to 1430 CFU 100 mL^{-1}. Parks in urban areas usually

have high populations of dogs, and their feces are frequently left on the ground rather than being properly disposed of, resulting in increased FIB concentrations in nearby waterways (Whitlock et al., 2002). Also, Woodruff et al. (2009) found domestic animals (i.e. dog, cat) contributed to *E. coli* contamination in both marine and freshwater. The study reported 64 out of 719 and 16 out of 265 *E. coli* isolates were contributed by domestic animals in freshwater and marine water, respectively. Geldreich et al. (1962) quantified FIB concentrations in fecal samples from cats and dogs. Cat feces contained 8×10^{6} MPN g^{-1} of fecal coliform and 2.7×10^{7} MPN g^{-1} of fecal streptococci, while dog feces contained 2.3×10^{7} MPN g^{-1} of fecal coliform and around 1×10^{9} MPN g^{-1} of fecal streptococci.

2.2.2.3 Sediment

Sediment is a non-point source of pollution (USEPA, 2018b). FIB concentrations have been measured up to 10^{6} CFU $100g^{-1}$ in the surface layer of sediment (Haller et al., 2009b). Bacteria can either associate with sediment particles, or remain unattached (Pachepsky et al., 2006; Thurston-Enriquez et al., 2005; Jamieson et al., 2004; Fiener and Auerswald, 2003). The unattached bacteria are buoyant and can be transported longer distances (Jamieson et al., 2005b), while bacteria associated with sediment particles can settle out of the water column more rapidly (Characklis et al., 2005).

Bacteria can survive for longer time periods in sediment than the overlying water (Craig et al., 2004; Haller et al., 2009b and Anderson et al., 2005) because sediment provides more favorable conditions, including higher carbon and nutrient concentrations (Jamieson et al., 2005a; Jamieson et al., 2005b), as well as protection from sunlight and protozoan grazing (Pachepsky and Shelton, 2011). Consequently, FIB concentrations in

the sediment can be 100 to 2500-fold higher than the water column (Brinkmeyer et al., 2015; Pandey and Soupir, 2013; Byappanahalli et al., 2012; Pachepsky and Shelton, 2011, Roslev et al., 2008 and An et al., 2002; Crabill et al., 1999). In a 3-year water quality monitoring study by Crabill et al., (1999), the mean water FIB concentrations ranged from 10^1 to 10^2 CFU 100 mL^{-1} while the mean sediment FIB concentrations ranged from 10^5 to 10^6 CFU 100 mL^{-1}, which was, on average, 2200-fold higher than the water column. Similarly, another study by Liao et al. (2014) reported a monthly geometric mean *E. coli* concentration in the sediment 40 to 350 times higher than *E. coli* concentrations in the water column, and Roslev et al. (2008) observed FIB concentrations 281 to 2500-fold higher in sediment than water column at a recreational beach.

Streambed sediments are a concern because when the FIB reservoirs get disturbed, FIB can resuspend to the water column and contribute to water quality impairments (Crabill et al., 1999). The bed-sediment can be disturbed by storm events (Pandey and Soupir, 2014), recreational activities, such as boating, bathing and swimming (An et al., 2002), livestock grazing or crossing the stream (Sherer et al., 1988 and Abia et al., 2017), dredging (Grimes, 1980), and the passage of boats or ships (Pettibone et al., 1996). Although resuspension occurs from bed-sediment where bacteria can survive for a long time, when FIB resuspend, it can be mistaken for recent fecal contamination (Curtis and Trapp, 2014). The FIB can survive in sediment for days to months, while survival in water is only a few hours to days (Haack, 2017).

2.2.3 Microbial Source Tracking (MST)

Microbial source tracking (MST) is used to trace the sources of fecal pollution. MST is a method developed to identify microbes of a specific host in a waterbody; thus,

unlike FIB, it can differentiate between different sources and characterize sources of fecal contamination (Kephart et al., 2019; Bradshaw et al., 2016). This technique helps water managers by enabling them to apply source-appropriate treatment approaches to improve water quality (Kephart et al., 2019). While there is no standard method for MST analysis, one of the most common methods used is real-time quantitative Polymerase Chain Reaction (qPCR) to identify and enumerate specific host-associated markers (Kephart et al., 2019; Boehm et al., 2013).

Higher concentrations of human and pet-associated genes are found in urban environments, while rural environments typically have a mix of livestock and wildlife. For example, Whitlock et al. (2002) performed a MST study at five locations mostly surrounded by residential area and urban facilities, including a failing wastewater treatment system. Anthropogenic sources were the predominant source for 1168 isolates out of 2398 isolates, followed by dogs which were the origin of 480 isolates. Failing wastewater treatment systems and the high population density residential area with septic tanks were the major sources of fecal contamination, while the prominence of dog isolates was explained by pets commonly found throughout the residential area and nearby parks. Similarly, higher concentrations of a human associated biomarker (HF183) were observed in water samples collected from a recreational beach (Wilson et al., 2016). Among the 30 water samples collected, 11 contained the HF183 marker and the highest *E. coli* concentration (9.2×10^5 CFU 100 mL^{-1}) indicated the presence of raw sewage, which was detected by the HF183 marker (Wilson et al., 2016). Interestingly, Whitlock et al. (2002) observed genes associated with wild animals dominated samples collected immediately following rain events. A significant positive relationship was measured

between fecal coliform counts and the percentage of isolates classified as wild animals, indicating wild animals contributed to elevated fecal coliforms in the watershed. On the other hand, human-associated genes dominated samples when fecal counts were low and during the transition to drier seasons, because lower water tables may draw fecal matter from failing wastewater systems resulting in more fecal coliform with human origin. These findings of temporal variability suggested seasonal variation impacts pollution sources which should be incorporated when developing a sampling strategy (Whitlock et al., 2002).

While urban areas are dominated by human associated genes, areas with agricultural land uses have higher fecal contamination by cow (CowM3) and ruminant (Rum-2-Bac) associated markers (Bradshaw et al., 2016). Both water and sediment samples contained significantly higher CowM3 and Rum-2-Bac concentration in the monitoring sites impacted by agriculture than monitoring sites impacted by forests and water pollution control plants (WPCP) (Bradshaw et al., 2016).

Furthermore, origins of FIB isolates in sediment samples have largely been identified as wildlife. For example, Wilson et al. (2016) found both sediment samples and suspended sediment samples were dominated by the wildlife associated marker (GFD). Among the 16 sediment samples analyzed, 14 samples contained the GFD marker. Historical beach grooming practices can worsen this problem by burying fecal matter which can persist and survive longer in sediment and be resuspended to the water column by bathers (Wilson et al., 2016).

2.3 FIB Concentration in Sediment

2.3.1 FIB concentration in sediment

Sediment is a reservoir for FIB (Haller et al., 2009a; Karbasdehi et al., 2017), and provides a more favorable environment for microorganisms where they can survive for longer periods of time than in the water column (Garzio-Hadzick et al., 2010; Haller et al., 2009a; Rehmann and Soupir, 2009). Observed FIB concentrations ranged from roughly 10^1 to 10^5 CFU $100g^{-1}$, 10^1 to 10^6 CFU $100g^{-1}$, and 10^1 to 10^6 CFU $100g^{-1}$ for *E. coli,* enterococci, and fecal coliforms, respectively (Table 2.1). The highest concentration of *E. coli* in MPN gdw^{-1} reported in past studies was 6.5×10^4 MPN gdw^{-1} at the Grand Glaize Beach, Lake of the Ozarks State Park, Missouri. The primary pollution source was recreational activities, but microbial source tracking also identified waterfowl as a prominent source (Wilson et al., 2016). Waterbodies that were affected by anthropogenic sources, such as agricultural activities, recreational activities, or industrial pollution, showed higher concentrations of FIB than waterbodies that were affected by natural sources (i.e. storm water runoff). For example, Haller et al. (2009a) measured 8.5×10^5 CFU $100g^{-1}$ in the Bay of Vidy, Switzerland where identified pollution sources included sewage water and a wastewater treatment plant, whereas Curtis and Trapp (2014) reported FIB concentrations of $150 – 7.9\times10^4$ MPN $100g^{-1}$ in their storm water runoff study.

Site specific factors, such as streamflow, sampling location, land use, sediment characteristics, and weather, impact FIB concentrations in sediment (Pandey and Soupir, 2013; Pandey et al., 2012). For example, it has been argued that FIB presence in sediment may cause little or no contamination to the overlaying water during low flow conditions,

but during high flows, contamination to the overlying water from sediment can be high due to resuspension, resulting in a potential risk to human health (Pandey and Soupir, 2013). Another study observed *E. coli* concentrations in water and sediment during baseflow conditions were 27 CFU 100 mL^{-1} and 5,441CFU 100g^{-1}, but after a rainfall event the *E. coli* concentrations of water and sediment increased by 88-fold and 1.6-fold, respectively (Pandey and Soupir, 2014). Past studies have also shown that rainfall has a significantly positive impact on elevated FIB concentration (Curtis and Trapp, 2014; Staley et al., 2013), and the association between elevated FIB concentration and rainfall was stronger than FIB concentration and land use (Staley et al., 2013). This is not surprising, as storm runoff can wash out fecal contamination on land and deliver it to nearby waterbodies (Brownell et al., 2007; Parker et al., 2010).

Higher FIB concentrations in sediment have been observed in urban environments when compared to rural environment. For example, Staley et al. (2013) studied microbial water quality in lakes with different land uses, and observed significantly higher FIB concentration in sediment from urban lakes than undeveloped or cattle impacted lakes. Impervious surfaces, such as concrete, in urban environments occupy more surface area, resulting in greater runoff which may lead to bacterial accumulation in streambed sediment (Staley et al., 2013). Another study by Pandey et al. (2018) measured sediment FIB concentrations that were one order of magnitude higher in sediments adjacent to mixed land uses (urban and rural) than recreational beaches. The study reported *E. coli* concentrations in the sediment ranged from 10^{10} to 10^{13} CFU 100 mL^{-1} in Squaw Creek, which is impacted by agricultural, residential, and industrial areas, and 10^{9} to 10^{12} CFU

100 mL^{-1} in the Yosemite River which is impacted by mostly recreational users (Table 2.1) (Pandey et al., 2018).

FIB concentrations also vary substantially by depth (Karbasdehi et al., 2017; Garzio-Hadzick et al., 2010). The top few centimeters of sediment play a crucial role in bacterial resuspension, as this layer is the most easily disturbed by events such as storms, livestock, and wildlife (Garzio-Hadzick et al., 2010; Pachepsky and Shelton, 2011). The FIB concentrations in streambed sediment vary with depth, with the highest FIB concentrations in the top few (i.e. < 3) centimeters (Karbasdehi et al., 2017; Brinkmeyer et al., 2015; Haller et al., 2009 a,b; Garzio-Hadzick et al., 2010; Desmarais et al., 2002). Sediment *E. coli* concentrations also decrease by one order of magnitude per 2 cm of sediment sampling depth (Haller et al., 2009b). Haller et al. (2009b) examined the difference of FIB concentrations at different depths within the sediment profile, and found *E. coli* and enterococci concentrations were 10^6 CFU $100g^{-1}$ in the surface layer of the sediment bed, while at 8-10 cm, it decreased two orders of magnitude with a range of 10^4 to 10^5 CFU $100g^{-1}$. Similarly, Desmarais et al. (2002) reported sediment *E. coli* concentrations were below the detection limit in sediment core samples below the top 5 cm, while, enterococci did not decrease with depth as drastically as *E. coli*.

One reason for the FIB abundance in the top layer of sediment is the presence of nutrients and the periodic renewal of bacteria from runoff (Garzio-Hadzick et al., 2010). In addition, Haller et al., (2009b) reported that sediment organic content decreased with increasing depth, with the organic content at 25% in the first two centimeters and decreasing to 15% at a depth of 10 cm.

There is high variability of FIB in stream sediment, and it is not unusual to have a three order of magnitude difference between the highest and lowest concentration of FIB in sediment from the same watershed or even the same sampling location (Pachepsky and Shelton, 2011; Cho et al., 2010a). For example, Cho et al. (2010a) measured *E. coli* concentrations in sediment that were five times higher in the upstream reach (~49×10^5 MPN kg^{-1}) than the downstream reach (8.4×10^5 MPN kg^{-1}) in a tributary of Beaver Dam Creek (Table 2.1). Also, replicates of sediment samples showed higher variability than replicates of water samples (Anderson et al., 2005). Despite these reports on the high spatial variability of FIB in stream sediment, past studies have not focused on understanding FIB concentration across the stream transect.

Table 2.1: This table presents concentrations of FIB in sediment from past literature. The pollution sources are mainly agricultural, recreational activities, human activities, and storm water runoff. The units used are inconsistent and presented per 100 mL, gram dry weight (gdw), or grams, thus limiting the comparability between studies.

Paper	Region	Pollution Source	Medium	Sampling Depth	range/ average	Units
Stocker et al., (2019), *E. coli*	Little Paint Branch Creek, Maryland, USA	Plant compost	Sediment	1 cm	13 - 238	CFU gdw^{-1}
Stocker et al., (2019), enterococci					16 - 543	CFU gdw^{-1}
Pandey et al., (2018)*, *E. coli*	Yosemite, Merced River, CA, USA	Recreational users	Sediment		5.7×10^{9} – 2.9×10^{12}	CFU 100 mL^{-1}
Pandey et al., (2018)*, *E. coli*	Squaw Creek, Iowa, USA	agriculture, animal feeding operation, industrial, residential area etc.	Sediment		7.8×10^{10} - 17.8×10^{12}	CFU 100 mL^{-1}
Kim et al., (2010)	Little Cove Creek watershed, Pennsylvania	agricultural area, livestock (cows, cattles and horses)	sandy and sandy to silty texture	1 cm	10 - 1×10^{4}	CFU100 mL^{-1}
Abia et al., (2015a)	Apies River, South Africa	Pretoria City	sediment	5 cm	(geomean) 0.59 – 4.53	Log_{10} MPN 100 mL^{-1}
Piorkowski et al., (2014b)	Thomas Brook Watershed, Nova Scotia, Canada	agriculture, agri Food	sandy loam	0 - 5 cm	30-13360 (6.0 ln CFU g^{-1})	CFU g^{-1}
(Perkins et al., 2014) *E. coli*	River Conwy, UK	No large point sources	River Sediment		0-2.4×10^{4}	CFU 100 g^{-1}
Perkins et al., (2014) Total Coliform	River Conwy, UK	No large point sources	River Sediment		0-5.4×10^{5}	CFU 100 g^{-1}
Perkins et al., (2014) enterococci	River Conwy, UK	No large point sources	River Sediment		0-1×10^{6}	CFU 100 g^{-1}

Cho et al., (2010a), *E. coli*	Mid Atlantic Coastal plain of Maryland, USA	agricultural field, deciduous forest	sediment	1 cm	8.39×10^{5} - 48.93×10^{5}	MPN kg^{-1}
Orear and Dalman, (2011) *E. coli*	Chattahoochee River, GA, USA	recreational sites, non-recreational sites	riverbed sediment		80 - 408	MPN $100ml^{-1}$
Kovacic et al., (2011), *E. coli*	Žrnovnica River, Croatia	Public Water Supply and agriculture area	sediment		4.9 – 68.9	CFU 100 mL^{-1}
Kovacic et al., (2011), Total Coliforms	Žrnovnica River, Croatia	Public Water Supply and agriculture area	sediment		481.4 – 41.05×10^{4}	CFU $100mL^{-1}$
Kovacic et al., (2011), Fecal Coliforms	Žrnovnica River, Croatia	Public Water Supply and agriculture area	sediment		5.5 – 59.4	CFU $100mL^{-1}$
Kovacic et al., (2011), enterococci	Žrnovnica River, Croatia	Public Water Supply and agriculture area	sediment		8.7 - 345	CFU $100mL^{-1}$
Wilson et al., (2016), *E. coli*	Grand Glaize Beach, Lake of the Ozarks state park, Missouri	recreational sites, humans, pets and wild animals	sediment	5 m	2 - 6.5×10^{4}	MPN gdw^{-1}
Ge et al., (2010), *E. coli*	Southwest shore of Lake Michigan	Chicago 63rd street Beach	sediment (submerged sand)		(median) 10^{3} - 10^{4}	CFU 100 mL^{-1}
Curtis and Trapp, (2014)	Withers Swash, Myrtle Beach, South Carolina	storm water runoff (commercial, facilities, park, campgrounds and residential developments)	sediment	5 cm	1.5 - 794.6	MPN g^{-1}
Ouattara et al., (2011)	Scheldt watershed, Belgium	High population density, intense industrial activities, agriculture and breeding	sediment	10 cm from the surface	2.1×10^{2} - 3.3×10^{5} (geomean)	*E. coli* g^{-1}

Abhirosh et al., (2010), Fecal coliform	Vembanadu Lake, India	agriculture (rice field), human dwellings, fishing, transportation and recreation	sediment		1.1×10^{5} - 9.88×10^{5}	MPN g^{-1}
Roslev et al., (2008), enterococci	Ringkobing Fjord, west coast of Jutland, Denmark	recreational beach	sediment	0 - 1 cm	1×10^{4} - 4.5×10^{5}	CFU 100 cm^{-3}
Roslev et al., (2008), *E. coli*	Ringkobing Fjord, west coast of Jutland, Denmark	recreational beach	sediment	0 - 1 cm	5.5×10^{2} - 2×10^{4}	MPN 100 cm^{-3}
Ge et al., (2012), *E. coli*	Chicago 63rd street Beach	beach	submerged sediment	submerged sediment	1585	CFU 100 mL^{-1}
Garzio-Hadzick et al., (2010), *E. coli*	OPE3 research field, USDA-ARS, Beltsville, MD	agricultural land	streambed sediment	5 cm	11 - 1099	MPN gdw^{-1}
Pandey et al., (2012), *E. coli*	Squaw creek watershed, Iowa	row-crop production	streambed sediment	2 - 3 cm	1.63×10^{3} - 3.43×10^{4}	CFU 100 mL^{-1}
Pandey and Soupir, (2013), *E. coli*	Squaw creek watershed, Iowa	row-crop production	streambed sediment		1×10^{12}	CFU 100 mL^{-1}
(Cho et al., 2010 b), *E. coli*	Gwangju Creek, Yeongsan River, South Korea	wastewater treatment plant, industrial area and Gwangju City	sediment	3 cm	1.6 - 7.2×10^{5}	MPN $100g^{-1}$
(Cho et al., 2010 b), enterococci	Gwangju Creek, Yeongsan River, South Korea	wastewater treatment plant, industrial area and Gwangju City	sediment	3 cm	1.5 - 6.6×10^{5}	MPN $100g^{-1}$
Borade et al., (2014), Total Coliform	West coast of Gujarat, India	Industrial zone	sediment		0-8000	CFU g^{-1}
Borade et al., (2014), Fecal Coliform	West coast of Gujarat, India	Industrial zone	sediment		0-7000	CFU g^{-1}
Borade et al., (2014), *E. coli*	West coast of Gujarat, India	Industrial zone	sediment		0- 4000	CFU g^{-1}

Stumpf et al., (2010) *E. coli*	New river water shade, Onslow County, NC, US	boating, bathing, commercial and recreational fishing, shellfish harvesting and military operatrions	sediment	19 - 168	MPN g^{-1}
Stumpf et al., (2010) enterococci	New river water shade, Onslow County, NC, US	boating, bathing, commercial and recreational fishing, shellfish harvesting and military operations	sediment	28 - 451	MPN g^{-1}
Brooks et al., (2016), *E. coli*	Clinton River, St. Clair, NC, US	agricultural land, urban development	sediment	0.14×10^{7} – 1.7×10^{7}	CE gdw^{-1}
Brooks et al., (2016), *E. coli*	Anchor Bay, St. Clair, NC, US	agricultural land, urban development	sediment	1.8×10^{6} - 8.5×10^{6}	CE gdw^{-1}
Brooks et al., (2016), enterococci	Clinton River, St. Clair, NC, US	agricultural land, urban development	sediment	0.03×10^{5} - 9.9×10^{5}	CE gdw^{-1}
Brooks et al., (2016). enterococci	Anchor Bay, St. Clair, NC, US	agricultural land, urban development	sediment	0.1×10^{5} - 2×10^{5}	CE gdw^{-1}
Haller et al., (2009a), Total coliforms	Bay of Vidy, Lake Bret and Versoix river located in Lake Geneva, Switzerland	sewage water, wastewater treatment plant, varying organic content and nutrients	sediment	1.9×10^{5} - 1.2×10^{6}	CFU $100g^{-1}$
Haller et al., (2009a), *E. coli*	Bay of Vidy, Lake Bret and Versoix river located in Lake Geneva, Switzerland	sewage water, wastewater treatment plant, varying organic content and nutrients	sediment	1 - 8.5×10^{5}	CFU $100g^{-1}$

Haller et al., (2009a), enterococci	Bay of Vidy, Lake Bret and Versoix river located in Lake Geneva, Switzerland	sewage water, waste water treatment plant, varying organic content and nutrients	sediment		9.8×10^{3} - 3.1×10^{5}	CFU $100g^{-1}$
Karbasdehi et al., (2017), Total Coliforms	Persian Gulf, Bushehr province, Iran	industrial pollution	sediment	0 - 20 cm	37.4×10^{3} - 105.9×10^{3}	MPN $100ml^{-1}$
Karbasdehi et al., (2017), Fecal Coliforms	Persian Gulf, Bushehr province, Iran	industrial pollution	sediment	0 - 20 cm	7.5×10^{3} - 17.4×10^{3}	MPN $100ml^{-1}$

* CFU m^{-3} to CFU 100 mL^{-1}

2.3.2 FIB Concentrations in Sediment vs. Water Column

Though monitoring microbial water quality is a common practice, monitoring FIB in sediment is rare. Several studies have compared the concentrations of FIB in sediments to water column concentrations and commonly observed higher FIB concentrations in the sediment than the water column (Karbasdehi et al., 2017; Perkins et al., 2014; Norman et al., 2013; Kovacic et al., 2011; Orear and Dalman, 2011; Abhirosh et al., 2010; Roslev et al., 2008). Pandey et al. (2018) measured *E. coli* concentrations in the sediment that were 47 to 386 times higher than the water column in the Merced River, California (Table 2.2). Similar results were observed by Karbasdehi et al. (2017), who found the total and fecal coliform concentrations were 10 to 100 fold higher in sediment than the surrounding seawater.

Conversely, some literature has demonstrated that water column *E. coli* concentrations can exceed sediment bed *E. coli* concentrations in some circumstances. One example is a study by Pandey and Soupir (2013) that reported 13% of samples had higher *E. coli* concentrations in the water column than the streambed sediment; however, the mean *E. coli* concentration in the sediment was one order of magnitude higher than the water column (Table 2.2). In another study by Pandey and Soupir, (2014), 44% of water samples had *E. coli* concentrations that exceeded the concentration of sediment *E. coli*.

Overall, there is a weak relationship between FIB in sediment and the water column during baseflow conditions (Pachepsky and Shelton, 2011). Byappanahalli et al. (2003) reported a low correlation between *E. coli* concentrations in the water versus those measured in the sediment. It was postulated that the reason for the low correlation

between the water and sediment during baseflow conditions is the lack of turbulence or resuspension during periods of baseflow, which results in the sediment having little or no effect on the bacterial load to overlaying water (Pachepsky and Shelton, 2011).

Not only are FIB concentrations in sediment during baseflow conditions generally higher than FIB concentrations in the water column, but the concentrations in the sediment during baseflow are also fairly consistent over time (Pandey and Soupir, 2014; Curtis and Trapp, 2014). For example, Karbasdehi et al. (2017) analyzed sediment samples in eight locations for two months and observed the FIB (e.g. total coliform, fecal coliform) concentrations ranged from 10^4 to 10^5 MPN 100 mL^{-1} and were consistently higher than water FIB concentrations.

Though *E. coli* concentrations have been found to be consistently higher in sediment during baseflow conditions, storm events or high flow events cause turbulence and disturb the bed sediment, resulting in the resuspension of particles and the associated bacteria to the water column (Pandey and Soupir, 2013; Nagels et al., 2002), thereby increasing FIB concentrations in the overlaying water (Pandey and Soupir, 2014). This is supported by Curtis and Trapp (2014) who sampled sediments and water during both baseflows and storm conditions and found the concentrations of *E. coli* in water during baseflow conditions were lower than those found in sediment, as previous studies demonstrated. However, *E. coli* concentrations in the water sampled during storm events increased, resulting in concentrations five times higher in the water column than the sediments. Pandey and Soupir (2014) had similar results when monitoring a storm event, where the *E. coli* concentrations in the water column increased 88-fold, but the concentrations in the sediment only increased 1.6-fold over baseflow samples. In a study

of seasonal variation by Borade et al. (2014), the *E. coli* and fecal coliform concentrations in water were greatest during the monsoon (August) season when compared with the postmonsoon (January) and premonsoon (May), whereas the sediment FIB concentrations were greatest during the premonsoon period. The decrease of FIB concentration in sediment during the monsoon and postmonsoon period could be due to in-stream resuspension (Bai and Lung, 2005). The stream current exerts stress on the streambed and when this stress is above a certain critical value, it mobilizes bed materials along the stream bed (Abia et al., 2017; Bose and Dey, 2013; Chang and Scotti, 2003), resuspending the bed material, including microbes, to the water column (McDaniel et al., 2013; Cervantes, 2012; Pachepsky and Shelton, 2011).

Table 2.2: Examples of bacterial concentrations in sediment vs the water column. The table contains information about study sites, the FIB measured in the study, and the measurement units that were used. In most cases the sediment contained higher concentrations of FIB than water column.

Paper	Location	Water	unit	Sediment	unit	FIB
Stocker et al., (2019)	Little Paint Branch Creek, Maryland, USA	8 - 514	CFU 100 mL^{-1}	13 - 238	CFU gdw^{-1}	*E. coli*
		29 - 885	CFU 100 mL^{-1}	16 - 543	CFU gdw^{-1}	enterococci
Pandey et al., (2018)*	Yosemite, Merced River, CA, USA	1.2×10^{8} – 7.5×10^{9}	CFU 100 mL^{-1}	5.7×10^{9} – 2.9×10^{12}	CFU 100 mL^{-1}	*E. coli*
Pandey et al., (2018)*	Squaw Creek, Iowa, USA	2.2×10^{10} – 2.2×10^{11}	CFU 100 mL^{-1}	7.8×10^{10} - 17.8×10^{12}	CFU 100 mL^{-1}	*E. coli*
Curtis and Trapp, (2014)	Withers Swash, Myrtle Beach, South Carolina	20 - >48,392	MPN 100 mL^{-1}	1.5 - 794.6	MPN g^{-1}	*E. coli*
Ouattara et al., (2011)	Scheldt watershed, Belgium	1.4×10^{3} - 4.6×10^{5}	CFU 100 mL^{-1}	2.1×10^{2} - 2.2×10^{5}	CFU g^{-1}	*E. coli*
Abhirosh et al., (2010),	Vembanadu Lake, India	5.1×10^{3} - 9×10^{3}	MPN 100 mL^{-1}	1.1×10^{5} - 9.88×10^{5}	MPN g^{-1}	Total Coliform
Roslev et al., (2008),	Ringkobing Fjord, west coast of Jutland, Denmark	4 - 1598	CFU 100 mL^{-1}	1×10^{4} - 4.5×10^{5}	CFU 100 cm^{-3}	enterococci
Roslev et al., (2008),		5 - 13,775	MPN 100 mL^{-1}	5.5×10^{2} - 2×10^{4}	MPN 100 mL^{-1}	*E. coli*
Ge et al., (2012)	Chicago 63rd street Beach	35 - 317	CFU 100 mL^{-1}	1585	CFU 100 mL^{-1}	*E. coli*
Pandey and Soupir, (2014)	Squaw creek watershed, Iowa	7598	CFU 100 mL^{-1}	3354	CFU/100g	*E. coli*
Pandey and Soupir, (2013)	Squaw creek watershed, Iowa	1×10^{7}	CFU m^{-3}	1×10^{8}	CFU m^{-3}	*E. coli*
Wilson et al., (2016)	Grand Glaize Beach, Lake of the Ozarks state park, Missouri	89 - 760	CFU 100 mL^{-1}	2 - 6.5×10^{4}	MPN gdw^{-1}	*E. coli*

Perkins et al., (2014),	River Conwy, UK	2.1×10^{1}	CFU 100 mL^{-1}	5.9×10^{3}	CFU 100 g^{-1}	*E. coli*
Perkins et al., (2014)	River Conwy, UK	3.0×10^{2}	CFU 100 mL^{-1}	1.3×10^{5}	CFU 100 g^{-1}	Total Coliforms
Borade et al., (2014)	West coast of Gujarat, India	ND**- 470	CFU mL^{-1}	0-8000	CFU g^{-1}	Total Coliform
Borade et al., (2014)	West coast of Gujarat, India	ND**- 360	CFU mL^{-1}	0-7000	CFU g^{-1}	Fecal Coliform
Borade et al., (2014)	West coast of Gujarat, India	ND**- 260	CFU mL^{-1}	0- 4000	CFU g^{-1}	*E. coli*
Stumpf et al., (2010),	New river watershade, Onslow County, NC, US	1.11×10^{3}	MPN 100 mL^{-1}	19 - 168	MPN g^{-1}	*E. coli*
Stumpf et al., (2010),	New river watershade, Onslow County, NC, US	3.87×10^{2}	MPN 100 mL^{-1}	28 - 451	MPN g^{-1}	enterococci
Karbasdehi et al., (2017),	Persian Gulf, Bushehr province, Iran	1238.1	MPN 100 mL^{-1}	37.4×10^{3} - 105.9×10^{3}	MPN 100 mL^{-1}	Total Coliforms
Karbasdehi et al., (2017),	Persian Gulf, Bushehr province, Iran	150.8	MPN 100 mL^{-1}	7.5×10^{3} - 17.4×10^{3}	MPN 100 mL^{-1}	Fecal Coliforms

* Not Detected (ND)

2.3.3 Impact of Streambed Disturbance on Water Quality

The highest concentrations of FIB in sediments are in the topmost layer of the streambed sediment (Garzio-Hadzick et al., 2010; Drummond et al., 2014). Not only does the top layer of sediment contain the highest concentrations of FIB, it is also the layer most readily available for resuspension into the water column (Garzio-Hadzick et al., 2010). The shear stress associated with high flow events begins with the top-most layer of sediment (Jamieson et al., 2005b); while other disturbances, such as those produced by animals crossing streams, also generally occur at the top layer. In fact, disturbances (e.g. recreational, storm events) often help explain increases in FIB concentrations (Abia et al., 2017; Crabill et al., 1999).

There are many ways the sediment bed can be disturbed, including storm events (Pandey and Soupir, 2014; Pandey and Soupir, 2013; Fries et al., 2006 and Jamieson et al., 2005b; Muirhead et al., 2004; Nagels et al., 2002); mechanical disturbances such as dredging (Grimes, 1980); raking (Abia et al., 2017; Stephenson and Rychert, 1982); recreational activities such as boating, bathing, or swimming (Abia et al., 2017; Roslev et al., 2008; An et al., 2002); the passage of boats or ships (Pettibone et al., 1996); animal access (Abia et al., 2017; Sherer et al., 1988); and artificial flood events (Bai and Lung, 2005; Muirhead et al., 2004; Nagels et al., 2002; Sherer et al., 1988).

The impact of sediment bacteria concentrations on the concentrations of FIB in the water column can be substantial (Abia et al., 2017; Pachepsky and Shelton, 2011; Stephenson and Rychert, 1982). Sediment disturbances reintroduce bacteria to the water column and can increase the water column bacterial concentrations by several fold, substantially contributing to water quality impairments (Stephenson and Rychert, 1982;

Cho et al., 2010a; Orear and Dalman, 2011; Abia et al., 2017). For example, Abia et al. (2017) performed several sediment bed disturbance analyses including mechanical disturbance, a high flow event, and raking, and observed 2 to 35-fold higher FIB concentration in the water column. Similarly, Stephenson and Rychert, (1982) simulated stream sediment disturbance by raking bottom sediments, and found a 10-fold increase in *E. coli* concentrations in the water column from the initial sample to the peak concentration.

2.4 FIB Survival in Sediment

Sediment is a favorable environment for bacteria to survive and persist in. FIB survive in the sediment for a longer period than in the overlying water (Garzio-Hadzick et al., 2010; Haller et al., 2009a; Jamieson et al., 2005b; Craig et al., 2004). The FIB can survive in water from a few hours to days, while in sediment, FIB can survive from days to months (Haack, 2017). For instance, Czajkowska et al. (2005) and Garzio-Hadzick et al. (2010) observed FIB, such as *E. coli*, survived in water for a month while this survival time increased to three or four months in sediment. The survival of bacteria in sediment depends on many factors including streamflow, sediment physical characteristics such as sediment texture, minerological composition, organic content, and ionic strength (Pandey et al., 2012, Pandey and Soupir, 2013; Droppo and Ongley, 1994; Mehta et al., 1989); physico-chemical factors such as pH, temperature, turbidity, and sunlight; and biological factors such as biofilms and other microbes, including predators and compititors (Byappanahalli et al., 2012; Ishii and Sadowsky, 2008).

2.4.1 Sediment Particle Size

Sediment texture greatly influences bacterial survival and persistence in bed sediment as well as instream resuspension (Droppo and Ongley, 1994; Mehta et al., 1989). FIB often have slower die-off rates in clay and fine particles than coarse particles (Garzio-Hadzick et al., 2010; Howell et al., 1996; Sherer et al., 1992). Garzio-Hadzick et al. (2010) observed the lowest inactivation, or die-off, rates in sediment containing higher amounts of finer particles. Similarly, Sherer et al. (1992) and Howell et al. (1996) observed higher FIB die-off rates in loamy textures than clayey textures. In a laboratory based microcosm study, researchers reported that sediment with 25% clay content increased *E. coli* survival as compared to sediment with high contents of sand (Burton et al., 1987).

In addition to higher survival, the ability of FIB to associate with particles is greater with fine particles than coarse particles (Pandey and Soupir, 2014; Cho et al., 2010a; Muirhead et al., 2004; Black et al., 2002; Burton A., et al., 1987). For example, Garzio-Hadzick et al. (2010) performed a microcosm study on the survival of manure borne *E. coli* in sediment using three different sediment textures. Two of the sediment textures were classified as loamy sand and contained about 84% sand with the rest being silt and clay (16%), and the third sediment texture was classified as sandy loam and sandy clay loam which contained 60% sand and 40% silt and clay. The study showed the highest *E. coli* concentrations and lowest *E. coli* inactivation rates in the sediment with finer particle size distribution (silt and clay 40%) (Garzio-Hadzick et al., 2010). Similarly, in a study on the impact of sediment composition on the spatial variation of indicator bacteria, Perkins et al. (2014) found a significant positive relationship between

FIB and fine size particles (i.e. silt and clay). Another study by Cho et al. (2010) found that fine particles (silt and clay) contained two to six times higher *E. coli* concentrations per unit mass than the total sediment contained per unit mass.

Fine particles protect bacteria from predators and provide favorable environmental conditions such as high moisture availability (Garzio-Hadzick et al., 2010; Cools et al., 2001). Also, fine particles are more favorable for *E. coli* participation in biofilm formation in sediment, which may be due to presence of smectite clay. Clay slurries developed substantial biofilm components and demonstrated increased FIB concentration in fine particles (Garzio-Hadzick et al., 2010; Banning et al., 2003).

However, this trend is not universal. Cinotto (2005) reported that larger particles, between 0.125 mm to 0.5 mm, contained the highest median *E. coli* concentration. Similarly, Lang and Smith (2007) studied the impact of soil type and moisture content on *E. coli* fate in agricultural soil, and found higher *E. coli* presence in sandy loam than in finer particles (i.e. silty clay). Higher FIB concentrations in sediments with larger particle sizes may be due to the permeability and nutrient accessibility facilitated by coarse particles (Cinotto, 2005).

2.4.2 Organic Content

In addition to particle size, the amount of organic matter also influences survival of bacteria in streambed sediments (Garzio-Hadzick et al., 2010; Haller et al., 2009a) as well as the persistence of FIBs, including total coliforms, *E. coli,* and enterococci (Haller et al., 2009a). Higher organic carbon content provides favorable conditions for the survival of *E. coli* in sediment (Craig et al., 2004; Curtis and Trapp, 2014), as high

organic-content sediments could have slow-release, polymeric nutrients which hinder cell die-off (Garzio-Hadzick et al., 2010).

Organic matter is often strongly correlated with *E. coli* concentrations (Perkins et al., 2014; Curtis and Trapp, 2014; Piorkowski et al., 2014a; Pandey and Soupir, 2014; Garzio-Hadzick et al., 2010). For example, Curtis and Trapp (2014) monitored Withers Swash in Myrtle Beach, South Carolina, which receives storm water runoff from different land uses including residential developments, parks, campgrounds, and commercial facilities, resulting in high concentrations of FIB. They found a strong positive correlation between particle size, organic content, and sediment *E. coli* concentration when sampling during a rainfall event. In other studies of *E. coli* survival, sediment that contained high organic content in addition to fine particles showed higher survival of *E. coli* (Garzio-Hadzick et al., 2010; Pandey and Soupir, 2014). This was demonstrated by Perkins et al. (2014) who found clay and silt sediments with higher organic matter content were associated with significantly higher FIB concentrations.

On the other hand, a study by Piorkowski et al. (2014a) on characterizing the spatial structure of sediment *E. coli* for sampling design reported a diverse relationship between *E. coli* concentration and organic matter content. In this study, three stream reaches were investigated, and the results showed three different relationships, including positive ($r = 0.53$), negative ($r = -0.36$), and no significant relationship, between *E. coli* abundance and organic content. Negative or no significant relationships between *E. coli* concentrations and organic content may be due to increased resource competition among members of the microbial community, resulting in decreased *E. coli* concentrations in fine particles (Surbeck et al., 2010; Banning et al., 2003).

2.4.3 Temperature and Seasonal Effect

Temperature is another major influential factor for FIB survival within sediment (Garzio-Hadzick et al., 2010; Abia et al., 2015a). The highest FIB survival and lowest FIB decay rates are observed in low temperatures (Garzio-Hadzick et al., 2010; Craig et al., 2004; O¨zkanca and Flint, 1997; Terzieva and McFeters, 1991). For instance, Garzio-Hadzick et al. (2010) monitored *E. coli* survival in sediment in a flow-through chamber with three different temperatures (4˚C, 14˚C and 24˚C). With the increase in temperature within the chamber, the *E. coli* inactivation rates in sediment also increased. Similarly, Craig et al. (2004) performed a microcosm experiment and monitored *E. coli* survival at three constant temperatures, 10°C, 20°C and 30°C. In this study the highest *E. coli* die-off was observed at the highest temperature, 30°C. In addition, Sjogren (1994) found the highest *E. coli* growth and survival at the lowest temperature monitored (5°C).

Not only can FIB survive in low temperature conditions, they have the ability to survive in sediment during winter conditions (Garzio-Hadzick et al., 2010; An et al., 2002). An et al. (2002) found the highest pathogen survival at 4°C.

Despite the ability of FIB to survive at low temperatures, high temperatures, around 37 °C, are optimal for FIB growth under suitable conditions such as high nutrient availability and low stress (Ishii et al., 2006; Whitman et al., 2003). For example, a microcosm study by Ishii et al., (2006) on the presence and growth of *E. coli* in temperate soils found that the *E. coli* strain grew to maximum cell densities up to 4.2×10^5 CFU g^{-1} in soil at 37 °C.

Temperature varies seasonally and therefore, seasons can impact FIB concentrations (Stocker et al., 2019; Crabill et al., 1999). For example, Stocker et al.

(2019) examined the seasonality of *E. coli* concentrations in water, sediment, and periphyton and reported that *E. coli* decreased significantly from summer to winter.

2.4.4 Other Influential Factors

While sediment physical characteristics and temperature are the most studied variables in connection to sediment FIB growth and survival, other studies have also examined the impact of turbidity, electrical conductivity (EC), salinity, water velocity, and nutrients (Abia et al., 2015a; Shelton et al. 2014; Piorkowski et al., 2014b; Garzio-Hadzick et al., 2010) .

Higher turbidity is associated with less FIB concentrations in sediment (Abia et al., 2015a; Garzio-Hadzick et al., 2010). For example, Abia et al. (2015a) found there was a strong negative correlation between *E. coli* counts in sediment and turbidity, which could be due to the resuspension of sediments and their associated bacteria back into the water column.

Both EC and dissolved oxygen (DO) showed a proportional relationship with FIB in sediment (Abia et al., 2015a; Garzio-Hadzick et al., 2010). Abia et al. (2015a) reported a positive correlation between *E. coli* concentrations in sediment with the water EC and DO, similar to the temperature trend.

An inverse relationship between salinity and FIB concentration has been observed in sediments (Karbasdehi et al., 2017; Atwill et al., 2007). This inverse relationship could be due to specific seawater characteristics, such as toxicity of inorganic salts and osmotic pressure (Gauthier et al., 1987). For example, in a study by Karbasdehi et al. (2017) on indicator bacteria in seawater and coastal seidment, an increase of indicator bacteria coincided with a decrease of salinity. Gauthier et al. (1987) also performed a microcosm

study to monitor *E. coli* recovery in filtered and unfiltered seawater. After 2-days, *E. coli* die-off was faster in the presence of seawater as marine flora were present in the water.

Water velocity also influences bacterial concentrations in streambed sediment (Piorkowski et al., 2014b). For example, Piorkowski et al. (2014b) measured water velocity both in baseflow and stormflow conditions and found increases in water velocity corresponded with increases in *E. coli* concentration, and the velocity had a greater influence on sediment *E. coli* concentrations than sediment particle size under both flow conditions.

Storm events can have a substantial impact on FIB in sediment (Pandey and Soupir, 2014), but not always. Curtis and Trapp (2014) collected sediment samples before and during a rainfall event and concluded that there was no effect on sediment *E. coli* concentrations.

Higher nutrient presence increases FIB in sediment. Shelton et al. (2014) observed the effect of nutrients (PO_4^{3-}, TOC, NO_3-N) on bacterial concentration in sediment and determined that both total coliforms and *E. coli* counts increased with increased nutrients. Brooks et al. (2016) reported that *E. coli* counts in sediments had significant correlations with nutrient loading, including the percentage of total carbon and the percentage of total nitrogen.

Furthermore, FIB concentrations in sediment are impacted by climatic variables as well as anthropogenic variables. For example, Brooks et al. (2016) reported that the *E. coli* and enterococci counts in sediments had significant correlations with air temperature and human population.

2.5 FIB Transport

To improve microbiological water quality, it is necessary to understand the transport of pollutants within the environment. Storm events, for example, contribute to pollutant transport to the water through elevated subsurface flow, runoff, and resuspension of bed sediments (Pandey and Soupir, 2013; Pandey et al., 2012; Cho et al., 2010a; Wu et al., 2009; Jamieson et al., 2005b).

2.5.1 Runoff

Runoff can significantly contribute to microbial pollution, resulting in sharp increases of bacteria concentrations in the stream (Kim et al., 2005; Reeves et al., 2004 and Kistemann et al., 2002). For instance, Reeves et al. (2004) monitored FIB in runoff from a highly urbanized watershed and found runoff contributed 99% of fecal indicator bacteria to the stream. Similarly, Stumpf et al. (2010) measured FIB loading in a highly urbanized watershed, and found the primary source of FIB in the stream was terrestrial runoff.

The strong positive correlation often found between bacteria concentrations and flowrate also supports the idea that storm events and the associated runoff greatly contribute to increased FIB in the water column (Kim et al., 2005, Crowther et al., 2002). For example, in a study by Kim et al., (2005) on the impact of point and non-point source pollution, a strong positive correlation (0.71) was observed between bacteria concentrations and flowrate. In addition, bacteria attached to particles which can be transported during high flow rate to receiving waterbodies (Crowther et al., 2002).

Elevated FIB concentrations in the stream can remain for a long time, contributing to water quality impairments. For example, Jenga et al. (2005) studied urban

runoff and observed 75%-80% of FIB stay unattached in the water column before dying off, and it takes three to seven days for the stream to return to its background FIB concentrations.

2.5.2 Sediment Disturbances

Runoff is not the only method of transporting FIB; they can also be transported via resuspension. Streambed sediments can be a reservoir for FIB, so disturbances of the sediment bed transport the bacteria to the water column, thus increasing bacterial loads (Wu et al., 2009). This process of bacteria moving from bed sediments to the water column is called resuspension. Streambed disturbances can be caused by, among other things, storm events through processes such as storm water runoff and increased shear stress due to increased flow (Abia et al., 2017; Crabill et al., 1999); recreational activities including boating, bathing, and swimming (Abia et al., 2017; An et al., 2002); and animals crossing rivers or streams (Abia et al., 2017; Sherer et al., 1988).

2.5.2.1 Storm Event Disturbances

The force from increased flow during storm events often results in increased fecal indicator bacteria concentrations in the water column due, in part, to resuspension. *E. coli* concentrations have been shown to be significantly higher during wet weather conditions than dry weather conditions, sometimes many orders of magnitude higher (Curtis et al., 2014; Huang et al, 2015). Curtis et al. (2014) also noted a decrease in *E. coli* concentrations in sediments when comparing pre-storm concentrations and concentrations during storm events, indicating the bacteria are flushed out of the sediments and into the water column (i.e. resuspended). In addition to reductions of FIB reservoirs in sediments, physical factors associated with storm events, such as high flow

rates, have a strong relationship with *E. coli* concentrations in the water column. Pandey et al. (2014) observed higher *E. coli* concentrations in the water column with increasing stream flow (R^2=0.56).

While much information can be obtained from natural storm events, these events can also transport FIB from other sources via overland flow. Artificial elevated flow events have been studied to remove the impact of overland flow and isolate the impacts of instream sediment stores (e.g. Cho et al., 2010; Wilkinson, 2995). Wilkinson (1995) observed an increase in fecal coliform concentrations between 5 and 25 times in the water column during an artificial flood experiment. Abia et al. (2017) also artificially elevated flow and observed similar increases in loading, ranging from 2.4 to 17.4-fold higher *E. coli* loads in the water column.

Resuspension due to storm events has also been examined through modeling work. Yakirevich et al. (2013) used a conservative tracer diflurobenzoic acid (DFBA) and found transient storage could be an important element of the in-stream mobilization and resuspension processes. Wilkinson et al. (1995) also observed *E. coli* concentrations in sediment decreased during high flow due to in-stream resuspension of particle-attached bacteria to the water column.

While many of the aforementioned studies found large contributions of bacteria to the water column from resuspension, not all studies have found resuspension to be a dominant factor in increased FIB loads measured during storm events (e.g. Stumpf et al. 2010).

2.5.2.2 Recreational Disturbances

Human activities on recreational beaches can increase fecal contaminants in the water column, High FIB counts are public health hazards, leading to an increased probability of illness in recreators (Varness et al., 1978; Dalman et al., 2007; Dalman et al., 2009; Phillip et al., 2009). *E. coli* concentrations can increase significantly in the water column due to resuspension from recreational activities, such as bathing (Wilson et al., 2016; Wilson et al., 2014; Abia et al., 2017), boating (An et al., 2002) and swimming (Wilson et al., 2016; Crabill et al., 1999). Some areas with high recreational use can result in water quality violations (Wilson et al., 2016). Orear and Dalman (2011) reported a 7.5-fold increase of *E. coli* concentrations at a recreational site.

While boating does not always put recreators in direct contact with the water, these activities can increase FIB concentrations, contributing to water quality impairments. An et al. (2002) found a strong positive correlation between water column *E. coli* concentrations and sediment resuspension due to boating activity. The authors also reported high *E. coli* concentrations at the boat docks and gasoline filling stations. Wilson et al. (2016) supported the observation that recreational users can cause the resuspension of polluted sediment based on their results which demonstrated significantly higher *E. coli* concentrations in the water column during weekends when compared to weekdays, with the highest spike of FIB in water observed over Memorial Day weekend which also had a decrease of *E. coli* concentrations in the sediment.

2.5.2.3 Animal Disturbances

When wildlife and livestock have direct access to the stream, their access can disturb stream bottom sediments, resuspending bacteria and contributing to increased

bacterial concentrations in the stream (e.g. Sherer et al.,1988; An et al., 2002; Stephenson and Rychert, 1982). Elevated FIB concentrations have been measured immediately following cattle crossing a stream and can remain elevated for an extended period of time, from weeks to months (e.g. Stephenson and Street, 1978). This extended period of elevated FIB concentrations after the cattle cross suggests resuspension from direct fecal deposits that settled in the streambed sediments, as the initial fecal plume would have flushed through the system rapidly. Other studies have simulated animals crossing a stream by artificially disturbing (e.g. raking) stream bottom sediments, and observed FIB increases many times their original values (Sherer et al., 1988; Abia et al., 2017). For example, Stephenson and Rychert (1982) simulated stream sediment disturbance by raking bottom sediments, and found a 10-fold increase in *E. coli* concentrations in the water column from the initial sample to the peak concentration.

2.5.2.4 Other Disturbances

The resuspension of fecal indicator bacteria is also caused by other, less studied disturbances, such as dredging (Grimes, 1980) and ship traffic (Phillip et al., 2009; Pettibone et al., 1996). Disturbances from ship traffic were studied by Pettibone et al. (1996) and Phillip et al. (2009), and both found an increase of FIB in the water column resulting from ship traffic. Pettibone et al. (1996) also reported a strong positive correlation between total suspended solids and bacterial loads in the water column, indicating similar transport mechanisms and resuspension. In addition to ship traffic, disturbances due to dredging have been shown to increase fecal coliform concentrations in the water column by 4 to 50 times (Grimes, 1975).

2.5.3 Shear Stress

Bacterial transport also depends on hydraulic parameters such as turbulence and bed shear stress (Walters et al., 2014b). Shear stress is the force per unit area, and bed shear stress exerts force parallel to the streambed on sediment particles and the associated sediment reservoir of bacteria. The shear stress resulting in particle entrainment (i.e. resuspension) is known as the moment of incipient motion. The shear stress at this threshold is the critical shear stress.

The critical shear stress is important in predicting resuspension of bacteria from streambed sediment. At the critical shear stress, bed material starts to mobilize due to turbulence in the streambed and water boundary layer (Abia et al., 2017; Bose and Dey, 2013). One of the major factors influencing the critical shear stress value is sediment texture. Many studies have reported the different values for critical shear stress based on sediment characteristics (e.g. Cho et al., 2010a; Jamieson et al., 2005b; Bai and Lung, 2005). For example, in a study on release of *E. coli* from bed sediment Cho et al. (2010a) observed higher critical shear stress in smaller size particles. This study measured shear stress in three stream reaches and reported the reaches mainly consisting of silt and clay had shear stresses of 18.7 N m^{-2} and 6.2 N m^{-2}, whereas the reach containing mostly sand particles had a shear stress of 3.4 N m^{-2}. Another study by Jamieson et al. (2005b) examined the resuspension of sediment *E. coli* and reported the critical shear stress for cohesive sediment ranged from 1.5 to 1.7 N m^{-2}.

During storm events, resuspension occurs as streamflow and bed shear stress increase. Sediment-associated bacteria resuspend at shear stresses ranging from 1.5 N m^{-2} to 1.7 N m^{-2}, which is similar to and sometimes higher than the critical shear stresses of

cohesive sediments (Walters et al., 2014b; Jamieson et al., 2005a). High bed shear stress can result in a substantial (150%) rise in total suspended solids in the water column and increase FIB persistence in the water column due to reduced light penetration (Walters et al., 2014b).

While shear stress with forces high enough to resuspend sediment and bacteria often occur during storm events (Park et al., 2017; Jamieson et al., 2005a), baseflow conditions with low shear stress can still result in resuspension (Walters et al., 2014b; Yakirevich et al., 2013). In an artificial high flow event, Yakirevich et al. (2013) observed that after the water pulse passed and the stream returned to baseflow, significant *E. coli* releases continued though shear stress was small. They hypothesized that this was due to the erosion of the boundary layer.

2.6 Resuspension

2.6.1 Resuspension Rate

The impact of sediment bacteria on water quality via resuspension has been well documented (Nagels et al., 2002; Sherer et al., 1988; McDonald et al., 1982). The magnitude and pattern of bacteria resuspension in waterbodies are, in part, dependent on sediment transport characteristics (Jamieson et al., 2005a). Disturbance and transport of sediment causes the associated movement of bacteria within the sediment. Though it is difficult to measure and harder to predict resuspension rates (Rehmann and Soupir, 2009), it is important to understand the persistence and resuspension rates of sediment bacteria because of their substantial impact on FIB in the water column. FIB concentrations can increase in the water column and decrease in the sediment bed due to the resuspension of bacteria (Curtis and Trapp, 2014; Pandey et al., 2014; Bai and Lung,

2005). Also, a large difference has been observed between the resuspension rates during high flow conditions and baseflow conditions. High flow events can result in resuspension rates multiple times higher than rates during baseflows (Bai and Lung, 2005; Pandey and Soupir, 2014; Curtis and Trapp, 2014). Previous researchers have measured, calculated, and predicted resuspension rates by monitoring waterbodies (Jamieson et al., 2005a; Weiskel et al., 1996), conducting laboratory flume studies (McDaniel et al., 2013; Cervantes, 2012), and building models (Park et al., 2017; Yakirevich et al., 2013; Cho et al., 2010a).

2.6.1.1. Resuspension from Waterbodies

Different hydrologic conditions, such as storm events, as well as sediment bed texture influence the resuspension of bacteria to the water column. For instance, Jamieson et al. (2005b) measured the resuspension rate for three storm events in a natural stream and found it ranged from 8,200 to 15,000 CFU $m^{-2}s^{-1}$ with an estimated critical bed shear stress of 1.5 to 1.7 N m^{-2}. The researchers reported calculated bed shear stress values similar to the values of critical shear stress for erosion of cohesive sediment. Also, the resuspension of sediment bacteria occurred on the rising limb of the storm hydrograph, indicating there was a finite supply of sediment bacteria for resuspension to the water column during individual storm events. In a coastal embayment study by Weiskel et al. (1996) the reported average resuspension rate was 5×10^4 CFU m^{-2} per high flow event.

2.6.1.2. Resuspension from Modeling Studies

Several modeling studies have also been used to estimate resuspension rates (Park et al., 2017; Pachepsky et al., 2017; Yakirevich et al., 2013; Cho et al., 2010a). High flow events had higher estimated resuspension rates than baseflows. For example, in a one-

hour artificial high-flow experiment, Cho et al. (2010) reported the *E. coli* estimated resuspension rate for a single event was 15,000 cells m^2 s^{-1}, similar to the resuspension rate measured by Jamieson et al. (2005b). Park et al. (2017) estimated much lower resuspension rates during baseflow conditions, with *E. coli* resuspension rates ranging from 1.5 to 6.3 CFU m^2s^{-1}. Similarly, Pachepsky et al. (2017) reported net resuspension rates of FIB ranging from 36 to 87 CFU m^2 s^{-1} during baseflow conditions.

Although high flow causes resuspension rates that are several fold higher than baseflow conditions, resuspension during low flow conditions is can be substantially contribute to FIB in the water column. For instance, an artificial high flow study by Yakirevich et al. (2013) reported significant resuspension of sediment bacteria continued even during baseflow conditions when the bed shear stress was small.

2.6.1.3 Resuspension from Laboratory Experiments

Only a few flume studies have been conducted to evaluate resuspension rates. In a flume study examining *E. coli* resuspension from direct fecal deposits, McDaniel et al. (2013) calculated resuspension rates ranging from 10^3 to 10^5 CFU $m^{-2}s^{-1}$ in steady state flows ranging from 6.8×10^{-3} to 17.6×10^{-3} m^3s^{-1}. Cervantes (2012) calculated much lower resuspension rates for both attached and unattached *E. coli* under similar flows. The unattached and attached *E. coli* resuspension rates for sand particles were 1.32×10^{-6} CFU $m^{-2}s\text{-}^{1}$ and 3.84×10^{-6} CFU $m^{-2}s^{-1}$, respectively, while the unattached *E. coli* resuspension rate for sand-silt particles was 1.03×10^{-6} CFU $m^{-2}s^{-1}$. No resuspension occurred for *E. coli* attached to sand-silt.

2.6.2 Calculation of Resuspension Rate

There are several ways of calculating or measuring resuspension rates of sediment bacteria. Jamieson et al. (2005b) calculated the resuspension rate of *E. coli* from bed sediment over three storm events in an alluvial stream. For that, researchers seeded a streambed with *E. coli* cells that were resistant to nalidixic acid (*E. coli* NAR) to examine the resuspension and persistence of sediment bacteria. The researchers calculated resuspension rate using Equation 2.1.

$$RS = \frac{C_{ECavg} \times Q_{avg}}{SA} \qquad \text{(Equation 2.1)}$$

Where RS is the resuspension rate in CFU $m^{-2}s^{-1}$, C_{ECavg} is the average concentration of *E. coli* NAR in CFU m^{-3} during the resuspension period, Q_{avg} is the average flow in m^3 s^{-1} during the resuspension period, and SA is the source cell surface area in m^2. This study is based on the theory that mostly cohesive sediments associate with bacteria (Auer and Niehaus, 1993); thus, though a stream contains a mixture of cohesive and non-cohesive sediment, the resuspension rate will largely be controlled by cohesive sediment.

Sediment characteristics may cause large differences in the parameters used to calculate resuspension (Cho et al., 2010a). For example, considerably different critical bed shear stress values have been estimated (Cho et al., 2010a). Both Jamieson et al. (2005b) and Bai and Lung (2005) conducted research on cohesive sediment, but used substantially different shear stresses (1.7 Nm^{-2} (Jamieson et al., 2005b) and 0.4 Nm^{-2} (Bai and Lung, 2005)) for measuring resuspension rates. Cho et al. (2010a) observed better model performance for predicting resuspension rates by using multiple sets of parameters

(eg. reach specific parameters) rather than a single set of parameters. In this study three stream reaches with distinctly different sediment particle sizes were chosen with shear stresses of 3.4, 6.2, and 18.7 Nm^{-2}. Equation 2.2 was used by Cho et al. (2010a) to estimate the resuspension rate:

$$\text{Resuspension rate, } \overline{R} = \frac{(N_t - N_i - B)}{t_R} \qquad \text{(Equation 2.2)}$$

Where N_t and N_i are the number of cells leaving and entering the stream reach, respectively, t_R is the duration of the release and B is the number of cells that settled back in the sediment bed.

Some studies also considered different flow conditions. For example, Park et al. (2017) examined *E. coli* release from bed sediment during baseflow conditions. In this study, two equations were used; the first equation assumes *E. coli* concentrations change following a log function (Equation 2.3) and the second equation is used to calculate the bacterial release rate from the bottom sediment (Equation 2.4).

$$\log_{10} C_B = c_1 \sin\left(c_2 \frac{j_{day} - c_3}{366} \pi\right) + c_4 \qquad \text{(Equation 2.3)}$$

Where C_B is the concentration of *E. coli* in streambed sediment; c_1, c_2, c_3 and c_4 are the regression coefficients 1.543, 2.194, 187 and 3.87, respectively, and j_{day} is the Julian calendar day of the year.

$$B_A = \gamma A_{streambed} C_B \qquad \text{(Equation 2.4)}$$

Where B_A is the number of bacteria released from bottom sediment by active transport in CFU d^{-1}, γ is the bacteria release factor in $Tm^{-2}d^{-1}$, and, $A_{streambed}$ is the area of the streambed in m^2.

Similarly, Pachepsky et al. (2017) studied FIB released from bed sediment resulting in the enrichment of FIB in stream water during baseflow conditions. This study used mass balance principles and followed the same volume of water through space and time. Using this concept, the specific net release rate was calculated using Equation 2.5.

$$RS = \frac{Rt_{tr}}{Lwt_{pas}} \quad \text{(Equation 2.5)}$$

where RS is the net release rate, R (Equation 2.6) is the difference in the number of FIB moving from the sediment into the water column and the number of FIB moving from the water column into the sediment per day, t_{tr} is the average transport time for the specified volume of water to travel from the upstream sampling location to the downstream sampling location, Lw is the sediment surface area, and t_{pas} is the average time of passage through input and output sampling points in the same volume of water.

$$R = \frac{N_{total,ISL}[\frac{N_{total,OSL}}{N_{total,ISL}} - 1]}{t_{tr}} \quad \text{(Equation 2.6)}$$

Where $N_{total,\ ISL}$ and $N_{total,\ OSL}$ are the total FIB numbers at the upstream and downstream sampling locations respectively.

Other studies have used variations of the above calculation methods. For instance, McDaniel et al. (2013) modified the equation presented by Jamieson et al. (2005b) and calculated resuspension rates using equation 2.7:

$$\mathrm{R} = (\mathrm{EC} - \mathrm{BG}) \times 10^6 \times \frac{\mathrm{Q}}{\mathrm{SA}} \quad \text{(Equation 2.7)}$$

Where R is the rate of resuspension (CFU m^3s^{-1}), EC is the velocity weighted *E. coli* concentration CFU m^{-3}, BG is the velocity weighted background concentration of *E. coli* (CFU m^{-3}), Q is the flow rate (m^3s^{-1}) and SA is the resuspension surface area (m^2).

2.7 FIB Attachment

2.7.1 Microbial Attachment

It is important to understand microbial attachment for monitoring bacterial transport. Generally, microbial attachment is categorized as (1) free or unattached bacteria or (2) bacteria attached to soil particles, to manure particles, or fragments of vegetation and residue (Pachepsky et al., 2006; Thurston-Enriquez et al., 2005; Jamieson et al., 2004; Fiener and Auerswald, 2003).

Until recently, few studies examined microbial attachment in natural environments. It was previously assumed that bacteria were unattached colloids (Jamieson et al., 2005b; Characklis et al., 2005); however, there is now a general consensus that bacteria can also attach to sediment particles in the stream environment (Jamieson et al., 2005b). The transport of attached bacteria depends on the characteristics of the particles they are attached to, such as the size of the particle and particle density (Guber et al., 2007; Jamieson et al., 2005b). Attached bacteria can settle out easily and can be removed by sedimentation (Pachepsky and Shelton, 2011), while the unattached fractions are more mobile as well as more difficult to trace and remove than the attached fraction (Characklis et al., 2005). This is because the unattached fractions can travel independently, are buoyant, and, therefore, travel farther in water (Jamieson et al., 2005b; Pachepsky and Shelton, 2011). Due to the impact on transport, it is important to differentiate between attached and unattached fractions to trace microbial loads and to

determine appropriate removal mechanisms (e.g. sedimentation) (Pachepsky and Shelton, 2011; Characklis et al., 2005). In addition, association with particles increases bacterial survival in natural water (Howell et al., 1996; Sherer et al., 1992; Burton et al., 1987). Hence, microbial attachment and partitioning not only impact bacterial fate and transport, but also the length of time these bacteria stay viable in the natural environment causing a potential threat to public health (Characklis et al., 2005).

The unattached FIB is often the dominant fraction in surface waters, but the fraction of attached bacteria can still be significant. For example, Characklis et al. (2005) reported that the mean attachment rate ranged from 20% to 35% for various FIB, including fecal coliforms, *E. coli*, and enterococci. Similarly, Schillinger and Gannon (1985) found 16%-47% of the total bacteria were attached to particles. However, Mote et al. (2012) reported higher attachment rates which ranged from 1% to 95% of the bacteria in the water column.

Attachment rates in runoff can also vary widely, with measured attachment ranging from 9% to 72%. For instance, Soupir et al. (2010) performed a study on the attachment of FIB to particles in runoff and reported 28-49% of *E. coli* and enterococci are associated with particles. Another study by Jeng et al. (2005) observed an average bacterial association with particles of 9.32%, 19.6%, 22% for enterococci, fecal coliforms, and *E. coli*, respectively, in storm water runoff.

The attachment of bacteria to particles also varies with flow conditions, though the direction is not consistent. In a study on attachment of FIB to particles, Fries et al. (2006) observed a higher percent of attachment in baseflow conditions (50%) when

compared to storm events (37%). However, Characklis et al. (2005) observed a higher bacteria attachment rate in storm events (30-55%) than baseflow (20-35%).

Furthermore, the percent of FIB attached to particles also varies between different bacterial species. Jeng et al. (2005) demonstrated that *E. coli* had higher association with suspended particles than other fecal coliforms or enterococci. They also observed enterococci had a high association with particles with diameters from 10 μm to 30 μm, while *E. coli* and other fecal coliforms were associated with a broader range of particle sizes. Another study by Krometis et al. (2007) partitioned different microbial organisms between those associated with settleable particles and those that were suspended (≤5μm). Throughout the course of a storm, they observed the highest association to particles was 65% by *Clostridium perfringens,* followed by fecal coliforms, *E. coli* and enterococci which had similar percent associations at 40%, while the least association occurred with total coliphage at 13%. Similarly, Characklis et al. (2005) reported a higher percentage of particle association for *Clostridium perfringens* at 50-70% in storm water than any other indicator organism measured, including fecal coliforms, *E. coli,* and enterococci which had percent attachments ranging from 30-55%. Cizek et al. (2008) also found higher attachment rates for *Clostridium perfringens* and *Giardia* (40-65%) than other FIBs, including fecal coliforms, *E. coli,* and enterococci which ranged from 15-35%.

2.7.2 Influential Factors of Microbial Attachment

There are several physical, chemical, and biological factors which influence microbial attachment in the environment. The most extensively studied factor is particle size (Walters et al., 2014a; Pandey and Soupir, 2014; Soupir et al., 2010). Most studies examining the relationship between particle size and attachment have observed strong

positive correlations between fine particles and bacteria (Walters et al., 2014a; Pandey and Soupir, 2014; Soupir et al., 2010). For example, Soupir et al. (2010) determined that *E. coli* and enterococci were attached to fine particles (8-62 μm) for at least 60% of all attached bacteria. This was supported by Walters et al. (2014a) who analyzed wastewater and observed FIB were associated with smaller particles at a higher rate than large particles. In this study, the association of *E. coli* and enterococci with smaller particles (diameter < 12 μm) was 91% and 83%, respectively, while less than 1% of bacteria were associated with large particles (> 63 μm). A batch experiment performed by Oliver et al. (2007) found 35% of *E. coli* attached to particles > 2 μm, and of this 35%, the most preferential particle size of bacterial association was 16-30 μm. One hypothesis for the greater attachment to smaller particles is the larger surface area of fine particles which provides more space for attachment (Soupir et al., 2010).

While less studied, organic matter can also impact FIB attachment (Guber et al., 2007). Guber et al. (2007) examined the impact of organic matter on FIB attachment to different particle sizes both in the presence and absence of bovine manure. This study found that in the absence of manure, FIB have higher attachment rates to silt and clay particles than to sand particles with no organic coatings. However, association of bacteria to sand with an organic coating had similar attachment rates as observed in silt and clay particles. In the presence of manure, the association of bacteria to silt, clay, and sand with an organic coating decreased significantly, but the association of bacteria did not decrease in sand fractions with no organic coatings (Guber et al., 2007). Similarly, Soupir and Mostaghimi, (2011) found low bacterial association with particles in runoff from a fresh manure source, averaging 4.8% for *E. coli* and 13% for enterococci.

2.7.3 Methods of Measuring Microbial Attachment

Several methods have been developed to separate attached and unattached bacteria, with the most common being filtration (Mahler et al., 2000; Henry, 2004), fractional filtration (Soupir et al., 2010; Jeng et al., 2005; Schillinger and Gannon, 1985), sedimentation (Kunkel et al., 2013; Oliver et al., 2007), and centrifugation (Cizek et al., 2008; Krometis et al., 2007; Fries et al., 2006; Guber et al., 2005; Characklis et al., 2005; Muirhead et al., 2005; Schillinger and Gannon, 1985).

Filtration is a technique which uses filters with pore sizes larger than the typical FIB. Usually, samples are passed through an 8 μm filter, with those passing through the filter classified as unattached (Mahler et al., 2000; Henry, 2004). The samples are also processed for total bacteria using standard methods. The difference between the unattached and total bacteria is measured and the attached bacteria concentration is the difference between the two. One limitation of this method is it cannot characterize the attachment of bacteria to different particle sizes (Henry, 2004). Another limitation is the potential retention of unattached bacteria in the filters.

Fractional filtration is similar to filtration, but uses multiple filter sizes to differentiate between attachment to different particle sizes (Soupir et al., 2010; Jeng et al., 2005; Schillinger and Gannon, 1985). In this method samples pass through several filters sequentially, and bacteria that are retained in the filter are classified as attached to a certain size of particle. Limitations of fractional filtration include the retention of unattached bacteria in the filters and clogging of the filters by particulate matter which may cause inaccurate classification (Henry, 2004).

Sedimentation, also referred to as the settling method, is another method used for assaying attachment of bacteria to different sizes of particles (Kunkel et al., 2013; Oliver et al., 2007). In this method, Stoke's law is used for calculating the settling velocity of different particle sizes and their associated bacteria. Bacterial attachment to different sizes of particles is determined by calculating the difference between the concentration before and after settling of each particle size.

The last separation method is centrifugation, which is widely used for separating unattached from attached bacteria (Cizek et al., 2008; Krometis et al., 2007; Fries et al., 2006; Guber et al., 2005; Characklis et al., 2005; Muirhead et al., 2005; Schillinger and Gannon, 1985). Samples are centrifuged at a certain rotation per minute (rpm), which varies by study. After centrifugation, the supernatant is used for determining unattached bacteria. Again, the attached bacteria concentration is determined by the difference between unattached and total bacteria. Centrifugation is limited in its ability to differentiate between clay particles and unattached bacteria, as the size of clay and unattached bacteria can be similar (Henry, 2004).

2.8 Best Management Practices

Microbial contamination is a major concern for water resources. Bacteria can enter the environment in two ways: i) direct deposition of fecal matter or point source discharges, and ii) bacteria transport via hydrological pathways such as soil seepage, runoff, or tile drainage (Kay et al., 2012 and Collins et al., 2007). Management practices have been designed to improve microbiological water quality, typically by limiting access of animals to waterways (Bragina et al., 2017; Smolders et al., 2015; Miller et al., 2010, Willms et al., 2002) or treating water before it reaches a waterbody (Sunohara et al.,

2016; Craggs et al., 2004a,b; Parkyn, 2004). Some examples include vegetative treatment systems (Harmel et al., 2018a), limiting contact between livestock and waterbodies by permanent fencing, bridging, buffer strips, or alternative water sources (Bragina et al., 2017; Smolders et al., 2015; Miller et al., 2010, Willms et al., 2002), and riparian area management (Parkyn, 2004).

A vegetative treatment system (VTS) is a wastewater treatment system which includes at least one vegetation treatment, often known as vegetative treatment areas (VTAs) or vegetative filter strips (VFS), to reduce the pollution risk from an open feedlot livestock system (USDA-NRCS, 2006). These treatment systems use sedimentation (i.e. solids settling), infiltration, and filtration to treat waters passing through them. In a study on the effectiveness of VTAs in reducing bacteria within runoff from a swine operation over a 4-year period, Harmel et al. (2018a) reported an average reduction of 73-94% for *E. coli*. Fajardo et al. (2001) simulated small-scale runoff from manure stockpiles and observed fecal coliform removal rates were 64-87% when water passed through a VFS. Similarly, Ikenberry and Mankin. (2000) examined runoff from pastureland and found 76.6% removal of fecal coliforms when the water was treated with a VFS. Constructed wetlands are another vegetative system for improving runoff water quality before entering receiving waterbodies. Davies and Bavor (2000) monitored and compared bacteria removal rates from constructed wetlands and a pollution control pond, and determined that constructed wetlands removed significantly more bacteria than the pollution control pond.

Restricting livestock access to streams is another management practice for improving microbiological water quality, as livestock have been linked to poor water

quality (Davies-Colley et al., 2004; Line, 2003). There are several ways of restricting livestock access to streams, such as stream fencing (Bragina et al., 2017; Miller et al., 2010), stream bridging (Smolders et al., 2015), and alternative water sources (Willms et al., 2002). Line (2003) measured the effectiveness of livestock exclusion by installing fencing. Researchers observed bacterial reductions of fecal coliforms of 65.9% and enterococci of 57%. Similarly, Muenz et al. (2006) analyzed three unfenced streams and two fenced streams to determine the effectiveness of fencing in an agricultural stream. They observed average fecal coliform and fecal streptococci concentrations of 410 CFU 100 mL^{-1} and 1239 CFU 100 mL^{-1}, respectively, in the three unfenced streams; whereas the average concentrations of fecal coliforms was 197 CFU 100 mL^{-1} and fecal streptococci was 927 CFU 100 mL^{-1} in the two fenced streams. Doran and Linn (1979) measured fecal coliform concentrations that were 5-10 fold higher in runoff from a grazed area than a fenced and ungrazed area (Doran and Linn, 1979). Davies-Colley et al. (2004) supported this finding with their observation that cows defecated 50 times more per square meter of the stream than elsewhere.

Riparian management is the last step of reducing contamination before it enters the stream (Parkyn, 2004). There are several forms of riparian management including grass filter strips, fenced or riparian wetlands, and filter strips with rotational grazing (Parkyn, 2004). These management practices can act as a filter and trap contaminants and sediments, as well as reduce the impact and magnitude of overland flow by increasing infiltration into the soil. In addition, a combination of riparian management practices, including livestock exclusion, provide higher reductions of microbial contaminants (Kay et al., 2012; Wilcock et al., 2009; Parkyn, 2004). For example, Wilcock et al. (2009)

observed a median *E. coli* reduction of 116 MPN 100 $mL^{-1}year^{-1}$ after installing two management practices, stream fencing and riparian management, to reduce dairy effluent and runoff from a pasture. Parkyn (2004) found installing both fencing and riparian buffer strips greatly reduced microbial contamination in a pastoral stream.

In addition, controlled tile drainage (CTD) can be used to limit pollutant transport from tile drainage systems. The CTD regulates the tile discharge through the use of a water flow control structure, and has both environmental and agricultural benefits including a potential increase in crop yields and a reduction of tile drainage pollution (Nash et al., 2015; Wilkes et al., 2014; Sunohara et al., 2014; Ghane et al., 2012; Skaggs et al., 2012). Sunohara et al., (2016) assessed the effectiveness of CTD for reducing *E. coli* and enterococci added to surface waters at a watershed scale and reported a 76% and 25% reduction of *E. coli* and enterococci, respectively, in drainage water over nine growing seasons. Frey et al. (2015) compared CTD and uncontrolled tile drainage for reducing microbial loads and found significant load reductions of FIB and *Campylobacter sp*. in CTD systems.

2.9 FIB in Streambed Sediment: Importance of The Current Study

Although surface water is routinely assessed to monitor water quality, streambed sediments are often overlooked as a source of FIB to the water column; however, it is well understood that streambed sediment harbors FIB and often contains higher FIB concentrations than the water column. Research has been done on FIB survival and persistence in sediment yet the variability of FIB concentrations in sediment are not well understood. More research should be done to monitor and quantify the variability of FIB

in streambed sediment to provide insight on this unconventional source of microbiological water quality impairment.

In addition, the variability of FIB in sediment is influenced by many factors, including asymmetric bacterial distribution in sediments and its association with sediment particles. In-depth knowledge about microbial attachment to sediment particles is useful to predict bacterial fate and transport and determine potential removal mechanisms. While research has been done on microbial attachment to settleable particles in the water column, the author is not aware of any published research on bacterial association with sediment particles in streambed sediment. Lack of knowledge on microbial attachment to sediment particles in bed sediment is an impediment to understanding and predicting the fate and transport of bacteria in waterways. Therefore, this work examined the microbial attachment to sediment particles in streambed sediment for better prediction of bacterial fate and transport in waterbodies and assess the contribution of sediment to poor water quality.

CHAPTER 3: VARIABILITY OF *E. COLI* IN STREAMBED SEDIMENT AND ITS IMPLICATION FOR SEDIMENT SAMPLING

Abstract

E. coli is the number one cause for water quality impairments in rivers and streams in South Dakota and the United States. Stream bottom sediments can be a reservoir for bacteria, including pathogenic organisms and fecal indicator bacteria (FIB), due to the favorable conditions provided by sediments for survival. Despite this, little is known about the variability of *E. coli* in sediments which should be considered when developing a sampling regime. This study examines the spatial variability and temporal stability of *E. coli* concentrations in stream bed sediment and the implications for sediment sampling and processing. Five locations were sampled for sediment *E. coli* along two tributaries to the Big Sioux River in eastern South Dakota, four along Skunk Creek (Sk1, Sk2, Sk3, and Sk4) and one on Six Mile Creek (SM). Sediment samples were collected by creating a five-by-five sample grid at each site for a total of 25 samples. Samples were processed two times, within 8-hour and 24-hour of sample collection, to assess the temporal stability of *E. coli* in sediment samples. *E. coli* concentrations in the sediment ranged from 4 to 997 CFU g^{-1} (8.9×10^{2} to 2.1×10^{5} CFU 100 mL^{-1}). All the Skunk Creek sites were dominated by sand particles, with the D50 value ranging from 0.32 to 0.35 mm, while the SM site was dominated by gravel particles (D50=6.72 mm). The Spearman correlation showed a significant correlation between particle size and *E. coli* concentration in bed sediment; however, the direction of the correlation was inconsistent between sites. The sample size analysis indicates the spatial variability of *E. coli* concentration in sediment is high and a single grab sample may not

be able to provide adequate representation of *E. coli* concentrations in sediment without substantial error. Sampling uncertainty was lowest for Sk1 (±192%). In addition, samples can be held up to 24-hour after sample collection in refrigerated conditions in a majority of cases (80%) without significant changes in *E. coli* concentrations. The findings provide insight for designing *E. coli* monitoring projects and promoting the awareness of unconventional sources of microbiological water quality impairment which are often overlooked.

Keywords

E. coli, sediment, spatial variability, temporal stability, fecal indicator bacteria, sampling, load, uncertainty

3.1 Introduction

Fecal indicator bacteria (FIB), including *E. coli*, are the leading cause of impairments in rivers and streams within the United States. *E. coli* alone is responsible for impairments in over 100,000 miles of rivers and streams in the U.S. (USEPA, 2016). Conventional sources include runoff from manure-applied agricultural fields, direct fecal deposition, leaching from failed septic systems, and waste water treatment plant outflows (Garzio-Hadzick et al., 2010). The presence of FIB in streams is used to indicate fresh fecal contamination (Garzio-Hadzick et al., 2010; Wade et al., 2003), and thus the risk of pathogen exposure (Haack, 2017). However, FIB have the ability to survive and persist in the environment for a long time (Anderson et al., 2005), meaning that their presence does not always indicate fresh contamination. FIB can live in the environment for a few hours (Haack, 2017), days (Haack, 2017; Anderson et al., 2005), weeks (Haller et al., 2009a;

Jamieson et al., 2005b), or even months (Haack, 2017; Garzio-Hadzick et al., 2010; Czajkowska et al., 2005) depending on the suitability of the environmental conditions.

There are different environmental factors that influence the survival and persistence of FIB in the environment, including temperature, nutrient availability, salinity, oxygen levels, predation, and UV radiation (Craig et al., 2004; Hughes, 2003; Thomas et al., 1999; Davies et al., 1995). Sediment is one such environment that provides favorable conditions for FIB survival (Jamieson et al., 2005b; McElhany and Pillai, 2011), with increased nutrient and carbon availability (Davies et al., 1995), reduced exposure to sunlight (Koirala et al., 2008), and protection from predatory protozoans (Jamieson et al., 2005b; Decamp and Warren, 2000). Survival times of FIB increase from days or weeks in the water column (Haack, 2017; Garzio-Hadzick et al., 2010; Czajkowska et al., 2005) to weeks or months in sediments (Garzio-Hadzick et al., 2010; Haller et al., 2009a; Anderson et al., 2005; Czajkowska et al., 2005).

Not only are sediment conditions favorable for longer survival, but higher concentrations of FIB are often observed in sediment when compared to the overlying water column (Kovacic et al., 2011; Edge and Boehm, 2011; Jamieson et al., 2005b; An et al., 2002). For example, Van Donsel and Geldreich, (1971) found fecal indicator bacteria concentrations were up to 1,000 times greater in the sediment than in the water column. Similarly, Pandey et al. (2018) reported the average *E. coli* concentration in sediment was 47 to 386 times higher than the average *E. coli* concentration in water column.

Sediment disturbance results in the resuspension of particles along with the reservoir of bacteria within the sediment. Disturbances can be caused by storm events

(Pandey and Soupir, 2014; Fries et al., 2006; Jamieson et al., 2005b; Crabill et al., 1999), recreational activities (Abia et al., 2017; An et al., 2002), animals crossing the river or stream (Abia et al., 2017; Sherer et al., 1988) and commercial activity (Pettibone et al., 1996; Irvine and Pettibone, 1993; Liou and Herbich, 1976). FIB concentrations in the water column can increase 8 to 88-times due to sediment disturbances (Abia et al., 2017; Pandey and Soupir, 2014), thus substantially contributing to water quality impairments (Abia et al., 2017; Nagels et al., 2002; Sherer et al., 1988).

To accurately predict FIB concentrations in the water column, the resuspension of FIB from streambed sediments must be considered (Rehmann and Soupir, 2009), as these reservoirs of FIB can substantially increase FIB concentrations in the water column. Artificial flood studies have been performed to isolate the impact of sediment disturbances on microbiological water quality (Muirhead et al., 2004; Nagels et al., 2002). For example, Muirhead et al. (2004) released water from a supply reservoir to generate an artificial flood and exclude runoff sources of fecal contamination. They measured *E. coli* concentrations in the water column up to two orders of magnitude higher than background concentrations, increasing from 10^2 to 10^4 CFU $100mL^{-1}$, indicating that bed sediments alone can result in concentrations that exceed water quality standards when disturbed. This release of FIB from the sediment to the water column may be incorrectly interpreted as recent fecal contamination rather than resuspension, which may lead to inaccurate perception of water quality.

To better understand sediments as a source of FIB, they need to be quantified and monitored; however, little information exists to help inform sampling design. Therefore, the goal of this study is to assess the variability of *E. coli* in streambed sediment and the

implications for sampling and reporting results. The main objectives of the present work are to (i) assess the spatial variability of *E. coli* in bed sediment, (ii) determine the number of samples required for a representative *E. coli* concentration in sediment, and (iii) evaluate some of the uncertainty in sampling and processing samples. This information is required to design sediment sampling regimes and to understand the impact of unconventional sources on water quality impairments, which are often overlooked.

3.2 Materials and Methods

3.2.1 Study Area

This study was conducted on two tributaries of the Big Sioux River located in eastern South Dakota (Figure 3.1), Skunk Creek and Six Mile Creek. Skunk Creek covers a total drainage area of 73,000 acres and the land use is dominated by agricultural production, primarily row crops (38% corn and 26% soybeans). Skunk Creek is impaired for *E. coli* which exceeds the standard for limited contact recreation. The single sample maximum standard for *E. coli* in limited contact recreation waters is 1,178 CFU 100 mL^{-1} (USEPA, 2016). To improve the water quality within Skunk Creek, the city of Sioux Falls has partnered with landowners to implement Seasonal Riparian Area Management (SRAM) along large stretches of the stream. Sediments in Skunk Creek are dominated by silt clay (Mehan et al., 2016). The average streamflow during the sampling period was 16.3 cfs (0.46 cms) (USGS gauging station 06481480).

The Six Mile Creek watershed covers a drainage area of 24,000 acres with 95% of the land use in the watershed consisting of cropland, grassland, and pastureland. The soil texture of the creek is dominated by gravel with sand. Six Miles Creek has been

identified for not meeting the water quality criteria and is impaired due to FIB (East Dakota Water Development District, 2005). The average streamflow during the sample period was 211.7 cfs (59.8 cms) (USGS gauging station 06480000).

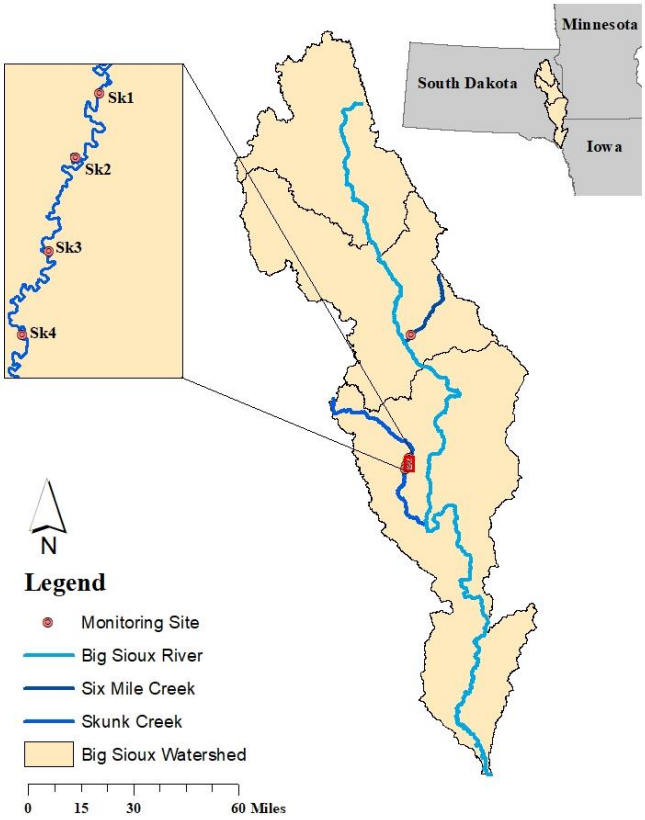

Figure 3.1: Five monitoring sites were selected in eastern South Dakota on two tributaries of the Big Sioux River. Four monitoring sites (Sk1, Sk2, Sk3 and Sk4) were located on Skunk Creek and one of the monitoring sites (SM) was located on Six Mile Creek.

Four monitoring sites are located along Skunk Creek, abbreviated Sk1, Sk2, Sk3, and Sk4. Sk1 is the most upstream site and has cattle access. Each consecutive site is located one mile (straight-line distance) downstream with Sk4 being the farthest downstream site. Nearly all the riparian area between Sk1 and Sk4 has been enrolled in SRAM. SRAM is a unique program designed to allow for limited use of riparian areas

during the recreation season. No grazing is allowed in the riparian area from April to September, but haying is permitted for partial use of the land. Grazing is permitted during the non-recreational season from October through March, provided the vegetation remains above four inches high. One monitoring site is located on Six Mile Creek, abbreviated SM. A swine facility is located near the monitoring site, providing a potential source of bacteria to the stream.

3.2.2 Sample Collection and Processing

All sites were sampled using a five-by-five grid resulting in a total of 25 samples for each site. The width of the grid was based on the width of the stream with five samples collected from one edge of the stream to the other, roughly equidistant across the stream cross section. Each of the five rows of the sample grid were spaced approximately five feet apart. This resulted in a total sample area ranging from 128 to 426 ft^2 (11.9 to 39.6 m^2). Sk2, Sk3, Sk4, and SM were sampled between September 13 and October 11, 2016; however, access to Sk1 was not granted until 2017 and sampling occurred on August 15, 2017. Samples were collected using a wide mouth bottle and transported to the laboratory on ice.

Analysis was conducted using a 1:11 sediment to phosphate buffer solution dilution ratio. Briefly, 200 mL of phosphate buffer solution was added to 20 g of sediment in a 250 mL conical flask. To detach and disperse the bacteria from the sediment, samples were shaken approximately 150 rpm for 45 minutes using an orbital shaker. The mixture was allowed to settle for 30 seconds at which point 50 mL of the supernatant was poured into a sterile test tube and used for *E. coli* enumeration.

E. coli concentrations were determined using standard membrane filtration methodology with modified mTEC agar (USEPA, 2002). Briefly, the supernatant was passed through a 0.45-micron filter, plated on modified mTEC agar, and placed in a water bath for 2 ± 0.5 hours at 35°C ± 0.5 °C to resuscitate any stressed bacteria prior to incubation. After removing the sample from the water bath, it was incubated for 22 ± 2 hours at 44.5°C ± 0.2 °C. Samples were processed twice, once within 8-hour and once within 24-hour of sample collection, to determine the temporal stability of *E. coli* concentrations in streambed sediments stored in refrigerated (~ 4°C or, 37 °F) conditions.

3.2.3 Sediment Particle Size Analysis

Sediment characterization was conducted for each sample to determine the relationship between particle size and *E. coli* concentrations. Particle size was characterized using a sieve analysis (ASTM, 2007), which covers the quantitative distribution of particle sizes greater than 75 μm in sediment. A total of nine sieves was used to characterize the distribution of particle sizes. Approximately 500 g of sample was oven dried at 105 °C for 24-hour. The oven dried samples were shaken in a sieve shaker for ten minutes. The weight of the sample in the sieves was measured and the total weight of particles falling within each size range was calculated using the difference in weights.

3.2.4 Uncertainty

Both random and systematic uncertainty were calculated for this work. The 25 sampling data points for each of the five monitoring sites were used to calculate the random uncertainty (equation 3.1).

$$\pm\%uncertainty = \frac{\Delta x_i}{x_i} \approx \frac{2SD(x_i)}{x_i} \quad (3.1)$$

Where x_i is the measured mean and SD is the standard deviation of the raw dataset.

Systematic uncertainty was calculated for the location within the stream (edge and middle) as well as the holding time (equation 3.2). For the calculation of the sampling location uncertainty, the 25 sediment samples were divided into two categories, the edge and the middle of the stream, for each monitoring site.

$$\%uncertainty = \frac{(b_i - a_i)}{a_i} \quad (3.2)$$

Where a_i is the "true" value and b_i is the measured value. For the location uncertainty calculation, the measure of central tendency for all 25 data points (*E. coli* concentrations) were used as the "true" value while the 8-hour holding time was used as the "true" value, for the holding time uncertainty analysis.

3.2.5 Statistical Methods

The normality of the data was evaluated using the Shapiro-Wilk test due to its suitability for data with small sample sizes ($n < 50$) (Ghasemi and Zahediasl, 2012). The original (i.e. non-log transformed) data from all the sites as well as the log transformed data from three out of the five sample sites were not normally distributed ($p < 0.05$). Therefore, the original data was used, and non-parametric tests were selected to evaluate the data. Historically, studies have often presented the mean FIB concentration in stream sediments (Wilson et al., 2016; Muirhead et al., 2004; Desmarais et al., 2002). However, the extreme rightly skewed data found in this work (skewness range was 1.5 to 3.6) demonstrate that other measures of central tendency may be more appropriate. To compare this work to previous studies as well as provide an appropriate measure of

central tendency for the skewed data, we have presented the mean, geometric mean, and median values.

The potential differences in *E. coli* concentrations among the monitoring sites as well as the location of sediment samples within the stream cross-section were analyzed using the Kruskal-Wallis test. This is a nonparametric test that can be used for independent datasets with different or equal sample sizes. The Bonferroni correction was used to determine the significant difference of *E. coli* concentrations between the sites. This is a post-hoc test for nonparametric data which adjusts the p-values when multiple pair wise tests are performed simultaneously on a single set of data. In this test, each significance level for each individual hypothesis is α/m, where α is the desired significance level and m is the number of hypotheses.

The Wilcoxon signed-rank test was used for analyzing the differences between the two sample processing times, 8-hour and 24-hour after sample collection. This test is a nonparametric test for paired datasets; in this case, the same sample processed at the two different time intervals.

While much research has been conducted on sediment FIB concentrations, little information has been provided on the appropriate number of samples to effectively represent the FIB concentration at a particular site. Equation 3.3 was used to calculate sample size with an increase of 15% due to the non-normal distribution of the data (Lehmann and D'Abrera, 1998).

$$n = \left[\frac{Z_{\alpha/2} \times \sigma}{E}\right]^2 \tag{3.3}$$

where n is the sample size, $Z_{\alpha/2}$ is the critical value, σ is the population standard deviation, and E is the margin of error.

All statistical analyses were conducted using R (version 3.3.1) and RStudio (version 1.0.143).

3.3 Results and Discussion

3.3.1 *E. coli* Concentrations and Loads

The measured sediment *E. coli* concentrations were highly variable, demonstrated by the high standard deviation relative to the mean concentrations (Table 3.1). Sediment *E. coli* concentrations had a three order of magnitude range. The minimum concentration measured at all five sites was 4 CFU g^{-1} located at Sk4 and the maximum concentration was 997 CFU g^{-1} at Sk1. Along Skunk Creek, the concentrations were highest at Sk1. Sk2 and Sk3 showed similar concentrations and Sk4 had the lowest concentrations. The high concentrations found at Sk1 are unsurprising given the accessibility of the site to cattle. Previous research has also found that cattle access to streams can result in *E. coli* concentrations in the sediment that are several fold higher than seen in areas with other land uses (Bragina et al., 2017; Stephenson and Rychert, 1982).

All measures of central tendency were over five times higher at Sk1 than Sk4. The reductions observed between Sk1, the cattle crossing, and Sk4, located three miles downstream, were significant ($p < 0.05$). The three miles between the two sites are almost entirely enrolled in SRAM, a best management practice to reduce FIB concentrations in the stream. This provides support for the theory that riparian management strategies, such as SRAM, can reduce FIB concentrations in sediments in

addition to the water column reductions observed by many previous studies (Bragina et al., 2017; Smolders et al., 2015; Parkyn, 2004).

Table 3.1: Table of statistics for *E. coli* concentrations in CFU g^{-1} for all sites. The highest and lowest *E. coli* concentrations were observed at Sk1 and Sk4, respectively.

	Sk1	**Sk2**	**Sk3**	**Sk4**	**SM**
Min	8	31	14	4	7
Max	997	788	899	212	701
Mean	240	158	147	45	63
Geomean	135	105	105	24	25
Median	157	84	115	19	17
Std Dev	230	171	167	55	144

The potential load from streambed sediments was also estimated by multiplying area weighted average concentration with sample area, sample depth and sediment density. The potential *E. coli* load ranged from 2.7×10^6 to 1.8×10^7 CFU m^{-2}. The cattle crossing at Sk1 had the highest load, which may be due to direct fecal deposits from cattle accessing the stream. Cattle have been reported to defecate 50 times more when crossing a stream than elsewhere (Davies-Colley et al., 2004). Muirhead et al. (2004) measured *E. coli* from sediments at cattle crossings and also found much higher levels (3-4 order of magnitude) than other locations; however, their estimated *E. coli* yield was an order of magnitude higher than estimated by this work at 10^8 CFU m^{-2}.

3.3.2 *E. coli* Variability

The highest *E. coli* variability was observed at Sk1 (Figure 3.2) and gradually decreased moving downstream. Every sample site had pockets of high *E. coli* concentration which were typically located at the edge of the stream (Figure 3.3), except for Sk1 where the high *E. coli* pockets were located in the middle of the stream, possibly due to direct fecal deposits from cattle. These pockets of high bacteria concentration caused the stream bottom sediment *E. coli* data to be skewed. Results indicated that the data were right skewed for all sites ($p < 0.01$). The skewness persisted in three out of the five sites even after $\log_{10}$ transformation. Given the skewed nature of the dataset, a measure of central tendency appropriate for skewed data should be used, such as geometric mean or median concentrations; however, past literature often reports arithmetic mean values (Wilson et al., 2016; Pandey and Soupir, 2013; Abhirosh et al., 2010; Muirhead et al., 2004; Desmarais et al., 2002) which may result in overestimation of FIB concentrations in the sediment. Wilson et al. (2016) noted this potential issue but opted to report the mean to preserve the effect of extreme values. The arithmetic mean, geometric mean, and median of all sites were presented herein for comparison to previous work, as well as presenting more appropriate measures of central tendency for these data.

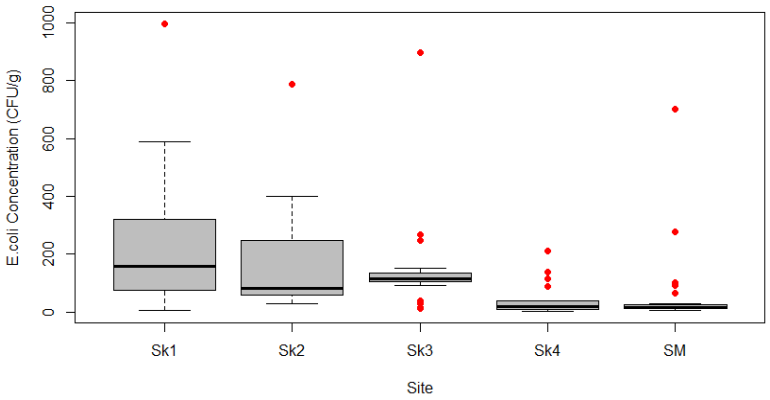

Figure 3.2: *E. coli* concentrations at all five monitoring sites varied from four to nearly 1,000 CFU g^{-1}. Outliers are indicated by red points.

Past studies have measured similar concentrations of *E. coli* in sediment. For example, Donderski and Wilk (2002) measured sediment *E. coli* in a river and found concentrations ranging from 1.2×10^{2} to 2.4×10^{2} CFU g^{-1} wet weight. Haller et al. (2009a) examined the influence of sediment characteristics on FIB persistence and found a wide range of concentrations, from 1×10^{0} to 8.5×10^{3} CFU g^{-1}. In a study by Perkins et al. (2014) sediment *E. coli* concentrations ranged from 0 to 2.4×10^{2} CFU g^{-1} wet weight. Higher concentrations than those measured in this study have also been reported, such as the range reported by Ouattara et al. (2011) who found the geometric mean concentration of *E. coli* ranged from 210 to 3.3×10^{5} CFU g^{-1}.

The arithmetic mean values of the *E. coli* concentrations were 40% – 152% greater than the geometric means and 28% - 271% greater than the median values, emphasizing the skewness of the data. Arithmetic mean considers every single observation of the dataset making it more sensitive to outlier. The skewed data (e.g. outlier) can drag the mean from the typical value resulting inability of mean to measure the best central location of the dataset. Thus, choosing an appropriate measure of central tendency is important and depends, in part, on the objectives of the study. Such as, Wilson et al. (2016) also reported skewed data, they opted to report the arithmetic mean value as they wanted to preserve the effect of extreme values. However, reporting the arithmetic mean of skewed data can result a misleading representation of the central location of the dataset.

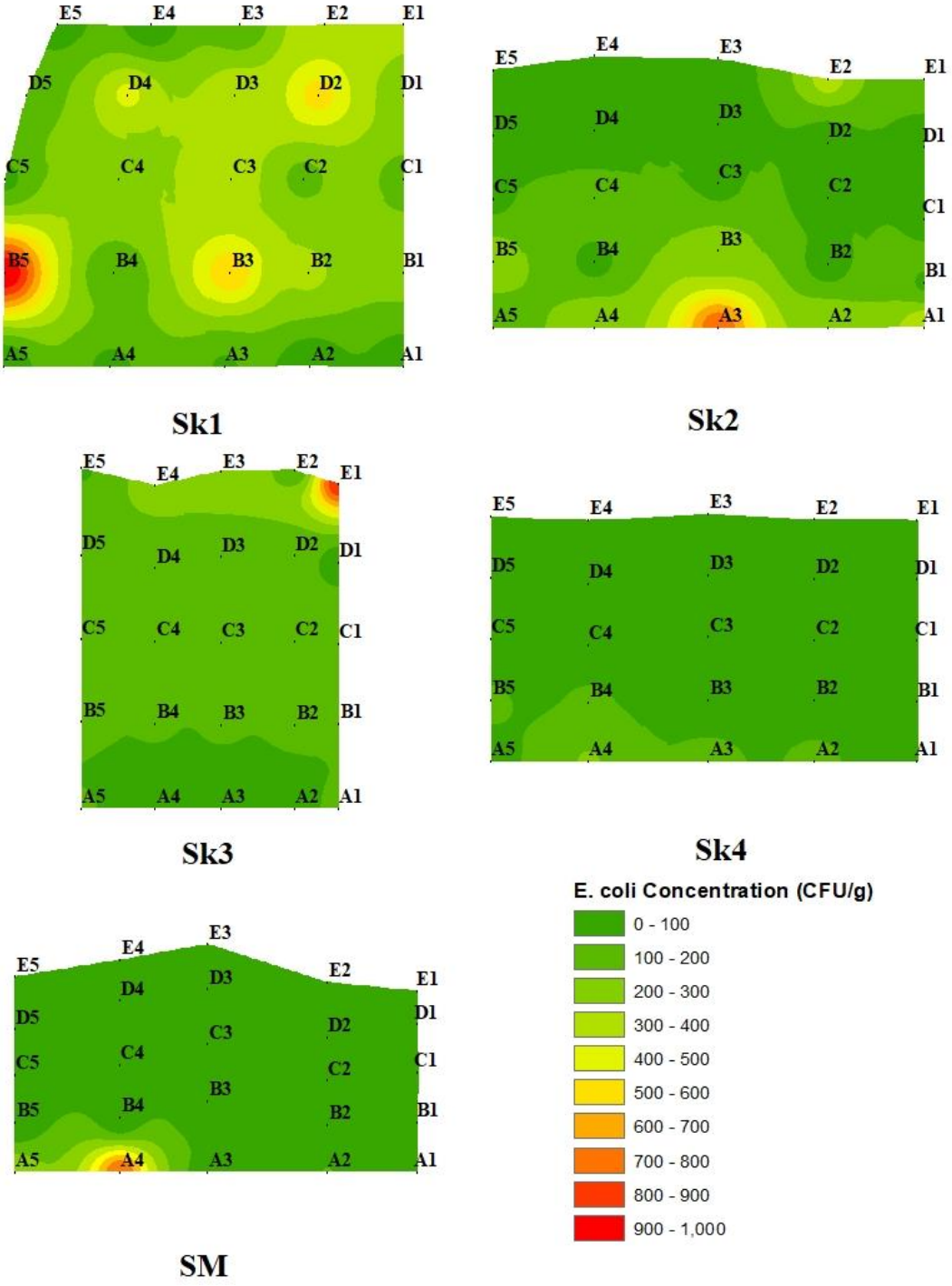

Figure 3.3: The aerial view of *E. coli* concentrations at the five monitoring sites shows pockets of high *E. coli* concentrations at all sites. Rows A and E are the banks of the stream and the flow is moving from column 5 to column 1.

3.3.3 Particle Size Relationship

Past studies have demonstrated strong relationships between sediment particle size and *E. coli* concentrations in the sediments (Pandey and Soupir, 2014; Garzio-Hadzick et al., 2010; Howell et al., 1996; Sherer et al., 1992). Due to this demonstrated relationship, sediment particle size was evaluated to determine if particle size has a significant impact on the *E. coli* concentrations at one instance of time within a single site.

Skunk Creek samples were dominated by sand sized particles (0.02 to 2 mm) whereas the Six Mile Creek samples were primarily gravel (2 to 6.3 mm) (Table 3.2). This is supported by the D_{50} which ranged from 0.32 to 0.35 mm at all Skunk Creek sites and was 6.72 mm at the Six Mile Creek site. All monitoring sites had a moderate to strong correlation between *E. coli* concentration and the percent of fine particles (< 0.075 mm); however, the direction of the correlation was inconsistent. Sk1 and Sk3 had significant negative correlations between fine particles and *E. coli* concentrations, whereas the other three sites (Sk2, Sk4, and SM) had significant positive correlations, where coarse particles (>2 mm) showed moderate to strong correlation with *E. coli* concentration but the direction was opposite. For instance, strong positive correlation was observed for sites Sk1 and Sk3 and strong negative correlation was observed for sites Sk2, Sk4 and SM.

Strong correlation between particle size and *E. coli* may suggest the importance of considering the variation of particles while sampling. More variation in the sediment composition may require a larger number of samples to accurately quantify the sediment source of *E. coli*. While, all Skunk Creek monitoring sites having almost consistent D50 and strong but inconsistent direction of ρ value may indicate homogeneity of sediment texture, velocity and shear stress, but statistical heterogenization of *E. coli* persistence within the stream reach.

Table 3.2: Characterization of sediment particle size. Skunk Creek was dominated by sand particles whereas Six Miles Creek was dominated by gravel particles. The Spearman-rho correlation between fine particles (< 0.075 mm) and *E. coli* concentration shows a strong relationship at all sites, though the direction was inconsistent.

	Soil Type (%)				
	Coarse Soil		Fine Soil	**D50 (mm)**	**Correlation (ρ)**
Monitoring Site	*Gravel (2 - 6.3 mm)*	*Sand (0.02 – 2 mm)*	*Silt & clay (< 0.02 mm)*		
Sk1	1	91	8	0.35	-0.56
Sk2	16	77	7	0.32	0.62
Sk3	4	85	11	0.34	-0.48
Sk4	2	93	5	0.34	0.7
SM	75	23	2	6.72	0.64

Past work has also demonstrated both significantly positive and significantly negative relationships between particle size and *E. coli* concentration (Curtis and Trapp, 2014; Pandey and Soupir, 2014; Piorkowski et al., 2014b; Garzio-Hadzick et al., 2010; Lang and Smith, 2007; Cinotto, 2005). However, these studies analyzed sediment characteristics depending on different geomorphological features, for multiple instances in time, or multiple sites. Cho et al. (2010a), Garzio-Hadzick et al. (2010), Pandey and Soupir (2014) and Perkins et al (2014) all observed increasing *E. coli* concentrations with increasing percent of fine particles. Other research has found that cohesive particles and

fine sediments contain more bacteria (e.g. Black et al., 2002; Muirhead et al., 2004). Higher FIB concentrations in sediments with fine particles has been hypothesized to be, in part, a result of the protection of bacteria from predators by the fine particles and favorable environmental conditions like higher moisture availability in clays (Garzio-Hadzick et al., 2010; Cools et al., 2001).

On the other hand, Lang and Smith, (2007) reported finer particles (i.e. silty clay) to contain fewer *E. coli* than coarser particles (i.e. sandy loam). Similarly, Cinotto, (2005) observed coarse or sand particles had a strong correlation with *E. coli*. Reports of large particles (0.125 – 0.5 mm) containing the highest median concentration of *E. coli* may be due to the permeability and nutrient accessibility facilitated by coarse particles (Cinotto, 2005).

3.3.4 Sampling Uncertainty

The 25 (5×5 grid) sampling mean *E. coli* data in bed sediment was used for measuring random sampling uncertainty for each monitoring site (Table 3.3). In each site the uncertainty varied considerably, and ranging from ± 192% to ± 458%, the lowest and highest uncertainty value observed at site Sk1 and SM, respectively. This high uncertainty value indicating the spatial variation of *E. coli* in sediment is quite high as compared to the arithmetic mean value. The high uncertainty

Table 3.3: Random sampling uncertainty for 25 sampling in five monitoring sites.

Sample Site	Uncertainty (%)
Sk1	192
Sk2	215
Sk3	227
Sk4	246
SM	458

value indicates that a single grab sample likely will not adequately represent the concentration of *E. coli* in sediment (Berry et al., 2007; Erkenbrecher, 1981).

The systematic uncertainty across the transect of the stream for the five monitoring sites was also measured (Table 3.4). In this case, the "true" value for each measure of central tendency was estimated using all 25 samples. The uncertainty in the arithmetic mean at the edge overpredicts the *E. coli* population, while the middle underpredicts the "true" arithmetic means for all sites except Sk1. The difference in streamflow across stream cross-section likely influences the *E. coli* concentration across the transect, with lower flows at the edge of the stream resulting in more bacterial deposition. Four out of the five sites had less uncertainty in the median and geometric mean than the arithmetic mean, as well as less uncertainty in samples taken from the middle of the stream as compared to the edge.

Table 3.4: Sampling location uncertainty within the stream cross section for five monitoring sites for both mean and median data. Here, sampling location per monitoring site divided in two section depending on stream cross section named as edge (A, E) and middle (B, C, D) of the stream.

Sample Site	Location Uncertainty (%)					
	Mean		Median		Geomean	
	Edge	Middle	Edge	Middle	Edge	Middle
Sk1	-56	37	-63	70	-63	95
Sk2	66	-43	159	-22	88	-34
Sk3	31	-21	2	0	-9	6
Sk4	48	-32	27	-15	39	-20
SM	109	-73	150	-10	101	-37

The uncertainty at Sk1 is inconsistent when compared to the other sites. This is the only site with direct cattle access which may have influenced the distribution of *E. coli* across the transect as well as the uncertainty. More work comparing the pattern of *E. coli* populations in stream sediments will need to be conducted to determine if these differences are consistent with direct cattle access.

The estimated uncertainty for sediment *E. coli* concentrations was much higher than previously reported for water (Harmel et al., 2018b; Quilliam et al., 2011). Harmel et al. (2018b) compiled data from previous literature and found the random uncertainty ranged from ±33% to ±101% for grab samples from the water column.

3.3.5 Sample Size

The sample size was determined for all five monitoring sites with variabilities ranging over an order of magnitude for a range of error margins (Table 3.5). The cattle crossing at Sk1 had the highest variance in *E. coli* concentrations and, therefore, the highest number of samples were required to characterize the site. Both the *E. coli* concentrations and variability in *E. coli* concentrations decreased as the sample sites moved downstream through the three miles of SRAM adjacent waters. The sample size ranged from four to 65 for a moderate margin of error (i.e. 60 CFU g^{-1}) and is much higher than the number of samples collected in several past studies (e.g. Pandey and Soupir, 2014; Shelton et al., 2014; Brinkmeyer et al., 2015; Jang et al., 2015; Bradshaw et al., 2016; Wilson et al., 2016). These results demonstrate that a single sample cannot adequately capture the variability of *E. coli* in streambed sediments without substantial error.

Table 3.5: Sample size analysis for all sites for various margins of error (E) at a 95% confidence.

Monitoring Site	**Variance**	**Sample Size, n**				
		E-20	E-40	E-60	E-80	E-100
Sk1	5.3×10^4	586	147	65	37	23
Sk2	2.9×10^4	321	80	36	20	13
Sk3	2.8×10^4	308	77	34	19	12
Sk4	0.3×10^4	34	8	4	2	1
SM	2.1×10^4	230	57	26	14	9

Previous studies have also reported high variabilities of *E. coli* in sediments (Berry et al., 2007; Erkenbrecher, 1981), and it is not uncommon to observe concentrations ranging from two to five orders of magnitude at the same site or in the same watershed (Pachepsky and Shelton, 2011; Cho et al., 2010a). Cho et al. (2010a) also noted that collecting grab samples could result in missed hotspots due to the high variability of *E. coli* in sediment.

Ideally, sample size should not be too small or too big, as both may point out the conclusion in wrong direction and decreases the ability of statistical power. For instance, too few samples may be statistically limited to reject the null hypothesis, while big data may cause bias of the output likely resulting something significant becomes insignificant (Faber and Fonseca, 2014).

3.3.6 Temporal Stability during Storage

In general, the time between water sample collection and analysis should be as short as possible to limit changes in microbial populations within the sample. However, sediments have been shown to be a more stable source of bacteria than water (Pachepsky and Shelton, 2011) with long survival times (Haack, 2017; Haller et al., 2009a; Garzio-Hadzick et al., 2010). No information is available on potential changes to FIB concentrations in sediment samples during storage; therefore, a comparison between short (i.e. < 8-hour) and long (~24-hour) storage time was conducted to determine the temporal stability of *E. coli* in sediment samples and the resulting uncertainty in storing these samples in a refrigerated (~ 4°C or, 37 °F) environment.

The *E. coli* concentrations in four out of five of the sample sites did not show significant differences when processed within 8-hour and 24-hour after sample collection. Sk2 was the only site that demonstrated a significant difference in concentrations between the short and long sample storage times. This indicates that sediment samples can be processed within 24-hour without significant changes to the *E. coli* concentrations in the majority (80%) of sample locations.

Table 3.6: Uncertainty resulting from sample storage in a refrigerated environment

Sample Site	Storage Uncertainty (%)		
	Mean	**Median**	**Geomean**
Sk1	-10	-0.4	4
Sk2	-17	2	-20
Sk3	33	-5	19
Sk4	62	-13	5
SM	-7	-32	-28
Average	12	−10	−4

The systematic uncertainty for different storage times was calculated for the mean, median, and geometric mean of the data (Table 3.6). The median provided the least amount of uncertainty for three of the five sites monitored, but the average uncertainty for all the sites was least for the geometric mean. In addition, the average uncertainty for all measures of central tendency was within ±12%. The median concentration of *E. coli* tended to be reduced over the holding period, as demonstrated in four out of the five sets of samples. However, both the arithmetic mean and the geometric mean values were split, showing no consistent pattern in the uncertainty between the sites. The average arithmetic mean concentration increased by 12%, while the geometric mean decreased by 4%. This is less change than observed in water samples reported by USEPA (2006) where *E. coli* concentrations decreased 20% on average after a 24-hour holding period. However, a study by the Texas Commission on Environmental Quality (TCEQ) only

reported a small decrease, 4% in *E. coli* concentrations after 24-hour as compared to an 8-hour holding time (TCEQ, 2008).

3.4 Conclusions and Recommendations

The current study focused on understanding the variation of *E. coli* concentration in bed sediment and its implications for sediment sampling. Higher *E. coli* concentrations in bed sediment were observed at the site with direct cattle access as compared to the cattle exclusion sites. High spatial variation of *E. coli* in bed sediment was observed throughout the stream cross section, with pockets of high concentrations at all sites resulting in skewed distributions. Significant relationships between *E. coli* and fine particles (< 0.075 mm) were observed at all sites; however, the direction was inconsistent. The lowest uncertainty in the *E. coli* samples was $\pm$192%. The high sampling uncertainty and sample size analysis implies that a single grab sample may not be able to adequately represent *E. coli* concentrations in the sediment without substantial error. Finally, sediment samples can be stored in refrigerated conditions up to 24 hours without significant changes in the *E. coli* concentrations in the majority of cases. The systematic uncertainty resulting from a 24-hour holding period was within ± 12% on average, depending on the measure of central tendency used. Additional work should include measurements from sites with different characteristics and land uses to determine the consistency of the results.

3.5 Acknowledgements

This research was funded by the US EPA, South Dakota Department of Environment and Natural Resources, and USDA Hatch projects SD00H604-15 and SD00H452-14 courtesy of the SDSU Agricultural Experiment Station. We appreciate the

support for this work including the Moody County Conservation District and East Dakota Water Development District who assisted with site access as well as Miranda Lebrun, Louis Amegblator and Sara Mardani who helped with sample collection and processing.

CHAPTER 4:EFFECT OF SEASONAL VARIABILITY AND STORM EVENTS ON *E. COLI* IN FRESHWATER SEDIMENTS: A CASE STUDY

Abstract

One often overlooked source of fecal indicator bacteria (FIB) to the water column is bed sediments. Sediments can provide a favorable environment for FIB to survive and persist, resulting in a reservoir of these bacteria which are available for resuspension during disturbances such as storm events, and can contribute to water quality impairments. The goal of this study was to assess the variation in streambed sediment reservoirs of *E. coli* over the recreation season and as a result of storm events, using a case study in eastern South Dakota. The upstream-most site (abbreviated Sk1) was the site of a cattle crossing, while the downstream sites were enrolled in a management practice that removed cattle from the stream (abbreviated Sk2, Sk3, and Sk4). Three to five samples were collected from May to October from each monitoring site for two years, 2017 and 2018. *E. coli* concentrations in the sediment ranged from 10^1 to 10^4 CFU g^{-1} (10^1 to 10^6 CFU 100 mL^{-1}). All sites demonstrated increases in sediment *E. coli* concentrations from early (May – July) season to late (August – October) season, with two of the sites showing significant increases. Only one storm event had a substantial hydrologic response, and sediment concentrations of *E. coli* significantly increased immediately following this event. Additional work should be conducted to determine the consistency of these results over a wider range of climatic and environmental conditions.

4.1 Introduction

Over 175,000 miles of rivers and streams in the United States are impaired due to the presence of fecal indicator bacteria (FIB), making it the leading cause of impairments in the United States (USEPA, 2019). FIB, such as fecal coliforms, *E. coli*, and enterococci, are used to identify fecal contamination, in part, because it was thought that these organisms did not survive long in the environment, and therefore represent recent fecal contamination. However, more recent research has demonstrated the ability of FIB to survive and persist in the environment (Ishii et al., 2006) and provided evidence for FIB strains adapted to environmental conditions (Pachepsky and Shelton, 2011 ; Ishii et al., 2006).

Sediments provide more favorable conditions than water, resulting in FIB decay rates that are an order of magnitude lower in sediment than water (Anderson et al., 2005; Jamieson et al., 2005b; Mallin et al., 2007). The decay rate depends on the bacterial adaptation and persistence in a particular environment (Pachepsky and Shelton, 2011). Sediment provides a favorable environment due to the availability of organic matter and nutrients (Jamieson et al., 2005a; Jamieson et al., 2005b), protection from predators (Jamieson et al., 2005a; Jamieson et al., 2005b; Decamp and Warren, 2000), and particles shielding the microbes from sunlight (Koirala et al., 2008). Studies comparing the survival in water as compared to sediment have consistently demonstrated longer survival times in sediment (Haack, 2017; Czajkowska et al., 2005; Garzio-Hadzick et al., 2010). For example, Czajkowska et al. (2005) found that *E. coli* survived 32 days in water, but over 90 days in sediment.

Not only can FIB survive for long periods of time within bed sediments, but some strains have adapted to various environmental conditions resulting in indigenous environmental populations (Pachepsky and Shelton, 2011 ; Ishii et al., 2006) in tropical (Fujioka, 2001; Fujioka et al., 1999; Byappanahalli and Fujioka, 1998), subtropical (Desmarais et al., 2002; Solo-Gabriele et al., 2000) and in temperate regions (Byappanahalli et al., 2006; Ishii et al., 2006). Ishii et al., (2006) examined the genetic relatedness of FIB, including *E. coli,* in three different soils and observed indigenous *E. coli* strains. Researchers reported a 92% similarity between soil-borne *E. coli* strains in the same locations for different seasons, indicating that these strains were naturalized members of the microbial community in the soil (Ishii et al., 2006).

While there is evidence for the survival of *E. coli* in stream sediments, their growth rate is temperature dependent (Garzio-Hadzick et al., 2010; Vital et al., 2008). The longest FIB survival times occur at low temperatures, about 4 to 5 °C (Garzio-Hadzick et al., 2010; An et al., 2002; Sjogren, 1994), while FIB die-off increases with increasing temperature (Garzio-Hadzick et al., 2010; Craig et al., 2004). However, past studies have also reported the optimum temperature for *E. coli* and enterococci growth is 37 °C with the presence of plentiful nutrients and low stress (Ishii et al., 2006; Whitman et al., 2003).

Seasonal changes in environmental conditions, such as temperature and moisture, can also influence FIB growth and survival. For example, FIB concentrations were reported to drop significantly from summer to winter in both water and sediment (Stocker et al., 2019; Crabill et al., 1999). Stocker et al. (2019) measured *E. coli* numbers in sediment during the summer and winter with values ranging from 105 to 238 CFU gdw^{-1}

in the summer and dropping to 13 to 29 CFU gdw^{-1} during the winter. Streamflow also has significant impact on in-stream *E. coli* concentrations and can vary by season. For example, higher *E. coli* concentrations have been found in streams during the wet season when compared to the dry season (Pandey and Soupir, 2014). During the wet season runoff from diffused land sources and resuspension of the sediment bed by storm events can move bacteria into the water column, thus increasing concentrations. Research has shown that sediment contains between 100 to 1000-fold more FIB than the water column (Karbasdehi et al., 2017; Norman et al., 2013; Van Donsel and Geldreich, 1971). While sediment usually contains higher FIB than the water during baseflow conditions (Bai and Lung, 2005 and Pandey et al., 2012), higher *E. coli* levels can occur in the water column during storm events (Pandey and Soupir, 2014).

Despite the potential for seasonal variation to significantly impact sediment FIB populations, previous work has largely focused on monitoring FIB variability in stream sediment for a few months to one season for a year (Stocker et al., 2019; Abia et al., 2015a; Pandey and Soupir, 2014; Curtis and Trapp, 2014; Borade et al., 2014; Orear and Dalman, 2011; Roslev et al., 2008; Shelton et al., 2008; Crabill et al., 1999) which limits our understanding of long term temporal trends, including seasonal patterns. It is important to understand seasonal patterns of FIB concentration in stream sediment for a long periods, as temperature has a strong effect on FIB survival in sediment (Garzio-Hadzick et al., 2010).

The present work provides a case study examining the variability in *E. coli* concentrations in streambed sediments both seasonally and surrounding storm events, to expand the understanding of the variability of FIB concentrations during a range of

temperature and streamflow conditions. The objectives of this study were to a) monitor seasonal patterns of FIB concentrations throughout the recreational season, b) assess FIB concentrations in streambed sediments and the water column during storm events, and c) determine the potential risk of bed sediment as a source to the water column.

4.2 Materials and Methods

4.2.1 Study Area

The study was conducted along a three mile stretch of Skunk Creek, a tributary of the Big Sioux River, located in eastern South Dakota. Skunk Creek drains 73,000 acres and the land is primarily in agricultural production, including row crops and pastureland. The average annual precipitation is 668 mm (Mehan et al., 2016) with the majority of the precipitation occurring from April to September and peaking in June. The average precipitation was 81 mm and the average temperature was 17.1 °C (62.8 °F) from 1998-2018 (NOAA: #USC00395090) during the recreation season. The average precipitation during the study period was around 84 mm, a slightly wetter year compared to the 20 year average, while the average temperature was 17.2 °C (63 °F) which was similar compared to the 20 year average (South Dakota Mesonet station CTNS2 at Colton, SD).

Skunk Creek is impaired for pathogens due to high concentrations of *E. coli* (USEPA, 2018b), and is a large contributor of microbial contamination to the Big Sioux River. The single sample maximum (SSM) standard for limited contact recreation, a designated use for Skunk Creek, is 1,178 CFU 100 mL^{-1}. Skunk Creek contributes approximately 59% of the flow in the Big Sioux River downstream of the confluence (McCutcheon et al., 2012). Thus, the water quality in Skunk Creek has a substantial impact on the water quality within the Big Sioux River, which has a stricter designation

of immersion (primary contact) recreation. Therefore, both designated uses are referenced herein.

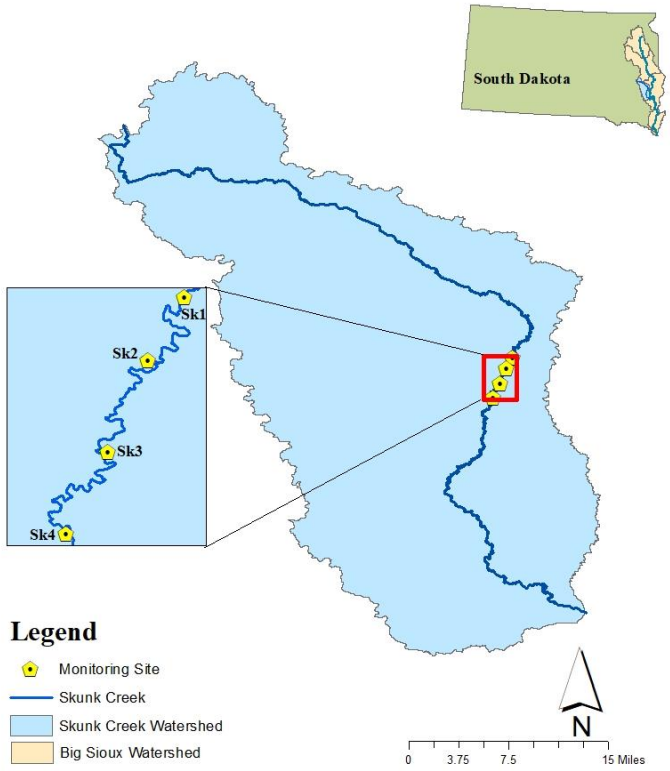

Figure 4.1: Samples were collected along Skunk Creek, a tributary to the Big Sioux River located in eastern South Dakota. Four sites were monitored each located one mile apart. The site farthest upstream (designated Sk1) is cattle crossing while the three downstream sites (Sk2, Sk3, and Sk4) are enrolled in a form of riparian management.

Monitoring occurred at four sites. The farthest upstream, abbreviated Sk1, is a cattle crossing (Figure 4.1). Three additional sites were monitored and are located a mile apart (straight line distance), abbreviated Sk2, Sk3, and Sk4 from upstream to downstream. The majority of the riparian area between Sk1 and Sk4 is enrolled in Seasonal Riparian Area Management (SRAM). SRAM is a form of riparian area management that does not allow cattle access to the stream during the recreation season (April – September), but does allow for occasional use of the land via haying. An

alternative water source is provided to the cattle and grazing can occur during the off season, starting October 1.

Streamflow data were collected from USGS gauging station 06481480 and precipitation was obtained from the South Dakota Mesonet (weather station at Colton, SD).

4.2.2 Sample Collection and Processing

Sediment samples were scooped into sterile 1 L wide-mouth bottles. During the monthly monitoring, three to five samples were collected at each site in 2017 and 2018 from May through October. Samples were collected from the edges and middle of the stream to cover the cross-sectional area.

Two study sites, Sk2 and Sk4, were selected for more detailed storm event analysis based on time constraints and site accessibility. A three by three grid was created for sediment sample collection for storm event monitoring. Samples were collected approximately one day before the event, one day after the event, and five to seven days after the event depending on weather conditions (Figure 4.2). A single water sample was also collected from both sites in a 1 L sterile wide-mouth bottle.

All samples were transferred on ice in a cooler to South Dakota State University where they were processed.

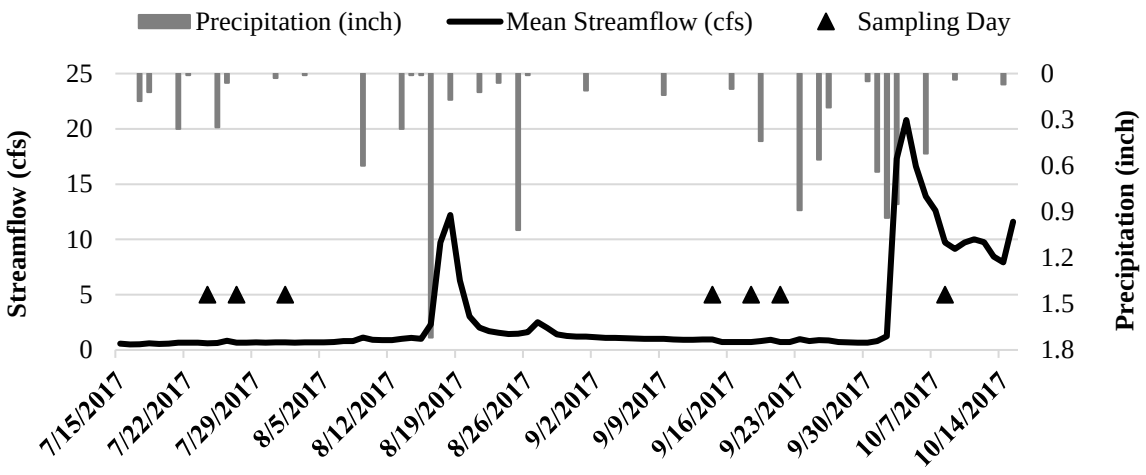

Figure 4.2: Sample collection prior to, during, and after storm events with the associated streamflow and precipitation. At the end of September there was continuous precipitation in Skunk Creek which resulted in a substantial hydrologic response.

Sediment samples were processed using a 1:10 dilution ratio of sediment: PBS (phosphate buffer solution). The mixture was shaken approximately 150 rpm for 45 minutes in an orbital shaker to disperse bacteria. After mixing the samples were allowed to settle for 30 seconds and the supernatant was used for sample analysis. *E. coli* concentrations were determined using standard membrane filtration methodology with modified mTEC agar (United States Environmental Protection Agency. USEPA, 2002). In short, the samples were filtered using 0.45 μm filters and placed on plates with modified mTEC agar. The plates were placed in a water bath for 2 ± 0.5 hours at 35°C ± 0.5°C to resuscitate any stressed bacteria prior to incubation. After removing the plates from the water bath, the samples were incubated for 22 ± 2 hours at 44.5°C ± 0.2°C.

4.2.3 Statistical Analysis

All statistical analyses were done using R software version 3.3.1 and RStudio version 1.0.143. Significant differences of sediment *E. coli* numbers within a season (early and late season) as well as storm events were determined using the Wilcoxon

Signed-Rank test. This test is nonparametric and used to rank observations of paired, or dependent, samples. In this case, the pairs were the sample locations processed at two different time intervals.

4.3 Results and Discussion

4.3.1 Seasonal Variability of *E. coli* in Sediment

The median *E. coli* concentrations ranged from 0 to 2.6×10^4 CFU g^{-1} in sediment throughout the recreation season. This is similar to previous studies which have reported concentrations ranging from 0 to 1.3×10^4 CFU g^{-1} in sediment in areas with non-point sources and human-associated pollution, such as agricultural runoff, recreational activities, wastewater treatment plants, or industrial outflow (Piorkowski et al., 2014b; Perkins et al., 2014; Curtis and Trapp, 2014; Borade et al., 2014; Haller et al., 2009a).

Sk1, the cattle crossing site, had the highest *E. coli* variation throughout the 2017 season with the concentrations ranging from 3.6×10^1 to 1.6×10^3 CFU g^{-1} (4×10^3 to 5.8×10^5 CFU 100 mL^{-1}) (Figure 4.3). The highest concentration was observed in 2017 in August at the cattle crossing. Livestock-derived manure is a well-documented source of

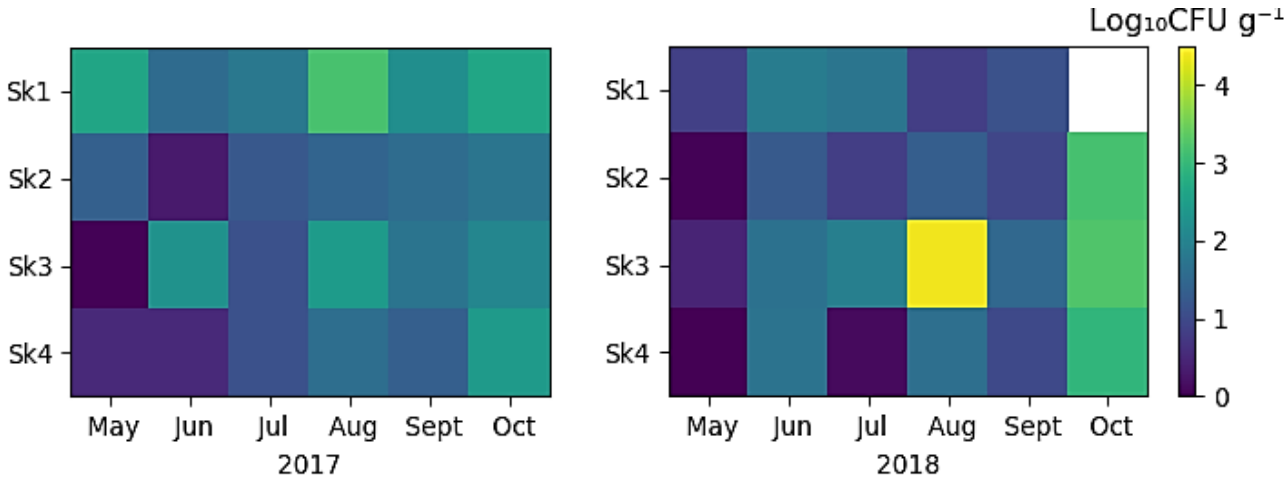

Figure 4.3: Seasonal variation of $\log_{10}$ median *E. coli* concentration from May to October for Sk1, Sk2, Sk3, and Sk4 in 2017 and 2018. Missing data are represented in white.

bacteria to stream, lake, and river sediment via runoff and direct fecal deposits (Smolders et al., 2015; Stott et al., 2011; Wilcock et al., 2007; Davies-Colley et al., 2004). Higher temperatures often result in higher *E. coli* survival and growth in sediment (Ishii et al., 2006; Berry and Miller., 2005), so the relatively higher temperatures observed mid to late season may have led to increased FIB in the sediments.

While the highest concentration and most variability in 2017 was observed at Sk1, in 2018, it was observed at Sk3, two miles downstream of the cattle crossing. The 2018 season also had much higher streamflow, with the average streamflow being eight times higher in the 2018 recreation season than the 2017 season (Table 4.1). The high streamflow observed in 2018 may have resulted in the bacteria being deposited farther downstream may have flushed the *E. coli* sediment stores at Sk1. Flushing bacteria from sediments during high flows was hypothesized by Muirhead et al. (2004), who conducted a series of artificial flood events, and observed a decrease in the amount of *E. coli* transported to the water column with each consecutive reservoir release. Pandey and Soupir (2014) supported these results with their study which concluded that the *E. coli* concentrations in sediment decrease during high streamflow. In addition, higher streamflow can reduce attachment and deposition of bacteria (Abia et al., 2015a), which may have also contributed to the lower concentrations observed at Sk1 during 2018.

Table 4.1: USGS station 06481480 mean streamflow (cfs) data for 2017 and 2018.

Year	Streamflow (cfs)		
	Mean	Early Season	Late Season
	May - October	May - July	August - October
2017	18.5	33.4	3.5
2018	151.7	104.7	78.4

Only the sediment concentrations at Sk1 decreased significantly ($p<0.05$) from 2017 to 2018. No significant differences were observed in the other three sites between the 2017 and 2018 seasons; however, relatively high concentrations were observed in October 2018 at the sites where data were collected. Sk1 was not monitored in October 2018 due to lack of site accessibility. Sites Sk2, Sk3, and Sk4 are enrolled in SRAM which allows producers to graze the riparian area during the off-season from October through April. While most years the producers keep their cattle out of the area and opt to hay it instead, the wet conditions in 2018 made some of the riparian areas inaccessible for haying. Therefore, at least one of the producers (Sk2) allowed cattle access to the riparian area and stream in October 2018, possibly leading to the higher concentrations observed at the SRAM locations.

The six months of monitoring were divided into early (May to July) and late (August to October) season to evaluate changes as the recreation season progressed. While all sites had higher *E. coli* concentrations in the late season as compared to the early season, only the increases at Sk3 and Sk4 were significant ($p<0.05$). Temperatures were suitable for *E. coli* growth and survival in sediment from June to September with average temperatures ranging from 17 to 23 ºC during study period. This is supported by Berry and Miller. (2005) Berry and Miller (2005) who demonstrated *E. coli* growth in manure rich soil at 19 ºC and Ishii et al. (2006) who found longer *E. coli* survival times at temperatures $\leq$ 25 ºC in unsterile and unamended soil Ishii et al. (2006). In addition, cattle access during the off season (October-March) may increase the FIB in sediment during late season. Due to wet conditions in October 2018, haying was not possible as the

site was inaccessible to the necessary equipment, resulting in at least one producer allowing the cattle to graze the land.

A similar seasonal trend of *E. coli* concentration in sediment was observed by Bragina et al., (2017) in a study on impact of cattle exclusion by fencing on reducing sediment *E. coli*. The study observed an increasing trend of *E. coli* in sediment from summer (July) to fall (October) in fenced tributary though the difference was not significant. The reason of higher FIB numbers during Fall likely is due to spreading of cattle slurry in the surrounding agricultural land earlier in October (Bragina et al., 2017).

4.3.2 Storm Events

Sediment *E. coli* was monitored at two sites (Sk2 and Sk4) surrounding a series of storm events in 2017. Despite following the weather for precipitation events, only one event that was monitored resulted in a substantial hydrologic response (October 8, 2017). Due to the lack of hydrologic change in the system, no significant changes resulted from the precipitation events monitored in August or September. However, the final storm event resulted in elevated streamflow, and a corresponding increase in sediment *E. coli* concentrations was observed at the two monitoring sites (Figure 4.4), starting at 10^1 CFU g^{-1} (10^3 CFU 100 mL^{-1}) prior to the event and increasing to 10^2 CFU g^{-1} (10^4 CFU 100 mL^{-1}), an increase of 1.5 to 8 times, following the hydrologic response. These increases at both Sk2 and Sk4 were significant ($p<0.05$) when compared to samples taken on September 21, 2017. While few studies have monitored sediment FIB concentration response to storm events, Pandey and Soupir (2014) reported higher flow rates were associated with higher *E. coli* concentrations in streambed sediments.

A similar response was measured in the water column, with concentrations starting between 37 to 287 CFU 100 mL^{-1} and increasing to 570 to 743 CFU 100 mL^{-1} (Figure 4.4), an increase of 2 to 20 times, following the hydrologic response. A higher range was reported in previous work where *E. coli* concentrations have increased two to three orders of magnitude in the water column after a storm event due to resuspension and runoff (Pandey and Soupir, 2014; Abia et al., 2015a).

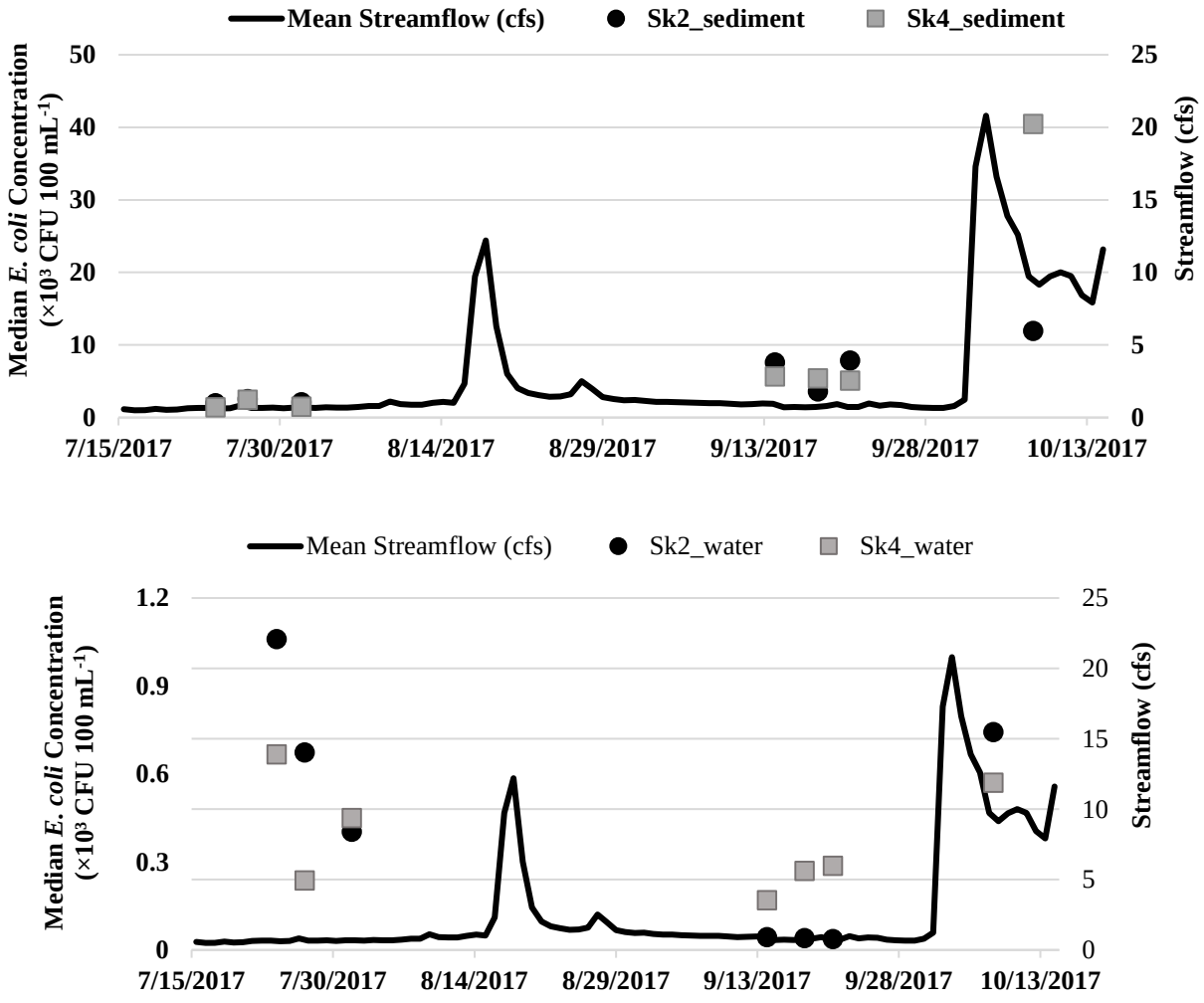

Figure 4.4: *E. coli* concentration in stream-bed sediment and water column at site Sk2 and Sk4 prior, during and after storm events. Storm events resulting in small streamflow changes do not have an appreciable effect on both water and sediment *E. coli* concentrations, while the high streamflow change observed in October had a significant impact on both water and sediment *E. coli* concentrations at both monitoring sites.

The *E. coli* concentration was significantly higher ($p<0.05$) in streambed sediments than in the water column both prior to and after storm events at both monitoring sites. Higher concentrations of FIB in sediment are consistent with previous work comparing water concentrations of FIB to sediment concentrations (Pandey et al., 2018; Abia et al., 2015a; Pandey et al., 2012; Bai and Lung, 2005).

4.3.3 Potential Impacts on Water Quality

The Skunk Creek watershed is listed as an impaired waterbody because it does not support limited contact recreation, one of its designated beneficial uses (East Dakota Water Development District, 2005). As Skunk Creek contributes a large proportion, 59%, of the flow in the Big Sioux River during the recreation season (McCutcheon et al., 2012), it is important to monitor microbiological water quality to understand FIB effect on a larger scale.

To understand how streambed sediments may contribute to water quality impairments if resuspended, the sediment was compared to the water standard for limited contact recreation, or 1,178 CFU 100 mL^{-1} (SD DENR, 2018). The exceedance was high for both recreation seasons monitored (Table 4.2). Approximately 88% and 80% of the sediment samples exceeded the standard for limited contact recreation during 2017 and 2018 monitoring periods, respectively. The high exceedance rate of limited contact recreation shows a potential threat to public health if, coming in to contact with these waters. Human association with these water via recreational activities may cause gastrointestinal illness (Dorevitch et al., 2012; Wade et al., 2003).

Table 4.2: The data of *E. coli* concentrations in sediment from May to October for a two year period compared to the standard for limited contact recreation of 1178 CFU 100 mL^{-1} (3.07 log_{10} CFU 100 mL^{-1}). All data were log_{10} transformed for better representation of the data. Here, *E. coli* concentration is expressed as log_{10} CFU 100 mL^{-1}.

Year	**2017**				**2018**			
Month	Sk1	Sk2	Sk3	Sk4	Sk1	Sk2	Sk3	Sk4
May	2.6	1.4	0	0.5	0.9	0	0.4	0
June	1.6	0.3	2.3	0.5	1.9	1.2	1.7	1.7
July	1.8	1.2	1.1	1.1	1.8	0.8	1.9	0.1
August	3.2	1.4	2.5	1.6	0.8	1.3	4.4	1.6
September	2.2	1.6	1.7	1.3	1.1	0.9	1.5	1.0
October	2.7	1.7	2.1	2.4	No Data	3.2	3.3	2.9
Scale Bar			Lowest		Standard		Highest	

The streambed can act as storage for bacteria and influence concentrations in the overlying water through resuspension (Pandey and Soupir, 2014; Jamieson et al., 2005b). Thus, increases in FIB concentrations in the water column can occur when there is disturbance of the sediment, including storm events (Pandey and Soupir, 2014; Pandey and Soupir, 2013; Fries et al., 2006 and Jamieson et al., 2005b; Muirhead et al., 2004; Nagels et al., 2002), mechanical disturbances such as dredging (Grimes, 1980), animal crossing (Abia et al., 2017; Sherer et al., 1988), and recreational activities (Abia et al., 2017; Phillip et al., 2009; Roslev et al., 2008; An et al., 2002; Pettibone et al., 1996).

4.4 Conclusions

The goal of this study was to provide a case study to begin to understand how *E. coli* concentrations in streambed sediments change over the recreation season and as a result of storm events. Understanding these variations will help inform how streambed sources of FIB might impact water quality. The results from this work suggest that sediment *E. coli* concentrations in streambed sediments increase from the early part of the

recreation season to the latter part of the recreation season. In addition, storm events that result in a hydrologic response have the potential to significantly increase sediment *E. coli* populations. While this case study provides initial evidence for the changes in *E. coli* concentrations in streambed sediments over time, additional studies are required to verify the findings herein. It is recommended that longer-term monitoring as well as monitoring in more varied stream conditions be conducted to determine the consistency of these results.

4.5 Acknowledgements

This research was funded by the US EPA, South Dakota Department of Environment and Natural Resources, and USDA Hatch projects SD00H604-15 and SD00H452-14 courtesy of the SDSU Agricultural Experiment Station. We appreciate the support for this work including the Moody County Conservation District and East Dakota Water Development District who assisted with site access, as well as Miranda Lebrun, Zeb Nelson and Suraiya Akter who helped with sample collection and processing.

CHAPTER 5: CHARACTERIZATION OF SEDIMENT FECAL INDICATOR BACTERIA POPULATIONS ALONG A REACH: A CASE STUDY IN SOUTH DAKOTA

Abstract

Fecal indicator bacteria (FIB) can be transported to waterbodies via runoff and direct fecal deposits, which can be minimized using several management practices including riparian area management, stream fencing, and stream bridging. However, bacteria can also survive and persist in stream bed sediments which can be resuspended into the water column, which is difficult to mitigate. This study focuses on characterizing the *E. coli* population in streambed sediments along a stream reach to understand the variability within a reach. Four sites, abbreviated Sk1, Sk2, Sk3, and Sk4, were monitored for two years along Skunk Creek which is located in eastern South Dakota. Sk1 is the upstream sampling location and is at the site of a cattle crossing, followed by Sk2, Sk3, and Sk4, each a mile apart. The majority of the three miles between Sk1 and Sk4 are managed using Seasonal Riparian Area Management, which restricts cattle access to the stream during the recreation season. Sk1 and Sk3 had the highest measured *E. coli* concentrations, with median concentrations of 53 and 85, respectively. Sk2 and Sk4 had significantly lower *E. coli* concentrations, with median concentrations of 23 and 21, respectively. Attachment rate of *E. coli* to settleable particles (> 0.004 mm) ranged from 37% to 78% and was highest at Sk2 and Sk3. Both microbial source tracking and phenotypic antibiotic resistance showed a similar pattern, with Sk2 demonstrating the highest fecal biomarker quantification and highest proportion of phenotypically antibiotic resistant *E. coli* isolates. While the organic content showed strong correlation with the *E.*

coli concentration in the middle of the stream at Sk1 and Sk2, organic content was not well correlated over the entire dataset. Additional work on bacterial attachment in stream sediment under different hydrological conditions, as well as more detailed monitoring using microbial source tracking might give additional understanding on reach-specific *E. coli* fate and transport.

5.1 Introduction

The microbiological water quality of surface water is a major concern in the United States, and is the number one cause of impairments in the country (USEPA, 2016). Fecal indicator bacteria (FIB), such as *Escherichia coli* (*E. coli*) are used to indicate the potential presence of pathogens from fecal contamination. The sources of FIB to the water column include runoff, direct fecal deposits, point sources (e.g. outfall from a wastewater treatment plant), and channel storage of bacteria associated with bed sediment which can be resuspended. Runoff can be reduced by various management practices, such as riparian area management (Parkyn, 2004), vegetative treatment systems (Harmel et al., 2018a), and controlled drainage (Sunohara et al., 2016; Wilkes et al., 2014; Sunohara et al., 2014). Direct fecal deposits from livestock can also be limited through management practices, such as exclusion by fencing and alternative water sources (Bragina et al., 2017; Smolders et al., 2015; Miller et al., 2010, Willms et al., 2002). However, channel storage is more challenging to address because it is already within the system and FIB can survive and persist in sediment for long periods of time (Haack, 2017; Garzio-Hadzick et al., 2010; Anderson et al., 2005). Survival of FIB in water is only a few hours to several days, whereas FIB in sediment can survive for days or months (Haack, 2017). In addition, sediment can contain FIB concentrations 100 to

1000 times higher than in the water column (Kovacic et al., 2011; Van Donsel and Geldreich, 1971). When the sediment bed is disturbed by events such as storms, recreational activities, or animal crossings, the bacteria stored in the sediment can be transported into the water column (Abia et al., 2017; Pandey and Soupir, 2014; An et al., 2002; Sherer et al., 1988).

Impaired waterbodies do not support their designated beneficial use. Several management practices have been developed to improve water quality with the goal of attaining water quality sufficient for the waterbody's designated beneficial use. Management practices focus on removing contaminant loads to the waterbody through a reduction in concentration, the amount of water a water body receives (Craggs et al., 2004a, Craggs et al., 2004b), or removing the direct contributions to the waterbody (e.g. direct fecal deposits from cattle) (Parkyn, 2004; Sunohara et al., 2016). Some management practices that have been evaluated include vegetative treatment systems (Harmel et al., 2018a), riparian area management (Parkyn, 2004), livestock exclusion (Bragina et al., 2017; Miller et al., 2010), livestock crossing bridges (Smolders et al., 2015), and alternative water sources for livestock (Willms et al., 2002). These management practices have the potential to substantially reduce *E. coli* loads. Harmel et al. (2018a) reported *E. coli* load reductions up to 94% when using a vegetated treatment area, while livestock exclusion via fencing can reduce the median *E. coli* concentration in water by over 100 MPN 100 mL^{-1} (Wilcock et al., 2009).

Channel storage can also be a major contributor to microbiological water quality impairments (Curtis and Trapp, 2014; Garzio-Hadzick et al., 2010; Haller et al., 2009a). FIB can settle out of the water column and accumulate in the bed sediment, which can act

as a reservoir for microorganisms (Pachepsky and Shelton, 2011; Haller et al., 2009b; Garzio-Hadzick et al., 2010). During resuspension, this bed sediment is transported along with microorganisms to the water column, resulting in poor water quality (Pandey and Soupir, 2014; Jamieson et al., 2005b). Bed sediment storage may also provide inaccurate information about microbiological contamination. It is assumed that *E. coli* in waterways indicates fresh fecal contamination; however, since FIB can survive and persist in sediments for long periods of time, this is not always the case. Re-entry of FIB to the water column via the resuspension of bed sediments can then be incorrectly interpreted as recent fecal contamination. This imprecise information may affect the water quality assessment results (Haller et al., 2009 a,b).

The current study was performed along a three mile stretch of a stream reach. Both cattle access and cattle exclusion practices were present in this stretch of stream. This study examines the characteristics and patterns of *E. coli* concentrations in bed sediment along the stream reach. The main objectives of this study were to (i) assess the differences of *E. coli* concentration in the streambed sediment among the monitoring sites, (ii) determine the degree to which *E. coli* in stream sediment associates with sediment particles and how it varies along a reach, and (iii) evaluate the differences in microbial characteristics within a reach.

5.2 Materials and Methods

5.2.1 Study Area

The study was conducted on Skunk Creek, a tributary of the Big Sioux River located in eastern South Dakota. Skunk Creek contributes about 59% of the flow in the Big Sioux River. Monitoring was conducted along a three mile (straight line distance) stretch of the creek at four monitoring sites, abbreviated Sk1, Sk2, Sk3, and Sk4 (Figure 5.1). The monitoring sites are located about one mile apart from each other. Sk1 is the upstream-most site and has cattle access. Sk2 is located a mile (straight line distance) downstream, followed by Sk3 and Sk4. Nearly the entire stretch of stream between Sk1 and Sk4 is enrolled in Seasonal Riparian Area Management (SRAM). SRAM is a pilot program as a part of the central Big Sioux watershed project, and consists of an incentive to remove cattle access to the stream via fencing during the recreation season, defined as April 1 to September 30. An alternative

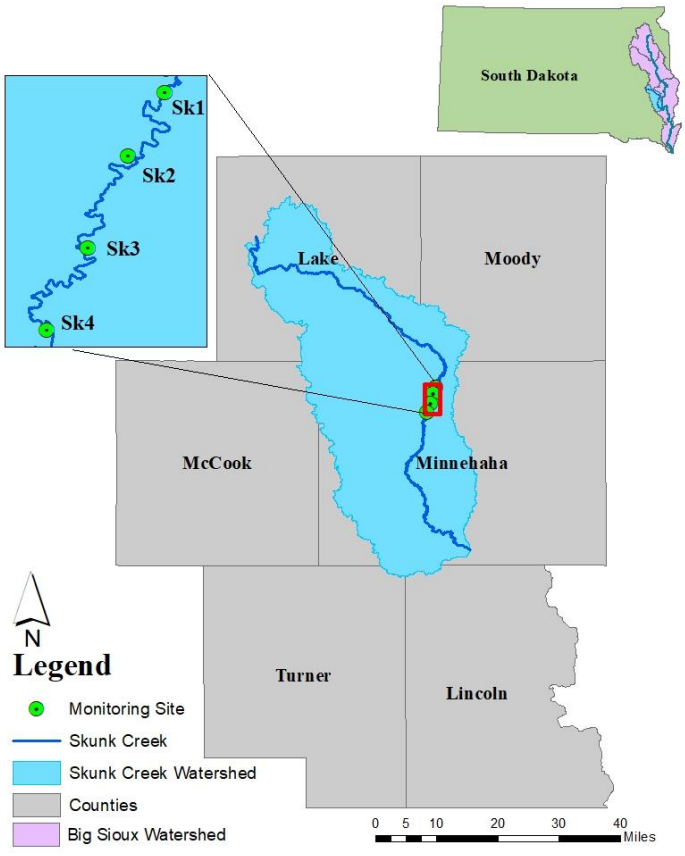

Figure 5.1: Four Monitoring sites in Skunk Creek, located in Minnehaha county, South Dakota. Skunk Creek is a Sub-watershed in Big Sioux Watershed contributing 59% of the flow in Big Sioux River.

water source is provided, and the producers are allowed to hay periodically during the recreation season and graze or hay the land on the off season, providing producers partial use of the land.

5.2.2 Sample Collection and Processing

Sediment samples were collected in a 1L, sterile, wide mouth bottle. Three to five sediment samples were collected from each monitoring site from May through October during 2017 and 2018, with the exception of Sk1 during October 2018 due to issues with site accessibility. Samples were transported in a cooler on ice to the Water Quality Laboratory in the Agricultural and Biosystems Engineering Department at South Dakota State University for processing. Sample processing followed the method outlined in Salam et al. (in review). Briefly, a 1:10 dilution ratio of sediment: PBS (phosphate buffer solution) was used and shaken approximately 150 rpm for 45 minutes in an orbital shaker. The sediment was allowed to settle for 30 seconds and the supernatant was plated using standard membrane filtration methodology with modified mTEC agar (USEPA, 2002). In short, the samples were filtered using 0.45 μm filters and plated on modified mTEC agar. The plates were placed in a water bath for 2 ± 0.5 hours at 35°C ± 0.5°C. The samples were then incubated for 22 ± 2 hours at 44.5°C ± 0.2°C.

To measure a single *E. coli* concentration per site for each sampling event, the three to five samples collected at each site for each sampling period were combined by reporting the geometric mean value. To calculate the geometric mean, *E. coli* concentrations with a zero value were ignored (Roenfeldt, 2018). This had minimal effect on the result as the total percentage of zero values was less than 5% of all data for each

monitoring sites. The geometric mean value was used for all discussions of the *E. coli* concentrations in the results section, the statistical analyses, and the analysis for each location.

5.2.3 Microbial Source Tracking (MST)

Microbial source tracking (MST) was performed by Source Molecular Corporation (Miami Lake, FL 33016). Detection and quantification of the fecal associated biomarker were done by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology. One sediment sample and one water sample from each site were collected and analyzed for general fecal contamination (GenBac3 biomarker) and ruminant contamination (Rum-2-Bac biomarker). The general fecal biomarker is designed for general fecal Bacteroidetes. Fecal Bacteroidetes is commonly found in the gastrointestinal tract of humans and warm-blooded animals (Scott et al., 2002; Dick and Field, 2004). It is used as an alternative to fecal indicator bacteria. Fecal Bacteroidetes are strict anaerobes and cannot survive for long periods of time outside their host. Therefore, the presence of fecal *Bacteroidetes* indicates recent fecal contamination (Scott et al., 2002; Dick and Field, 2004). The ruminant gene biomarker reacts with genes found in microbes originating from the gastrointestinal tract of animals from the ruminant taxa. 16S rRNA gene sequencing was used for both general and ruminant fecal ID source tracking analysis (Table 5.1).

Analyze for qPCR were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA). All assays were run in duplicate. Quantification was achieved by extrapolating target gene copy numbers from a standard

curve generated from serial dilutions of known gene copy numbers. For quality control purposes, a positive control and a negative control were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Table 5.1: The biomarker, gene, primer and probe sequence information for general and ruminant fecal ID used by "Source Molecular Corporation".

Assay name	**Biomarker**	**Gene**	**Primer & probe sequence**
General	GenBac3	16S rRNA	Forward primer: GGGGTTCTGAGAGGAAGGT Reverse primer: CCGTCATCCTTCACGCTACT Probe: CAATATTCCTCACTGCTGCCTCCCGTA
Ruminant	Rum-2-Bac	16S rRNA	primer: BacB2-590F: ACAGCCCGCGATTGATACTGGTAA Bac708Rm: CAATCGGAGTTCTTCGTGAT probe: BacB2-626P: (FAM)ATGAGGTGGATGGAATTCGTGGTGT

*FAM, 6-carboxyfluorescein; BHQ-1, black hole quencher, 1.

5.2.4 Organic Content

Loss on ignition was used to determine the organic content of the sediment following a method described by Sutherland (1988). A total of 107 samples from the 2018 monitoring season were measured for organic content. Briefly, 100 g of sample were placed in a crucible and dried at 105°C for 24 hours. After drying the samples, the crucible was placed in a furnace for 4 hours at 550°C. The weight was measured before and after drying and ignition. The change in weight over the pre-ignition weight was used to determine the percent organic content.

5.2.5 Attachment Rate

The samples taken for monthly monitoring during September and October 2018 (35 samples in total) were analyzed for bacterial attachment rates. A sedimentation method

was used to differentiate between *E. coli* attached to settleable particles (≥ 0.004 mm in diameter) and *E. coli* attached to non-settleable particles (≤ 0.004 mm in diameter) or unattached to particles (Oliver et al., 2007). Briefly, 30 g of sediment were mixed with 270 mL of PBS in a 500 mL graduated cylinder and covered it with parafilm. The cylinder was inverted approximately 30 times for homogenous mixing. The first sample was taken immediately following the mixing to measure the total concentration of *E. coli* in the sediment. The sample was then allowed to settle of settleable particles for two hours and fifty minutes, at which time a second sample was collected from the top of the graduated cylinder. The time for settleable particles to settle was calculated using Stokes' Law. Processing the sample after settling estimated the concentration of *E. coli* attached to very fine particles or unattached to particles altogether, hereafter referred to as unattached. The fraction of *E. coli* attached to settleable particles, hereafter referred to as attached, was calculated by subtracting the unattached concentration from the total concentration. Samples were plated using standard membrane filtration methodology on modified mTEC agar as described above.

5.2.6 Antibiotic Resistance (ABR)

Selected *E. coli* isolates from 25 samples collected at Sk1 and Sk2 were tested for antibiotic resistance (ABR). A total of 463 isolates were tested using a modified Kirby-Bauer method (CLSI, 2011), 202 from Sk1 and 261 from Sk2. Among 261 of *E. coli* isolates for site Sk2, 190 isolates were tested for ampicillin as this antibiotic added later for ABR analysis. Each isolate was streaked onto tryptic soy agar (TSA) plates and isolated colonies were used to culture the bacteria in tryptic soy broth (TSB). These cultured bacteria were spread onto Muller-Hinton agar plates after which with five

antibiotic discs were applied onto the agar. The five antibiotics selected based on their detection in terrestrial and aquatic environments (Kemper, 2008) were: penicillin (P, 10 U), ampicillin (Amp, 10 µg), erythromycin (E, 2 µg), tetracycline (TE, 30 µg), and sulfisoxazole (G, 0.25 mg). The plates were incubated for 24-48 hours at 37°C. After incubation, inhibition zones were measured, and all isolates identified as 'resistant' and 'intermediate' were grouped into the 'resistant' category similar to Sidrach-Cardona et al., (2014) and Reinthaler et al. (2003). The 'intermediate' forms were grouped with 'resistant' because they were somewhat resistant to the antibiotic (Reinthaler et al., 2003).

5.2.7 Statistical Analysis

All statistical analyses were completed using R software (version 3.3.1) and RStudio (version 1.0.143). The data were found to be non-normally distributed when analyzed using the Shapiro-Wilk test. Therefore, nonparametric tests were used to analyze the data. Differences between sites were evaluated using a Bonferroni correction with the Kruskal-Wallis test. This is a nonparametric post hoc test, where the p-value is adjusted when multiple pairwise tests are performed together on a single dataset. To determine the relationship between organic content and *E. coli* concentrations, a Kendall-tau correlation test was performed. This test represents the degree of agreement between two variables. The Fisher exact test was used to analyze the ABR data as it is used for categorical data.

5.4 Results and Discussion

5.4.1 *E. coli* Concentrations

In general, *E. coli* concentrations ranged from zero or nearly zero CFU g^{-1} to 10^3 or 10^4 CFU g^{-1}, depending on the site (Table 5.2). There was, however, one outlier at Sk3 during August 2018 of 10^6 CFU g^{-1}. The second highest value for Sk3 was in the 10^4 CFU g^{-1} range, similar to Sk2. This outlier resulted in higher measurement of central tendency and standard deviation for Sk3 when calculated over the two-year sample collection period (Table 5.2). The highest geometric mean of *E. coli* concentration observed at Sk3 followed by Sk1 for the 2017-2018 sample period. Where the geometric means for Sk2 and Sk4 are similar, and smaller than those for Sk1 and Sk3. The Wilcoxon Signed Rank test was used to assess the differences in the geometric means for the two-year sample period between all sites. Sk1 and Sk3 were both significantly ($p < 0.05$) higher than Sk2 and Sk4, while Sk1 and Sk3 nor Sk2 and Sk4 were significantly different. This suggests that site specific conditions can result in spatial hotspots of elevated FIB in sediment along a stretch of stream.

Table 5.2: Sediment *E. coli* concentration (CFU g^{-1}) statistics for the two-year monitoring period from 2017 to 2018

	Sk1	Sk2	Sk3	Sk4
Min	1	0	0	0
Max	2.7×10^3	1.4×10^4	1.4×10^6	1.7×10^3
Mean	2.3×10^2	4.1×10^2	2.6×10^4	1.5×10^2
Geomean	53	23	57	21
Median	53	16	115	26
Std Dev	5×10^2	2.1×10^3	1.6×10^5	3.3×10^2

Elevated concentrations at Sk3 may have been due, in part, to deposition as evidenced by the formation of a sandbar in the middle of the stream that was visible during normal flow conditions. According to USGS streamflow data (USGS gauge: #06481480), the

streamflow was approximately 8-fold higher in 2018 than 2017. Storm events likely washed in the bacteria which may be more prone to settle out at the Sk3 site due to the stream morphology. Additionally, there may be other sources than cattle present at Sk3 which could contribute to elevated concentrations of FIB in the sediment. For example, there have been anecdotal reports of beaver activity along this stretch of stream.

Sk1 also had significantly higher concentrations of *E. coli* in the sediment when compared to Sk2 and Sk4, likely due to direct access of livestock resulting in direct fecal deposits at the site. These results are similar to previous studies that have found *E. coli* concentrations were several fold higher in sediment adjacent to pastureland than areas with other land use (Bragina et al., 2017; Stephenson and Rychert, 1982).

The other two monitoring sites, Sk2 and Sk4, showed significantly lower *E. coli* concentration than Sk1 and Sk3. These sites were under SRAM, thus limiting cattle access to the stream reducing the potential for direct fecal deposits in the stream as compared to Sk1. Past studies also examined the impact of limiting cattle access on the concentration of FIB both in water and sediment as well as on water quality. Management practices that limit cattle access to the stream, such as streambank fencing (Smolders et al., 2015), stream bridging (Bragina et al., 2017), alternative water source (Willms et al., 2002) and buffer strips (Miller et al., 2010), reduce direct contact between livestock and waterways and significantly improve water quality.

E. coli concentrations in stream sediments measured in this study were generally on the high end of the range reported in previous studies. Perkins et al. (2014) examined the influence of sediment composition on indicator bacteria and reported mean *E. coli*

concentrations from 0 to 2.4×10^2 CFU g^{-1} (reported as 2.4×10^4 CFU $100g^{-1}$). Similarly, Borade et al. (2014) measured seasonal variations in FIB distributions and observed the highest average *E. coli* concentration in the sediment was 10^3 CFU g^{-1}. While Sk3 had a measured mean *E. coli* concentration higher than these studies, a study by Ouattara et al. (2011) demonstrated higher *E. coli* concentrations in the sediment near a sewer overflow, with a geometric mean ranging from 2.1×10^2 to 3.3×10^5 CFU g^{-1}.

5.4.2 Microbial Source Tracking

A limited microbial source tracking analysis was performed to quantify general fecal contamination and ruminant fecal contamination in the sediment and water column. Gene concentrations in the sediment were converted from a mass to a volume basis using the density measured at each site by following Archimedes' principle (Abia et al., 2015b) to compare sediment concentrations with concentrations found in the water column. Concentrations of the general *Bacteroidetes* (GenBac3) in sediment ranged from 7×10^5 to 3.8×10^7 copies 100 mL^{-1} and 1.9×10^5 to 1.0×10^6 copies 100 mL^{-1} in the water column, indicating higher contamination in sediment. The sediment GenBac3 quantification was 1.3 to 38 times higher than for the water column. Site Sk2 had the highest concentrations for both markers in both matrices though it was under SRAM (Table 5.3), while Sk3 had the lowest concentration in the water samples and Sk4 had the lowest concentration in the sediment samples. The concentration of the ruminant fecal marker in sediment ranged from below the detection limit to 2.6×10^6 copies 100 mL^{-1} and 6.1×10^4 to 1.0×10^5 copies 100 mL^{-1} in water. Three of the four sites had measured Rum-2-Bac concentrations one order of magnitude higher in the sediment than the water column, ranging from 19 to 29 times higher (Table 5.3).

Table 5.3: Microbial Source tracking number for 'General Bacteroidetes' and 'Ruminant Fecal' in all four monitoring sites for both sediment and water samples. Site Sk2 showed highest marker quantified for both markers and for both water and sediment. Here, 'Detected but Not Quantifiable' is abbreviated 'DNQ'.

Monitoring Site	Marker Quantified ($\times 10^5$ copies/ 100 mL)			
	GenBac3		Rum-2-Bac	
	Sediment	Water	Sediment	Water
Sk1	30.7	6	8.8	0.3
Sk2	381	10	26.2	1
Sk3	14.4	1.9	3.9	0.2
Sk4	7	5.6	DNQ	0.6

5.4.3 Sediment Characteristics

Streambed sediment characteristics, such as particle size and organic matter, play an important role in bacterial transport, resuspension (Droppo and Ongley, 1994; Mehta et al., 1989), and persistence (Haller et al., 2009). The particle size distribution for all Skunk Creek sites were similar, with the D50 value ranging from 0.32 to 0.35 at all locations (Salam et al., In Review). Sk3 had the highest amount of fine particles (11%) while Sk4 had the lowest (5%).

The mean organic content ranged from 4.2% to 8.2% at all sites, with Sk3 having the highest organic content and Sk2 having the lowest organic content (Table 5.4). Over the year of sediment organic content monitoring, the organic content decreased from May through September, but increased substantially in October (Figure 5.2). The SRAM program allows for seasonal grazing of the riparian

Table 5.4: Statistical summary table of percent organic content for sediment samples taken at the four sites during six months of recreational period (May to October) in 2018.

	Sk1	Sk2	Sk3	Sk4
Min	0.4	0.8	0.6	0.9
Max	11.6	21.9	28.8	21.7
Mean	4.8	4.2	8.2	6.1
Geomean	2.9	2.6	5.3	4.2
Variance	13.2	25.7	46.7	30.6

area from October until April. Producers typically opt to hay the land, even in the off season; however, 2018 was a wet year and the riparian area was inaccessible to the necessary equipment. Therefore, at least one producer (Sk2) did use the riparian area

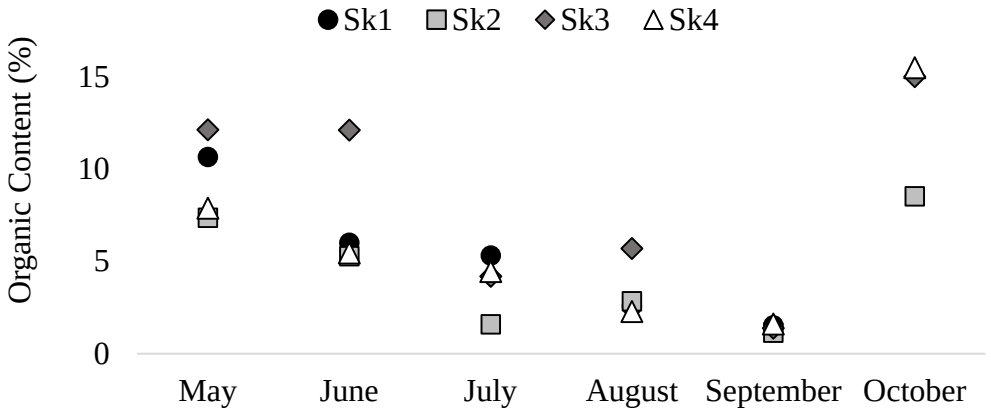

Figure 5.2: Percent organic carbon from May to October 2018 for all four monitoring sites. The data show a decreasing trend from May to September while October had the highest %OC for all monitored sites.

along Skunk Creek for grazing in October 2018. In addition, the mean streamflow (USGS gauge: #06481480) during the day of sampling in October 2018 was 93.7 cfs, substantially higher than the flow observed during the day of sampling in the preceding month which averaged 11.5 cfs. As high flow increases, runoff as well as organic matter increases in the stream because bacteria enter the stream and fecal organic matter can enter the stream via runoff (Piorkowski et al., 2014b; Pachepsky and Shelton, 2011). The combination of these factors may have led to the elevated organic content observed during October.

There was no statistically significant correlation between organic matter and *E. coli* concentrations when using all data points collected (−0.13 to +0.2). However, the data were also split between those taken at the edge of the stream and those taken in the middle. Interestingly, there was a significant correlation ($p < 0.05$) between sediment

organic content and *E. coli* concentration in the middle of the stream at the Sk1 (Kendall-$\tau = 1$) and Sk2 (Kendall-$\tau = 0.7$) sites, but not at the edges of the stream. Since cattle are allowed access to the stream at Sk1, cattle-derived manure may play a role in the significant correlation between *E. coli* and organic content in the middle of the stream.

Although a significant positive relationship between organic content and *E. coli* has been observed often (Garzio-Hadzick et al., 2010; Pandey and Soupir, 2014; Curtis and Trapp, 2014; Haller et al., 2009a; Haller et al., 2009b), it is not universal. Negative relationships and no relationships between *E. coli* and organic content have been reported (Piorkowski et al., 2014a); possibly because the increased availability of organic contents may increase the competition from other microflora while having little impact on *E. coli* survival and persistence in bed sediment (Surbeck et al., 2010; Banning et al., 2003).

5.4.5 Attachment Rate

Attachment rates for *E. coli* in the sediment ranged from 37% to 78% (Table 5.5) for all sites during the two-month period of analysis (September and October 2018). Sk4 had the least amount of attachment, while Sk2 and Sk3 had the highest attachment rates for September and October, respectively. Higher attachment rates were observed in October for all three sites monitored during this month (Table 5.5). Data for October 2018 are missing for Sk1 due to lack of site accessibility.

Table 5.5: Percent (%) *E. coli* concentration (Mean ± Standard Deviation) associated with in-streambed sediment particles for two months (September – October)

Site	September	October
Sk1	59 ± 10.3	-
Sk2	64.4 ± 10.5	69.7 ± 13.4
Sk3	50.7 ± 33.3	78.2 ± 14.1
Sk4	37.4 ± 33.7	49.1 ± 34.2

The average stream flow (USGS 06481480) during the day of sample collection was eight times higher in October (93.7 cfs) than September (11.5 cfs). This higher flow may have contributed to the higher attachment rates seen in October. For one, the higher flow may have washed out more of the loosely attached or free *E. coli* from the sediment reservoirs. Secondly, higher attachment rates are often observed in the water column during high flows than baseflow conditions (Characklis et al., 2005; Soupir et al., 2010) when particulates may have settled out in the sediment.

In addition, the attachment rates were higher for the sediment than previously reported for the water column (Amegbletor, 2018) at this location. A study by Amegblator, (2018) reported average attachment rates in the water column were 19% during baseflow and 25% in storm flow at Sk2, whereas the average measured attachment rate for sediment at Sk2 found in this study was 67%.

To the authors' knowledge, no previous work has been completed assessing the attachment rate of bacteria in sediment environments; however, several studies have examined attachment in different sources of water. Attachment rates reported for the water column are often similar or lower than this study measured in the sediment. For example, Soupir et al. (2010) measured attachment rates of 28% and 49% for *E. coli* and enterococci, respectively, in runoff samples. However, occasionally storm events can result in high attachment rates, similar to what was found in the sediments herein (Characklis et al., 2005; Jeng et al., 2005). For example, Characklis et al. (2005) observed higher FIB (fecal coliforms, *E. coli,* and enterococci) attachment rates in storm event, ranging from 30 to 55%, than baseflow conditions which ranged from 20 to 35%. Attachment depends on particle characteristics such as particle size and density, type of

microbes and microbial characteristics (Liang et al., 2017; Guber et al., 2007; Jamieson et al., 2005b; Characklis et al., 2005). While the data in this study are limited (n = 35), it provides an initial estimate for attachment in stream sediments and possible changes due to flow conditions. More work is needed to expand the understanding of bacterial attachment in stream sediments.

Understanding bacterial attachment is critical for understanding bacterial fate and transport (Jamieson et al., 2005a; Pachepsky and Shelton, 2011). Bacteria associated with particles can settle out easily and can be removed by sedimentation (Pachepsky and Shelton, 2011), while unattached bacteria are buoyant and travel farther (Kunkel et al., 2013; Jamieson et al., 2005b; Pachepsky and Shelton, 2011). In addition, FIB survive longer in water when they are attached to particles (Howell et al., 1996; Sherer et al., 1992; Burton et al., 1987). Thus, bacterial attachment to particles not only impacts transport, but it also increases the survival time of these bacteria in natural waters (Characklis et al., 2005).

5.4.6 Antibiotic Resistance (ABR)

A total of 463 *E. coli* isolates were selected from the sediment at two monitoring sites, 202 from Sk1 and 261 from Sk2, for phenotypic antibiotic resistance (ABR) analysis. Penicillin acted as a positive control for the experiment and was not used in the analysis for percent resistant or percent multi-resistance because it is expected that Gram-bacteria, such as *E. coli*, are phenotypically resistant to it. *E. coli* isolates from both sites were nearly all (95.5%) resistant to penicillin, as expected.

For all 463 *E. coli* isolates from both sites, 99.6% were resistant to at least one antibiotic and 81.4% were resistant to more than one antibiotic, or multi-resistant. Despite Sk1 being located at the cattle crossing, more phenotypic resistance was generally found at the downstream location at Sk2 (Table 5.6), including phenotypic resistance to ampicillin, tetracycline and sulfizoxazole. Significantly more isolates demonstrated phenotypic resistance for ampicillin, tetracycline and sulfizoxazole at Sk2 than Sk1 with the percent of isolates phenotypically resistant increasing from 48 to 90%, 31 to 56% and 26 to 77%, respectively.

Table 5.6: Phenotypic antibiotic resistance results for selected *E. coli* isolates from two monitoring sites, Sk1 and Sk2.

	Sk1 (%)		**Sk2 (%)**		
Antibiotics	Resistant	Susceptible	Resistant	Susceptible	**p-value**
Ampicillin (Amp)	47.5	52.5	90	10	**2.20E-16**
Erythromycin (E)	97.5	2.5	95	5	0.23
Tetracycline (TE)	30.7	69.3	55.9	44.1	**1.127e-07**
Sulfisoxazole (G)	26.2	73.8	77.4	22.6	**2.2e-16**

Similar antibiotic resistance results for *E. coli* isolates from environmental samples have been observed. Sidrach-Cardona et al. (2014) performed a study on a river that was impacted by an antibiotic-production plant as well as a wastewater treatment plant and *E. coli* isolated from sediment samples were 85 to 100% resistant to erythromycin. Ampicillin and tetracycline resistance were also observed at rates of 44 to 100% and 48 to 92%, respectively. Watkinson et al. (2007) found 63% of *E. coli* isolates from environmental water samples were resistant to sulfisoxazole. In addition, in a study on association of multiple antibiotic resistance of *E. coli*, Parveen et al. (1997) found that

82% of *E. coli* isolates from both point and non-point source pollution are resistant to one or more antibiotics, such as ampicillin, tetracycline and sulfathiazole.

5.5 Conclusions

Sediment reservoirs of bacteria are one potential source of fecal indicator bacteria to the water column that is rarely considered. The information from this study expands the understanding of *E. coli* sediment reservoirs. The highest *E. coli* concentrations in sediment were observed at two miles downstream of the cattle crossing site (Sk3), followed by the cattle crossing site (Sk1) over the two years of monitoring. Of all the isolates tested for phenotypic antibiotic resistance, 99.6% were resistant to at least one antibiotic and 81.4% were resistant to more than one antibiotic, or multi-resistant. Significantly more *E. coli* were phenotypically resistant to ampicillin, tetracycline and sulfisoxazole at one mile downstream of the cattle crossing site (Sk2) than the cattle crossing site itself (Sk1). No statistical difference was observed between the rates of phenotypic resistance to erythromycin between the two sites. The MST data were limited, but results showed the highest concentrations of general and ruminant biomarkers were at Sk2. Sediment had higher concentrations of both the general and ruminant biomarkers than measured in the water column at all four sites. Higher bacterial attachment rates were observed in sediment than was previously reported in the water column in this reach. The attachment rate of sediment *E. coli* ranged from 37% to 78%. Although overall the sediment organic content showed little relationship with sediment *E. coli* concentrations, there was a significant correlation of sediment organic content and *E. coli* concentration in the middle of the stream at the cattle crossing and one mile downstream of the cattle crossing. No significant relationships were measured

between the sediment organic content and sediment *E. coli* concentration at the two sites farthest downstream. It is recommended that additional work be performed on monitoring the sediment *E. coli* in different stream reaches with different geographical locations or pollution sources as well as attachment rate analyses for FIB in sediment samples during different hydrological conditions.

5.6 Acknowledgements

This research was funded by the South Dakota Department of Environment and Natural Resources (SDDENR), through the US EPA 319 program. It was also supported by USDA Hatch projects SD00H604-15 and SD00H452-14 courtesy of the SDSU Agricultural Experiment Station. We appreciate the support for this work and the help from Dr. McDaniel's research group, including Pavan Kulkarni, Brian Bose, Zeb Nelson, Moriah Flanagan and all members of the group who assisted with the project.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary and Conclusion

Streambed sediment is a potential reservoir of fecal indicator bacteria (FIB) which can be a source to water column via resuspension. Sediment sampling was performed at five monitoring sites in eastern South Dakota to identify spatial, seasonal, and reach-specific differences in sediment *E. coli* concentrations. *E. coli* concentrations in bed sediments were monitored across the stream transection throughout the recreational period within a range of temperature and streamflow conditions. In addition, this study evaluated the effect of a cattle exclusion-based management practice on reducing *E. coli* in sediments.

The spatial variation of *E. coli* in stream sediment was high. The highest concentration and variability of *E. coli* was observed at the site with direct cattle access. Results from the location uncertainty analysis shows that sites without cattle access had the least amount of error when were used median data. The high spatial variability of *E. coli* concentrations in stream sediment resulted in a need for a larger sample size to achieve a representative *E. coli* concentration in sediment. No significant changes in *E. coli* concentrations were observed in the majority (80%) of cases when samples were held for 24 hours when compared with samples that were only held for 8 hours.

Streambed characteristics, such as particle size and organic content, of the bed sediment showed diverse relationships with *E. coli* concentrations. Skunk Creek was mostly dominated by sand particles whereas Six Mile Creek was dominated by gravel particles. Particle size was significantly correlated with *E. coli* concentrations in sediment, but, the direction of the correlation differed between sites. However, the

organic content had little correlation with *E. coli* concentration in stream sediment in most cases, with the exception of a strong positive correlation in the middle of the stream at the cattle crossing and one mile downstream from the cattle crossing.

The seasonal monitoring showed higher *E. coli* concentrations in the sediment during the latter part of the recreation season (August to October) as compared to the early season (May to July) at all sites along Skunk Creek. Streamflow and stream morphology likely affect *E. coli* concentrations in the sediment. Only one storm event monitored had a substantial hydrologic response, and it resulted in a significant increase in *E. coli* concentration in both the water column and the sediment.

Higher antibiotic resistance and the highest fecal biomarker concentration were observed one mile downstream of the cattle crossing. Higher attachment rates were measured in the sediment as compared to attachment rates in the water column as measured by a prior study. *E. coli* attachment in sediment ranged from 37% to 78%. In addition, a higher attachment rate within the sediment was observed in the sites that were one- or two-miles downstream sites from the cattle crossing site.

6.2 Implication

This work provided insight into the extent and impact of sediment, an often-overlooked source, on water quality degradation. Work was presented on *E. coli* concentrations in stream sediments over space and time which is an important factor in understanding microbiological impairments in water resources. The information herein is useful to design water quality assessments and monitoring projects. The preliminary investigation on the impact of sediment *E. coli* concentrations along the stream reach and the sediment attachment rates may increase insights into microbial fate and transport

which may assist with water quality modeling studies. Modeling contaminant transport is useful for decision makers to aid in planning and implementing management practices in a more effective way.

6.3 Recommendation for Future Works

It is recommended that additional work be done to extend the knowledge on *E. coli* concentrations in sediment, including:

1. Analysis from multiple sites with diverse characteristics including different land uses to verify the results;
2. Longer term monitoring of seasonal variations of *E. coli* concentrations in sediment;
3. Additional tests on sediment attachment rate associated with FIB are required for extending knowledge of the sediment's effects on microbial fate and transport; and
4. More in-depth source tracking with specific biomarkers to identify source-specific microbial contamination in the stream.

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