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Evaluating *E. Coli* Particle Attachment and the Impact on Transport During High Flows

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EVALUATING *E. COLI* PARTICLE ATTACHMENT AND THE IMPACT ON TRANSPORT DURING HIGH FLOWS

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This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science in Agricultural and Biosystems Engineering degree and is acceptable for meeting the thesis requirements for the degree. Acceptance of this thesis does not imply that the conclusion reached by the candidate are necessarily the conclusion of the major department.

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LIST OF ABBREVIATIONS

Abbreviation	**Full Name**
ATTAINS	Assessment TMDL Tracking and Implementation System
CAFO	Confined Animal Feeding Operation
CFU	Colony Forming Unit
CTD	Controlled Tile Drainage
CU	Clay and Unattached
EFDC	Environmental Fluid Dynamics Code
EMC	Event Mean Concentration
EPA	Environmental Protection Agency
FIB	Fecal Indicator Bacteria
FVF	Fine and Very Fine Silt
GI	Gastrointestinal Illness
GM	Geometric Mean
HCGI	Highly Credible Gastrointestinal Illness
HSPF	Hydrologic Simulation Program-Fortran
MC	Medium and Coarse Silt
MPN	Most Probable Number
NTU	Nephelometric Turbidity Units
RWQC	Recreational Water Quality Criteria
SSM	Single Sample Maximum
STV	Statistical Threshold Value

SWAT	Soil and Water Assessment Tool
TMDL	Total Maximum Daily Load
TSS	Total Suspended Solids
UCTD	Uncontrolled Tile Drainage
VTA	Vegetative Treatment Area
VTS	Vegetative Treatment System
WWTP	Waste Water Treatment Plant

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ABSTRACT

EVALUATING *E. COLI* PARTICLE ATTACHMENT AND THE IMPACT ON TRANSPORT DURING HIGH FLOWS

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Fecal indicator bacteria, including *E. coli*, are the leading cause of water quality impairments within assessed waters in the United States. The source of *E. coli* includes WWTP, leaking sewers, animal manure, wildlife, livestock, and stream bed sediment. Storm events contribute to bacteria loading within waters through wash-in of land sources of bacteria and resuspension of bacteria within sediments. Bacteria introduced into the water column are either attached to particles or are unattached (or free-living). The goal of this study was to examine the attachment of *E. coli* to different particle sizes, including their impact on contributing to water quality impairments during storm events. A series of storm events and baseflow conditions were monitored within an impaired stream (Skunk Creek) located in eastern South Dakota. Samples were taken during storm events over a 5-hour duration via autosampler while baseflow samples were taken via grab sampling. In addition, flow and water quality parameters (i.e. turbidity and temperature) were monitored, and the bed shear stress was estimated. These variables were used in a correlation analysis to determine their relationship with *E. coli*, including the prediction of *E. coli* within the water column during storm events. Unattached *E. coli* dominated total *E. coli* concentration across both storm and baseflow events (i.e. at least 75% of total *E. coli* concentrations). The water quality standard during baseflow conditions was satisfactory while storm events consistently exceeded the standard. Total, settleable and free-living *E.*

coli concentrations ranged from $7 \times l0^2$ to $22 \times l0^3$ CFU 100 mL^{-1} , $4 \times l0^1$ to $66 \times l0^2$ CFU 100 mL^{-1}, and $5\times l0^2$ to $15 \times l0^3$ CFU 100 mL^{-1}, respectively. The high levels and exceedance rate of free-living *E. coli* mean that sedimentation of the settleable fraction of *E. coli* would not be adequate to reduce bacteria to within the microbiological water quality standard. Many instream water quality models assume that the total bacteria concentration within the water column can be predicted by modeling bacteria as free-living; this assumption was tested by assessing the statistical difference between total and unattached bacteria. The findings revealed that free-living *E. coli* concentrations were equal to total *E. coli* concentrations 5 out of 8 times (63%), meaning that over one third of events would not be accurately modelled with only unattached bacteria. Thus, increased understanding of attachment and incorporation of bacteria partitioning between attached and unattached (free-living) into water quality models could improve model performance and predictive capabilities. The correlation analysis revealed a weak ($p > 0.05$) relationship between flow, temperature, turbidity, shear stress and *E. coli* fractions. Regression models developed to predict total *E. coli* and those attached to different particle fractions during storm events performed poorly (R^2 = 0.09-0.22). The results presented in this study will further the understanding of fate and transport of bacteria within water as well as provide information that can be incorporated into the development of microbial water quality models.

CHAPTER 1: GENERAL INTRODUCTION

1.1 Introduction

The major cause of water quality impairments of surface waters within the US is fecal indicator bacteria (*E. coli*, fecal coliform, and enterococci) (USEPA, 2018). Out of 187,088 miles of streams and rivers found to be impaired by pathogens within the US, *E. coli* alone was found to be responsible for impairment in about 111,827 miles (USEPA, ATTAINS (Assessment, Total Maximum Daily Load (TMDL) Tracking and Implementation System, 2018). Within the US, bacterial water quality standards, including those for recreation, are determined based on the extent of fecal contamination by examining the level of total or fecal coliform bacteria, *Escherichia coli*, or enterococci (US EPA 1986).

The presence of fecal indicator bacteria (FIB) is not limited to the detection of fecal contamination, but also includes the detection of other pathogens (Ishii and Sadowsky, 2008). Although *E. coli* originates from the gut of mammals including human beings, the bacterium is transported into the environment through the release of fecal matter (Ishii and Sadowsky, 2008), where it can survive and persist. Sources of *E. coli* within the environment include livestock, wildlife, leaking sewers, Waste Water Treatment Plant (WWTP), animal manure, runoff from agricultural land, and Concentrated Animal Feeding Operations (CAFOs) etc.

E. coli finds its way into surface waters through a number of pathways; including the direct deposition of fecal matter into the water column (Collins et al., 2007), wash-in of fecal indicator bacteria stores from diffused and land sources by runoff (Davies-Colley et al., 2008a; Stout et al., 2005), WWTP (Baudart et al., 2000; Garcia-Armisen and Servais, 2007; Haller et al., 2009), leaky septic tanks (Weiskel et al., 1996), and through the resuspension of stores of bacteria within sediment either during natural disturbance of these stores (Jamieson et al., 2005b; Stephenson and Rychert, 1982) or artificial disturbance (Abia et al., 2017; An et al., 2002; Grimes, 1980; Muirhead et al., 2004a; Stephenson and Rychert, 1982).

Storm events have been implicated in several water quality studies for elevated bacteria levels and loading into surface waters (Ballantine and Davies-Colley, 2013; Davies-Colley et al., 2008a; Krometis et al., 2007a; McKergow and Davies-Colley, 2010). In addition, increased flow during storm events is linked with the resuspension of bacteria from sediment into the water column within streams and rivers. Resuspension of sediment-borne microorganisms (including pathogens) into the water column could increase the health risk when using these waters Apart from wash-in mobilized by runoff from fecal pollution from land sources and within catchment, storm events provide additional input for bacteria into the water column via resuspension (McDonald and Kay, 1981). Once bacteria are transported into the water-sediment environment, they undergo series of processes including settling (sedimentation) into stream bed, die-off, growth, survival, attachment, and resuspension.

The fate and transport of bacteria within the water-sediment interface are affected by whether the cells are attached to particles or remain free-living. Moreover, attachment to particles plays a strong role in controlling the transport of FIB in this system as well. There have been contradictory reports on the partitioning of bacteria between attached (particle-associated) and unattached (free-living) phases within a water column (Jamieson et al., 2004, Wilkinson et al., 1995). Thus, the incorporation of bacteria attachment into water quality models to predict bacteria fate is usually based on assumptions, since this phenomenon is poorly understood.

1.2 Goal and objectives

The overall goal of this study was to further the understanding of the fate and transport of *E. coli* during storm events. The objectives of this study were to:

I. Measure *E. coli* concentrations and attachment rates to particle and unattached fractions;
II. Evaluate the relationship between particle size association of *E. coli*, water quality, and hydrological parameters;
III. Estimate the load contribution by attached and unattached fractions of *E. coli*; and
IV. Estimate the transport distance of *E. coli* by particle size.

1.3 Hypotheses

The hypotheses for this study were:

I. *E. coli* concentrations associated with various particles will significantly differ from each other.
II. The attached fraction of *E. coli* will not be significantly different from the total *E. coli* concentration.
III. *E. coli* fractions will be significantly correlated with water quality and hydrological parameters.

CHAPTER 2: LITERATURE REVIEW

2.1 Fecal Indicator Bacteria as a Threat to Water

2.1.1 Fecal Indicator Bacteria

Fecal indicator bacteria (FIB) have been studied extensively both in temperate (e.g. Pachepsky and Shelton, 2011; Ferguson and Signoretto, 2011) and tropical environments (e.g. Rochelle-Newall et al., 2015). FIB refers to a group of microorganisms that reside in the gut of warm-blooded animals and include *Escherichia coli*, fecal coliforms, and Enterococcus spp. These organisms find their way into the environment through fecal matter and indicate fecal contamination (Bolster, 2009, Ishii and Sadowsky, 2008; Rochelle-Newall et al., 2015). Although other microorganisms (e.g. viruses, protozoa, algae, and helminths, intestinal worms) cause water borne disease, more attention is given to FIB (Tallon et al., 2005; Chapra, 1997) because these organisms are easier to isolate and detect, are usually present in greater numbers than pathogens, and are much safer to work with than pathogens (Mubiru et al., 2000; Tate et al., 2000). Thus, FIB is preferred as surrogates for the detection of other pathogenic bacteria in environmental samples, such as water and soil (Berg, 2001; Elmund et al., 1999, Rochelle-Newall et al., 2015).

According to Bitton, G. (2005) and Ishii and Sadowsky (2008), an ideal indicator bacterium should be one that is found in the gut of warm-blooded animals, be present only when there are also pathogens and be absent when there are no pathogens, have similar survival patterns to pathogens in the environment, not be able to proliferate in the environment, be easily detected and enumerated using cheap methods, and be non-pathogenic in nature.

2.1.2 FIB and waterborne illness

Epidemiological studies have confirmed a strong relation between presence of fecal indicator bacteria and occurrence of highly credible gastrointestinal illness (HCGI) in both freshwaters (Stevenson, 1953; Dufour, 1984; Wade et al., 2006) and marine water (Cabelli, 1983; Cabelli et al., 1979; Colford et al., 2007). A higher risk of "highly credible" gastrointestinal infection caused by enterococci and *Escherichia coli* can occur at densities as low as MPN counts of 10 cells per 100 mL within recreational waters (Cabelli et al., 1982). Cabelli et al. (1982) compared the ratio of swimmer to non-swimmer symptoms and concluded that recreation in even lightly contaminated marine waters posed a danger for gastroenteritis. In addition, Haile et al. (1999) conducted an epidemiological study to assess the risk posed to a person who swam in marine waters harbouring total and fecal coliform, enterococci, and *Escherichia coli*. A higher risk of disease symptoms, including upper respiratory and gastrointestinal illness, was observed for swimmers in waters with a high level of one indicator bacterium and a low ratio of total to fecal coliforms.

In another study by Marion et al. (2010), a strong relationship between FIB and illness was found by conducting a comprehensive beach cohort study to examine relationships between water quality indicators and associated adverse health outcomes. Water use, including wading, playing, or swimming, in waters harbouring FIB resulted in a significant risk factor for gastrointestinal (GI) illness, with an adjusted odds ratio (AOR) of 3.2. In addition, an elevated *Escherichia coli* density was found to be significantly associated with elevated GI illness risk, where the highest *E. coli* quartile was associated with an AOR of 7.0 (CI 1.5, 32).

Studies in the UK also found a relation between health risk and bathing in FIB contaminated waters. Beach studies conducted in two different sites produced significant results. Swimmers were found to be more susceptible to minor infections and symptoms related to gastroenteritis than non-swimmers (Walker, 1992).

These studies demonstrate that FIB can be used as a surrogate for pathogens when examining health risks associated with impaired microbial water quality both in fresh and marine waters.

2.1.3 Water Quality Standards

Recreational and drinking water standards for FIB have been developed due to the association of illness with waters contaminated with fecal material. Standards have been developed for FIB in waters which are used to determine if a water is qualified to serve its designated use. Apart from standards suggested by the USEPA; state, territorial, and authorized tribal groups also set their own standards. The United States Environmental Protection Agency (USEPA) defines water quality standards as "provisions of state, territorial, authorized tribal or federal law approved by EPA that describe the desired condition of a waterbody or the level of protection or mandate how the desired condition will be expressed or established for such waters in the future".

The various designated uses of waterbodies typically described by the USEPA include; waters for protection and propagation of fish, shellfish and wildlife, recreation, public drinking water supply, and waters for agricultural, industrial, navigational and other purposes. Under the Clean Water Act, the EPA is required to develop criteria for ambient water quality that fairly convey the scientific knowledge of the effects of pollutants associated with both human health and the environment. States may adopt these criteria or use them as a guide in developing their own criteria. Criteria exist for aquatic life, biological organism presence, human health, microbial (recreational), and suspended and bed sediment.

Water quality criteria are developed and set by states, territories, and authorized tribes to protect the use to which the water body is assigned. Typically, water quality criteria are stated in two forms: (1) a numerical threshold value that should not be exceeded, or (2) a narrative describing the desired conditions of a water body to be met before its use. The USEPA has developed FIB criteria to protect both recreational waters and drinking water sources. Typically, enterococci and *E. coli* are used by the USEPA in defining Recreational Water Quality Criteria (RWQC). The 2012 RWQC states two numerical threshold for bacteria (enterococci and *E. coli*) namely a geometric mean (GM) and a statistical threshold value (STV). In addition, the new criteria are divided into "recommendation 1" and "recommendation 2" which represents an estimated illness rate of 36 out 1000 persons and 32 out 1000 persons, respectively. Based on recommendation 1, a geometric mean of 35 CFU 100 mL^{-1} and 126 CFU 100 mL^{-1} for enterococci (marine and fresh water) and *E. coli* (fresh waters), respectively should not be exceeded.

In South Dakota, FIB standards are provided for waters that are designated as limited contact recreation, immersion recreation, or domestic water supply. Currently, for limited contact recreation, the *E. coli* concentration should not exceed 1178 CFU 100 mL^{-1} in any one sample and 630 CFU100 mL^{-1} for the 30-day geometric mean (SD DENR, 2018).

2.1.4 Impairment of Water Quality due to FIB

A water body is said to be impaired if it fails to meet the criteria established for its designated use. Pathogens are the leading cause of impairments for surface waters in the United States based on a nationwide surface water quality summary (US EPA, 2008). A national summary of water quality impairment causes from ATTAINS (Assessment TMDL Tracking and Implementation System) showed that pathogens alone are responsible for impairments in 178,755 miles of streams and rivers (US EPA, 2018). Furthermore, the US National Water Quality Inventory Reports to Congress from 2000, 2002, and 2004 also reported pathogens as the leading cause of water quality impairments in the assessed rivers and streams across the nation (USEPA, 2000; USEPA, 2002; USEPA, 2004).

2.2 Sources of FIB

The USEPA categorizes pollution into two main groups namely point and non-point sources. The Clean Water Act defines point sources as “any discernible, confined and discrete conveyance, including but not limited to any pipe, ... This term does not include agricultural storm water discharges and return flows from irrigated agriculture.” Unlike point sources, which are easily traced to a specific or direct source, non-point sources are difficult to identify and are sometimes termed as “diffused sources” (USEPA, 2018).

2.2.1 Point Sources

Numerous studies have reported high concentrations of fecal indicator bacteria concentrations at various point source outlets (Table 2.1). Point sources of FIB include storm drains and storm water falls (Brownell et al., 2007; Dickerson Jr et al., 2007; Fujioka, 2001; Geldreich et al., 1968; Jiang et al., 2007; Lee et al., 2012; Lewis et al., 2005; Marino and Gannon, 1991; Marsalek, 1979; Marsalek et al., 1992; Marsalek and Rochfort, 2004; McCorquodale et al., 2004a; McCorquodale et al., 2004b; Petersen et al., 2005; Sercu et al., 2011), sewage effluent (Fujioka, 2001; Kay et al., 2008; Petersen et al., 2005), sewage (wastewater) treatment plants (Baudart et al., 2000; Garcia-Armisen and Servais, 2007; Haller et al., 2009; Petersen,et. al 2005; Parveen et al., 1997; Sorensen et al., 1989; Templar et al., 2016), leaking sewer systems (McLellan et al., 2007; Sercu et al., 2011; Weiskel et al., 1996) and industrial and municipal effluents (Geldreich, 1966)

Levels of FIB at point source outlets tend to be high, because point source outlets are localized (or more concentrated) sources whereas non-point sources are spread out and vary spatially at their source. Findings from previous studies reported that FIB levels were at least 10^2 CFU 100 mL^{-1} regardless of the type of point source (Table. 2.1). For instance, Lewis et al., (2005) found that fecal coliform level within gutters and drains ranged from 6.9×10^1 to 1.5×10^2 CFU 100 mL^{-1}, and 3.1×10^3 to 1×10^6 CFU 100 mL^{-1} respectively. In another study, Hyer, 2007 recorded fecal coliform levels from 7.5×10^5 to 4.1×10^6 CFU 100 mL^{-1} in a sewer line. The tendency of the high levels of FIB recorded at various point sources could be linked to the reason that FIB within these sources are conveyed through conduits and channels which makes them localized.

Table 2.1 Examples of FIB concentrations observed across various point sources

Author	Region	Source	FIB	Range or Average	Units
Reeves et al., 2004	Southern California	Coastal Outlet	Total Coliform	2×10^2	GEOMEAN MPN $100mL^{-1}$
		Coastal Outlet	*E. coli*	2×10^1	GEOMEAN MPN $100mL^{-1}$
		Coastal Outlet	Enterococci	3×10^1	GEOMEAN MPN $100mL^{-1}$
		Tidal Channel	Total Coliform	19.5×10^3	GEOMEAN MPN $100mL^{-1}$
		Tidal Channel	*E. coli*	2×10^2	GEOMEAN MPN $100mL^{-1}$
		Tidal Channel	Enterococcus	3×10^2	GEOMEAN MPN $100mL^{-1}$
Lewis et al., 2005	Tomales Bay Watershed, California	Gutter	Fecal Coliform	6.9×10^1 to 1.5×10^2	CFU $100mL^{-1}$
		Storm Drains	Fecal Coliform	3.1×10^3 to 1×10^6	$CFU100mL^{-1}$
Marino and Gannon, 1991	Ann Arbor, Michigan	Storm Drains	Fecal Coliform	1×10^5	$CFU100mL^{-1}$
		Storm Drains	Fecal streptococci	1×10^5	CFU $100mL^{-1}$
Schillinger and Gannon, 1985	Ann Arbor, Michigan	Storm Drains	Fecal Coliform	2.4×10^5	log CFU $100mL^{-1}$
Schiff and Kinney, 2001	San Diego, California	Storm Drains	Enterococcus	1×10^4	MPN $100mL^{-1}$
Stein and Tiefenthaler, 2005	Southern California	Storm Drains	Total Coliform	1×10^6	MPN $100mL^{-1}$
		Storm Drains	*E. coli*	$< 1 \times 10^2$ to 1.4×10^5	MPN $100mL^{-1}$
		Storm Drains	Enterococcus	1×10^1 to $>2.4 \times 10^5$	MPN $100mL^{-1}$
		Storm Drains	Total coliforms	$<1\times 10^2$ to 2.4×10^5	MPN $100mL^{-1}$
Hyer, 2007	Virginia	Sewer line	Fecal coliforms	7.5×10^5 to 4.1×10^6	CFU $100mL^{-1}$
Irvine et al., 2011	Western New York	Storm water Outfall	*E. coli*	1.4×10^4 to 2.8×10^4	CFU $100mL^{-1}$
Sauvé et al., 2012	Montréal, Canada	Storm water Outfall	Fecal coliforms	2 to 6.1×10^5	CFU $100mL^{-1}$
Ellis and Butler, 2015	London, UK	Storm water Outfall	*E. coli*	44×10^4	MPN 100 mL^{-1}

2.2.2 Non-Point Sources

Non-point sources of water pollution are sources which cannot be traced to a single or a direct source. These sources are not concentrated at a point and are therefore referred to as diffused sources. Non-point sources of FIB include; surface runoff (Jeng et al., 2005a; Lewis et al., 2005), soil leaching (Garcia-Armisen and Servais, 2007), soil amendment (Jiang et al., 2007), foreshore-beach sand (Boehm, 2007; Haack et al., 2003; Kinzelman et al., 2004; Wheeler Alm et al., 2003), human bather shedding (Elmir et al., 2007), and animal fecal input (Calderon et al., 1991; Field and Samadpour, 2007; Jiang et al., 2007; Wright et al., 2009).

Characterizing non-point sources of FIB is difficult due to high spatial and temporal variability of these sources (Bradford et al., 2013). Non-point sources of FIB are often driven by runoff resulting from precipitation. As runoff moves over the land, it mobilizes the stores of fecal matter and deposits them into surface waters. Both subsurface drainage and leaching of soil also provide a means of transport for non-point sources of FIB.

The USEPA ranks agriculture as the second most probable source of microbial impairments in assessed rivers and streams (USEPA, 2018). Sources of FIB from agricultural settings include fecal matter and wastewater generated from CAFOs (Bradford and Segal, 2009; Bradford et al., 2008), livestock (grazing or feeding operations), and runoff from manure applied field (USEPA, 2018). Unrestricted access to streams by livestock has been linked to increase in bacteria levels during water quality studies (Line, 2003; Miller et al., 2010; Muenz et al., 2006). For instance, Vidon et al. (2008a) observed that *E. coli* concentrations increased within a stream by 36-fold over a 12-month period after allowing cattle to access the stream.

2.2.2.1.1 Livestock

Non-human sources of fecal contamination, such as fecal matter from livestock, have been identified as a possible source of *E. coli* (Webster et al., 2004). For instance, Valcour et al., (2002) and Michel (1998) found a strong association between the incidence of *E. coli* and cattle density (i.e. total number of cattle per hectare) using a spatial regression technique. In another study, Hancock et al. (1998) analysed fecal samples from 12 livestock farms and detected the prevalence of *Escherichia coli* O157 ranging from 1.1 to 6.1% among the herds of cattle. Similarly, LeJeune et al., (2004) studied the prevalence of *E. coli* within fecal samples from cattle and reported 13% (636 of 4790) of fecal samples having *E. coli.*

Unrestricted or direct access of cattle to waterbodies has been linked to fecal contamination leading to cases of elevated *E. coli* concentrations in the water column (Byers et al., 2005; Davies-Colley et al., 2004; Gary et al., 1983). Davies-Colley et al. (2004) studied the impact of a herd of 246 dairy cows accessing a stream. They found that, upon crossing the stream, there was a sharp increase in *E. coli* concentrations that reached as high as 50×10^3 CFU 100 mL^{-1}, compared to background concentrations which of 3×10^2 CFU100 mL^{-1} . In addition, they found that the herds defecated 50 times more while crossing the stream than on the way leading to the stream. In another study, Vidon et al. (2008a) investigated the changes in water quality including *E. coli* levels on a 1005 metres long pastoral stream due to access by cattle on the upper 130m of the reach. After a year of monitoring water quality, it was found that *E. coli* levels increased by 36-fold during the summer and fall. Furthermore, Johnson et al. (1978) studied the levels of fecal coliform and fecal streptococci in a stream due to the impact of grazing cattle. After monitoring water quality during grazing and non-grazing periods, they found that there was approximately a five-fold and two-fold increase in fecal coliform and fecal streptococci, respectively, during the grazing period as compared to the non-grazing period.

In another study, Aitken (2003) assessed the risk associated with livestock intensity within a farming catchment and its impact on FIB contamination. Findings revealed that FIB in streams within the sub-catchment with high livestock intensity were 4 to 8-fold higher compared to those within the sub-catchment which had low livestock intensity.

These studies have shown that livestock contribute FIB loads directly by defecating while wading in the stream, and indirectly by defecating on pastures or cropland that can lead to feces being washed off the land during precipitation events. Thus, livestock is a potential source of elevated bacteria levels in surface waters.

2.2.2.1.2 Manure

The negative impact associated with pathogens and FIB within animal manure has long been studied (Burkholder et al., 2007; Gerba and Smith, 2005; Mawdsley et al., 1995; Pell, 1997). Manure from livestock contains high levels of bacteria including pathogens (Crane et al., 1983; Oun et al., 2014). For instance, Witzel et al. (1966) analysed cattle manure and found 3.4 -5 .6 x 10^5 MPN g^{-1}, 3.2 -5.6 x 10^5 MPN g^{-1}, and 3.5 - 17 x 10^6 MPN g^{-1} of total coliforms, fecal coliforms, and fecal streptococci, respectively. Maki and Picard (1965) performed a similar analysis on cattle manure and found fecal coliforms and fecal streptococci levels as high as 6 x 10^5 g^{-1} and 3.1 x 10^5 MPN g^{-1}, respectively.

Contamination from animal manure occurs through several ways including; leaching from land-applied manure, runoff from land applied manure, feedlots and animal housing, and manure storage units (Oun et al., 2014).

Jenkins et al. (2006) studied impact of poultry manure application on the microbiological status of runoff from agricultural land. On average, runoff was found to contain 5.2, 2.9 and 1.1 $\log_{10}$ MPN 100 mL^{-1} of total coliforms, *E. coli* and fecal enterococci respectively. Culley and Phillips (1982) studied the bacteria concentrations in runoff from cropland receiving liquid dairy manure. Total coliform, fecal coliform, and fecal streptococci levels found within the runoff water ranged from 91×10^3 to 214×10^3 MPN 100 mL^{-1}, 12×10^3 to 19×10^3 MPN 100 mL^{-1}, and 53×10^3 to 72×10^3 MPN 100 mL^{-1}, respectively. In another study, Thurston-Enriquez et al. (2005) assessed the impact of three different animal manure; fresh cattle manure, aged cattle and swine slurry manure applied on cropland. Results revealed that FIB (*Escherichia coli*, enterococci, and *Clostridium perfringens* along with coliphage) loads released from the manure upon rainfall ranged from 5.52×10^5 to 4.36×10^9, 3.92×10^4 to 4.86×10^8, and 9.63×10^5 to 3.05×10^8 CFU for the plot treated with fresh cattle, aged cattle, and swine slurry manure, respectively.

Bacterial contamination due to from tile drained water from manure applied fields has been implicated as a source of FIB contamination (Ball Coelho et al., 2007; Geohring et al., 1998; Palmateer et al., 1993). Patni et al. (1984) studied bacteria concentrations within tile drainage water from three manured cropped fields over a 4-year period. Concentrations of fecal coliforms (FC) and fecal streptococci (FS) found in tile water were 3–5 orders of magnitude lower than in applied manure. In a similar study, following swine manure application on a tile drained field over three-years, Pappas et al. (2008) observed peak fecal coliform (FC), enterococcus (EN), and *Escherichia coli* (EC) densities in subsurface tile water of 9.6×10^2 , 8.2×10, 12×10^2 CFU 100 mL^{-1}, respectively.

Furthermore, elevated FIB concentrations are observed in tile drainage water shortly after application to the field, in some cases within an hour of application (Geohring et al, 1998).

2.2.2.2 Wildlife and Pets

Several studies have analyzed and identified fecal matter from wildlife (Allen et al., 2011; Guenther et al., 2010; Hancock et al., 1998; Jardine et al., 2012; Literak et al., 2010; Navarro-Gonzalez et al., 2013; Pesapane et al., 2013) and pets (Geldreich et al., 1962) to quantify their potential contribution of FIB. For instance, Renter et al. (2001) analysed fecal samples from free-ranging deer within south-eastern Nebraska for *E. coli* O157:H7 and found 0.25% of (7 out of 1426) samples tested positive for the presence of this strain of *E. coli.* In another study, Pavlova et al. (1972) found fecal matter from both rabbit and rat with fecal streptococci levels of 8.5×10^5 MPN g^{-1} and 3.9×10^6 MPN g^{-1}, respectively.

Fecal samples from cats and dogs were analysed by Geldreich et al. (1962) to detect the presence of bacteria. Fecal matter from cats was found to contain fecal coliform and fecal streptococci concentrations as high as 7.9×10^6 MPN g^{-1} and 2.7×10^7 MPN g^{-1}, respectively. Similarly, dog feces were also found to contain fecal coliforms (2.3×10^7 MPN g^{-1}) and fecal streptococci (9.8×10^8 MPN g^{-1}).

While fecal analyses have estimated the level of some FIB within fecal matter of wildlife and pets, improved indirect methods such as bacteria source tracking have been used to trace sources or origin of FIB contamination in surface waters (Anderson et al., 2005).

For instance, Whitlock et al. (2002) studied the sources of fecal pollution within Stevenson Creek in Clearwater, Florida and found wild animal feces as the dominant source, and lesser amount of pet (dog) feces, using bacteria source tracking. In another study, Ahmed et al. (2005) used bacteria source tracking to trace the sources of fecal contamination following the detection of *E. coli* and *Enterococcus* in a local creek. Based on 10 host organism sources studied, dog feces were found to contribute 7% of *Enterococcus* contamination, while duck feces contributed 9% of *E. coli* contamination. Furthermore, Woodruff et al. (2009) performed bacteria source tracking in Washington's lower Dungeness watershed and Dungeness Bay to determine the sources of fecal coliform pollution that impacted water quality for more than a decade. Out of the 1164 *E. coli* samples tested, wild mammal sources represented about 26% of isolates collected (i.e. raccoons, rodents, deer, elk, beaver, otter, rabbit and marine mammals), while domestic animals (dog) represented only 4.3%.

2.2.2.3 Sediments

Sediments are a major source of bacteria to the water column. Sediment reservoirs of bacteria are often categorized as non-point sources (US EPA, 2018). Bacteria find their way into sediment through; (1) runoff carrying particle-associated bacteria from both agricultural (Crowther et al., 2002; Lewis et al., 2005) and urban catchment, (2) through direct deposition of fecal matter from livestock and wildlife (Collins and Rutherford, 2004; Davies-Colley et al., 2004) and (3) leaky sewers (McLellan et al., 2007; Sercu et al., 2011), septic tanks systems (Weiskel et al., 1996), and waste water treatment plant (Sorensen et al., 1989; Templar et al., 2016) Eventually, sources of FIB inputs entering receiving waters settle out of the water column and are stored in the bottom sediments where they can survive for long periods and can potentially proliferate (Craig et al., 2004; Haller et al., 2009; Anderson et al., 2005, Lee et al., 2006). Thus, bottom sediments serve as reservoirs of FIB within waterbodies (Jamieson et al., 2003; Jeng et al., 2005a; Whitman et al., 2006) and as a potential source of fecal bacteria for the overlying water.

With sediments identified as a potential source for bacteria, some studies have quantified these stores. For instance, Muirhead et al. (2004) quantified stores of *E. coli* within sediments by creating three artificial floods on three successive days in the Topehaehae stream located in New Zealand. After assuming that each individual flood generates a constant proportion of the previous flood *E. coli* yield, they estimated sediment stores of *E. coli* to be as high as 10^8 CFU m^{-2} .

Bacteria concentrations within sediments are often several folds higher when compared to the concentrations within the water column (An et al, 2002; Brinkmeyer et al., 2015; Byappanahalli et al., 2003; Byappanahalli et al., 2012; Irvine and Pettibone, 1993; LaBelle et al., 1980; Matson et al., 1978; Pachepsky and Shelton, 2011; Van Donsel and Geldreich, 1971, Pandey and Soupir, 2013). For instance, Crabill et al. (1999) observed that FC levels within sediments of a creek were, on average, 2200 times greater than that of the water column. Liao et al. (2014) also found that the monthly geometric mean of sediment *E. coli* concentrations was 40 to 350 times that of the water column. In another study, Buckley et al. (1998) reported that total coliform concentrations in sediments were approximately 1000 times higher than that of the water column.

These reservoirs of bacteria are a concern because bacteria within the sediments can be mobilized into the water column through resuspension during storm events (e.g. Weiskel et al., 1996), increased flow during dam or reservoir discharge (e.g. McDonald et al., 1982), recreational activities (An et. al 2002), the passing of livestock within a stream (e.g. Sherer et al., 1988), and passage of boats (An et al., 2002). The resuspension of bacteria into the water column from sediment is linked to the deterioration of water quality (Crabill et al., 1999; McDonald et al., 1982).

Sediment stores of bacteria recorded by researchers have been found to vary largely between locations. For instance, previous studies have observed *E. coli* levels as low as 1 MPN GDW^{-1} and as high as 10^8 MPN GDW^{-1} within sediment of surface water (Table 2.2).

Table 2.2 Examples of FIB concentrations observed within sediments of various surface waters

Author	Region	Medium	FIB	Range or average	Units
Haller et al., 2009	Geneva, Switzerland	River, sediment	*E. coli*	10 to 10^7	CFU GWW^{-1}
			Enterococcus	10 to 10^7	CFU GWW^{-1}
Desmarais et al., 2002	Florida, Fort Lauderdale	River Sediment	*E. coli*	14×10^3	MPN GDW^{-1}
		River Sediment	Enterococcus	11×10^3	MPN GDW^{-1}
Irvine and Pettibone, 1993	New York, Buffalo River	River sediment	Fecal coliform	10^2-10^4	GEOMEAN MPN GDW^{-1}
		River sediment	Fecal streptococci	10^1-10^2	GEOMEAN MPN GDW^{-1}
Lee et al., 2006	Santa Monica, California	Bay Sediment	*E. coli*	10^4–10^8	MPN100 GDW^{-1}
Garzio-Hadzick et al., 2010	Beltsville, Maryland	Stream sediment	*E. coli*	10^1 to 10^3	MPN GDW^{-1}
He et al., 2007	San Diego, California	Creek sediment	Fecal Coliform	15×10^2	MPN GDW^{-1}
			Enterococcus	36×10^2	MPN GDW^{-1}
			Total Coliform	85×10^3	$MPNGDW^{-1}$
Stephenson and Rychert, 1982	Boise, Idaho	River sediment	*E. coli*	6×10^2 to 45×10^2	MPN GWW^{-1}
Liao et al., 2014	Blacksburg, Virginia	Creek sediment	*E. coli*	33×10^2 to 95×10^3	CFU GDW^{-1}
			Enterococcus	3×10^2 to 59×10^2	CFU GDW^{-1}
Byappanahalli et al., 2003	Dunes Creek, Michigan	Creek sediment	*E. coli*	1 to 1×10^2	MPN GDW^{-1}
	Warren Dunes, Michigan	Creek sediment	*E. coli*	68 to 10^2	MPN GDW^{-1}
Donovan et al., 2008	Newark, New Jersey	River Sediment	Fecal coliform	33×10^2	CFU GWW^{-1}
			Enterococcus	9×10^1	CFU GWW^{-1}
Evanson &Ambrose, 2006	Southern California	Wetland Sediment	Total Coliform	12×10^2	GEOMEAN MPN $5GDW^{-1}$
			E. coli	20×10^2	GEOMEAN MPN $5GDW^{-1}$
			Enterococcus	70×10^2	GEOMEAN MPN $5GDW^{-1}$

GWW = gram wet weight, GDW =gram dry weight.

2.3 Survival of FIB within sediment

Bacteria are able to survive within sediments for days (Gerba and Mcleod, 1976; Goyal and Adams, 1984, Anderson et al., 2005; Craig et al., 2004), weeks (Haller et al., 2009; Jamieson, 2005), or longer (Garzio-Hadzick et al., 2010), which is often longer than survival times in the water column. For instance, Czajkowska et al. (2005) found that *E.coli* survived up to 32 days within water, while survival within sediment exceeded 90 days. Garzio-Hadzick et al. (2010) supported these findings with a microcosm study which revealed *E. coli* within overlaying water survived up to 30 days, but up to 120 days in sediment.

The survival of FIB is dependent on several factors including physio-chemical (abiotic) (e.g. temperature, sunlight, dissolved oxygen, pH, humidity, and salinity) and biological (biotic) factors (e.g. the presence of other competing organisms and predators, presence of biofilm (Byappanahalli et al., 2012; Ishii and Sadowsky, 2008). In addition, the ability of FIB to access and compete for available or limited nutrients and organic matter within their environment also affects how long they survive in both favourable and unfavourable conditions.

2.3.1 Texture influence on the growth and survival of bacteria in sediment

Particles size within the sediment has been linked to FIB survival (Garzio-Hadzick et al., 2010, Decamp and Warren, 2000; Grimes, 1980; Howell et al., 1996; Sherer et al., 1992). Burton et al. (1987) conducted a laboratory microcosm study to determine the survival rate of *E. coli* in different sediment textures varying from high clay content (75%) to high sand content (98%). Results revealed that *E. coli* survived longer in sediments containing at least 25% clay, with a strong positive correlation ($r_s = 0.80$) between bacteria and the survival times in sediments with at least 25% clay content. On the other hand, sediment with high sand content showed high die-off of bacteria and short survival periods.

Garzio-Hadzick et al. (2010) found similar results when they studied the survival of *E. coli* in loamy sand and sandy clay loam-textured sediments (based on USDA texture class). Sediments with high fine particle content were found to have higher bacterial survival rates (i.e. slower inactivation) compared to others. The authors linked this phenomenon to the significantly higher organic carbon content (5.14%) observed in sediments with a greater amount of fine particle size when compared to the organic carbon content in other sediments (1.35% and 1.78%). In addition to differences in organic carbon content, fine–textured sediments can offer bacteria protection from microbial predators (M Davies and J Bavor, 2000) allowing for longer survival periods.

2.3.2 Organic Matter Content and Other Nutrient

The presence of organic matter in the right quantity may also enhance the survival of FIB. Survival rates of FIB within sediments were found to improve with increasing nutrient and organic carbon availability (Gerba and McLeod, 1976; LaLiberte and Grimes, 1982; Blumenroth and Wagner-Dobler, 1998; Craig et al., 2004).

For instance, Lee et al. (2006) performed a microcosm study on sediments in the presence and absence of natural organic matter, to determine the importance of organic matter on the survival of FIB in the overlaying water. Concentrations of bacteria were examined over one day in both experiments. *E. coli* in sediments with organic matter reached as high as 1.5×10^5 MPN 100 g^{-1} wet sediment, while sediment without organic matter content fell below detection limit (64 MPN 100 g^{-1} wet sediment), indicating extended survival and persistence of FIB are dependent, at least in part, on sediment organic matter content.

Craig et al. (2004) performed similar a study, but solely on sediment with organic carbon content from three different sites under three temperature ranges of 10, 20, and 30°C. Overall, *E. coli* in two of the sediments with higher organic carbon content (i.e. 0.35% and 2.38%) experienced significantly higher survival with decay rates ranging from 1.15 to 7.69 days and 1.72 to 7.14 days, respectively. On the other hand, sediment with less organic carbon content (0.05%) had decay rates ranging from 0.90 to 3.13 days, demonstrating shorter survival periods.

2.3.3 Temperature

The impact of temperature on the die-off of FIB within sediment appears to be more pronounced as compared to other environments (Pachepsky and Shelton, 2011). *E. coli* survival rates have been found to be inversely proportional to sediment temperature (Craig et al., 2004, Faust et al., 1975). Craig et al. (2004) determined that at 10°C, *E. coli* was likely to survive for more than 28 days, but the survival time dropped to 7 days when temperatures reached 30°C. Similarly, Garzio-Hadzick et al. (2010) studied the survival of *E. coli* in sediment mixed with dairy manure under three temperatures (4, 14, and 24°C). For the three different sediments samples studied, *E. coli* inactivation at 4°C was the slowest, ranging from 0.0169 to 0.0233 per day, followed by 0.0754 to 0.138 per day, and 0.110 to 0.346 per day for temperatures of 14 and 24°C, respectively.

Furthermore, in attempts to mimic surival of bacteria within sediments, soil is sometimes used as a medium to replace sediment. For instance, Sjogren (1994) tested the survival of *E. coli* in sandy-loam podzol soils ('Webb' soil and 'Rich' soil) taken from two different locations. Microcosm experiments were carried out on the soils at temperatures of 5, 10, 20, and 37 °C. Survival time was highest under 5 °C for both soil types, with an estimate of 23.3 months within the 'Webb' soil, and 20.7 months within the 'Rich' soil.

2.4 Transport of FIB

2.4.1 Transport in Runoff

FIB can survive for long periods of time in the environment and can be mobilized from their sources into surface waters, thus contributing to water quality impairments. Generally, storm events are associated with inputs of FIB into overlying water, through (1) runoff carrying particle-attached and unattached bacteria from within catchment (also known as wash-in of bacteria from overland flow), and (2) through the resuspension of bacteria from sediment reservoirs due to the bed shear stress exerted by flow (Jamieson, et. al 2005).

Runoff contributes significantly to water quality deterioration within receiving waters, sometimes days after the occurrence of a storm event (Jeng et al., 2005a). Several studies have quantified concentrations of FIB associated with runoff from catchments (Reeves et al., 2004, Kim et al., 2005). For instance, the work of Reeves et al. (2004) estimated that, annually, over 99% of fecal indicator bacteria (*Escherichia coli*) loading was contributed by runoff from a highly urbanized watershed in Talbert California into surface waters nearby. Jeng et al. (2005a) studied wet weather runoff entering the Lake

Pontchartrain estuary in New Orleans. Runoff samples were found to harbour as high as 50×10^3, 14×10^3, and 24×10^3 MPN 100 mL^{-1} of fecal coliform, *Escherichia coli*, and enterococci, respectively. Levels of indicator organisms were elevated within both the water column and the sediment. In addition, they estimated that it would take 3 to 7 days for levels of bacteria within the water column to return to background concentrations after the impact of runoff. Similarly, Kistemann et al. (2002) reported an increase in concentrations of indicator organisms (*E. coli*, coliforms, fecal streptococci, C. perfringens) within the overlying water in three different tributaries in Germany following runoff from storm events.

2.4.2 Transport via Resuspension

The entry of bacteria into the water column is not limited to inputs from runoff from within a catchment or direct inputs of fecal matter from livestock and wildlife. Bacteria attached to particles and free-living bacteria also have the potential to enter the water column from sediment reservoirs though resuspension (Jamieson et. al, 2005a).

Resuspension is an important mechanism whereby bacteria within sediment reservoirs are mobilized into the water column. When stream bottom sediments are disturbed, both attached and unattached bacteria are suspended into the water column. Resuspension leads to an increase in water column FIB concentrations and the subsequent degradation of water quality. Resuspension has been studied via naturally occurring storm events (Fries et al., 2006; Jamieson et al, 2005a; Nagels et al., 2002; Pandey and Soupir, 2013; Stephenson and Rychert, 1982); mechanical disturbance of sediment (Grimes, 1980; Seyfried and Harris, 1990; Stephenson and Rychert, 1982) such as raking of the sediment bed (Abia et al., 2017; Gary and Adams, 1985; Sherer et al., 1988); recreational activities (An et al., 2002b); the passage of boats or ships (Pettibone et al., 1996); and artificial flood events (Gannon et al., 1983; McDonald et al., 1982; Muirhead et al., 2004; Nagels et al., 2002).

Field studies have quantified the contribution of bacteria sediment reservoirs to water column bacteria concentrations by estimating resuspension rates. For instance, Cho et al. (2010) generated an artificial flood within a first order creek in Maryland and found a resuspension rate of about 15×10^3 MPN m^{-2} s^{-1} for *E. coli*. Similarly, Jamieson et al. (2005a) estimated resuspension rates for *E. coli* within Swan Creek in Canada. Unlike Cho et al. (2010), resuspension was determined for several storm events. Resuspension of *E. coli* coincided with an increase in total suspended solids and was estimated to be 11×10^3, 8.2×10^3, and 15×10^3 CFU m^{-2} s^{-1} across three storm events. Finally, the authors concluded that resuspension of *E. coli* was limited to solely the rising limb of the storm hydrograph, indicating that a finite supply of the bacteria may be available for resuspension during individual storm events.

Laboratory experiments have also been conducted to estimate resuspension rates through flume studies (e.g. Cervantes, 2012). For instance, McDaniel et al. (2013) used a recirculating flume to mimic resuspension in a shallow stream. Resuspension was estimated for direct fecal deposits at various flow rates over time. Overall, resuspension rates ranged from 8.5×10^2 to 2.15×10^5 CFU m^{-2} s^{-1}. The authors reported that these values were in ranges of values determined in previous studies of Cho et al. (2010), and Jamieson et al. (2005a).

2.4.3 Artificial Floods

To study the resuspension of FIB during high-flow scenarios, such as storm events or flooding, researchers have released large amounts of water into water bodies either through reservoir releases (Muirhead et al., 2004; Nagels et al., 2002) or by discharging a large quantity of water from water tanks into a stream or river (Cho et al., 2010). These artificial floods serve as a suitable means to assess the impact of instream stores of bacteria on water quality by eliminating the contributions of fecal bacteria from runoff that occurs during natural storm events.

Results obtained from artificial flood events were found to be similar to that of natural storm events (Nagels et al., 2002). In both events, peak *E. coli* concentrations precede peak flow and had similar order of magnitude increases in *E. coli* concentrations from baseflow levels. Bacteria concentrations can increase by several folds during artificial flood events (Muirhead et al., 2004). For instance, McDonald et al. (1982) found that bacteria increased by more than 10-fold in response to increased flow after a series of water releases from a reservoir.

Aside from their use as an alternative for studying the dynamics of bacteria during high flow conditions such as natural flooding, results from artificial flood experiments have been used to validate results from modelling the release and transport of attached and unattached bacteria (*E. coli*) within streams (Bai and Lung, 2005; Cho et al., 2010). For instance, the work of Wilkinson et al. (1995) used data from artificial flooding experiments conducted within three river sites in England to create a conceptual model of the entrainment (resuspension) of particle-attached fecal coliform bacteria from stream bed sediment. Similarly, Bai and Lung (2005) used results from artificial flood experiments conducted by Muirhead et al. (2004) to test the resuspension of sediment-associated fecal bacteria (*E. coli*) under flood conditions by using the framework of the Environmental Fluid Dynamics Code (EFDC) model. Results showed that the model was capable of individually simulating contributions of particle-attached fecal bacteria from either the sediment bed or watershed individually.

2.4.4 Shear stress

Flow within a channel that is parallel to the streambed exerts bed shear stress on sediment particulates and the reservoir of bacteria. When shear stress is high enough to initiate the movement of particles into the water column, resuspension of bacteria (both sediment-attached and unattached) occurs. Shear stress beyond which resuspension will occur is known as “critical shear stress”. High flow during storm events results in increased bed shear stress which causes the resuspension of both sediment and bacteria. For instance, Jamieson et al. (2005a) reported shear stress ranging from 1.5 to 1.7 Nm^{-2} was linked with the resuspension of sediment-attached bacteria on the rising limb of the storm hydrograph. The authors reported that these shear stress values were similar to critical shear stress values for cohesive sediments.

The critical shear stress that triggers the resuspension of sediment-associated bacteria varies from one reach to another based on bed material properties, such as texture. Cho et al. (2010) estimated critical shear stress at different reaches of the Beaver Dam Creek tributary, with sediment particle fractions that ranged from predominantly sandy to a high fraction of silt and clay. Shear stress as high as 3.4 $N\ m^{-2}$ was associated with the reach having high sand content, while the other two reaches had sediment containing mainly silt and clay which was associated with a shear stress of 18.7 and 6.2 N m^{-2}, respectively.

2.4.4.1 Impact of sediment resuspension on the water column

As described previously, the resuspension of bacteria from sediment can occur either naturally (e.g. during storm events) or through manmade activities such as recreational activities and crossing of streams by livestock. An increase in water column FIB concentrations of several fold has been observed following the resuspension of sediment bacteria in both field (Cho et al., 2010; Jamieson et al., 2005a; McDonald et al., 1982; Abia et al., 2017a; Muirhead et al., 2004) and laboratory experiments (McDaniel et al., 2013; Abia et al., 2017).

To study the impact of microbial resuspension from sediments on water column *E. coli* concentrations, Abia et al. (2017) examined increases in flow via flume experiments, as well as simulated disturbances (e.g. mechanical agitation through stirring) of sediment in both a flume and within the natural environment (Apies River, South Africa). Results revealed increases in *E. coli* concentrations within the water column of 3.6 to 35.8, 2.4 to 17.4, and 6.5 to 7.9 times higher than the initial concentration following mechanical sediment disturbance in flume, increased flow, and mechanical disturbance (raking and cattle crossing) within the river bed, respectively. McDonald et al. (1982) performed an artificial flood experiment by releasing water into the Washburn River in England following several rainless days and observed 10-fold increases in water column *Escherichia coli* and total coliform concentrations resulting from resuspension. Similarly, both Muirhead et al. (2004) and Nagels et al. (2002) carried out artificial flood experiments within streams in New Zealand to study the effect of instream stores of bacteria on water column bacteria concentrations in the absence of wash-in of bacteria from the catchment. Results from both studies reported an increase in *E. coli* concentrations in the water column by two or more orders of magnitude from background levels, due to the resuspension of sediment bacteria. These studies demonstrate that resuspension of sediment bacteria stores is a major contributor to the degradation of water quality within surface waters.

2.5 Bacterial attachment

2.5.1 Attachment in the environment

Bacteria exist in one of two states, either attached to particles or unattached (planktonic bacteria). The attached fraction of bacteria refers to the ratio of particle-associated bacteria to the total bacteria concentration usually expressed in a range of 0 to 1. It is important to know the attached fraction of bacteria because these fractions will more easily settle out of the water column into sediments (Pachepsky and Shelton, 2011). In addition, Jeng et al. (2005b) and Schillinger and Gannon (1985) noted that the settling of particle-associated bacteria is linked to an increase in bacteria concentrations within sediments. On the other hand, the unattached bacteria fraction tends to remain in the water column for longer periods. Knowing the fraction of bacteria attached to settleable particles is important in determining the impact of microbial removal through sedimentation (Characklis et al., 2005).

Researchers have largely reported unattached bacteria as the dominant fraction, though the attached fraction is not negligible. However, some studies have found that less than half (20 to 35%, and 16 to 47%) of the total FIB concentration is attached to particles

(Characklis et al., 2005; Schillinger and Gannon, 1985). Attachment influences the transport of bacteria from land sources through runoff into receiving waters. Soupir et al. (2010) reported that about 28 to 49% of *E. coli* and enterococci were attached to particulates in runoff. Jeng et al. (2005b) examined attachment of indicator organisms within urban storm water runoff associated with estuarine sediments, and found that 19.6%, 22%, and 9.32% of fecal coliform, *E. coli*, and enterococci were associated with suspended particles, respectively. In addition, Schillinger and Gannon (1985) found the attachment rate of fecal coliform to suspended particles ranged between 15.9 to 16.8%. On the other hand, Mote et al. (2012) reported particle attachment of enterococci in estuarine water samples as low as 1% and as high as 95%, indicating that under certain circumstances, the dominant proportion of FIB can be attached.

Attachment of bacteria to particles has been reported to vary between storm and baseflow. Characklis et al. (2005) studied the attachment of various indicator bacteria to settleable particles in storm and baseflow water samples from three locations in and around Chapel Hill, North Carolina. The attachment of bacteria to settleable particles differed between the baseflow and storm water samples, with 30–55% of indicator organisms attached to settleable particles in storm water, while baseflow samples reported 20–35% attachment. Similarly, Fries et al. (2006) studied attachment of bacteria during both baseflow and storm events. About 37% of bacteria were found to be associated with particles in storm water samples, while nearly 50% of particle-associated bacteria were found in baseflow samples.

Attachment rates and partitioning behaviours vary between species of bacteria. For instance, Characklis et al. (2005) found that Clostridium perfringens spores exhibited a high proportion of attachment to settleable particles (50–70%) in storm water compared to fecal indicator organisms (fecal coliforms, *Escherichia coli*, enterococci) which had attachment rates of 20–35%. Similarly, Krometis et al. (2007) studied attachment of indicator bacteria (fecal coliforms, *Escherichia coli*, and enterococci), Clostridium perfringens spores, and total coliphage to denser settleable particles in storm water samples over three storm events. On average, attachment was highest for Clostridium perfringens spores (65%), followed by fecal indicator bacteria (40%) and then total coliphage (13%). Furthermore, Jeng et al. (2005b) studied attachment among three indicator organisms, fecal coliform, enterococci, and *E. coli*, and found that enterococci preferentially attached to the suspended particles with a diameter range of 10 µm to 30 µm, while fecal coliform and *E. coli* displayed a broader particle diameter range when attaching to particles.

These studies demonstrate that FIB are partitioned between the attached and unattached phase.

2.5.2 Factors affecting attachment

The partitioning behaviour and attachment of bacteria to particles is affected by a range of factors including biological, physical, and chemical factors of the environment in which they persist. FIB have been found to be disproportionally associated with certain particle sizes. Walters et al. (2013) determined the association of *E. coli* and enterococci to a range of particle sizes (≤ 12, 12-63, 63-1000, > 1000 µm) found in municipal wastewater. The majority of *E. coli* (90.6 %) and enterococci (83.0%) attachment was found in particles ≤ 12µm in diameter, followed by particle size ranges of 12-63, 63-1000, and > 1000 µm. Similarly, Guber et al. (2007) studied the attachment of fecal coliforms to various sand particles sizes (0.0625–0.125, 0.125–0.25, and 0.25–0.5mm), silt particles (0.002 to 0.05 mm), and clay particles (<0.002 mm) both in the presence and absence of bovine manure. The results revealed that in the absence of manure, bacterial attachment was higher in the silt and clay fractions as compared to sand particles that had little or no organic coating. On the other hand, the presence of manure decreased bacteria attachment in silt, clay, and coated sand significantly; however, attachment to sand without coating did not decrease. Furthermore, the work of Soupir et al. (2010) studied the attachment of bacteria (*E. coli* and enterococci) to various particle size ranges (> 500 μm, 63-499 μm, and 8-62 μm) in runoff samples collected from soil boxes treated with cowpat. At least 60% of all attached *E. coli* and enterococci were associated with particles in the8 to 62-µm particle size range.

Both biological and chemical factors have been shown to affect bacteria attachment, including presence of biofilms (Rochelle-Newall et al., 2015), changes in ionic strength of the medium (Otto et al., 1999; Zita and Hermansson, 1994), physio-chemical strength of the substrate surface available for attachment (Regina et al., 2014), and presence and concentration of total suspended solids (TSSs) (Byamukama et al., 2005). Guber et al. (2005) used batch experiments to study the effect of the presence of manure on the attachment of *E. coli* to soil particles and confirmed that increasing manure content of the soil decreased the attachment of bacteria. In further studies, Guber et al. (2007) again found that the presence of bovine manure decreased the attachment of fecal coliforms (FC) to soils, including clay and silt fractions, and coated sand fraction.

While individual factors are important to understanding the preferential attachment of FIB to various particles, the interaction of these physical, chemical, and biological factors may result in higher variability in attachment among FIB.

2.5.3 Methods of measuring attachment

Partitioning between bacteria attached to various particles size ranges and unattached bacteria can be studied using simple methods (e.g. Soupir et al., 2010) or by a multi-step method that utilizes both chemical and physical means (Soupir et al., 2008) to partition between attached and unattached bacteria (Figure 2.1)

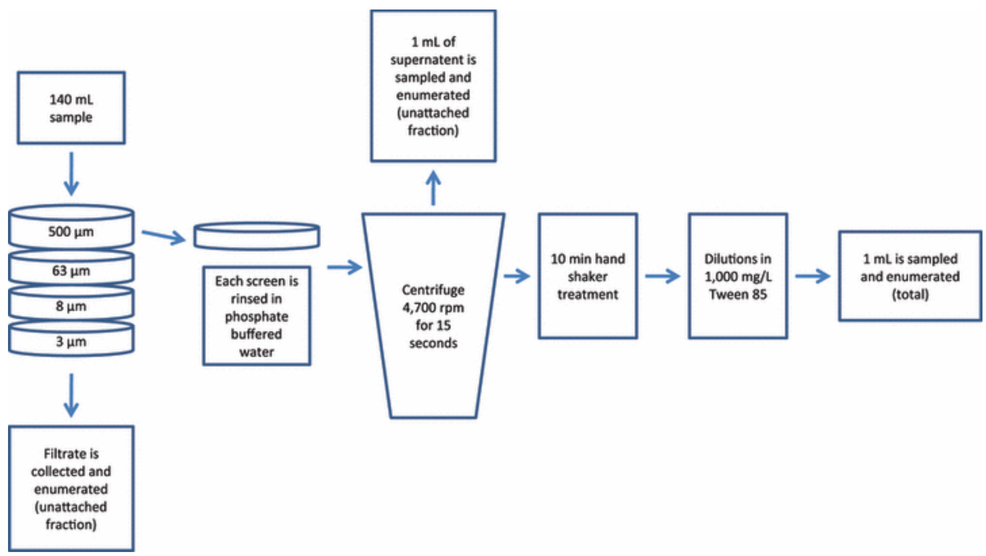

Figure 2.1 : Flow chart depicting the separation technique on attached and unattached bacteria

Source: (Soupir et al., 2008)

Common techniques used to separate unattached and attached bacteria include filtration, fractional filtration, settling (sedimentation) (Oliver et al., 2007), and centrifugation (Characklis et al., 2005; Cizek et al., 2008; Fries et al., 2006; Garcia-Armisen and Servais, 2009; Krometis et al., 2007; Soupir et al., 2010; Walters et al., 2013). In some cases, a combination of techniques is used.

Filtration has been used widely due to its simplicity (Henry, 2004; Mahler et al., 2000; Qualls et al., 1983). The drawback of this technique is that it cannot be used to partition bacteria into various particle size groups. In order to separate bacteria attached to particles, the sample (i.e. total bacteria concentration) is passed through an 8 μm filter to extract the particle-associated bacteria. The filtrate is processed and enumerated as the unattached bacteria, while the unfiltered sample is processed and enumerated as the total bacteria concentration (i.e. both attached and unattached bacteria). The difference between the total and the unattached fraction is the attached fraction.

Fractional filtration, also known as sequential filtration, is another technique used in determining attachment (Auer and Niehaus, 1993; Jeng et al., 2005b; Schillinger and Gannon, 1985; Soupir et al., 2010). Unlike filtration, this method is used to determine association of bacteria to various particle size ranges. Compared to simple filtration, fractional filtration is lengthier. For this technique, the sample is run through multiple filters in series, and the cells of bacteria trapped on the filter are assumed to be associated with particles of that size.

Another technique used in estimating attachment is settling (Kunkel et al. 2013, Oliver et. al, 2007). This technique takes advantage of Stoke's law. By calculating the settling velocity of a particle's size to which bacteria attach to, the time for the particle to settle out of the water column is then estimated. After thoroughly mixing sample in a graduated cylinder and allowing for the settling time for a particle to elapse, a portion of the sample is collected using a pipette, making sure that the sample is taken above the settling distance. The attachment of bacteria to each particle size is determined by calculating the difference between the concentrations determined before and after the settling time for each particle size.

The fourth technique for estimating attachment that is widely used is centrifugation (Characklis et al., 2005; Cizek et al., 2008; Fries et al., 2006; Guber et al., 2005; Krometis et al., 2007; Muirhead et al., 2005; Sayler et al., 1975; Schillinger and Gannon, 1985). Following centrifugation of samples at a specific revolution per minutes (rpm), the supernatant is processed and enumerated to determine the unattached bacteria. To find the fraction of attached bacteria, the difference between the total concentration and unattached fraction is determined. Henry (2004) stated that one flaw of centrifugation may result through the inclusion of clay attached bacteria in the category of unattached bacteria due to similarity in size of both clay-attached and unattached bacteria. It is, therefore, necessary to determine appropriate centrifuge settings to separate attached bacteria from unattached bacteria using this technique.

Pachepsky and Shelton (2011) hypothesized that; differences in estimates of bacteria attached to suspended particles observed in different studies is likely to result from the method used in analysing these attachment rates

2.5.4 Incorporating attachment of bacteria into water quality models

The attachment of bacteria to particles influences their transport and persistence within the environment. According to Russo et al. (2011), the modelling of suspended bacteria transport is performed using two methods. One is modelling all bacteria as unattached or free-living cells, while the other partitions them between unattached and sediment-associated bacteria.

Although the attachment of fecal bacteria to suspended particles in the water column has significant implications on the fate and transport of bacteria in water bodies (Pachepsky and Shelton, 2011), most models developed to predict microbiological water quality assume bacteria are solely unattached or free-living cells (Jamieson et al., 2004; Wilkinson et al., 1995). Thus, the inclusion of particle-associated bacteria (attachment) will likely lead to the improvement of these models.

Few studies have attempted modelling transport of bacteria by incorporating the attached fractions. The work of Bai and Lung (2005) successfully modelled the transport of sediment-associated bacteria by incorporating sediment process within the Environmental Fluid Dynamics Code (EFDC) model. The fraction of particle-associated bacteria in the water column was modelled using $K_{p,}$ the partition coefficient (L mg^{-1}); and m, the sediment concentration in the water column (L mg^{-1}); while particle-associated bacteria within the sediment were modelled using β_B, the bulk density of the sediment (mg L^{-1}); $\mathcal{E}$, the porosity of the sediment; and K_p.

Similarly, several SWAT (Soil and Water Assessment Tool) studies incorporated partitioning of bacteria between attached and unattached (or free-living) while predicting in-stream bacteria level (Kim et. al 2010, Kim et. al 2017). For instance, Kim et al. (2010) made modification to the bacteria transport within the original SWAT 2005 to simulate *E. coli* within three reaches of Little Cove Creek watershed in southern Pennsylvania. They included a model to simulate transport of sediment-attached bacteria similar to that used by Bai and Lung (2005) in grouping water column bacteria as either attached to particles or free living. Overall, in comparing the modified SWAT model that incorporated attachment to the original model, the modified model performed better at the three sites with NSE (Nash-Sutcliff model efficiency) of -0.2, -0.7, and 0.2 versus -64.9, -112.2, and -94.3 respectively for the original model with no modification. A successful model was created to inform swimming advisories based on bacteria concentrations for Lake Pontchartrain following storm water. A constant bacterial attachment rate of 0.09 was assumed and used for bacteria associated with suspended solids (McCorquodale et al., 2004b).

Attachment rates used in modelling differ based on study location. For instance, Steets and Holden (2003) simulated the fate of runoff associated with FC through a coastal lagoon in California using a mass balance-based, mechanistic model. The authors assumed the attachment of bacteria to suspended sediments to be 0.90. Similarly, Pandey and Soupir (2013) modelled the impact of sediment *E. coli* on the resuspension and transport of water column *E. coli.* The authors assumed the attachment rate for *E. coli* as 80-90% of the total *E. coli* in the water column based on Hipsey et al. (2008). Overall, in comparing the predicted *E. coli* with observed *E. coli* data, the model performed well and reported a skill of 0.78, NSE coefficient of 0.55 and an R^2 of 0.85.

While incorporating the attachment of bacteria to suspended particles yields better simulation results, most studies make assumptions of these attachment rates rather than using measured attachment rates from the studied system.

2.6 Best management practices for the reduction of bacteria and their impact on water quality

Collins et al. (2010) stated that pathways of bacteria transmission occur directly or indirectly. They defined direct pathways as "those by which fecal matter is deposited directly into waterways or are so close in proximity to waters such that potential for wash-in is very high"; on the other hand, indirect pathways were defined as "those which involve transport of fresh or aged fecal matter via surface runoff and subsurface seepage or drainage"

Management practices designed to improve water quality work in two ways. One is to reduce the delivery of the loads of bacteria into receiving water sources using engineered systems that intercept, capture, and treat bacteria-contaminated water from indirect pathways prior to releasing into receiving waters (e.g. Craggs et al., 2004a; Craggs et al., 2004b), or, secondly, by eliminating or reducing the access of direct pathways to water sources (e.g. Parkyn, 2004; Sunohara et al., 2016). Examples of management practices that are designed to improve microbiological water quality include vegetative treatment systems (e.g. vegetative treatment areas, constructed wetlands), riparian area management, and permanent fencing to exclude the direct access of livestock to waterways.

Vegetative treatment systems (VTS), or vegetative treatment areas (VTA), have been used extensively as an easily adopted and inexpensive means of improving water quality. The USDA-NRCS (2006) defines VTS as "plant-based treatment systems (typically perennial grass or forage crops) intended to reduce environmental risk associated with runoff and other process waters from an open lot livestock system. These systems perform treatment functions including solids settling, soil infiltration, and filtering (soil biological and chemical treatment), thus, the term *treatment* is used as opposed to *filter”*. Harmel et al. (2018) evaluated the efficiencies of VTAs in reducing bacteria within runoff from a small-scale swine operation in three counties in central Texas over a 4-year period. Overall, the runoff data showed that VTAs significantly reduced *E. coli* loads with treatment efficiencies ranging from 73 to 94%. Wetlands are another form of vegetative system adopted to improve the quality of runoff water entering receiving waters by reducing pollutant loads, including bacteria. The processes behind the removal of bacteria in constructed wetlands includes filtration, solar irradiation, sedimentation, aggregation, oxidation, antibiosis, predation, and competition from other microorganisms (Gersberg et al., 1987). Davies and Bavor (2000) demonstrated the reduction of bacteria levels in storm water that was routed through a wetland. Over a 6-month period of comparing bacteria removal performance of a constructed wetland and a water pollution control pond, they found that bacteria removal in the wetland was significantly higher ($p < 0.05$) and more effective than that observed in the water pollution control pond.

Aside from the use of plant-based systems to treat bacteria-laden water, techniques such as restricting livestock access to streams using fences and bridges for cattle crossings offer suitable alternatives in reducing the impact of direct microbial pathways. Assessing the impact of the installation of livestock exclusion fencing on stream water quality was performed by Line (2003). Microbiological analysis over the 5-year period after fencing exclusion showed 65.9% and 57.0%, reduction in fecal coliform and enterococci levels, respectively. The bacteria levels were significantly reduced, indicating that livestock exclusion through fencing was effective at reducing bacteria levels in the stream and improving water quality at large. A similar study was conducted by Muenz et al. (2006) to assess stream health for two buffered (fenced from cattle access) and three unbuffered (unfenced streams; cattle have access to streams) streams in an agricultural catchment. Overall, both average fecal coliform (410 CFU 100 mL^{-1}) and fecal streptococci (1239 CFU 100mL^{-1}) counts for the three unbuffered streams were higher compared to the average fecal coliform (197 CFU 100 mL^{-1}) and fecal streptococci (927 CFU 100 mL^{-1}) of the two buffered streams, indicating the water quality benefits of stream fencing. Furthermore, Doran and Linn (1979) found that fecal coliform levels were 5 to 10 times more in runoff collected from an unfenced pasture compared to a fenced pasture within eastern Nebraska during a three-year study.

Parkyn (2004) reviewed the effectiveness of riparian buffer zones and noted that adopting both fencing and riparian area management using riparian buffer strips largely reduces microbial contamination to pastoral streams. The buffer strips reduce the impact and the magnitude of surface runoff, thus providing some time for infiltration and at the same time trapping fecal matter and particle-attached bacteria. For instance, Wilcock et al. (2009) observed a reduction in median annual *Escherichia coli* concentrations at a rate of 116 MPN 100 mL^{-1} per year within a pastoral stream in the Waiokura catchment in New Zealand after reducing diary effluent discharges and adopting riparian management involving permanent livestock exclusion from stream banks and riparian buffers to mitigate runoff from pasture.

Studies have shown that tile drains serve as a conduit for transport of pollutants including microbial exports into surface waters (e.g. Joy et al., 1998; Lapen et al., 2008; Pappas et al., 2008), thus controlling the drainage provides a means of mitigating negative impacts of tile drainage on water quality. Controlling drainage in tiles within an agricultural catchment has been used as a management practice to reduce loading fecal indicator bacteria such as *E. coli* and enterococci into surface waters at a watershed scale (Sunohara et al., 2016). Water quality targets were met during the study period spanning from 2005 to 2013, representing nine growing seasons with 76% and 25% reduction of *E. coli* and enterococci in drainage water. Recent studies by Wilkes et al. (2014) have also demonstrated the effectiveness of controlled tile drainage in improving water quality. The study monitored the microbiological status within two agricultural watersheds, one with controlled tile drainage (CTD) and the other with uncontrolled tile drainage (UCTD) over a 7-year period. Significantly lower (at p=0.06 level) waterborne pathogen (bacterial and viral pathogens) and, coliphage loading were observed in stream discharge from the watershed with CTD compared to the watershed with UCTD systems. Furthermore, Frey et al. (2015) found that CTD systems employed on macro porous field plots significantly reduced loads of fecal indicator bacteria and Campylobacter spp. in tile drainage water that may reach surface waters as compared to UCTD.

2.7 Bacteria attachment (partitioning) :Gaps in knowledge and future work

The attachment of bacteria within the environment affects the fate and transport of the bacteria. Several studies used various techniques to estimate the partitioning of bacteria between attached and unattached including filtration (and or fractional filtration), centrifugation (Characklis et al., 2005; Cizek et al., 2008; Fries et al., 2006; Henry, 2004; Jeng et al., 2005; Krometis et al., 2007; Sayler et al., 1975; Schillinger and Gannon, 1985; Soupir et al., 2010; Soupir et al., 2008), and settling (sedimentation) (Kunkel et al., 2013). However, there still exist contradictory report on fraction of bacteria that exist as attached or unattached. For instance, a couple of studies reported less than 50% of total bacterial concentration as attached (Characklis et al., 2005; Cizek et al., 2008; Fries et al., 2006; Krometis et al., 2007; Soupir et al., 2010) while others reported more than 50% of attachment (Characklis et al., 2005; Krometis et al., 2007). Furthermore, Pachepsky and Shelton, (2011) hypothesized that the discrepancy in estimates of partitioned bacteria across various studies could be due to the technique used. Currently no study has been done to compare results from various techniques used in estimating partitioning of bacteria. Thus, analyzing the significance difference in results across different techniques could offer some ideas about how some of these discrepancies can be corrected.

While the partitioning of bacteria is receiving growing attention, the representation of this phenomenon in water quality models to predict in-stream bacteria is still very poor. Most models till date assume attachment instead of estimating in situ attachment which could be a fair representation of natural condition within the studied system. It is therefore laudable that future should compare results between using assumed and estimated (or in-situ) attachment coefficient.

Furthermore, attachment rate among various particle size could also be incorporated into mechanistic and watershed scale models since current efforts only attempts partitioning mainly between attached and unattached fraction. Involving attachment as a distributed parameter among various particle size rather than as a lumped parameter-i.e. as attached and unattached could offer water quality managers to target a more specific bacteria load contributed by bacteria attached to a particular size.

CHAPTER 3: FATE AND ATTACHMENT OF *E. COLI* DURING STORM EVENTS

A paper to be submitted

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Abstract: Storm events contribute to the deterioration of microbiological water quality status within receiving surface waters. The transport and fate of bacteria within the sediment and water column is affected by environmental factors including the partitioning of bacteria between free-living and particle associated bacteria. The goal of the study was to improve the understanding of the fate and transport of *E. coli* partitioned between attached and unattached (or free-living) phase during high flow regime. Baseflow conditions and a series of storm events were monitored for *E. coli* alongside water quality and hydrologic parameters. Satisfactory water quality was observed during baseflow, but storm events lead to poor water quality due to elevated *E. coli* concentrations that resulted in high exceedance rates. A significant fraction of *E. coli* within the water column during both storm events and baseflow conditions were free-living or associated with very fine particles (≥ 70% of total *E. coli*). The high concentrations of free-living bacteria (5×10^2 – 15×10^3 CFU $100mL^{-1}$) indicate that sedimentation of the settleable fraction of *E. coli* would not be adequate to reduce bacteria to within the microbiological water quality standards. Many water quality models assume bacteria are unattached; to test this

assumption, a Mann Whitney U-test test was performed to determine if there is a significant difference between unattached and total *E. coli* during storm events. This revealed that the free-living *E. coli* concentration was significantly different than the total *E. coli* concentration in three out of the eight storm events evaluated (38%). Water quality and hydrologic parameters, including turbidity, temperature, flow, and bed shear stress, showed a weak ($p > 0.05$) relationship with *E. coli*. A regression model was developed to estimate the concentration and, therefore, risk of *E. coli* in Skunk Creek; however, this model failed to adequately predict storm event *E. coli* ($R^2 = 0.09$-0.22) even when partitioned between the different particle fractions. The findings of this study demonstrate the importance of partitioning between particle associated and free-living bacteria when predicting bacteria concentrations in the water column as well as the need for determining site-specific attachment rates to determine appropriate management practices for bacteria reduction.

3.1 Introduction

Fecal indicator bacteria (FIB), including *E. coli*, are used to detect the presence of other microorganism including pathogens, (Ishii and Sadowsky, 2008) and are recognized as major contributors to water quality impairments in both marine and freshwater across the United States (US EPA, 2011). In addition, the presence of these bacteria has been associated with public health risks and the occurrence of water-borne diseases (Cabelli et al., 1979; Cabelli et al., 1982; Dufour, 1984; Prüss, 1998; Wade et al., 2006; Wade et al., 2003). For example, Cabelli et al. (1982) found that a higher risk of “highly credible” gastrointestinal infection was associated with enterococcus and *Escherichia coli* concentrations as low as 10 MPN 100 mL^{-1} within recreational waters.

FIB can be transported into receiving waters through several pathways, including direct fecal deposits from livestock, wildlife, and pets; runoff; and point sources, such as septic tanks and wastewater treatment plants. The proximity of land sources including pasture land, and homes with pets, etc.) of fecal matter impacts their susceptibility to be mobilized and transported into receiving waters (Collins et al., 2010).

Upon entering surface waters, bacteria eventually settle out of the overlying water into the sediment bed where they can survive and grow (Carrillo et al., 1985; Davies et al., 1995; Hendricks, 1971; Jamieson et. al, 2005a; Sherer et al., 1992). Streambed sediments can act as a reservoir for FIB (Byappanahalli et al., 2003; Gary and Adams, 1985; Gerba and Mcleod, 1976; Obiri-Danso and Jones, 2000; Shiaris et al., 1987) which can be transported into the water column through resuspension (Jamieson et al., 2005a; McDonald et al., 1982; Nagels et al., 2002; Sherer et al., 1988). Storm events are one way through which these reservoirs of bacteria can be mobilized and are linked to significant increases in *E. coli* concentrations within the water column due to resuspension (Fries et al., 2006; Jamieson et. al , 2005a; Krometis et al., 2007b; McKergow and Davies-Colley, 2010; Nagels et al., 2002). For example, Jamieson et al. (2005a) seeded sediment with a tracer bacterium within a creek and recovered these bacteria within the water column over several storm events due to resuspension.

The contributions of bacteria that occur during storm events lead to the deterioration of microbiological water quality. Not only do storm events resuspend FIB into the water column, they also contribute FIB to surface waters through runoff (Jeng et al., 2005a; Reeves et al., 2004). This phenomenon occurs when land sources (e.g. manure applied fields, feedlots, CAFOs) of FIB are mobilized via the impact of runoff (or wash-in). For instance, McKergow and Davies-Colley (2009) and Davies-Colley et al. (2008) estimated that about 98% and 95% of annual bacteria loading occurred within storm event, respectively.

Bacteria in the sediment environment or water column exist either attached to particles or remain free-living, which affects thier fate and transport in the environment. For instance, within the water column particle-attached bacteria are less mobile and settle out faster (Fries et al., 2006). Similarly, the particle sizes to which *E. coli* attaches will influence how far they are transported downstream. On the other hand, free-living or unattached *E. coli* are buoyant and remain in the water column longer, and are carried farther distances downstream.

Although past studies have looked at *E. coli* concentrations during storm events, there is limited information on the attachment rates during high flow and the size of particles *E. coli* is typically attached to when transported in the water column. Understanding attachment rates of *E. coli* during storm events will provide vital information that can be incorporated into water quality models used to predict bacteria concentrations in surface waters. In addition, selecting and designing best management practices (BMPs) to reduce *E. coli* will benefit from the expanded knowledge of bacteria transport dynamics.

The goal of this study is to understand the attachment of *E. coli* to various particle sizes affecting its fate and transport. The objectives of this work include: (a) to evaluate *E. coli* concentrations and their attachment rates, (b) evaluate the relationship between both attached and unattached *E. coli* concentrations and water quality parameters, (c) evaluate the impact of attached and unattached *E. coli* concentrations on water quality status, and

(d) develop a regression model to predict *E. coli* partitioned between the different particle fractions.

3.2 Materials and Methods

3.2.1 Study site

The study was conducted on Skunk Creek, a tributary to the Big Sioux River located in southeastern South Dakota. The Skunk Creek watershed extends across Moody, Lake, and Minnehaha Counties and drains an area of approximately 1613 km^2 (SD DENR, 2004) (Figure 3.1). The land use in the watershed is predominantly agricultural with row crop production dominating the landscape (64%), followed by hay and pasture (17%), and grassland (6%) (NLCD 2011). About 6.5% of the watershed comprise of urban developed area (Rajib et al., 2016).

Skunk Creek contributes a significant proportion of the flow to the Big Sioux River, at times making up nearly the entire flow. Thus, water quality issues within Skunk Creek greatly impact the water quality in the Big Sioux River. The designated use of Skunk Creek includes warm water marginal fish life propagation, limited contact recreation, fish and wildlife propagation, recreation, stock watering, and irrigation (SD DENR, 2004). Major causes of water quality impairment within Skunk Creek watershed include *E. coli*, fecal coliforms, and Total Suspended Solids (TSS) (US EPA, 2018). According to the US EPA Water Quality Assessment Report for 2016, limited contact recreation was not supported within the Skunk Creek due to high concentrations of *E. coli*. The primary source of the bacteria in the watershed is believed to be livestock, although human, pet, and wildlife sources also contribute a portion of the total load (SD DENR, 2008) . To reduce *E. coli* concentrations within the creek, riparian area management and seasonal riparian area management practices have been implemented; however, *E. coli* persists at high levels, often above the standard.

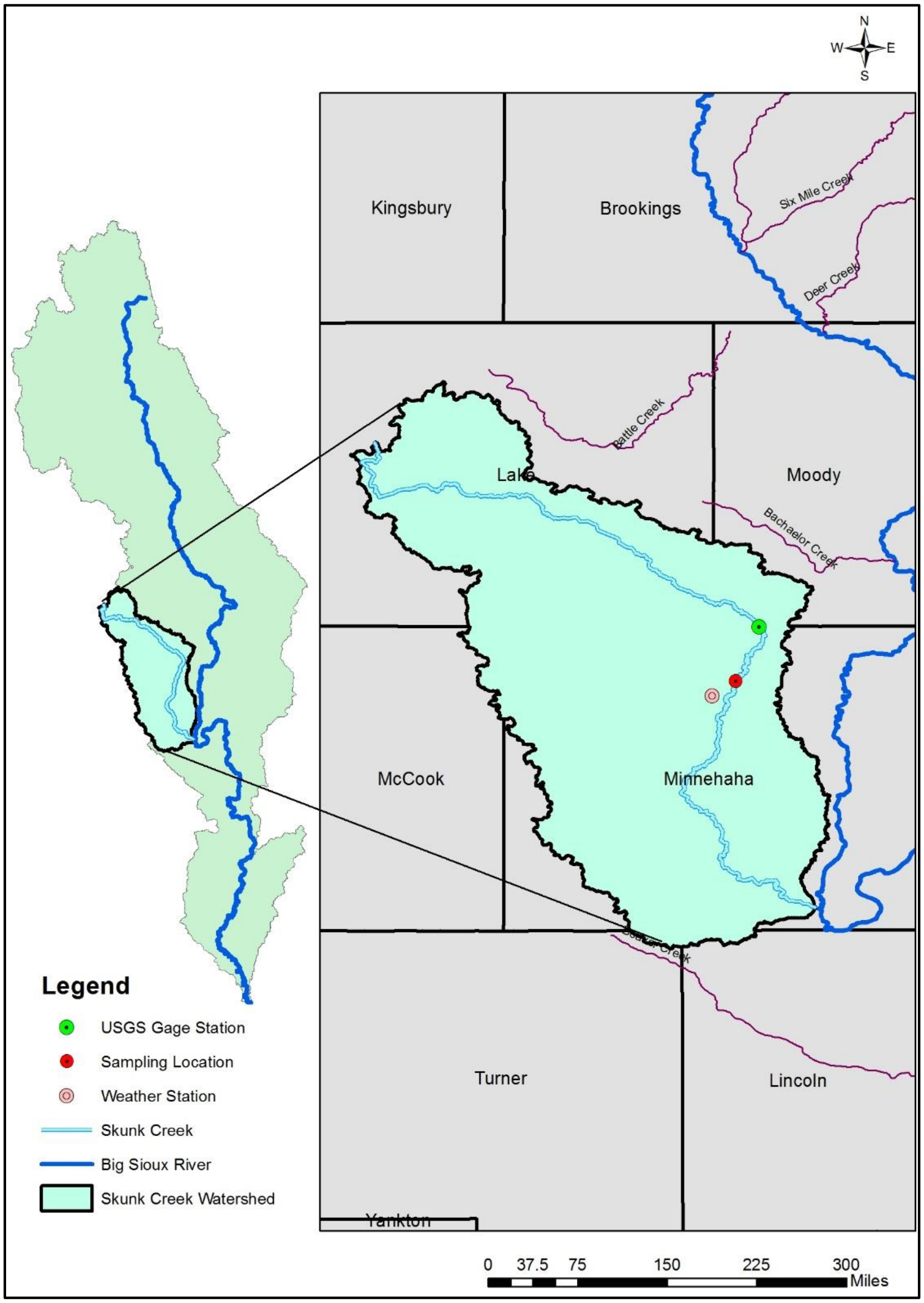

Figure 3.1 The Skunk Creek Watershed is located within the Big Sioux Watershed in eastern South Dakota.

3.2.2 Sample collection and processing

Both storm event samples and dry weather (baseflow) samples were collected for *E. coli* analysis. During the study period, discrete water samples were collected for eight individual storm events using a Teledyne 6712 ISCO refrigerated auto sampler (ISCO Inc., Lincoln, NE USA). Before each storm event, the autosampler was packed with one-liter bottles, which were sterilized through autoclaving. The sampler collected water samples every 30 minutes over a five-hour period. Turbidity and temperature were also monitored using an ISCO turbidity meter and temperature sensor respectively. Dry weather samples were collected by grab sampling using sterilized polypropylene bottles.

Both storm and grab samples were transported on ice to South Dakota State University-Water Research Laboratory for microbiological analysis of *E. coli* concentrations and attachment rates. Attachment was assessed by particle size ranges using sedimentation in graduated cylinders by employing Stokes's Law. Samples were plated within 24 hours on Modified mTEC agar (USEPA, 2002) using standard membrane filtration. Briefly, samples were filtered through 0.45μm filters and placed into a water bath for 2 ± 0.5 hours at 35°C ± 0.5°C. The plates were then placed in the incubator for 22 ± 2 hours at 44.5°C ± 0.2°C. Samples were plated in triplicate and colony counts were averaged.

3.2.3 Analysing *E. coli* attachment using Stoke's law

Each sample bottle was inverted several times (more than twice) to thoroughly mix any settled particles, thereby ensuring that the sample was homogenized prior to sedimentation. Immediately after inverting the samples, they were poured into 500 mL graduated cylinders and a sub-sample was immediately collected for enumeration of total *E. coli* concentration.

The *E. coli* was partitioned into three particle size ranges according to the American Geophysical Union (AGU) Sediment Classification System Ranges were medium and coarse silt (0.016 mm $\leq$ d $\leq$ 0.062 mm), fine and very fine silt particles (0.004 $\leq$ d $\leq$ 0.016 mm), and clay and unattached bacteria (d < 0.004 mm). Clay-sized particles were grouped with unattached bacteria because the diameter of *E. coli* (1-2.5 μm) (Holt et al., 1994) is similar to the size of clay particles (0.24 to 4 μm).

The settling velocities (Equation 1) for each particle size range were used to calculate the sampling times for each size fraction at a depth of 9 cm from the surface of water samples in the graduated cylinders. The settling velocities were computed using Stoke's Law:

$$V_S = \frac{g}{18}\left(\frac{\rho_s - \rho_w}{\mu}\right) d^2 \qquad (1)$$

where v_s is the settling velocity, ρ_s is the particle density (estimated at 2.65 gcm^{-3}), ρ_w is the density of water (1g cm^{-3}), μ is the dynamic viscosity of water (g cm^{-1} sec^{-1}) , and d is the particle diameter (cm). The minimum diameter for each particle size range was used in calculating settling velocities. A similar method was employed by Liu et al., (2011).

After each particle size range settled out of the column, a portion of the sample was collected with a pipette and plated using standard membrane filtration as described above.

3.2.4 Data Used and Estimation of Storm Event Variables

3.2.4.1.1 Rainfall Data

Precipitation data were obtained from the South Dakota Mesonet for the study period (Summer 2016 to Summer 2017). The weather station at Colton, Minnehaha County, South Dakota (N43.7687, W96.8897) was used to calculate storm precipitation amount and intensity.

3.2.4.1.2 Shear Stress

Bed shear stress is the stress exerted by the flow of water parallel to the streambed within stream channel. The stress exerted on the sediment reservoir of bacteria causes resuspension of bacteria into water the column. Bed shear stress was computed using Equation (2) according to Jamieson et. al (2005):

$$\tau_b = yS^{\frac{1}{4}}\left(\frac{n}{A}\right)^{\frac{3}{2}}Q^{\frac{3}{2}} \qquad (2)$$

where τ_b is the bed shear stress (Nm^{-2}), y is the specific weight of water (Nm^{-3}), S is the slope ($m\ m^{-1}$), n is Manning's roughness coefficient, A is the cross-sectional area of flow (m^2), and Q is flow (m^3s^{-1}). Manning's roughness coefficient was estimated as 0.045 based on channel characteristics (i.e. winding, with some pools, weeds and stones) (Ward and Trimble, 2003). The estimated slope of channel bed was 0.0006 $m\ m^{-1}$ according to USGS StreamStats Web Application Version 4.0 (Ries III et al., 2008).

3.2.4.1.3 Bacteria load and Equivalent Background Period Loading

The baseflow *E. coli* load represents the average of the estimate of loads of bacteria within the water column during dry weather periods. Baseflow load (BL) was computed as follows (Equation 3):

$$BL = Q_B\, C_B\, \Delta t \qquad (3)$$

where, Q_B is average baseflow for study period ($m^3\ s^{-1}$), C_B is average baseflow *E. coli* concentration (CFU 100 mL^{-1}); Δt = period of storm event sampling (s), and BL is base flow loads for the same duration of the storm event being monitored (CFU).

Baseflow was separated from total stream flow for the study period using the Web-based Hydrograph Analysis Tool (WHAT) (Lim et al., 2005). WHAT has been used in previous studies in separating baseflow from total stream flow (Ahiablame et al., 2017; Zhang et al., 2013). The baseflow separated was used to estimate *E. coli* load during the baseflow period.

To estimate the bacteria load for each storm event (i.e. the event load, EL), the bacteria concentrations for samples collected at each time interval, were multiplied by the corresponding flow and time, and the result was summed over for each storm event monitoring duration (Equation 4):

$$EL = 10^4 \sum_{i=1}^{N} Q_i\ C_i\ \Delta t \qquad (4)$$

where C_i is the ith discrete bacteria concentration (CFU 100 mL^{-1}); Q_i is the ith discrete discharge ($m^3\ s^{-1}$); N is the total number of discrete concentrations measured for a storm event and; Δt is the time interval of each measurement (s).

Equations 3 and 4 were combined to estimate the equivalent background period (EBP) (Equation. 5). The EBP represents the length of time required for baseflow to yield the same load as a storm event (Krometis et al., 2007b). A similar technique was used by Liao (2015) and Krometis (2007) in estimating EBP for enterococcus and *E. coli*:

$$EBP = EL/DL \qquad (5)$$

3.2.4.1.4 Event Mean Concentrations

The event mean concentrations (EMC) are the flow weighted concentrations of *E. coli* present within discrete water samples over the monitoring duration for each storm event. EMC for each storm event was calculated to compare *E. coli* concentrations from individual storm events (Equation 6):

$$EMC = \frac{\sum_{i=1}^{n} C_i \; Q_i}{\sum_{i=1}^{n} Q_i} \qquad (6)$$

3.2.4.1.5 Estimating transport distance of *E. coli* by particle size.

Estimate of how far *E. coli* associated with various particle sizes was estimated by combining Stokes's law, stream flow, and width of stream (Equation 7):

$$D_T = \frac{Q}{V_s W} \qquad (7)$$

where Q is stream flow ($m^3\ s^{-1}$); V_s is particle settling velocity ($m\ s^{-1}$); and W is stream width (m)

3.2.5 Statistical Analysis

All statistical analyses were performed using SAS 9.1 SAS Institute Inc., The SAS system for Windows Release 9.1, Cary, N.C., 2001.

The significance differences ($p \leq 0.05$) between the mean bacteria concentrations attached to the various particle size ranges, including medium and coarse silt (MC), fine and very fine silt (FVF), and clay and unattached (CU), were determined using ANOVA for each storm event. Prior to the ANOVA test, the homogeneity of variance (HOV) was tested using the Levene Test, Bartlett's Test, and the Brown-Forsythe Test to determine if the variances of the various *E .coli* fractions were equal. Groups of means whose variance were not equal was tested using Welch's ANOVA. Tukey's Honest Significant Difference (HSD) was used as a post hoc test to group the mean bacteria concentrations of the three factions across each storm event.

The significant difference between the unattached and total bacteria concentrations was tested using the Mann-Whitney U test- a non-parametric test since bacteria concentrations data were not normally distributed.

A non-parametric correlation analysis using a two-tailed Spearman's Rank was used to analyze relationships between bacteria concentrations associated with various particle fractions (MC, FVF and CU), as well as the total bacteria concentration with water quality parameters and hydrological parameters. The bacteria concentration was tested for normality using both graphical method (Q-Q plots) and numerical methods (Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling test). Spearman's correlation was used because the data were not normally distributed.

A correlation analysis was also performed to determine the relationship between the amount of rainfall recorded for each storm event and the *E. coli* EMC.

A multiple linear regression model was developed for each bacteria fraction to predict *E. coli* concentrations during storm events using measured water quality parameters (turbidity and temperature) and hydrological parameters (flow and shear stress). Both independent variables and dependent variables were log10 transformed, to reduce skewness and improve normality of data sets. Prior to the regression analysis, multicollinearity between the independent variables was assessed by calculating Variance Inflation Factors (VIF) (Ott and Longnecker, 2001) to ensure that the developed models did not include redundant variables. The regression equations developed for estimating *E. coli* concentrations were of the form:

$$\log(E.coliConc) = log\beta_0 \ + \ \beta_1 \log(\ X_1) \ \ + \ \beta_2 \log(\ X_2) \ \ + \cdots \ \beta_n \log(\ X_n)$$

where $E.coliConc$ is the *E. coli* concentration (CFU 100 mL^{-1}); β_0 is the regression constant; β_1, β_2…… β_n are regression coefficients; and X_1, X_2, …. X_n are the predictor variables. Since the equations were developed with log10 transformed variables, the final equations are expressed as:

$$E.coliConc = \beta_0 {X_1}^{\beta_1} {X_2}^{\beta_2} \dots \dots \dots . \ {X_n}^{\beta_n}$$

3.3 Results and Discussion

3.3.1 Statistics of storm events and baseflow *E. coli* fractions

The mean concentrations of *E. coli* associated with the particle fractions varied across the storm events. The highest concentration was associated with the clay and unattached fractions hereafter referred to as unattached, *E. coli* (9.3 to 92.8 × 10^2 CFU 100 mL^{-1}) followed by the medium and coarse silt fraction (0.8 to 17.9 × 10^2 CFU 100 mL^{-1}) and then fine and very fine silt fraction (0.8 to 8.6 × 10^2 CFU 100 mL^{-1}) (Table 3.1). The clay and unattached bacteria are hereafter referred to as unattached bacteria.

To further explore intra-storm trends, the mean *E. coli* concentration associated with each particle was tested for significant differences. Generally, across each storm, there was a significant difference between at least two groups of *E. coli* fraction (ANOVA, $p<0.05$, Table 3.1). However, the Tukey HSD multiple comparison test (Table 3.1) revealed no significant difference between the mean *E. coli* concentration associated with the medium and coarse silt and the fine and very fine silt. On the other hand, the unattached *E. coli* fraction was consistently significantly greater than both the medium silt and very fine silt fractions across all the storm events.

Although clay attached *E. coli* was grouped with unattached *E. coli* in this study, previous studies reported that bacteria have high affinity for attachment to finer and cohesive particles, such as clay (Auer and Niehaus, 1993; Gannon et al., 1983). For instance, Gannon et al. (1983) studied the association of fecal coliforms to various particle sizes, including clay sized fractions. Across a series of storm events, they found that clay-sized particles consistently reported highest concentration ranging from 24- 130 CFU 100 mL^{-1}, followed by silt-sized fraction with concentration within 0-9 CFU 100 mL^{-1}.

In addition, the average *E. coli* concentrations across storm events showed greater variability than those observed within baseflow. Both average medium and very fine silt *E. coli* were at least 0.8×10^2 CFU 100 mL^{-1} while average unattached *E. coli* were at least 6.2×10^2 CFU 100 mL^{-1}, across storm events (Table 3.1).

Table 3.1 : Bacteria concentration (Mean ± Standard Deviation) (10^2 CFU 100 mL^{-1}) associated with various particle size ranges across storm and baseflow events

Event	Medium and Coarse Silt	Fine and Very Fine Silt	Clay and Unattached
S-1	5.9 ± (7.9) b	6.4 ± (5.2) b	34.7 ± (11.9) a
S-2	0.8 ± (0.6) b	0.8 ± (0.4) b	6.2 ± (0.4) a
S-3	17.9 ± (18.1) bǂ	8.6 ± (9.8) b	92.8 ± (43.8) a
S-4	6.9 ± (5.7) b	3.7 ± (3.7) b	63.1 ± (9.5) a
S-5	6.9 ± (5.7) b	3.2 ± (2.8) b	38.4 ± (25.3) a
S-6	3.9 ± (2.8) bǂ	6.2 ± (4.4) ab	48.7 ± (49.2) a
S-7	0.9 ± (0.7) b	1.0 ± (0.7) b	9.3 ± (1.1) a
S-8	6.3 ± (5.9) b	3.0 ± (2.9) b	44.9 ± (13.2) a
BF	0.1 ± (0.7)	0.9 ± (0.7)	10.2 ± (11.6)

S = Storm Event; BF = Baseflow Event, Values followed by the same letter are not significantly different within each storm event according to Tukey HSD multiple comparison test ($p < 0.05$) after ANOVA test.
ǂNumber of samples (n) = 6, due to overflow from autosampler.

Estimating loads and the equivalent background (baseflow) period for each storm event helped define the magnitude and impact of storm events on bacteria loading compared to the baseflow period. The *E. coli* load ranged from 1.2^{10} to 1.5^{12} CFU, 1^{10} to 1^{12} CFU, and 2^{9} to 4^{11} CFU, over the storm event monitoring duration (i.e. over 5 hours for storm events 1, 2, 4, 5, 7 and 8, and over 3 hrs. for storm events 3 and 6) for total, unattached, and settleable *E. coli*, respectively (Figure 3a). The unattached *E. coli* load consistently dominated the total *E. coli* load within each storm event as it constituted a significantly high proportion of total *E. coli* concentration.

The results of the EBP of indicates that although storm events were occasional events, several periods of baseflow loading would be required to equal *E. coli* loading during these events. For instance, across five of the eight storm events (Figure 3.2b) , it is noted that among *E. coli* fractions including total *E. coli* at least two periods of baseflow were required to produce similar storm event bacterial loading. This suggests that water quality monitoring studies solely dependent on baseflow monitoring cannot fairly represent microbiological status of assessed waters. This finding corroborates previous work of Cizek et al., (2008); Krometis et al.,(2007); and Liao et al., (2015)

Since it is required by the Clean Water Act that programs such as Total Maximum Daily Load (TMDL) be established to enable impaired waters to meet set standards, storm events should also be targeted.

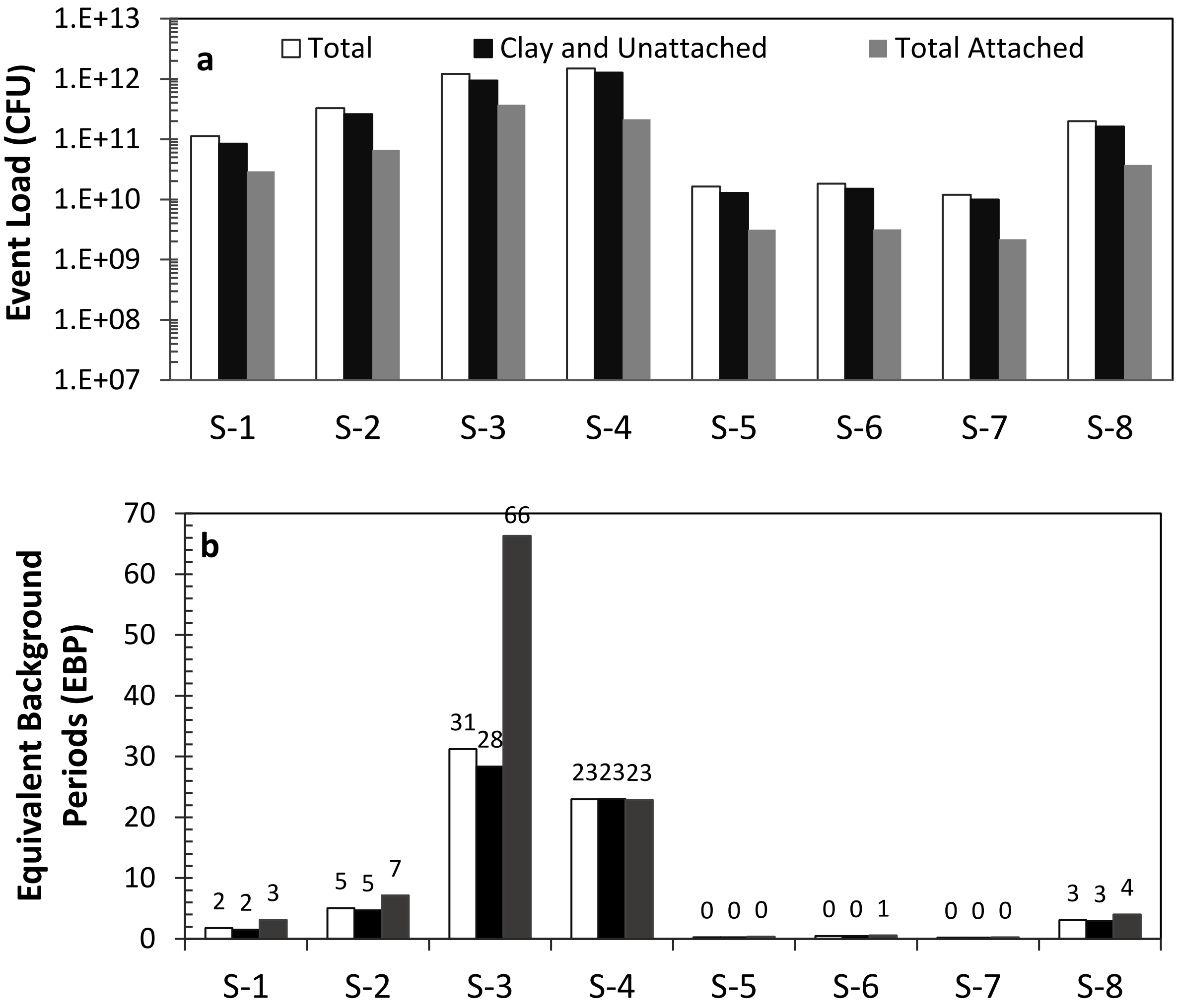

Figure 3.2 (a) Event loads associated with *E. coli* fractions across each storm event and (b) the equivalent background period (EBP) for *E. coli* fractions for each storm event.

3.3.2 Attachment of *E. coli* to particle fractions

The percentage of *E. coli* associated with various particle fractions was expressed as a proportion of the total bacteria concentration. It was necessary to estimate attachment since contradictory reports exist on the fraction of bacteria that occur as unattached (or free-living) and attached to particles (Jamieson et al., 2004). The average percentage of *E. coli* associated with the clay and unattached fraction was highest among the three fractions, with at least 70% of the bacteria associated with this fraction across all storm events (Table 3.2 and Fig 3.3b). Attachment rates among the silt fractions were similar, with the average percent attachment ranging from 8.7 to 15.2% for medium and coarse silt, and 5 to 13.6% for fine and very fine silt (Table 3.2).

Although the average percent attachment to particle sizes for baseflow were somewhat lesser compared to those across storm events, the baseflow average percent attachment to medium and coarse silt (9.1%), fine and very fine silt (9.7%), and clay and unattached (81.2%) were within the ranges found in storm events (Table 3.2). The average attachment rates in storm events for medium and coarse silt ranged from 8.7 to 15.2%, fine and very fine silt attachment that ranged from 5 to 13.6%, versus clay and unattached that ranged from 75.6 to 85.8% (Table 3.2).

To simplify the attachment analysis, particle fractions were categorized into two groups according to size: (1) medium and coarse silt along with the fine and very fine silt, hereafter referred to as the settleable fraction; and (2) clay and unattached, hereafter referred to as the unattached fraction.

Across all storm samples, at least 75% of the *E. coli* were unattached, while at least 62% of the bacteria in baseflow samples (n=7) were unattached. Similar results were observed by Jeng et al. (2005a) who found 75-80% of indicator bacteria (*Escherichia coli*, fecal coliforms, and enterococci) within storm events samples were unattached. The average settleable fraction, on the other hand, constituted at least 15% of the bacteria concentrations across both storm flow and baseflow conditions. This pattern compared favorably with that of Cizek et al. (2008) who found attachment rates of 15 to 30% for FIB (*E. coli*, fecal coliforms, and enterococci) associated with settleable particles during storm events. Krometis (2007) reported similar findings, with less than half (40%) of total FIB associated with settleable particles within storm events samples.

Although five out of eight storm events had a slight increase in the average percent (20-24%) of the settleable fraction of *E. coli* over that of baseflow (Figure 3.3a). Overall, the baseflow and storm event settleable *E. coli* fractions were not significantly different (ANOVA, $p > 0.05$).

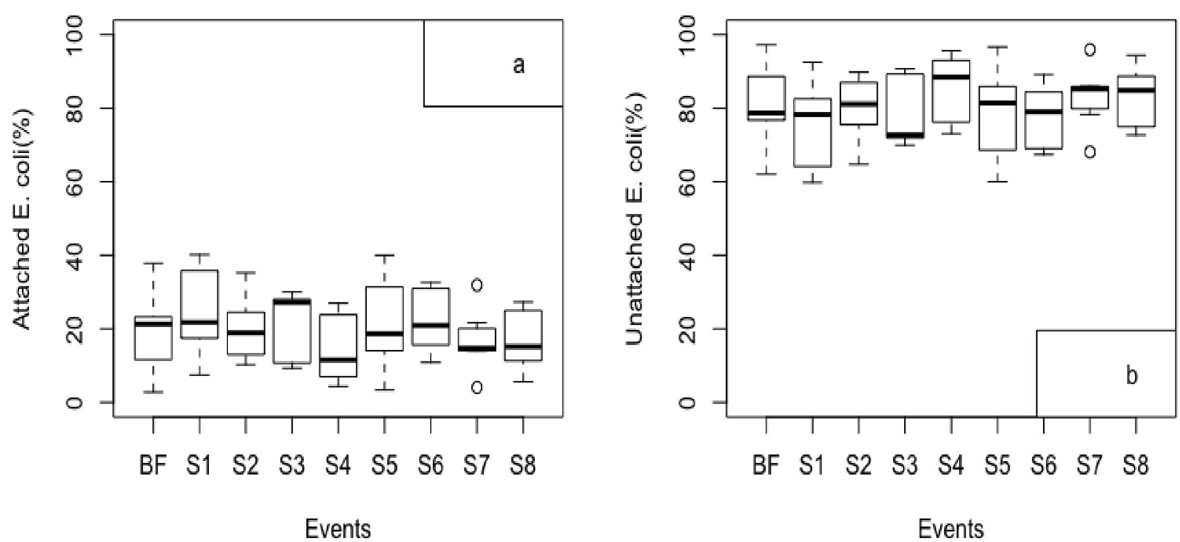

Figure 3.3 The distribution of the (a) settleable (attached) and (b) unattached *E. coli* over storm and baseflow events.

Table 3.2 Percent of bacteria (Mean ± Standard Deviation) associated with various particle sizes across each storm event and all baseflow samples

Event	Medium and Coarse Silt (%)	Fine and Very Fine Silt (%)	Clay and Unattached (%)
S1	10.8 ± 8.5	13.6 ± 11.1	75.6 ± 11.7
S2	10.1 ± 7.5	9.6 ± 5.1	80.3 ± 8.2
S3	14.2 ± 9.4ǂ	7.9 ± 10.2ǂ	77.9 ± 9.4ǂ
S4	9.2 ± 7.0	5.0 ± 5.2	85.8 ± 8.67
S5	15.2 ± 10.70	6.9 ± 5.6	77.9 ± 11.7
S6	9.1 ± 6.6ǂ	12.8 ± 9.5ǂ	78.0 ± 8.7ǂ
S7	8.7 ± 5.8	7.9 ± 6.3	83.4 ± 7.1
S8	10.8 ± 7.3	6.1 ± 6.6	83.1 ± 7.6
BF	9.0 ± 5.9	9.7 ± 8.6	81.3 ± 11.8

ǂNumber of samples n = 6, due to overflow from autosampler. S = Storm Event.
BF = Baseflow (n = 7)

3.3.3 Event Mean Concentrations of Storm Events

The EMCs of *E. coli* across the storm events ranged from 7.8×10^2 to 1.2×10^4 CFU 100 mL^{-1} (Figure 3.4). Event three had the highest EMC, while S2 had the lowest EMC. Correlation analysis showed that although the *E. coli* EMCs were positively correlated with both the total amount of rainfall (0.18) and the average rainfall intensity (0.12); however, these relations were not statistically significant ($p > 0.05$).

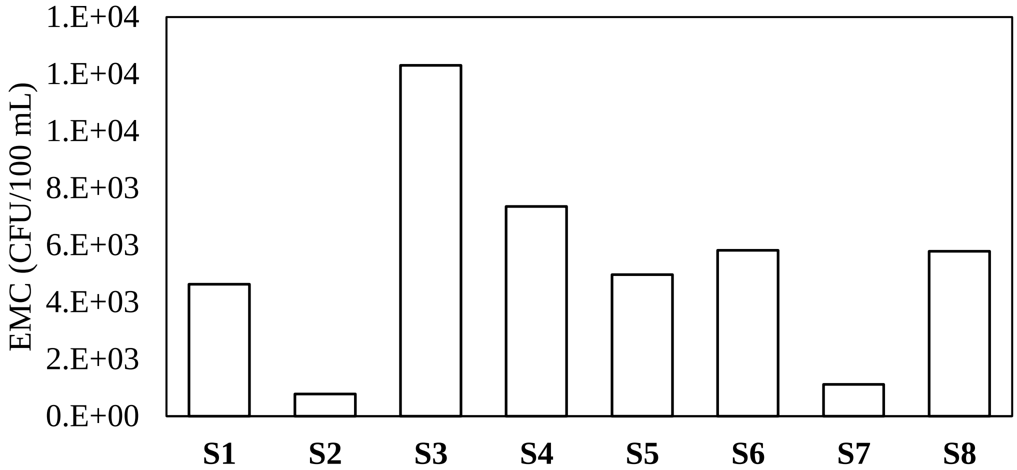

Figure 3.4 Event mean concentration of *E. coli* across all storm events

3.3.4 Comparison of total and unattached *E. coli*

Most in-stream water quality models assume bacteria within the water column are free-living despite the consensus that a portion of the bacteria are associated with particles (Jamieson et al., 2004; Wilkinson et al., 1995). To test the hypothesis that *E. coli* concentrations within the water column can be predicted by solely modeling the bacteria as unattached, a Mann-Whitney test was performed. The null (H_0) hypothesis was the total *E. coli* is equal to unattached.

Three out of eight storm events (38%) had unattached *E. coli* concentrations that were significantly different from the total *E. coli* concentrations within the water column (Table 3.3), although flow during all the storm event sampling durations were significantly different ($p < 0.05$). However, it should be noted that if solely attached bacteria were modelled to predict instream water column bacteria levels this could underpredict total bacteria load as well as the risk associated with such impaired water. This is because attached bacteria have the tendency to settle out of the water column faster compared to unattached ones that persist in water column for longer period. For instance, Rehmann and Soupir, (2009) reported that assuming total *E. coli* as attached *E. coli* resulted in a model that underpredicted *E. coli* levels within the water column. The authors identified attached fractions of bacteria as one source of discrepancy in the model developed. Therefore, modeling both attached and unattached bacteria could lead to improved predictions of bacteria during storm events.

Table 3.3 Three out of eight storm events had unattached bacteria concentrations that were significantly (p < 0.05) different than the total concentrations as shown by the p-values for each storm event

Storm Events	*p*-value
S-1	0.073
S-2	<.0001
S-3	0.330
S-4	0.015
S-5	0.120
S-6	0.480
S-7	0.002
S-8	0.159

3.3.5 Impact of storm event and baseflow on water quality standard

Skunk Creek is currently listed as impaired for limited contact recreation, which has a single sample maximum (SSM) *E. coli* limit of 1,178 CFU 100 mL^{-1}. Skunk Creek is a major tributary to the Big Sioux River which is impaired for *E. coli,* and is designated as primary contact recreation which has a SSM of 235 CFU 100 mL^{-1}. Exceedance for the total, settleable, and unattached fractions of *E. coli* for both storm and baseflow samples were estimated based on the SSM standard for primary contact recreation and limited contact recreation (Table 3.4). During the recreational season, the percentage of flow contributed by Skunk Creek to the Big Sioux River ranges from 45% in July and September to 67 % in May, averaging 59% over the entire recreation season (SD DENR, 2012). Thus, since water quality within Skunk Creek substantially impacts that of the Big Sioux River, therefore water quality analysis was conducted on the standards for both Skunk Creek and the Big Sioux River.

In comparing the total *E. coli* concentrations across storm events (n = 8) to the *E. coli* standards, 74% and 100% of total number (n =72) of samples exceeded the limited and primary contact recreation standards, respectively. On the other hand, 32% and 76% of *E. coli* attached to settleable particles were above the limited and primary contact recreational standard, respectively. The unattached *E. coli* showed a similar pattern of exceedance as observed with the total *E. coli* across with 72% and 97% of samples exceeding limited and primary contact recreational standard for *E. coli*, respectively.

The unattached *E. coli* has a greater tendency to contribute to water quality impairments with exceedance rates for limited contact recreation ranging from 9 to 1108% , whereas settleable *E. coli* exceedance rates ranged from 2 to 463% (Table 3.4). Although sedimentation of settleable bacteria contributes to the reduction of microbial contamination in the water column (Jeng et al., 2005b), this would not be enough to reduce *E. coli* concentrations to within the standard on Skunk Creek. In studying the removal of bacteria from the water column through sedimentation, Davies and Bavor (2000) found that the inefficiency in the reduction of bacteria from the water was due to the bacteria associated with the clay sized fraction (< 2 μm) which is similar in size to unattached *E. coli*. Moreover, Jeng et al. (2005b) found that three to seven days were needed for the elevated water column *E. coli* to return to background levels.

Table 3.4 Percent exceedance of total, settleable, and clay and unattached *E. coli* concentrations across storm events and baseflow conditions according to the SSM for primary and limited contact recreation

			Standard = 1178 CFU 100 mL^{-1}.			Standard = 235 CFU 100 mL^{-1}.		
	Event	Total No. of Samples	No. of samples > Standard	Min Exceedance by %	Max Exceedance by %	No. of samples > Standard	Min Exceedance by %	Max Exceedance by %
Total	S-1	10	10	129	619	10	1049	3503
	S-2	10	0	-	-	10	199	270
	S-3	6	6	443	1773	6	2623	9290
	S-4	10	10	435	706	10	2581	3943
	S-5	10	10	13	885	10	465	4836
	S-6	6	6	49	1255	6	645	6694
	S-7	10	1	21	21	10	313	504
	S-8	10	10	217	605	10	1489	3432
	BF	7	2	21	211	6	120	1460
Unattached	S-1	10	10	98	339	10	893	2099
	S-2	10	-	-	-	10	140	187
	S-3	6	6	299	1210	6	1900	6467
	S-4	10	10	307	613	10	1943	3474
	S-5	10	9	90	746	8	169	694
	S-6	6	6	22	1108	6	513	5957
	S-7	10	1	21	21	10	236	406
	S-8	10	10	138	463	10	1091	2723
	BF	7	1	203	203	6	104	1418
Settleable	S-1	10	6	13	180	10	28	1304
	S-2	10	-	-	-	2	1	30
	S-3	6	5	22	463	6	326	3645
	S-4	10	4	27	87	10	28	836
	S-5	10	4	16	58	8	169	694
	S-6	6	2	2	47	6	32	638
	S-7	10	-	-	-	3	1	57
	S-8	10	2	53	87	10	28	836
	BF	7	-	-	-	3	2	46

Exceedance rates for the SSM during baseflow conditions were much lower compared to those across storm event samples for all *E. coli* fractions. For instance, the maximum exceedance rate for total *E. coli* across most storm events (6 of 8) based on SSM for limited contact recreation was at least three-fold greater than that of the baseflow conditions. The limited and primary contact recreation SSMs were exceeded two out of seven and six out of seven samples, collected during baseflow conditions with a maximum exceedance of 221% and 1460% respectively for total *E. coli* (Table 3.4). The baseflow unattached *E. coli* showed nearly the same exceedance rate, according to SSM limited (1 out of 7 samples) and primary (6 out of 7 samples) contact recreation, as total *E. coli*. However, among the settleable fraction, there was no exceedance of the SSM standard for limited contact recreation, while 3 of 7 samples exceeded the SSM standard for primary contact recreation.

3.3.6 Correlation between *E. coli* concentrations, water quality, and hydrological variables

No significant correlations ($p > 0.05$) were observed between the *E. coli* concentrations, water quality parameters (turbidity and water temperature), and hydrologic factors (flow, shear stress) (Table 3.5).

Table 3.5 Spearman's Rank Correlation ($p \leq 0.05$) coefficient between *E. coli* concentrations, water quality parameters, and hydrological factors

	Turbidity	TC	MC	FVF	SF	CU
Flow ($m^3 s^{-1}$)	-0.47	NS	NS	NS	NS	NS
Water Temperature (°C)	0.99	NS	NS	NS	NS	NS
Turbidity (NTU)	NA	NS	NS	NS	NS	NS
Bed Shear Stress ($N\ m^{-2}$)	-0.32	NS	NS	NS	NS	NS

NTU = Nephelometric Turbidity Unit, TC = Total *E. coli*, MC = Medium and Coarse Silt, FVF = Fine and Very Fine Silt, SF = Settleable Fraction (MC + FVF)

NS = Not Significant, NA = Not Applicable.

The lack of correlation between *E. coli*, water quality, and hydrologic parameters reflects the variability of bacteria concentrations which are impacted by several factors including but not limited to temperature (Chahinian et al., 2012; Ishii et al., 2006), plant-microbe interaction (Carr et al., 2005; Cinotto, 2005), predation (Davies et al., 1995; González et al., 1990; Huws et al., 2008; Iriberri et al., 1994), salinity (Goyal et al., 1977; He et al., 2007; Lipp et al., 2001), and resuspension and redistribution of sediments stores during and following rainfall (Pachepsky and Shelton, 2011).

Temperature influences the survival and die-off rates of FIB within sediment and water (Bradford et al., 2013; Garzio-Hadzick et al., 2010; Vidon et al., 2008b), thus contributing to the concentration of bacteria present. However, previous studies have revealed mixed results on whether water temperature relates strongly with water column bacteria concentrations. For instance, Gentry et al. (2006) reported a significant negative correlation ($p < 0.05$, $r^2 = -0.30$), whereas Vidon et al. (2008b) reported significant positive correlations in two different creeks ($p < 0.01$ $r^2 = 0.7$, and 0.71), while Islam et al. (2017) reported no significant correlation between water temperature and *E. coli* concentrations. Therefore, the lack of correlation found in this study is supported by previous work.

Although turbidity is sometimes used as a surrogate for FIB within the water column, there have been mixed findings with regards to this variable based on flow regime. Davies-Colley et al. (2008a), He et al. (2007), Mallin et al. (2001), and Reeves et al. (2004) found significant positive correlations between turbidity and water column FIB concentrations during baseflow conditions, and Davies-Colley et al. (2008b) found positive correlations during storm events. However, in other studies (Gentry et al., 2006; Vidon et al., 2008b), no significant correlation between turbidity and water column FIB concentrations was observed. Vidon et al. (2008b) found no significant correlations ($p > 0.05$, $p > 0.01$) between turbidity and water column *E. coli* concentrations within two different creeks during both baseflow and storm flow conditions. The dominant presence of unattached *E. coli* in the Skunk Creek watershed could have led to the weak relationship between turbidity and *E. coli* concentrations. This relation is supported by Pachepsky and Shelton (2011) who explained that turbidity should be significantly correlated with *E. coli* concentrations within water if most of the total bacteria concentration are attached.

Streamflow was not significantly correlated with *E. coli* concentrations, which contrasts with Pandey and Soupir (2014) and Tiefenthaler et al. (2011) who found significant positive correlations. The lack of significant correlation between *E. coli* and flow could be due to a few factors. First, a portion of Skunk Creek is accessible to livestock and wildlife that directly deposit fecal matter into its waters thus contributing to water column and sediment stores of *E. coli*. The direct input of fecal matter from these animal sources likely does not correlate with streamflow. Secondly, sediment resuspension during storm events (Jamieson et. al, 2005a; Sherer et al., 1988) is linked to increased flow, but, the impact of flow on water column bacteria could be limited by how much bacteria is available for resuspension. Jamieson (2005) studied the impact of the release of in-stream *E. coli* stores on water column *E. coli* concentrations over three storm events within a creek. It was observed that a finite supply of *E. coli* is available for resuspension and could be depleted. This means that, even though flow might increase, once bacteria stores are depleted this might not lead to a corresponding increase in water column *E. coli*. This phenomenon could result in a lack of relation between flow and water column *E. coli*. In addition, the ‘flushing’ effect of elevated streamflow on sediment reservoirs of bacteria from the onset of storm event and various stages of the storm hydrograph could lead to high fluctuations in *E. coli* concentrations that are likely not to follow the flow pattern, thus resulting in a weak relationship between streamflow and water column *E. coli* concentrations.

Although shear stress impacts the erosion of sediment (Partheniades, 1965) and bacteria resuspension from the stream bed (Jamieson et. al, 2005a), it did not have a significant relationship with the different *E. coli* fractions. McDaniel et al. (2013) reported similar findings in a laboratory study where a flume was used to mimic the resuspension and deposition of *E. coli* in a stream. Like this study, their work showed both total and particle-attached *E. coli* were not significantly correlated with bed shear stress ($p > 0.05$).

3.3.7 Predicting stormflow *E. coli* concentration

The parameters considered for developing regression models were flow, temperature, turbidity, and shear stress. These variables have been identified to impact the concentration of bacteria within the water column (Pachepsky and Shelton, 2011).

Since more than one independent variable was used in creating the regression analysis, models (Table 3.6) were selected based on; (1) a variance inflation factor of less than 10 for each independent variable, and (2) statistically significant ($p < 0.05$) independent (predictor) variables. The variance inflation factor quantifies the severity of multicollinearity between independent variables (Ott and Longnecker, 2001).

Regression results showed that turbidity and shear stress were found to significantly contribute to the regression models in predicting *E. coli* concentrations and should be considered when developing a regression model to estimate *E. coli* during storm flows. Turbidity was a significant ($p < 0.05$) predictor of *E. coli* in all models with its coefficient being consistently positive across them. (Table 3.6), indicating that an increase in turbidity would result in an increase in *E. coli* concentration. Similarly, bed shear stress had a positive coefficient across all the models, meaning an increase in bed shear stress would result in a corresponding increase in *E. coli* concentrations.

Although both turbidity and shear stress significantly ($p < 0.05$) contributed to *E. coli* regression models, the coefficients of determination (R^2) were generally weak (0.09 to 0.22) in predicting the various *E. coli* fractions. These results indicate that storm-specific hydrologic parameters and water quality parameters were not sufficient to explain the variability of *E. coli* in the water column during storm events.

Table 3.6 Results of regression analysis to predict storm flow *E. coli* concentration

Selected Models	R^2
logTC = 1.05 + 1.99log(Turbidity) + 0.10log(Shear Stress) $TC = 11.22 \times Turbidity^{1.99} \times Shear\ Stress^{0.10}$	0.22
logMC = 0.18 + 1.77log(Turbidity) $MC = 1.51 \times Turbidity^{1.77}$	0.09
logFVF = 0.14 + 1.66log(Turbidity) $FVF = 1.38 \times Turbidity^{1.66}$	0.09
logCU = 0.98 + 1.98log(Turbidity) + 0.11log(Shear Stress) $CU = 9.55 \times Turbidity^{1.98} \times Shear\ Stress^{0.11}$	0.21
logSF = 0.65 + 1.64log(Turbidity) $SF = 4.47 \times Turbidity^{1.64}$	0.13

TC = Total *E. coli* concentration, MC = Medium and Coarse *E. coli* concentration, FVF = Fine and Very Fine *E. coli* concentration, CU = Clay and Unattached *E. coli* concentration, SF = Particle attached fraction = MC + FVF

3.3.8 Impact of particle size on travel distance of fraction of *E. coli*

The distance over which the bacteria travel is dependent on the size of the particle it is attached to. For instance, small-sized particles would travel farther and, therefore, stay within the water column longer compared to large-sized particles. This phenomenon is reflected in the estimated particle travel distance of the various *E. coli* fractions (Table 3.7). The estimated travel distance for fine and very fine silt across each storm event were at least 10-folds that of medium and coarse silt. Similarly, unattached bacteria had the potential to travel 10 times or more the distance travelled by bacteria attached to fine and very fine silt. Across all fractions of *E. coli*, the unattached bacteria had the potential to travel long distances (> 0.4 miles) and contribute to water quality impairments for an extended period.

Table 3.7 Estimated travel distance (miles) (Min-Max) for *E. coli* associated with particle fraction across each storm event

Storm Event	Medium and Coarse Silt	Fine and Very Fine Silt	Clay and Unattached
S1	0.009 – 0.01	0.13 – 0.17	2.1 – 2.7
S2	0.08 – 0.09	1.3 – 1.4	21.2 – 21.3
S3	0.03 – 0.04	0.6 – 0.7	8.9 – 0.8
S4	0.04 – 0.05	0.70 – 0.73	11.3 – 11.7
S5	0.0015 – 0.013	0.022 – 0.2	0.4 – 3.2
S6	0.002 – 0.0023	0.032 – 0.034	0.51 – 0.55
S7	0.003 – 0.005	0.04 – 0.07	0.70 – 1.08
S8	0.004 – 0.02	0.06 – 0.24	0.90 – 3.85
BF	0.002 – 0.047	0.025 – 0.72	0.41 – 11.5

3.4 Conclusions

This study examined the fate and attachment of *E. coli* to various particle sizes as well as their impact on water quality during both storm and baseflow events within an impaired stream. The study also assessed the relationship between water quality hydrologic variables and *E. coli* in predicting *E. coli* concentrations.

Unattached dominated the total *E. coli* concentration across both storm events (60– 97% of the total *E. coli*) and baseflow samples (62– 97% of the total *E. coli*). With unattached *E. coli* forming the majority of the total *E. coli* concentration, further analysis to test the assumption that the total bacteria concentration can be modeled as free-living was performed. The unattached *E. coli* were significantly different in three out of eight storm events, or 38% of storm events. Thus, partitioning between attached and unattached bacteria is recommended when predicting in-stream bacteria levels.

A comparison of *E. coli* to the SSM for primary and limited contact recreation across both storm and baseflow events was performed. Total and unattached *E. coli* posed a similar and severe threat to water quality, as *E. coli* levels among these fractions exceeded set the standards most of the time across both baseflow and storm event. In addition, settling of attached *E. coli* would not be enough to achieve the set water quality for Skunk Creek during a storm event. Generally, *E. coli* levels during storm events pose a health risk for human use based on designated use of this water.

Bacterial loading among *E. coli* fractions indicated that at least two periods of baseflow could be required to equal the same period for storm event *E. coli* loading.

Attempts to predict *E. coli* levels were not successful, as regression models performed poorly and could not adequately predict *E. coli* concentrations (R^2 = 0.09 – 0.22). Thus process-based modelling at the watershed scale is recommended to model water column *E. coli* during high flows such as storm events.

For future and adequate prediction of *E. coli* levels during storm events, a process-based modelling approach using watershed scale models such as SWAT or HSPF is recommended. The impact of storm events on bacterial loading could be further studied by undertaking scheduled sampling of baseflows prior to occurrence of storm events, in order to estimate *E. coli* levels contributed by storm events via resuspension and runoff. In addition, it is also suggested that tracer studies be undertaken to compare results with estimated travel distance of *E. coli* attached to particles.

3.5 Acknowledgements

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CHAPTER 4: CONCLUSIONS AND FUTURE WORK

4.1 Conclusion

A series of storm events and baseflow events were monitored to evaluate the fate and transport of *E. coli* within an impaired stream located in eastern South Dakota. *E. coli* concentrations were partitioned into those associated with settleable particles (attached) and those that were associated with clay and unattached. The impact of *E. coli* fractions on the water quality standard during both storm and baseflow events was assessed, including their relationship with water quality parameters (turbidity and temperature) and hydrologic parameters (flow and bed shear stress).

Among *E. coli* associated with particle fractions, the average *E. coli* concentration associated with coarse silt and fine silt were not significantly different, whereas the unattached concentrations were significantly higher. Partitioning of *E. coli* between unattached and settleable *E. coli* showed that unattached *E. coli* constituted a substantial portion of the total *E. coli* concentration across both storm events (> 75%) and baseflow (> 62%). Unattached bacteria consistently exceeded the SSM standard for *E. coli*. Thus, sedimentation of settleable *E. coli* would not be enough to reduce *E. coli* concentrations to meet the standard. The total, settleable and unattached *E. coli* load ranged from 1.2^{10} to 1.5^{12} CFU, 2^{9} to 4^{11} CFU, and 1^{10} to 1^{12} CFU respectively across storm events. The EBP of loading showed that at least two periods of baseflow would be required to equal *E. coli* loading across most of the storm events. Further analysis to test the assumption that bacteria concentrations within the water column could be modeled solely as unattached bacteria revealed that this assumption was not appropriate for nearly 40% of storm events.

The correlation analysis showed a weak relationship between water temperature, turbidity, flow, and bed shear stress. Attempts to model and predict *E. coli* concentrations during storm events as a function of water quality and hydrologic parameters using a regression analysis were poor (R^2 = 0.09-0.22).

4.2 Recommendations for Future Work

In this study, baseflow event samples for *E. coli* were analysed randomly for dry weather periods. For future work, baseflow samples could be taken on a day or a few hours prior to storm events for *E. coli* analysis, to enable estimation of additional input of *E. coli* into the water column via resuspension and runoff.

In addition, prediction of *E. coli* concentration using regression analysis performed poorly, therefore process-based models such as Soil Water Assessment Tools (SWAT) and Hydrological Simulation Program-FORTRAN (HSPF) could be used.

Furthermore, tracer studies could be undertaken to compare results with estimated travel distance of particle-attached *E. coli.* The impact of changing stream characteristics on travel distance of *E. coli* fractions could also be investigated.

APPENDIX A: *E. COLI* CONCENTRATION

The data below is the *E. coli* (CFU 100 mL^{-1} **)** associated with particle fractions across both storm events and baseflow events

Table A1-: *E. coli* concentration across storm and baseflow events

Events	MC	FVF	CU	Total
S1	133	1300	2567	4000
	567	767	5033	6367
	233	100	4133	4467
	200	100	2400	2700
	233	267	2367	2867
	533	1033	2333	3900
	367	133	2333	2833
	267	1533	3800	5600
	2800	500	5167	8467
	633	700	4567	5900
S2	120	70	620	810
	7	120	660	787
	30	47	673	750
	10	103	663	777
	120	43	590	753
	173	63	613	850
	40	57	647	743
	60	130	587	777
	183	123	563	870
	73	3	627	703
S3	1667	33	4700	6400
	867	133	8367	9367
	167	2467	6833	9467
	1300	1200	6400	8900
	1400	33	13967	15400
	5333	1300	15433	22067
S4	333	767	8400	9500
	1400	133	6133	7667
	500	33	6833	7367
	133	167	6600	6900
	1933	267	5967	8167
	433	67	6667	7167
	933	900	5733	7567
	200	167	6367	6733

	500	1000	4800	6300
	533	200	5633	6367
S5	100	33.3	3866.7	4000
	1433	200	9967	11600
	100	833	6267	7200
	1467	167	3567	5200
	467	200	3200	3867
	400	233	2533	3167
	1267	600	2800	4667
	333	700	2867	3900
	1167	200	2233	3600
	190	30	1107	1327
S6	433	1300	14233	15967
	400	733	6133	7267
	900	300	3767	4967
	67	900	2000	2967
	333	400	1633	2367
	213	97	1440	1750
S7	20	210	1190	1420
	27	130	957	1113
	17	23	943	983
	63	167	830	1060
	60	77	833	970
	137	13	910	1060
	187	50	940	1177
	123	33	963	1120
	180	10	990	1170
	177	193	790	1160
S8	133	800	2800	3733
	500	167	6633	7300
	133	167	5067	5367
	967	833	4800	6600
	433	133	3433	4000
	767	33	3167	3967
	400	67	3667	4133
	200	400	3767	4367
	733	333	5533	6600
	2100	100	6100	8300
BF	150	90	807	1047
	20	10	487	517
	33	67	3567	3667
	120	223	563	907

	23	107	480	610
	190	147	1090	1427
	30	7	177	213

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