December 2015

Transport Mechanisms for Human Fecal Indicator Bacteria in an Urban Stormwater Basin in Southeastern Wisconsin

Chelsea M. Corson
University of Wisconsin-Milwaukee

TRANSPORT MECHANISMS FOR HUMAN FECAL INDICATOR BACTERIA IN AN URBAN STORMWATER BASIN IN SOUTHEASTERN WISCONSIN

by

Chelsea Corson

A Thesis Submitted in

Partial Fulfillment of the

Requirements for the Degree of

Master of Science

in Freshwater Sciences and Technology

at

The University of Wisconsin – Milwaukee

December 2015

ABSTRACT

TRANSPORT MECHANISMS FOR HUMAN FECAL INDICATOR BACTERIA IN AN URBAN STORMWATER BASIN IN SOUTHEASTERN WISCONSIN

by

Chelsea Corson

The University of Wisconsin-Milwaukee, 2015
Under the Supervision of Professor Sandra Mclellan

Discharge of stormwater runoff to receiving waters is a known source of human pathogens; however the primary mechanisms by which these pathogens enter the stormwater system have yet to be quantified. This study builds upon and utilizes prior research findings in an attempt to explain the influence of the age of the pipes within stormwater and sanitary conveyance systems, rainfall and hydrogeological characteristics, and select infrastructure variables that contribute to the observed contamination of an urban stormwater basin in Southeastern Wisconsin.

Over the course of approximately two years from 2012 to 2014, a total of 260 samples from 22 stormwater manholes and two terminal outfalls and 47 groundwater samples from three monitoring wells were collected and assessed by culture based methods, PCR and quantitative PCR (qPCR) to test for traditional and alternative indicators of fecal pollution within a 170-acre study area in the City of Wauwatosa, Wisconsin.

Results indicated that all 22 manholes, both outfalls and each groundwater monitoring well location had the HF183 (human) *Bacteroides* genetic marker detected in at least one sample, suggesting sewage contamination is nearly ubiquitous within the 170-acre stormwater basin

selected for this study. Although 90% of the study site manholes tested positively (i.e. >1,000 copy number (CN)/100 ml) in more than 50% of the samples collected, positive results from the monitoring wells were somewhat less consistent, as only 20% of all samples collected were identified as positive for human *Bacteroides*. Detection of human fecal indicator bacteria (FIB) correlated with age of pipe, seasonality, rainfall duration and volume, antecedent conditions and certain infrastructure conditions. The pervasive nature of human FIB within this study area suggests that the suspected presence of leaking laterals and multiple breaches within the sanitary sewer system's structure are pathways for sanitary sewage contamination to enter the stormwater conveyance system.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

BLD – below level of detection which, quantitatively is 225 CN/100 ml

bgs – below ground surface

cfs – cubic feet per second

CFU – Colony Forming Units

CN – Copy Number

CSO – Combined sewage overflow

CWA – Clean Water Act

DA – Drainage Area (m^2)

DNA – Deoxyribonucleic Acid

E. coli – *Escherichia coli*

EMC – Event Mean Concentration

EPA – Environmental Protection Agency

FC – fecal coliform

GIS – Geographic Information System

GPS – Global Positioning system

HB – human *Bacteroides* (aka human *Bac*)

MAFM – Meinecke Avenue Flood Mitigation Project

M09020N1 – Stormwater basin study area (terminal outfall FMRMN44)

ml - milliliter

MMSD – Milwaukee Metropolitan Sewerage District

MRK – Milwaukee Riverkeepers

msl – mean sea level (feet)

Lachno – human *Lachnospiraceae* (aka human *Lachno*)

Q – discharge of impervious surface overland flow (cfs)

C – runoff coefficient (0.04)

i = intensity in/hr

A = area (acres)

PCR – polymerase chain reaction

qPCR – quantitative polymerase chain reaction

RWQC – Recreational Water Quality Criteria

TMDL – Total maximum daily load

USGS – United States Geological Survey

up-the-pipe – samples collected from the manhole pipe within the stormwater pipeline

WDNR – Wisconsin Department of Natural Resources

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my advisor, Professor Sandra Mclellan for her continuous support of my master's studies, immense knowledge and encouragement through challenging issues. I am extremely grateful to my committee members Professor Hector Bravo and Professor Timothy Grundl for their expertise and assistance with this project.

I am fully indebted to Deborah Dila, who provided endless technical support and unwavering encouragement throughout this project. I also thank my labmates: Melinda Bootsma, Danielle Cloutier, Jen Fisher, Amber Koskey, Hayley Templar and Katherine Halmo for their support and assistance in both the laboratory and the field.

I would also like to thank the City of Wauwatosa, specifically Margaret Anderson, P.E. and William Wehrley, P.E. for their cooperation and collaboration in providing infrastructure data and allowing us to complete our work in the Wauwatosa area; without their efforts this research would not be possible. The Milwaukee Riverkeepers, Milwaukee Metropolitan Sewerage District (MMSD), and the United States Geological Service (USGS) provided personnel and expertise. I am especially grateful to Joe Rath from Milwaukee Riverkeepers for his time and assistance with ArcGIS.

I would like to express my gratitude to the University of Wisconsin's School of Freshwater Sciences for allowing me to fulfill a life-long goal of conducting my graduate studies in a subject I find both fascinating and challenging.

I would like to thank my boss, DJ Burns for his patience, support and flexibility in allowing me to complete my studies with SFS while maintaining my position at Drake. Finally, I thank my family for their patience and support and my dad for instilling in me the constant pursuit of knowledge through education.

Chapter 1 – Introduction

Human sewage contamination in urban surface waters poses a significant impairment to water quality and is a major environmental and public health concern. It is estimated that over 54% of the global population resides in urban rather than rural areas and approximately 60% world's population lives within five miles of a surface freshwater body (Kummu et al. 2011). It is for this reason that one of the most serious and complex human health issues in connection to lakes and rivers, is exposure to pathogens and other bacteria due to a lack of clean drinking water. At a local level, high fecal bacteria levels undermine the integrity of Wisconsin rivers and lakes and are becoming of increasing concern as they impede water quality goals regulated by the Clean Water Act (CWA), contribute to total maximum daily load (TMDL) exceedances, diminish recreational value and indicate a failing wastewater conveyance and treatment system; all of which can ultimately lead to significant environmental, public health and economic consequences.

As this study makes clear, the limitations of traditional water quality monitoring techniques have been well documented, as culture-based methods do not differentiate between human and zoonotic origin. The current Recreational Water Quality Criteria (RWQC) guidelines promulgated by the United States Environmental Protection Agency (EPA) in 2012 employ conventional culture-based methods to assess human health risk. When RWQC is implemented based on inaccurate identification of fecal pollution sources, false positives can ultimately lead to costly infrastructure improvements that may not appropriately address the problem. The benefits of using molecular based methods has proven successful for the detection of potential human pathogens in Lake Michigan surface water (Bower et al. 2005; Newton et al. 2011; Newton et al.

2013; Sauer et al. 2011). Furthermore, the use of human fecal pollution genetic markers, such as human *Lachnospiraceae* (human *Lachno*) and human *Bacteroides* (human *Bac*), may be a more feasible alternative to assess pathogen risk (Newton et al. 2011). The study conducted by Newton et al. in 2011 demonstrated that human adenovirus (a known human pathogen that is not easily quantifiable) is likely present in surface water when combined human fecal indicator bacteria (FIB) such as *Lachnospiraceae* and human *Bacteroides* abundance is high.

As mentioned above, the discharge of stormwater runoff to receiving waters is a known carrier of human pathogens (Gaffield et al. 2003; Arnone & Walling 2007; Sauer et al. 2011; Sidhu et al. 2013), however the primary mechanisms by which these pathogens are mobilized into the stormwater system have yet to be quantified. Stormwater samples collected by the Mclellan lab from 2008 to 2012 identified the human *Bac* genetic marker in 97% of the samples collected from select outfalls along the Menomonee River. One of the major outfalls located along the Menomonee River watershed, M09020N1 (sample ID FMRMN44) services more than 1,000 homes and drains over 170-acres in the City of Wauwatosa (Figure A.1 in Appendix A). This outfall had tested positive for the human *Bac* marker in 100% of the stormwater samples collected from the terminal outfall in 2008 to 2012, and 100% positive in "up the pipe" (i.e. stormwater samples collected from within the manhole) samples (Sample ID SMN44A/C) collected in 2011. This outfall has been identified as a Tier 1 (>80% of all samples positive for human *Bac*), Category A/A (average human *Bac* >100,000 copy number (CN)/100 milliliter (ml) and positive 100% of the time) priority outfall by the Mclellan lab. (Mclellan & Dila 2013).

This study, hereafter referred to as the Storm/Sanitary Reconstruction Assessment, evaluated the primary mechanisms by which human fecal indicator bacteria (FIB) enter the stormwater system and are delivered to the Milwaukee River Basin by examining a source specific outfall (FMRMN44) located in the City of Wauwatosa that had routinely been identified as a major contributor of human FIB to the Menomonee River. The knowledge gained from this research will be especially useful for municipalities, water resource managers, urban planners, and policy makers seeking to rectify aging and failing sanitary lines.

Objectives/Specific Aims

The initial phases of Storm/Sanitary Reconstruction Assessment involved the identification and characterization of the specific mechanisms hypothesized to be responsible for the transfer of human fecal contamination to stormwater systems. The principal goal was to locate 'hot-spots' of fecal contamination occurring *in-situ* by sampling up-the-pipe from select manholes within the study area and analyzing the samples for human FIB. Transport mechanisms were explored by selecting a broad range of diverse sample sites throughout the stormwater-shed based on how well they represented a variable condition, which can generally be categorized as a physical or infrastructure condition and/or a geographical/hydrogeological condition. We evaluated trends in the data to identify which mechanism(s) contributed to the increased likelihood of human FIB contamination. Our primary objectives and general hypotheses included the following:

1) Characterize potential mechanisms of human FIB contamination by sampling a range of infrastructure conditions.

In terms of infrastructure, we considered the following potential mechanisms:

a) *Aging and dilapidated sanitary and/or storm water lines may act as a conduit for exfiltration, and thus will demonstrate higher concentrations of FIB than newly lined or newly reconstructed storm or sanitary lines.*

b) *Physical characteristics (depth of pipe, diameter, proximity to laterals and/or sanitary) of subsurface conveyance systems (sanitary lines, laterals and stormwater pipelines) may have a systemic effect on the integrity of the stormwater system, regardless of reconstruction efforts. Characterizing the physical nature of a section of pipeline where elevated concentrations of human FIB were detected can help to identify a breach in the system.*

2) Evaluate potential for subsurface groundwater flow infiltration

The next phase of our objectives assessed surrounding hydrogeological conditions:

a) *Stormwater pipes that are in close proximity to sanitary sewer laterals or pipes and in contact with fluctuating groundwater elevation are more likely to demonstrate higher levels of human FIB in aging systems due to the presence of bacteria in the vadose zone from leaking sanitary pipes.*

b) *Diffuse laterals contaminate the shallow subsurface in close proximity to the stormwater conveyance system, increasing the likelihood for contaminant migration through groundwater.*

3) Quantify FIB under specific conditions and estimate load contribution by source

a) *Precipitation, in conjunction with other mechanisms, may drive FIB concentration. Rainfall characteristics such as volume, antecedent conditions, duration, intensity and seasonality will be evaluated.*

Section 1.1 – Background & Significance

Human Health Impacts & Stormwater

Waterborne illnesses due to contact from pathogens and harmful bacteria are considered a serious public health concern, particularly for communities that reside by and rely on local waters. Urban stormwater is recognized as a major source of water quality impairment in rivers and streams worldwide as stormwater provides a direct connection to surface waters for nutrient loading, toxic organics, heavy metals, asphalt and surface debris, and micro-organisms from both human and animal sources (Bannerman et al. 1993; Gaffield et al. 2003). The Milwaukee River Basin is known to be heavily impacted by stormwater and sewage contamination both historically and presently (Bower et al. 2005; Mclellan et al. 2007; Newton et al. 2011; Sauer et al. 2011) and the detection of fecal bacteria in Lake Michigan surface water is of particular concern, as it serves as a drinking water resource for over 10 million people (Bailey et al. 2012).

The Milwaukee River Basin alone is home to 1.3 million people and is divided into six watersheds that ultimately drain to Lake Michigan. The most densely populated watersheds in the basin include: the Milwaukee (particularly the southern reaches), Menomonee and Kinnickinnic watersheds, which are named after the three rivers that converge at the Milwaukee estuary within the inner harbor. Together the three rivers encompass over 850 square miles of drainage area and contain 90% of the basins population.

It is generally understood that failing and aging sewer infrastructure, broken sanitary lines, and illicit cross-connections can act to mobilize the exfiltrated sewage to storm water lines. An additional common challenge in many older urban cities is the historic use of combined sewage systems. These combined systems were originally designed to collect and treat storm and sanitary flows which would then be discharged to a nearby waterbody. However, during periods of extreme wet weather, the volume of water received by the combined system can reach capacity, at which point a combined sewage overflow (CSO) discharges untreated sanitary and stormwater to the nearby waterbody. CSO's are a well known source of human pathogens (Bower et al. 2005; Arnone & Walling 2007; Newton et al. 2011; O'shea & Field 1992) and the source is readily identifiable as untreated sewage. However, in separate systems where CSOs are not possible, the source of fecal waste is less discernable. Laboratory results analyzed by the Mclellan lab indicate that stormwater samples collected from the greater Milwaukee area indicate a strong correlation with sanitary sewage cross-contamination, even in the absence of CSO's (Mclellan & Sauer 2009; Sauer et al. 2011; Newton et al. 2011; Salmore et al. 2006), suggesting that fecal pollution in stormwater is a chronic problem in Milwaukee waters.

A municipal separate storm sewer system (also known as an MS4) is a conveyance system (including roads with drainage systems, municipal streets, catch basins, curbs, gutters, ditches, man-made channels, or storm drains) that routes untreated rainwater from a designated area to a terminal outfall, which is typically adjacent to a major river or waterbody. Most of Milwaukee County operates on a separate stormwater and sanitary system, and a valuable tool for analyzing the potential contaminants from untreated stormwater entering receiving waters is sampling from the stormwater outfalls located adjacent to the waterbody. The likely types of pollutants can most often be correlated with land use, and in urban areas non-point source loading of human derived sewage has been identified as a ubiquitous contributor to TMDL exceedances and contamination to surface water bodies (Gaffield et al. 2003; Mclellan et al. 2007; Salmore et al. 2006). Several studies have demonstrated that sanitary leakage (a phenomena called exfiltration) can contaminate underlying groundwater (Ellis et al. 2003; Blackwood et al. 2005; Hunt et al. 2010) and local stormwater pipes (Sercu et al. 2011); the risk factors that increase the likelihood for sewage exfiltration into storm sewers were identified in these studies and others (Sercu et al. 2011; Ariaratnam et al. 2001; Rutsch et al. 2008) as: aged sanitary clay/brick pipes, placement of sanitary sewers above the storm drains, and locations where sanitary and storm pipes run parallel within 16 feet of each other. In a 2012 Progress Report prepared by Bradbury, Borchardt and Gotkowitz entitled: *Evaluation of Sanitary Sewers as a Source of Pathogen Contamination of Municipal Water Supply Wells* (Bradbury et al. 2012), the authors describe the potential for infiltration and exfiltration due to water table fluctuations. In Figure 1 (occurrence A) below, Bradbury et al. illustrates that during periods of low rainfall and a lower water table, a leaking sanitary sewer may result in exfiltration into the surrounding vadose zone. Their report goes on to suggest that exfiltration continues to occur through periods of high recharge and a rise in groundwater elevation, which can result in waste leaking directly to the groundwater system (Figure 1, occurrence B and C). The most interesting phenomena presented in this report however, is depicted in occurrence D; where the sanitary pipe is submerged, exfiltration is no longer possible, and groundwater (that is potentially contaminated with exfiltrated sewage) is infiltrating the system. If this model proves true, then we could speculate the same scenarios would occur in stormwater systems.

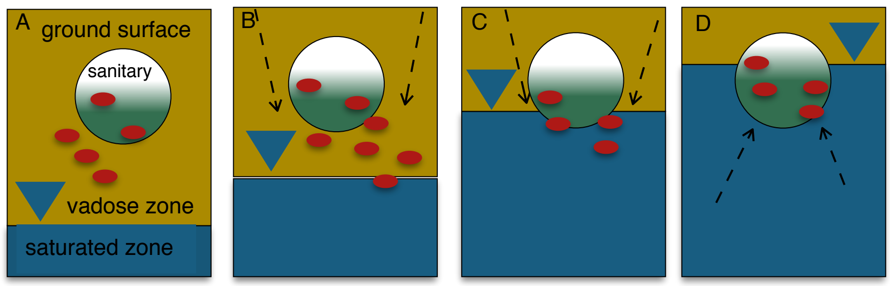

Figure 1. Illustration of exfiltration and infiltration scenarios under various hydrogeological conditions. Adapted from Bradbury et al. (2012).

Section 1.2 – Preliminary Work

Stormwater monitoring utilizing traditional culture-based methods to detect fecal contamination along the banks of the Milwaukee, Menomonee and Kinnickinnic Rivers has been conducted by the Mclellan lab in conjunction with the Milwaukee River Keepers (MRK) since 2008. These culture-based methods identify FIB such as *Escherichia coli* (*E. coli*), total fecal coliforms and enterococci and aid in the detection of potential pathogenic organisms, but they lack the ability to identify the host source. Molecular approaches such as quantitative polymerase chain reaction (qPCR) help to distinguish between human and animal sources of fecal pollution and serve as a more appropriate method to gauge human health risks associated with sanitary sewer conveyance systems.

HF183 Human *Bacteroides* Genetic Marker

The HF183 human *Bacteroides* marker, first described by Dick and Field (Dick & Field 2004) is the most widely used human marker today due to its powerful sensitivity and specificity in detecting a positive result for human fecal contamination (Harwood et al. 2014). A 2009 study by Ahmed et al. found that the human marker HF183 (i.e. human *Bac*) was able to discriminate between human and animal feces 99% of the time (Ahmed et al. 2009), and Sidhu et al. (Sidhu et al. 2013) found the human *Bacteroides* marker to demonstrate a high concurrence with human adenovirus.

Lachnospiraceae Genetic Marker

Lachnospiraceae is the second most abundant bacterial group in human fecal samples making up nearly a tenth of the total population in wastewater samples (Mclellan et al. 2010; Newton et al. 2011). In a study conducted by Newton et al. in 2011, human fecal pollution was detected using assays for human *Bacteroides* and an abundant member of *Lachnospiraceae* (human *Lachno*) in samples collected over a range of precipitation events (dry, rain, and combined sewage overflow (CSO) periods) from Milwaukee's inner harbor. Further, both assays showed strong correlation during and after CSO events (Pearson's $R = 0.97$, $P \leq 0.01$; non-CSO Pearson's $R = 0.87$, $P \leq 0.001$, respectively). The tightness of the correlation observed between the human *Bacteroides* and human *Lachno* assays, as summarized by Newton, strongly suggests that the two bacterial indicators identify human sewage in the harbor (Newton et al. 2011).

Previous research has established benchmark values for human markers in untreated sewage and thresholds for sewage detection. A paper published by the Mclellan lab (Sauer et al. 2011) identified the human specific genetic marker, human *Bacteroides,* in stormwater samples collected from Milwaukee Rivers at concentrations indicative of sewage contamination. Sauer found that the ratio of the average human *Bacteroides* to total *Bacteroides* spp. found in untreated sewage was 4.8 10^7 and 9.8 10^8 CN per 100 ml, respectively, which corresponded to approximately 5% of the total *Bacteroides* spp. being accounted for as human *Bacteroides*. The established threshold used by the Mclellan lab of 1000 CN/100 ml to identify a 'positive' human

Bac value, is somewhat conservative and corresponds to approximately 0.003% untreated sewage. Our threshold value for human Lachnospiraceae of 1500 CN/100 ml, also corresponds to approximately 0.003% untreated sewage.

Menomonee River Water Quality

The Menomonee River Watershed drains approximately 136 square miles across four counties and is comprised of 42% urban land use, 22% grassland, 17% agriculture, 8% forests and the remaining balance as protected wetlands (Wisconsin Department of Natural Resources 2010). The Menomonee River is a known impaired waterway and recognized by the EPA and the WDNR as having been impacted by several water quality impairments including: nutrients, total suspended solids, and bacteria. Figure 2 depicts the Greater Milwaukee Watershed Region, which includes the Menomonee watershed (highlighted in blue).

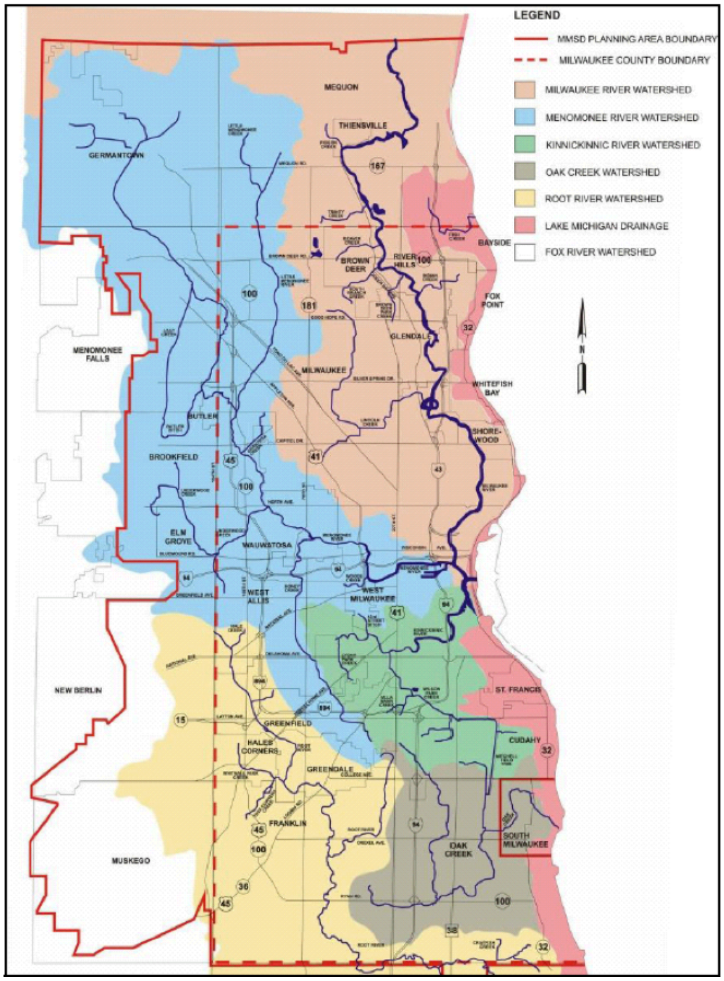

Figure 2. Major watersheds of southeastern Wisconsin – Milwaukee River watershed (orange), Menomonee River watershed (blue), Kinnickinnic River watershed (green), Oak Creek watershed (gray), Root River watershed (yellow) and Lake Michigan drainage (pink). Image courtesy of the Milwaukee Metropolitan Sewerage District (MMSD).

According to a 2007 *Regional Water Quality Management Plan* prepared by the Southeastern Wisconsin Regional Planning Commission, levels of fecal coliform in the Menomonee River often exceeded recreational water quality standards in the years 1998 to 2001 (Faraone et al. 2013). A report conducted as part of the Milwaukee Metropolitan Sewerage District's (MMSD) *Greater Milwaukee Watersheds Pathogen Source Identification* (Mclellan & Sauer 2009), identified several areas of persistent fecal bacteria in stormwater outfalls along the Menomonee River. In addition, a 2012 water quality report of the Milwaukee River Basin prepared by the

Milwaukee Riverkeepers (MRK) in collaboration with MMSD and the Mclellan lab revealed that the Milwaukee, Menomonee and Kinnickinnic River Watersheds are continually plagued by poor water quality. The study designated a "failing" grade to the Menomonee River for bacteria testing in 2011 and 2012; a designation indicating that based on monitoring data, water quality goals for FIB were meet in less than 50% of the samples collected (Milwaukee River Keepers 2012).

Extensive fieldwork was performed from 2008 to 2012, and included both inline and up the pipe composite stormwater samples from over 200 outfalls in the Milwaukee River Watersheds (Figure 2). Sixty-two (62) of the 200 outfalls sampled were collected from the Menomonee River tributary; samples were analyzed for molecular human indicator bacteria and/or microbiological parameters (Mclellan & Dila 2013). In terms of microbiological parameters, *E. coli*, enterococci, total coliforms were analyzed. Quantitative PCR analysis for the molecular markers, human *Bacteroides* and *Lachnospiraceae* were also analyzed for select samples. Of the 62 outfalls analyzed, 76% were identified as being positive (i.e. samples that demonstrated qPCR copy numbers greater than 1000 CN/100 ml sample) for the human *Bac* marker (Table 1).

	All Outfalls Tested				Outfalls tested more than once for HB		
Receiving Waters	# of Samples	# of Outfalls	**E.** ***coli*** **Average** (Geomean)	**Enterococcus Average** (Geomean)	# of Outfalls[1]	# That Tested **HB** Positive ≥ 50% of the Time	**HB** Average[2] (Geomean)
Menomonee River	212	62	30,840 (2,085)	43,523 (3,919)	39	30	23,445 (9,701)
Honey Creek	137	38	23,111 (4,439)	246,727 (10,742)	21	10	10,446 (5,330)
Underwood Creek	90	26	16,229 (947)	30,480 (2,092)	22	14	47,101 (13,370)
Kinnickinnic River	57	38	180,433 (772)	18,825 (939)	3	3	6,827 (5,293)
Holmes Ave Creek	55	31	1,928 (146)	2,512 (532)	5	1	949 (614)
Wilson Park Creek	13	13	10,340 (307)	2,822 (138)	0	NA	NA

Table 1. Summary of Mclellan lab results from the stormwater study conducted from 2008-2012. Adapted from McLellan & Dila 2013. a) Average and geomean concentrations identify outfalls that tested positive more than 50% of the time. b) The number of outfalls tested more than once for human *Bac* genetic marker is depicted on the right.

The sample collection and analysis previously conducted along the Menomonee River identified outfall M09020N1 (Sample ID FMRMN44) as a high priority site based on several factors: 1) Average human *Bac* counts are considered high (Average = 97,707 CN/100 ml) 2) 100% of samples submitted for analysis of human *Bac* were positive 3) the site was identified as having dry-weather flow and being human *Bac*-positive during dry-weather flow conditions and 4) up-the-pipe analysis also indicate 100% positive for human *Bac* with an average human *Bac* of 36,570 CN/100 ml.

The study conducted by Sauer in 2011, described in previous sections, employed qPCR to identify human genetic markers in subsequent fecal contamination surveys of stormwater outfalls along the Milwaukee, Menomonee and Kinnickinnic Rivers. According to their findings, of each of the outfalls tested, the human *Bacteroides* genetic marker was detected in at least one sample, with Menomonee River outfalls testing positive for FIB in 97% of the samples collected (Sauer et al. 2011). The results of the preliminary stormwater investigations conducted by the Mclellan lab and others highlight the need for additional research to characterize and identify the primary source(s) and mechanisms by which, fecal contamination enters the Milwaukee River watershed.

The diagram below (Figure 3) depicts a generalized summary of the integrity of the stormwater system in place at Menomonee River near study site outfall FMRMN44 (WA01). The lines adjacent to the Menomonee River indicate an outfall location. The geomean human *Bac* concentrations are provided next to the outfall sample site ID and the drainage area for the specific stormwater basin is identified within the parentheses. Stormwater outfall FMRMN44 and FMRMN39 are highlighted by a red bar, indicating that their geomean concentration for human *Bac* exceeds 10,000 CN/100 ml.

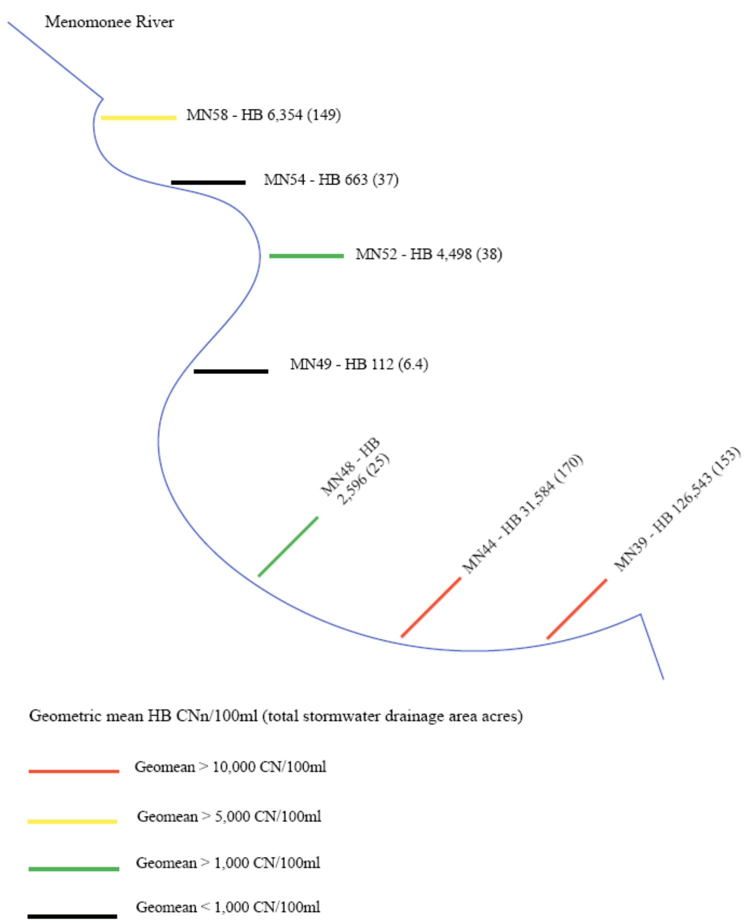

Figure 3. Subway style map outlining adjacent outfalls geometric mean human *Bac* and total stormwater drainage area for each outfall (samples collected between 2008-2010).

The City of Wauwatosa's Stormwater Relay Project allowed for a unique opportunity to analyze a high priority outfall before and after sanitary/storm reconstruction and due to the nature of the system, also assisted in the development of our specific aims for this project. This study was designed to incorporate the previous work conducted by the Mclellan lab in collaboration with MMSD and MRK, which targeted specific outfalls as potential human *Bac* 'hot-spots' in the Menomonee River. Specifically, stormwater basin M09020N1 in the City of Wauwatosa was selected as a suitable study area due to its history of positive sample collections from the terminal outfall (FMRMN44).

Section 1.3 – Study Area Description

Milwaukee County, including Wauwatosa is considered to have a humid continental climate with four distinct seasons characterized by large seasonal temperature differences, with warm to hot (and often humid) summers and cold (sometimes severely cold) winters. Precipitation is usually well dispersed throughout the year with an average rainfall amount of approximately 31.94 inches per year (National Oceanic & Atmospheric Administration (NOAA) 1981-2010). During the 2013-2014 sampling period, lower than average precipitation volumes were observed, specifically in the summer months when precipitation is typically at its peak.

Sampling Year, Month		Observed Precipitation (in)	Average Precipitation (in)	Difference (in)
2013	January	1.37	1.38	- 0.01
	February	0.22	1.30	- 1.08
	March	0.20	1.62	- 1.42
	April	2.32	3.36	- 1.04
	May	2.72	3.29	- 0.57
	June	2.21	4.04	- 1.83
	July	0.45	3.70	- 3.25
	August	0.27	3.74	- 3.47
	September	0.40	3.20	- 2.80
	October	0.64	2.41	- 1.77
	November	0.12	2.35	- 2.23
	December	0.13	1.55	- 1.42
2014	January	0.03	1.38	- 1.35
	February	0.15	1.30	- 1.15
	March	0.53	1.62	- 1.09
	April	1.35	3.36	- 2.01
	May	0.46	3.29	- 2.83
	June	4.27	4.04	0.23
	July	0.73	3.70	- 2.97
	August	1.01	3.74	- 2.73
	September	0.31	3.20	- 2.89
	October	0.83	2.41	- 1.58
	November	0.43	2.35	- 1.92
	December	0.45	1.55	- 1.10

Table 2. Observed and average (1981-2010) rainfall data for Wauwatosa, Wisconsin (National Oceanic & Atmospheric Administration (NOAA). The average annual rainfall total for Wauwatosa, Wisconsin is 31.94 inches/year. The observed rainfall for 2013 (11.05 in/year) and 2014 (10.55 in/year) represents only 33% of the average accumulated rainfall for this area.

The selected study area in Wauwatosa, Wisconsin consists of a separate stormwater and sewage system dating back to as early as 1920-30's (personal correspondence with Maggie Anderson, City Engineer). In June 2008 the City of Wauwatosa (the City) experienced flash flood conditions and low-lying areas suffered major damages from flooding. The economic costs of flooding take a toll on the municipality and its residents, but flooding is also a major concern in terms of environmental impacts and indicates a larger issue. Land elevation plays a pivotal role in flood conditions, as does the land surface and subsurface features in their ability to redirect and transport flow. A surge of stormwater has the potential to fill subsurface sanitary pipes, under these conditions; stormwater pipes may act as a secondary conduit to transport water away from the residence so as to decrease the likelihood for basement backups. Figure B.1 in Appendix B provides photographs of the outfall during dry-weather conditions (B.1.a) and following heavy rain (B.1.b).

In 2012 the City approved of an approximately 15 million dollar stormwater mitigation project (Meinecke Avenue Flood Mitigation Project) along 90th Street and Meinecke Avenue in an effort to mitigate the issues with flooding and high levels of fecal bacteria observed in the terminal outfall at 90th Street (FMRMN44). The sanitary lines formerly in place were known to be of older construction, likely brick or clay and installed in the 1920's. Additionally, several areas within the storm basin were identified as having shared utility trenches, which is believed to contribute to the transfer of bacteria via leaking sanitary pipes to the stormwater. The Meinecke Avenue Flood Mitigation Project addressed key areas along 90th Street and Meinecke where sanitary and stormwater pipes were completely removed and replaced with larger diameter pipes in an effort to alleviate flooding and basement back-ups. The new relay pipes are proposed to be backfilled with granular gravel and overlaid with new asphalt pavement. Figure B.2 in Appendix B provides photo documentation of the utility reconstruction project. Photograph B.2.a shows the 12-foot diameter stormwater pipe being placed along 90th Street. Photograph B.2.b shows the newly laid sanitary pipe trench with the new residential connections hooking in along 90th Street. The sanitary pipe trench is depicted as having been filled with granular crushed limestone.

The Mclellan lab, with the cooperation of the City of Wauwatosa set out to investigate one of the primary areas of concern identified in the 2009 *Greater Milwaukee Watersheds Pathogen Source*

Identification Report. Stormwater outfall FMRMN44 is recognized as a high priority site for human specific bacterial cross-contamination. Results from the 2008-2012 stormwater sampling and analysis of outfall FMRMN44, indicate concentrations of human *Bac* ranging from approximately 6200 CN/100 ml to greater than 3 million CN/100 ml (Mclellan & Sauer 2009) and exhibited a positive result (greater than 1000 CN/100 ml) for human *Bacteroides* 100% of the time.

In June 2012, the City of Wauwatosa commenced the repair, extension and relining of select storm and sanitary sewer lines within the basin study area as part of the reconstruction project. The project encompassed the area including: Menomonee River Parkway from Swan Boulevard to 90th Street, 90th Street from Menomonee River Parkway to Meinecke Avenue, Meinecke Avenue from Swan Boulevard to 80th Street, 86th Street from Meinecke Avenue to Wright Street and Wright Street from 86th Street to 81st Street. Figure 4 depicts the study area and the general extent of the utility reconstruction project.

The Storm/Sanitary Reconstruction Assessment developed by the Mclellan lab allowed for an excellent before and after analysis of human FIB presence in an area of known contamination in an effort to evaluate the efficacy of sanitary and stormwater utility rehabilitation. The select stormwater basin study area serves as an ideal location for hydrological and land-use influences as it encompasses over 170 acres of high density residential housing and contains a significant elevation gradient from the northern start of the stormwater basin (approximately 753 feet mean sea level (msl)) to the terminal outfall adjacent to the Menomonee River (approximately 660 feet msl).

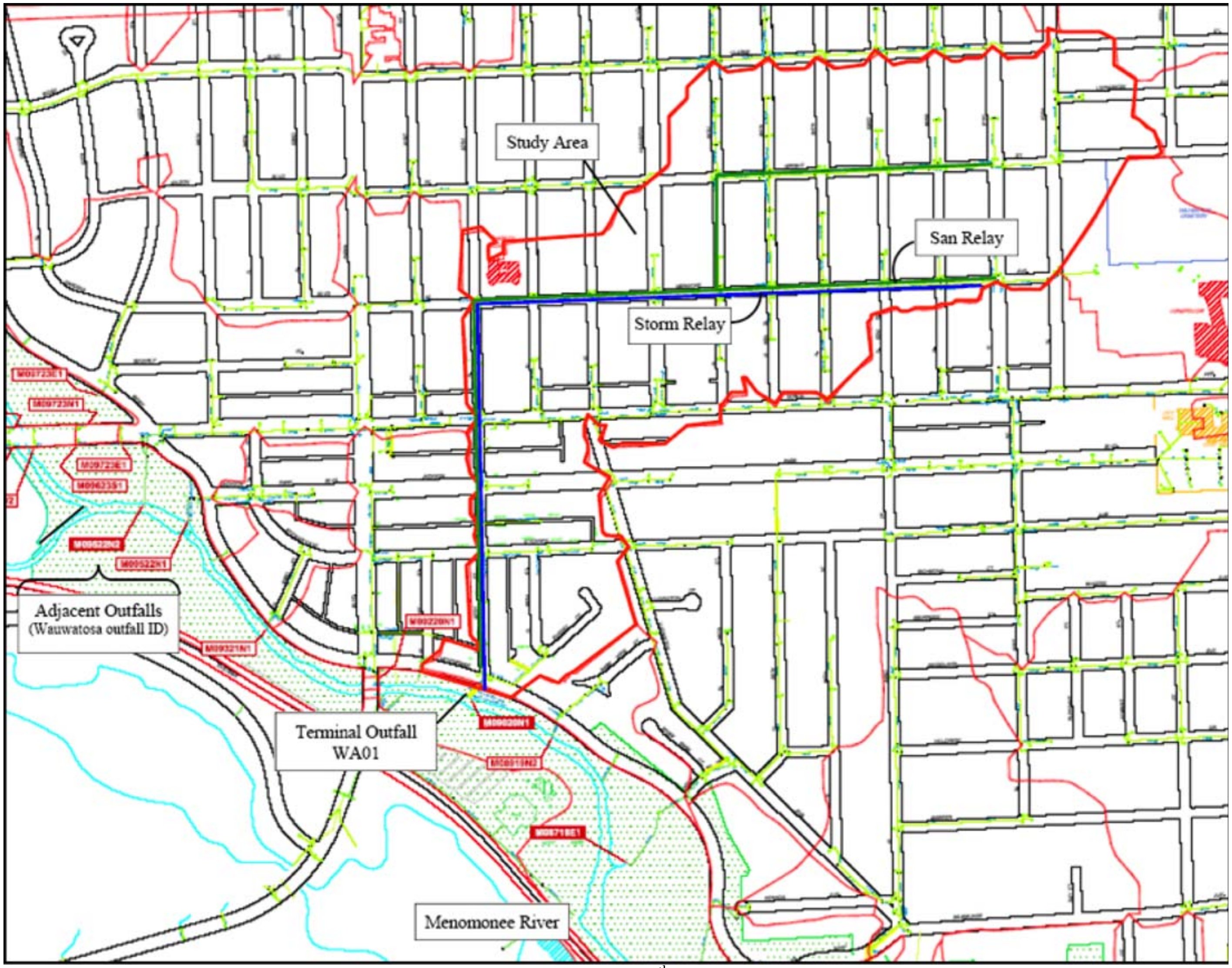

Figure 4. General study area. Stormwater Relay Project area along 90th and Meinecke outlined in red. The portions of sanitary pipeline reconstructed along 90th, Meinecke and parts of Wright Street are highlighted in green, and the stormwater relay along 90th and Meinecke is highlighted in blue. The pale green lines in the right-of-way represent the stormwater lines in the study basin (base map provided by City of Wauwatosa).

Site Geology & Hydrogeology

A topographic contour map obtained via the City of Wauwatosa's public Geographic Information System (GIS) database was used to determine general land features in the area, evaluate local topography and surface water features, and to estimate shallow groundwater flow direction. The topographic map identifies the study area as generally flat and level with a 2% slope declining generally to the south, towards the Menomonee River.

Surface soils in the vicinity of the study area are designated as the Ozaukee-Morley-Mequon Association, described as well-drained to somewhat poorly drained soils that have a subsoil of silty clay loam and silty clay. The Ozaukee-Morley-Mequon soils are underlain by reddish brown silty to gray silty clay, sands, and sands gravel identified as glacial till of the Oak Creek Formation (Mickleson et al. 1984).

Soil boring and well installation logs were reviewed during a Wisconsin Dept. of Natural Resources (WDNR) file review (January 2013) of contaminated properties located within the vicinity of the study area in Wauwatosa, Wisconsin. According to the information contained on the soil boring and monitoring well installation logs prepared by others (Drake Environmental Inc. and Giles Engineering, Inc.), surficial soils in the area generally consist of Oak Creek formation glacial till. The upper 20 feet of subsurface sediments were described as consisting of an "upper and lower dense clay separated by fine to coarse grain sand and gravel" which are indicative of glacial till and fluvial deposits of the Oak Creek formation. The soil boring logs also indicate that saturated alluvial deposits were observed in several soil borings between 9 and 11 feet below ground surface (bgs), suggesting that a shallow groundwater table is present.

In Southeastern Wisconsin, shallow groundwater elevation is often correlated with local topography, wetlands, and nearby rivers and lakes that typically act as local groundwater discharge areas. Based upon local topography and geologic records, shallow groundwater is anticipated to flow west-southwest toward the Menomonee River.

Section 1.4 – Site Sampling Design

The experimental design for this project was created in an effort to evaluate the influence of select mechanisms *in-situ* that are considered to be a factor in the transport and mobilization of human fecal bacteria to the stormwater system. A total of 244 stormwater samples, 47 groundwater monitoring well samples and 18 adjacent stormwater samples were collected during qualifying rain events (rainfall >0.10 inches) from December 2012 to June 2014. Stormwater samples were collected from select manhole locations in a singular stormwater basin in the Village of Wauwatosa, Milwaukee County, Wisconsin (Figure 5). A total of 23 stormwater sample sites (WA01 – WA22, and adjacent outfall FMRMN39) were selected based on several criteria that encompassed several categories; age of construction; pipe diameter; shared trench location; type of backfill material; sample site elevation; depth to stormwater pipe; residential density; outfall location/type, drainage area, and whether the sample location represents a mainline or smaller diameter artery line to the stormwater system. Sampling was conducted during qualifying rainfall events (rainfall > 0.10 inches) and snowmelt to provide for a diverse sampling pool and aided in the cross-examination of multiple factors that were thought to have the potential to contribute to the presence of human fecal bacteria in the stormwater system.

Study site manholes were categorized by age of pipe, based on whether they were in a location that received utility reconstruction, partial reconstruction or no reconstruction. Manhole sample sites that were within a system that had New Sanitary and New Storm replaced were categorized as "New/New", manholes that were located in an area that received New Sanitary but the Old Storm remained in place were categorized as "New/Old", and manhole sites that were located in areas where the Old Sanitary and Old Storm were still in place and active were categorized as "Old/Old".

Each major pipeline street generally consisted of one of two categories: New/New (90th Street and Meinecke Avenue) or New/Old (Wright Street), however it is important to note that the first manhole site along Wright Street is situated in an area of Old/Old and has been categorized as such. Also, manhole sites WA15 and WA14 are also located in Old/Old pipelines and they have been identified as such as well.

The design of the stormwater system is an important factor as well, when evaluating pollutant load and mixing contributions. The stormwater pipeline along Wright Street (containing sample sites: WA18, WA17 and WA16) is designed to flow from Wright Street to 85th Street and south along 85th Street, where the stormwater pipes deliver stormwater south along 85th to the Meinecke Avenue pipeline (at WA11). Manhole site WA11 then represents stormwater from the Wright Street pipeline and the area of Meinecke east of WA11. Figure 5 illustrates the stormwater system outlines and the manhole study sites selected for this project.

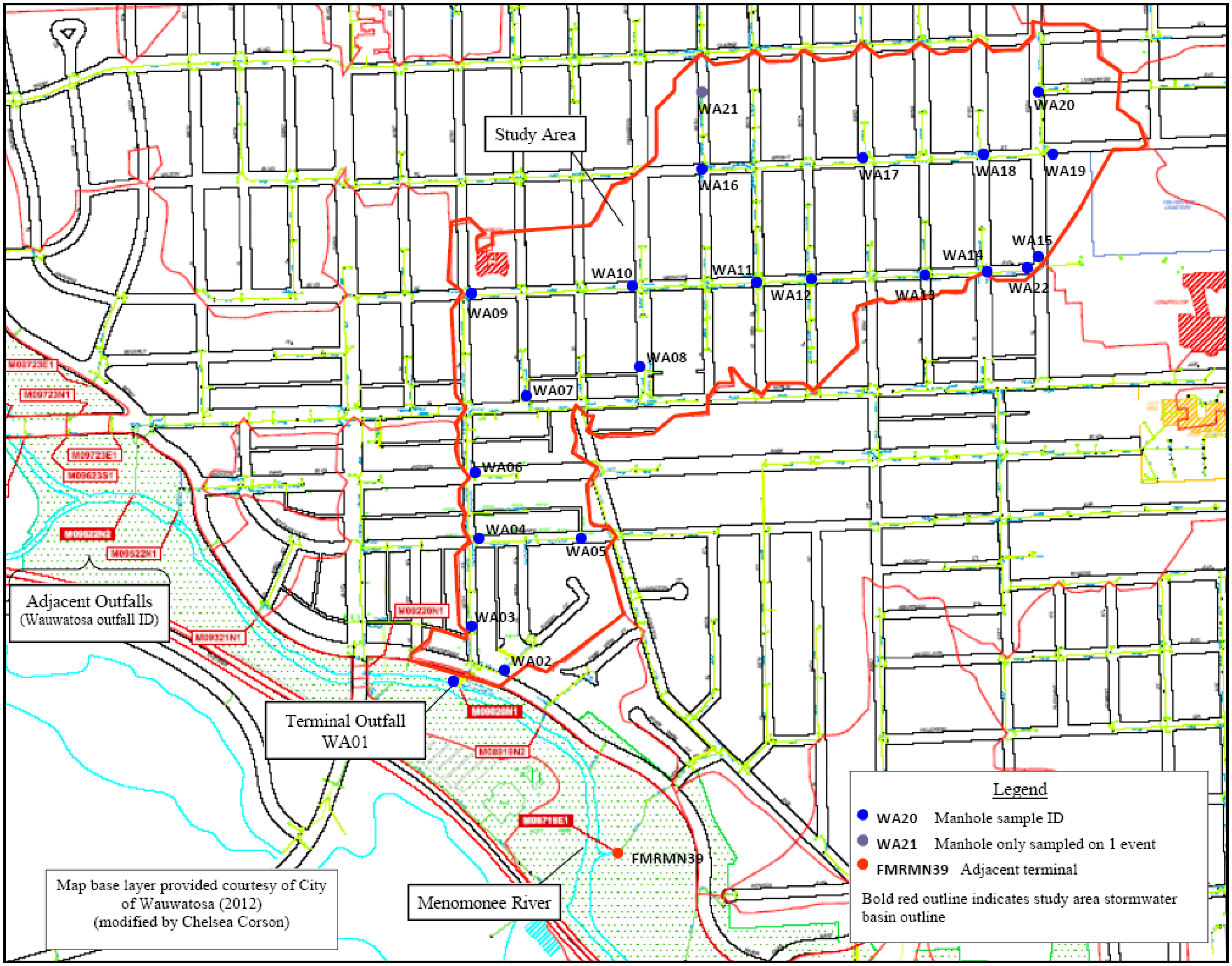

Figure 5. Storm/Sanitary Reconstruction Assessment study area with manhole locations. The pale green lines in the right-of-way represent the stormwater lines in the study basin (base map provided by City of Wauwatosa).

Chapter 2 – Experimental Design and Methodology

The specific methodology employed during the Storm/Sanitary Reconstruction Assessment in Wauwatosa, Wisconsin is described in the sections below.

Section 2.1 – Sample Collection

Outfall FMRMN44 (Storm Assessment ID WA01) has historically been identified as an outfall demonstrating dry-weather flow; however for the purposes of this investigation, We focused on precipitation driven contamination, which was needed in order to collect an appropriate sample volume (1000 ml). A minimum rainfall accumulation of 0.10-inches was used as an indication of a sampling event. Composite stormwater manhole ("up the pipe") samples were collected *in-situ* during qualifying rain events and included: 21 manhole locations within study area boundaries (WA02 – WA22), 1 terminal outfall location representing terminal outfall for study area (WA01) and 1 off-site terminal outfall representing the off-site manhole study area (FMRMN39). Inline samplers were also installed at each of the locations to evaluate first flush conditions. Inline samplers consisted of a 1000 ml HDPE Nalgene™ Storm Water Sampler, with EPDM gasket, polypropylene ball valve and closure to collect first flush grab samples. Composite stormwater samples were collected utilizing a sampling rod designed with an adjustable arm and a fixed 500 ml polypropylene bottle that can be anchored to various depths within the stormwater manhole. MMSD assisted the Mclellan lab with the initial deployment and Mclellan lab personnel collected the remaining samples during (or very shortly after) a qualifying rainfall event.

Flow samples were collected *in situ* utilizing the weighted in-line samplers, which were installed on November 9, 2012. Composite flow samples were collected during rainfall events or very shortly after utilizing the extended sampling pole, fastened with a 500 ml Nalgene plastic bottle. Each sample bottle was rinsed three times prior to sampling. Most samples were collected within 3 to 5 deployments allowing for a composite sample for a given time period. Composite sampling allowed us to analyze a sample that was representative of the flow at time of collection and removed the possibility of collecting a "slug" of contamination in a single deployment. Inline and flow samples were collected from each of the sites and transferred to a clean 1000 ml Nalgene™ bottle provided by Mclellan lab and stored on ice until further analysis.

Section 2.2 – Monitoring Well Installation

Monitoring wells were installed during utility reconstruction activities on November 9, 2012. Each well was constructed with 2-inch diameter PVC with an approximate 8 to 16-foot screen and fitted with a 2 ½ foot collection chamber and end point. Monitoring wells were placed adjacent to the newly replaced sanitary pipe and installed within gravel backfill to a depth of 11 feet bgs at sites WAMW1 and WAMW2, and to 16 feet bgs at monitoring well location WAMW3. Upon completion of well installation, each well was capped with a Morrison Plug and encased by a steel flush mount housing at an elevation compatible with the planned future road surface elevation. A 2.5-foot PCV collection chamber, affixed to the base of the well screen allowed for us to capture approximately 1.5 liters of water for collection and subsequent analysis. The wells were assembled by Mclellan lab and installed by Globe Contractors, Inc. of Pewaukee, Wisconsin (Wauwatosa's utility contractor). A cross section diagram depicting the general features and subsurface design of monitoring well WAMW3 is depicted in Figure 6 below. Photographs depicting the general locations of the wells installed at 83rd and Wright Street (B.3.a) and at 86th and Wright Street (B.3.b) are provided in Appendix B.

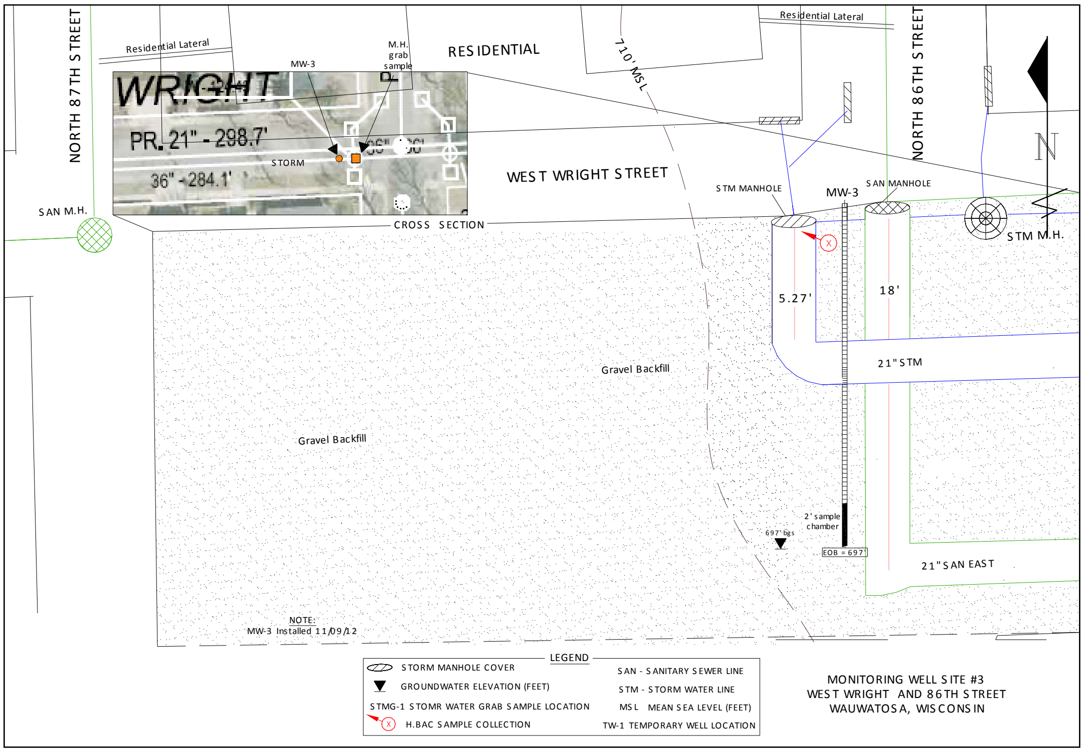

Figure 6. Monitoring well WAMW3 cross section diagram depicting the general subsurface features of the well location.

Water elevations were collected from each of the three (3) monitoring wells during and/or shortly after a qualifying rain event to determine if enough water was present for sample collection. If an adequate volume of water was present, samples were collected from the wells utilizing a sterilized 3-foot weighted polypropylene water collection bailer (Aqua Bailers® Knoxville, TN). Following sample collection, the samples were transferred to a clean 1000 ml Nalgene™ bottle and stored on ice for further analysis.

Section 2.3 – Data collection

Impervious surface drainage areas were calculated utilizing Google Earth™ aerial imagery (Google Earth 2015). Impervious surface area was calculated by measuring the length and width of the impervious surface area (i.e. street(s)) that drained to each manhole using Google Earth™ . Detailed topography and general land use features were evaluated using the City of Wauwatosa's public GIS database (published by Ruekert and Mielke, Inc. 2015). A contour map depicting the general topography of the study site and surrounding area is included in Appendix A, Figure A.2. An aerial photograph of the study area and the drainage area (DA) outlines used to calculate each manhole sample location is included in Appendix A, Figure A.3. Table 3 below, provides a summary the calculated impervious surface area for each manhole.

Percent slope was evaluated using the City of Wauwatosa online GIS topographic imagery to determine downgradient flow direction.

2% slope = [Rise = 660/753 = 93 feet Run = 4,585 (distance from WA20 to WA01)

Percent slope = (93 ft/4,585 ft) x 100 = 2%]

Mass balance calculations were provided by Professor Tim Grundl and were performed in Excel® utilizing the DA calculated for each specific manhole (DA_x) and the event concentration (Cx) human *Bac* (HB) concentration for select manholes located within the three major streets that represent the study area: Meinecke Avenue, Wright Street and 90th Street. In each of the mixing analyses performed, manhole sites were evaluated from east to west or north to south as the stormwater basin is designed for flow in these directions. The mixing calculations were performed by calculating the fractional proportion of DA (DA_{x-1}) for each manhole at each location along the pipeline and subtracting that value from the observed concentration of human *Bac* (by date). The calculation below is modified from Professor Grundl's mixing calculation:

Equation 1

$$DA_x = \sum_{1}^{(n-1)} (DA_0) + (DA_n)$$

Equation 2

$$(C_{mix}) = \frac{(C_{x-1}) - (C_0)(DA_0)}{DA_x}$$

Where,

C_0 = original concentration of HB at point a (point a being the start of the pipeline of interest)

C_x = concentration of HB at manhole x

C_{mix} = concentration of HB after DA is added (mass balance)

DA_0 = original drainage area at point a

DA_n = drainage area to n point

DA_x = fraction of manhole flow $[DA_0/(\sum DA_0 : DA_n)]$

note: the fraction of $[DA_x]$ plus the fraction of $[DA_0]$ is equal to 1.

A Garmin eTrex® handheld Global Positioning System (GPS) was used to locate sample site elevations and geographic coordinates. Infrastructure information pertaining to pipe diameter, depth, rim elevation, length and location were provided by the City of Wauwatosa's Engineering Department and are summarized in Table 3, below.

Manhole Sample ID	Site Elevation (ft msl)	Storm Pipe Diameter (in)	Sanitary Pipe Diameter (in)	Storm Pipe Depth to Bottom (ft bgs)	Artery or Mainline	Manhole Drainage Area (m^2)	Cumulative Drainage Area (m^2)	Age of Pipe	Sewershed ID	Latitude	Longitude
WA01	660	54	-	0	Mainline	3,984	88,706	New/New	WA3032	43.056046	-88.024802
WA02	673	12	8	4.5	Artery	3,500	3,500	Old/Old	WA3032	43.056239	-88.024371
WA03	682	54	30	14.5	Mainline	25,029	81,222	New/New	WA3032	43.056964	-88.024983
WA04	696	15	10	5.9	Artery	2,297	2,297	New/Old	WA3032	43.058392	-88.024758
WA05	698	12	10	4.9	Artery	-	-	Old/Old	WA3032	43.058422	-88.022669
WA06	699	12	10	7.5	Artery	2,232	2,232	New/Old	WA3032	43.059376	-88.024836
WA07	700	12	8	3.6	Artery	1,938	1,938	Old/Old	WA3007	43.060503	-88.023750
WA08	710	12	8	4.5	Artery	1,245	1,245	Old/Old	WA3007	43.061015	-88.021075
WA09	705	54	30	16.3	Mainline	9,030	48,481	New/New	WA3007	43.062312	-88.024958
WA10	708	48	27	17.3	Mainline	7,614	39,451	New/New	WA3007	43.062358	-88.021163
WA11	709	48	18	11.8	Mainline	3,359	31,837	New/New	WA3007	43.062365	-88.018460
WA12	709	36	12	9.3	Mainline	4,966	12,981	New/New	WA3007	43.062458	-88.017481
WA13	719	24	8	7.2	Mainline	4,396	8,015	New/New	WA3007	43.062449	-88.014977
WA14	728	18	0	7.1	Mainline	936	3,619	Old/Old	WA3006	43.062436	-88.013631
WA15	731	18	8	6.8	Mainline	2,683	2,683	Old/Old	WA3006	43.062521	-88.012458
WA16	713	21	21	8.3	Mainline	6,000	15,497	New/Old	WA3007	43.064206	-88.019801
WA17	717	12	8	4.6	Mainline	4,114	9,497	New/Old	WA3007	43.064377	-88.016234
WA18	732	21	10	5.6	Mainline	5,383	5,383	Old/Old	WA3007	43.064268	-88.013769
WA19	743	15	8	5.2	Artery	-	-	Old/Old	WA3006	43.064275	-88.012420
WA20	753	-	-	6	Artery	-	-	Old/Old	WA3006	43.065342	-88.012394
WA21	715	-	-	6	Artery	-	-	Old/Old	WA3006	-	-
WA22	730	-	-	6	Artery	-	-	Old/Old	WA3006	-	-

Table 3. Summary of infrastructure and physical data

Overland flow discharge calculations were performed for the study site area using the Rational Method: Q = CiA, where Q = discharge in cubic feet per second (cfs), C = runoff coefficient, i = intensity in/hr, and A = area. The runoff coefficient was determined to be 0.4 for residential areas (Shoblom 2014). The stormwater basin area is measured to be approximately 170 acres. Hourly rainfall amounts were provided by MMSD. Rainfall intensity was calculated for each hour by: rainfall (in)/(time 2 – time 1). Streamflow discharge volumes (cfs) were provided by the USGS (Station ID 4087120).

Pollutant Load calculations were calculated by dividing the mean human Bac concentration (CN/100 ml) by the impervious surface drainage area (meters squared (m^2)).

Seasonal analysis was performed based on the astronomical universal time, where samples collected within the time period of December 21 to March 20 were categorized as winter, March

21 to June 20 were categorized as spring, June 21 to September 21 were categorized as summer and September 22 through December 20 were categorized as fall samples.

Section 2.4 – Laboratory Methods

Microbiological analysis of stormwater samples

All water samples were analyzed for fecal coliform (FC), enterococci, and *E. coli* within 12 hours of collection using the USEPA 9222 D membrane filter method for FC enumeration (USEPA 2006), or the USEPA method for *E. coli* enumeration (USEPA 2002). Samples were vacuum filtered through a 0.45 μm pore size 47 mm nitrocellulose filter and placed on DifcoTM m FC agar, modified m-TEC, or MEI agar. Due to the unknown concentration of fecal contamination present in the samples, graduated volume(s) of sample to be filtered will vary from 100 ml, 10 ml and 1ml. If contaminant concentrations appear to be high, filtration volumes may be adjusted. Following filtration procedures, plates were incubated for 18 hours at 44.5°C and colony forming units (CFUs) were counted and recorded.

Molecular analysis of stormwater samples

Human *Bacteroides* genetic marker was evaluated by qPCR. For molecular analyses, duplicate 200 ml samples were vacuum filtered through a 0.22 um polycarbonate filters. The filters were placed onto sterile, DNase/RNase-free microcentrifuge tubes and stored at -80° C. DNA extractions were performed on the filters using the UltraClean™ Soil DNA Isolation Kit (Mo Bo Laboratories, Inc., Solana Beach, CA) following the protocol for maximum yield, with extracts stored at -20° C.

Polymerase Chain Reaction (PCR)

The *Bacteroides* human specific genetic marker (HF183F/708R) was based on the 16S rDNA gene (Bernhard & Field 2000; Bower et al. 2005). All primers were synthesized by Integrated DNA Technologies (Skokie, IL). The 25μl PCR reaction consisted of the Qiagen Taq PCR Master Mix Kit (2X-concentration: 5U/µl Taq; 3mM MgCl2 and 400 µM of each dNTP), each primer at a final concentration of 0.30µM and 1μl of sample DNA. The PCR conditions were 1 cycle 94oC; four minutes; 35 cycles: 94ºC, 30s; 59ºC, 30s; 72ºC, 30s; 1 cycle 72ºC, 6 minutes and a 10ºC, hold. All PCR reactions were run in the MJ Research PTC-Quad thermal cycler (now BioRad; Hercules, CA). The PCR products were visualized on 2% agarose gels using a 100 bp (base pair) DNA ladder.

Quantitative Polymerase Chain Reaction (qPCR)

All samples were assessed for fecal pollution utilizing a quantitative Polymerase Chain Reaction (qPCR) assay for human specific *Bacteroides*, which is a modification of previous published methods (Dick & Field 2004; Kildare et al. 2007) using the HF183 primer (Bernhard & Field 2000). Quantitative PCR assay for Lachnospiraceae was assessed via Newton et al. 2011. Assays were run on an Applied Biosystems One Step instrument with Applied Biosystems gene Taqman® Gene Expression Master Mix. Standard curves were generated.

The ratio of host-specific alternative indicator to total fecal pollution will provide information about the relative amounts of human sewage contamination present. Concentrations of human *Bac* and human *Lachno* greater than 1,000 CN/100 ml were documented as a positive result.

Section 2.5 – Statistical Analyses

Due to the variation in sample concentrations, log base 10 ($\log_{10}$) transformations were applied to all bacteria data ($\log_{10}(C_0)$, where C_0 is the original concentration. Data analysis was completed using Excel®, R version 3.1.2 (R Development Core Team 2014) open source programming language and the R Commander version 2.1-7 (Fox 2005) graphical user interface. Pearson's correlation analysis was used to test for associations between standard and human fecal bacteria indicator levels as well as associations between human *Bac* and human *Lachno* levels. We also set thresholds of > 1000 CN/ 100 ml of human *Bac* and 1500 CN/100 ml for human *Lachno* as "positive" and > 10,000 as highly contaminated. Samples were identified as below the limit of detection (BLD) at 225 CN/100 ml. To account for statistical errors, BLD samples were given the placeholder value of 225, and samples quantified as "zero" were placed at 1 CN/100 ml. Samples that were unable to be quantified or analyzed were given a "not applicable" (NA) descriptor.

Due to the unequal variance of the sample population, Welch's two-sample t-test was used on $\log_{10}$ transformed data to evaluate significant differences in the mean value of FIB observed in the stormwater samples and an independent variable. Paired sample t-tests were used to evaluate the differences in mean concentration of human FIB markers (human *Bac* and human *Lachno*) by date based on age of pipe. Differences of the various mechanisms and their relationship to bacteria concentration were evaluated using one-way analysis of variance (ANOVA). R programming software and Excel® were used for statistical analyses. ANOVA models were evaluated for categorical variables, logistic regression was used for quantitative variables. Table

4 provides a summary of the mechanisms explored, the general hypothesis and the statistics employed to evaluate the results.

Mechanism Category	Mechanism Tested	Hypothesis	Statistical Analysis
Infrastructure	Before/after	[FIB] in the before samples > after	Welch's two sample t-test
	Age of pipe	[FIB] in old pipe > new pipe samples	One-way ANOVA and Paired t-test
	Pipeline type	[FIB] in artery manhole samples > mainline manhole samples	Welch's two sample t-test
	Depth of pipe	[FIB] in shallow pipe samples > deeper pipe samples	Welch's two sample t-test
	Pipe diameter	[FIB] in smaller diameter pipes > larger diameter pipes	Logistic regression
	Manhole drainage area	[FIB] will be proportional to manhole drainage area	Mixing calculations
Rainfall/Hydro geological	Rainfall characteristics	Heavier rains will "dilute" [FIB] within system	Logistic regression
		Antecedent rainfall & increased rainfall duration will create super-saturated conditions, raising the groundwater table and allow for increased infiltration of residual HB	
	Season	[FIB] will fluctuate by season, demonstrating increased concentrations in wetter months	One-way ANOVA
	Discharge	[FIB] will be diluted with increasing overland flow discharge	Logistic regression
	Groundwater (wells)	[FIB] will be detected in the groundwater via exfiltration laterals and sanitary pipes	Presence/absence and % positive

Table 4. Summary of the mechanisms evaluated, the general hypothesis and the statistics used for each

Simple logistic regression analyses were performed in R to evaluate the relationship between human *Bac* and/or *Lachno* and an explanatory variable. The response variable, in this case human FIB, was translated to binary format where a positive result received a 1 designation and a negative result received a 0. The simple logistic function can be written as:

$$\hat{p} = \frac{\exp(B_0 + B_1 X)}{1 + \exp(B_0 + B_1 x)} = \frac{e^{B_0 + B_1 x}}{1 + e^{B_0 + B_1 x}}$$

where, ρ-hat is the probability that Y=1 (positive result), *e* is the base of the natural logarithm and β are the coefficients in the logistic model, with β_0 as the Constant and β_X is the explanatory variable Because the relation between x and P is nonlinear, β does not have a straightforward interpretation as it does in ordinary linear regression. Logistic regression is based on the probability of an event occurring, so to interpret the odds of an event occurring versus it not occurring, the probability (or the ratio of odds) is calculated by taking the natural log of the log odds which is: exp(β). A Wald Goodness of Fit test was performed for each of the analyses run and significance against the null hypothesis is represented by a p-value. Values exceeding the 95% confidence interval are denoted. For further explanation of logistic regression, Ariaratnam (Ariaratnam et al. 2001) provides an excellent description for application in evaluating the probability of an infrastructure deficiency (dependent binary variable) when in the presence of other explanatory variables.

Section 2.6 Human Markers in Stormwater Systems

The Mclellan lab has incorporated molecular analysis using two known human genetic markers; human *Bacteroides* and *Lachnospiraceae*, in combination with the typical microbiological techniques not only to delineate the extent of FIB in surface water, but also to better identify the source.

Human *Bacteroides* and *Lachnospiraceae* concentrations were evaluated by pair (i.e. each sample tested for both markers were observed side by side). Sample concentrations that appeared to be "unmatched" (i.e. pairs indicating one marker was positive while the other was negative, BLD, or zero) were removed from the analysis. In general, the levels of the human indicator that was detected were low or near the detection limit. The remaining paired human markers were considered a "match". Pearson's correlation of the matched human *Bac* and human *Lachno* sample concentrations indicated a well matched data set with a correlation of 0.68.

Bacteria members enterococci, *E. coli* and fecal coliforms were also measured via traditional culture-based methods. Correlation of the culture bacteria to the genetic markers, human *Bac* and human *Lachno* were also measured using Pearson's correlation. Based on the results of the

Pearson's correlation tests, none of the culture-based fecal indicators demonstrated a strong correlation with human *Bac* or human *Lachno*, with human *Lachno* being the least correlated with the plated bacteria. Table 5 below summarizes the basic statistics for the culture based fecal indicator bacteria and the results of the Pearson's correlation.

Micro Stats (CFU/100ml)	Enterococci	E.coli	FC
Count	213	213	213
Median	9,400	2,100	17,700
Mean	58,098	17,779	192,633
Geomean	5,529	1,414	11,674
Percent Positive	0.77	0.68	0.82
Pearson's cor: HB	0.25	0.20	0.21
Pearson's cor: Lachno2	0.07	0.06	0.05

Table 5. Summary of microbiological data and their relative correlation with human markers *Bacteroides* and *Lachnospiraceae*.

The purpose of this study was to evaluate human indicator bacteria within the stormwater system. It is well known that culture based methods alone fail to identify the source of FIB when detected; and as evidenced by the correlation tests mentioned above, these methods demonstrate a very low correlation to human specific genetic markers. Molecular based methods, utilizing qPCR is more sophisticated and accurate when attempting to identify a host source. Quantitative qPCR has been in practice for over a decade and the genetic markers used in this study have been shown to correlate very well with human sewage (Newton et al. 2011; Harwood et al. 2014). Due to the significant lack of association between the cultured bacteria and the molecular markers in this study, the primary findings in the following sections will focus on human *Bacteroides*, with *Lachnospiraceae* used as a confirmation indicator. The purpose of using a confirmation indicator is to avoid false positives, where a sample appears to be above the positive threshold, but is cross-reacting with another source. The presence of a confirmation indicator such as human *Lachnospiraceae* suggests a strong correlation with presence of sanitary sewage, as described above (Newton et al. 2011; Harwood et al. 2014).

Chapter 3 – Results and Discussion

The Storm/Sanitary Reconstruction Assessment study area is located in the eastern portion of the City of Wauwatosa and consists of a separate storm and sanitary system, much of which is constructed of clay and/or brick and dates back to as early as 1920-30's. Without a concerted effort made to inspect, repair and maintain such systems, the likelihood of this infrastructure to remain intact is highly improbable; yet it remains common practice to neglect these unforeseen deficiencies as municipalities are continually strained by limited budgets, public influence and resource allocation.

Conventional gravity drained storm and sanitary systems are relatively modest in design; however external environmental factors such as: horizontal stress from fluctuating groundwater elevations, increased land use causing vertical stress load, tree-root intrusion, surface and subsurface soil movement, rainfall and even seasonal changes resulting in the freezing and thawing of the shallow subsurface make is challenging to assess the integrity of these systems, as each section is uniquely influenced by these factors. When a municipality is faced with these issues, the question becomes: where do we start?

Assessment and remediation of entire systems would be extremely expensive, not to mention time consuming and disruptive. As discussed in Chapter 1, stormwater is recognized as the primary non-point source polluter to surface waters in the United States. The leaking that results from aging and dilapidated sanitary systems are often overlooked as they are not directly visible. Excessive disrepair of these systems can result in the exfiltration of sanitary waste to the surrounding subsurface. If stormwater systems are equally run-down, the potential for sanitary sewage to infiltrate a storm system increases. As such, properly identifying and characterizing the physical and/or environmental mechanisms that contribute to the co-mingling of sanitary waste to stormwater systems is a crucial element in municipal resource allocation. This study set out to investigate these mechanisms in an effort to aid in the prioritization of systems in need of repair.

Study Design

A total of 309 samples were collected from the study site area, the terminal outfall, groundwater monitoring wells and adjacent study areas. Table 6 summarizes the type of sample collected and the total number for each type.

Sample Type Description	# of samples collected (N)
Study site samples collected	N:244
Samples removed due to "unmatched" HB and Lachno2	N:26
Study site samples considered a "match" and evaluated	N:218
Terminal outfall (WA01) samples	N:21
Manhole samples	N:197
Baseline samples	N:6
Samples collected from inline samplers secured within select manholes	N:29
Flow samples collected from manholes during a qualifying rain event (> 0.10")	N:168
Monitoring well samples collected	N:47
Adjacent outfall (FMRMN39) samples	N:3
Adjacent to study site manhole samples collected (WAC)	N:15

Table 6. Summary of sample data

Section 3.1 – Infrastructure Mechanisms

In an effort to examine the before construction and after construction FIB concentrations, preliminary results (collected from 2008 to Fall 2012) were categorized as 'pre-construction' samples (terminal outfall WA01, manhole WA03 and adjacent outfall FMRMN9), and all corresponding samples collected after the completion of the storm and sanitary relay project (2013 to 2014) were identified as 'post-construction' samples. Details describing the methodology and the specific infrastructure conditions analyzed can be found in Chapter 2. For ease of reference, an enlarged site diagram depicting the manhole locations within the study area boundaries is provided in Appendix A Figure A.1.

Section 3.1.1 – Before and After Conditions

To compare the before and after construction concentrations, stormwater samples were collected from terminal outfall WA01 (formerly sample ID FMRMN44) and at a location up-the-pipe at manhole sample ID WA03 prior to the initiation of the stormwater relay construction project and for approximately two years following the completions of the construction activities. We found there was a ten-fold reduction in the mean concentrations of human *Bac* in the terminal outfall in the samples collected during the before and after time period. Although only one sample was collected and analyzed for human *Lachno* at the terminal outfall before construction, the concentration in the before analysis (315,210 CN/100 ml) decreased to a mean concentration of 5,685 (CN/100 ml) (N:21). Up-the-pipe manhole samples collected from WA03 resulted in a decreased concentration by 50% for human *Bac* and a decrease by 2 orders of magnitude for human *Lachno*.

A simple comparison of mean human *Bac* values in the pre-construction stormwater samples to the post-construction stormwater samples exhibited the following: pre-construction human *Bac* concentrations at WA01 were on average 97,707 CN/100 ml and 100% positive; post-construction human *Bac* concentrations were on average 8,754 CN/100 ml and only 60%

positive. In the up-the-pipe samples collected from WA03, pre-construction human *Bac* averages were approximately 36,570 CN/100 ml and 100% positive, while post-construction samples were on average 15,257 CN/100 ml and only 50% positive. Table 7 below summarizes the results of the before and after analysis.

Sample [HB] CN/100ml	Before Construction	After Construction	Number of samples (N)	T-Test p-value
Terminal Outfall WA01				
Mean HB (geomean)	97,707 (31,584)	8,754 (1,435)	Before (N:4) After (N:21)	**0.014**
% Positive HB	100%	60%		**Decrease**
Mean Lachno2 (geomean)	315,210*	5,685 (1,202)	Before (N:1) After (N:21)	**Decrease**
Up-the-pipe Manhole WA03				
Mean HB (geomean)	36,570 (26,198)	15,257 (3,057)	Before (N:6) After (N:8)	**0.013**
% Positive HB	100%	50%		**Decrease**
Mean Lachno2 (geomean)	846,409 (260,213)	2,393 (1,061)	Before (N:6) After (N:8)	0.0001

Table 7. Summary of before and after statistics for study site basin. The asterisks indicates that the sample mean could not be calculated as this sample was only analyzed for the specific parameter once.

One-tailed student t-tests were performed in Excel® comparing the before and after construction samples on the WA01 samples and the WA03 samples separately. Both sample sites demonstrated significant difference between the pre-construction samples to the post-construction samples with p-values of: 0.014 (WA01) and 0.013 (WA03).

Adjacent outfall FMRMN39 was also sampled during the pre-construction timeframe between 2008 and 2012. Although this outfall does not represent a storm basin that received utility rehabilitation, we collected samples from this outfall during the study time period (approximately 2012 to 2014) to evaluate weather the observed differences at our study site manhole were the result of real change or a system wide change. In contrast to the results observed in our study site, adjacent outfall FMRMN39 exhibited average concentrations of human *Bac* to be 129,879 CN/100 ml and 100% positive in the 'before construction' timeframe versus an average concentration of human *Bac* to be approximately 2,000,000 CN/100 ml and 100% positive in the "after construction" timeframe, suggesting an increasing trend.

The results of the preliminary before and after investigation of the stormwater collected at the terminal outfall demonstrate a ten-fold decrease in human *Bac* concentrations. The significant differences observed between the pre-construction and post-construction stormwater samples suggest that the storm and sanitary relay project significantly decreased the volume of human *Bac* released to the Menomonee River from this storm basin. However, sporadic concentrations of human *Bac* exceeding 60,000 to >100,000 CN/100 ml were still detected in some samples in post-construction samples collected from the newly laid stormwater pipeline and as described above, the post-construction stormwater samples collected from terminal outfall WA01 exhibited a positive, albeit lower concentrations, human *Bac* concentrations (i.e.: human *Bac* > 1,000 CN/100 ml) in 60% of the samples collected.

The following sections summarize the activities conducted from December 2012 to June 2014 and represent only post-construction sample results. The mechanisms that act to mobilize and transport FIB to the stormwater pipeline and ultimately the Menomonee River are explored through means of infrastructure, and physical and hydrogeological pathways.

Manhole Sample ID	Mean	Geomean	Standard Error	Max	Min	1st Quartile	Median	3rd Quartile	Sample Count	% Positive	Age of Pipe	Site Elevation (ft msl)
WA01	8,754	1,435	3,099	49,164	1	415	3,013	6,892	21	62%	New/New	660
WA02	14,274	5,431	5,064	59,535	346	2,347	7,337	27,229	13	85%	Old/Old	673
WA03	15,257	3,057	8,595	60,741	225	819	1,681	18,592	8	50%	New/New	682
WA04	13,225	2,441	11,780	60,313	468	556	1,940	2,849	5	60%	New/New	696
WA05	21,504	2,505	20,937	63,377	296	567	838	32,108	3	33%	Old/Old	698
WA06	36,332	5,889	22,986	157,071	484	975	6,187	44,317	7	71%	New/New	699
WA07	8,903	1,779	4,435	50,252	1	957	5,521	7,021	11	73%	Old/Old	700
WA08	2,943	1,436	1,565	6,946	225	578	2,301	4,666	4	50%	Old/Old	710
WA09	22,684	6,670	10,611	54,900	556	1,431	12,798	46,259	6	67%	New/New	705
WA10	11,411	2,044	8,491	53,260	225	340	2,951	7,945	6	50%	New/New	708
WA11	10,497	2,319	5,779	54,799	251	585	1,448	9,177	10	50%	New/New	709
WA12	6,006	1,301	2,723	27,340	1	733	2,143	8,411	10	70%	New/New	709
WA13	5,329	1,319	3,458	49,748	225	324	1,347	3,509	14	50%	New/New	719
WA14	5,541	527	2,645	36,983	1	330	2,125	4,155	14	57%	Old/Old	728
WA15	2,040	139	702	15,182	1	1	1,222	2,111	23	52%	Old/Old	731
WA16	9,992	1,339	5,284	76,012	1	318	2,333	4,014	15	60%	New/Old	713
WA17	26,677	2,950	15,452	172,165	1	1,325	4,040	27,601	11	82%	New/Old	717
WA18	18,957	8,560	5,234	60,951	302	2,645	13,488	28,425	14	93%	Old/Old	732
WA19	6,940	1,848	2,051	23,689	1	685	4,044	10,292	15	67%	Old/Old	743
WA20	2,946	2,882	430	3,682	2,192	2,579	2,965	3,324	3	100%	Old/Old	753
WA21*	2,129	-	-	2,129	2,129	-	-	-	1	100%	Old/Old	715
WA22	1,294	1,014	624	2,513	454	685	915	1,714	3	33%	Old/Old	730

Table 8. Summary of Select Statistics for human *Bacteroides* (CN/100 ml) by Site Code
*only one sample was collected from this location (WA21)
- indicates statistic not applicable

Section 3.1.2 – Age of Pipe Analysis

The age of pipe categorization was determined based on the Storm/Sanitary Reconstruction Project scope, which included removing all older sanitary and stormwater pipelines along 90th Street to Meinecke Avenue and along Meinecke to 81st Street and replacing them with newer and larger pipelines. The Storm/Sanitary Reconstruction Project scope also included the removal and replacement of older sanitary pipelines along 84th and 86th Street through Meinecke and Wright Street, and along Wright Street from 86th Street to 81st Street. The older stormwater lines remained in place along Wright Street. Table 8, above summarizes the age of pipe categorization for each specific manhole sampling location and provides a summary of statistics for the sample locations over the course of the entire sampling period.

Manhole sampling locations throughout the stormwater basin were selected based on various site evaluation criteria that can generally be categorized as a physical or infrastructure condition and/or a geographical/hydrogeological condition. Details regarding the site evaluation criteria and variable conditions can be referenced in Table 3 in Chapter 2, Section 2.2. Figure A.3 in Appendix A depicts the general study site layout and approximate locations of the sample sites in basin M09020N1.

With regard to the Age of Pipe categorization (an infrastructure associated condition), manhole sample sites were characterized as falling within one of the three groups: New Sanitary/New Storm, New Sanitary/Old Storm, and Old Sanitary/Old Storm. Human *Bacteroides* tested positively in over 50% of the samples collected from all three (3) groups, with the Old/Old category testing positive in 72% of the samples collected. Likewise, human *Lachno* tested positive in 48 – 54% percent of the samples collected from each category. Due to the high variability in concentration observed among sample sites, and as indicated by the high standard error for each category, geomean values were calculated for both human markers in each category. Human *Bacteroides* and human *Lachno* concentrations were log transformed to fit a normal distribution for statistical analysis. Table 9 below summarizes the results of the human *Bac* and human *Lachno* (CN/100 ml) concentrations observed by Age of Pipe.

Age of Pipe	Mean human *Bac* (CN/100ml)	Geomean human *Bac* (CN/100ml)	human *Bac* Standard Error (mean)	% Positive human *Bac*	Mean human *Lac2* (CN/100ml)	Geomean human *Lac2* (CN/100ml)	human *Lac2* Standard Error (mean)	% Positive human *Lac2*
New/New	10,001	1,874	1,957	57%	3,982	577	816	48%
New/Old	20,099	2,393	6,597	68%	8,149	722	3,126	47%
Old/Old	10,144	2,282	1,782	72%	15,533	1,292	6,180	54%

Table 9. Summary of Age of Pipe statistics for human FIB markers. *WA15 was removed from the old/old category as it does not reflect the conditions of the other manhole sample site for this evaluation.

The bar chart depicted in Figure 7 summarizes the mean and geomean concentrations of human *Bac* and human *Lachno* among the age of pipe groups. As depicted on the Figure, the blue circle indicates percent positive human *Bac,* and the orange bars indicate the mean human *Lachno* concentrations. Note that although the geomean concentrations of human *Bac* across groups are very similar, the percent positive for human *Bac* and the mean concentration for human *Lachno* follow a similar increasing trend from New/New to Old/Old.

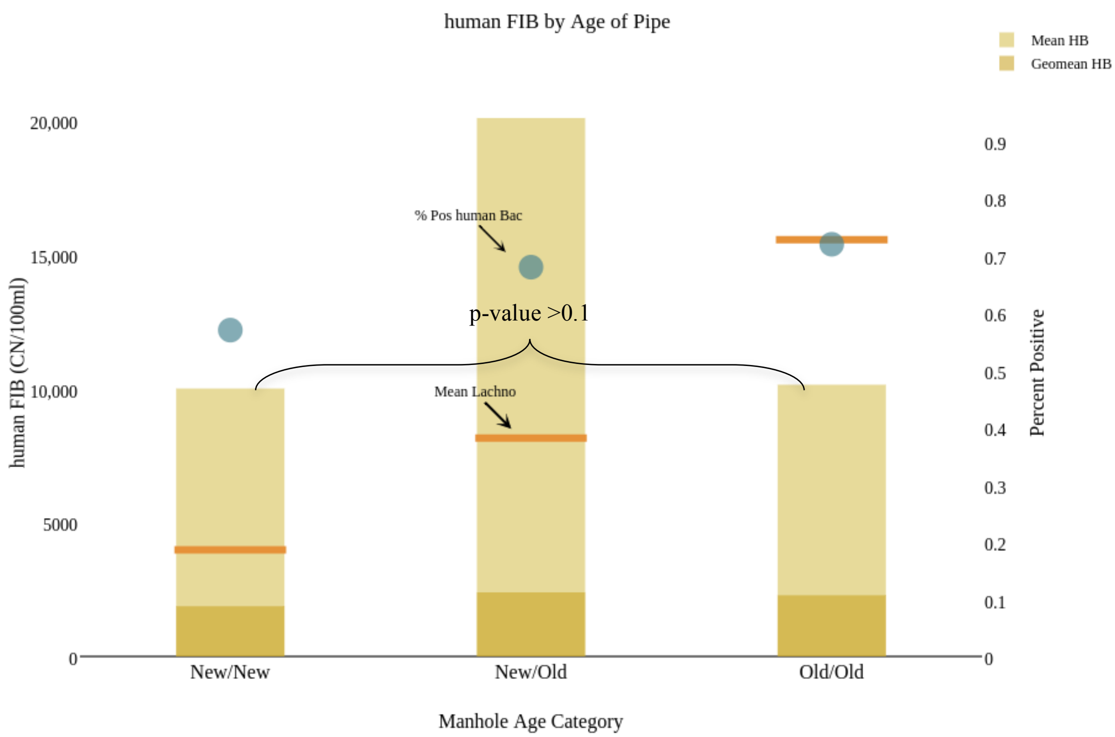

Figure 7. Mean, geomean and percent positive human *Bac* and mean *Lachno* [conc] by Age of Pipe.

Importantly, we observed that there was high variability in all the sites that corresponded with sample day. This indicated that precipitation or other hydrological conditions were a larger driving factor than sample site infrastructure conditions. Figure 8 below depicts the increasing and decreasing trends in human FIB concentrations measured in both the old/old and new/new pipe categories paired by date. Human FIB concentrations fluctuate by sample date, regardless of the age of pipe. The age of pipe concentrations observed for human *Lachno* increase from lower concentration at New/New pipes to higher concentrations at Old/Old pipes, but there appears to be a larger driving factor influencing the overall event concentrations.

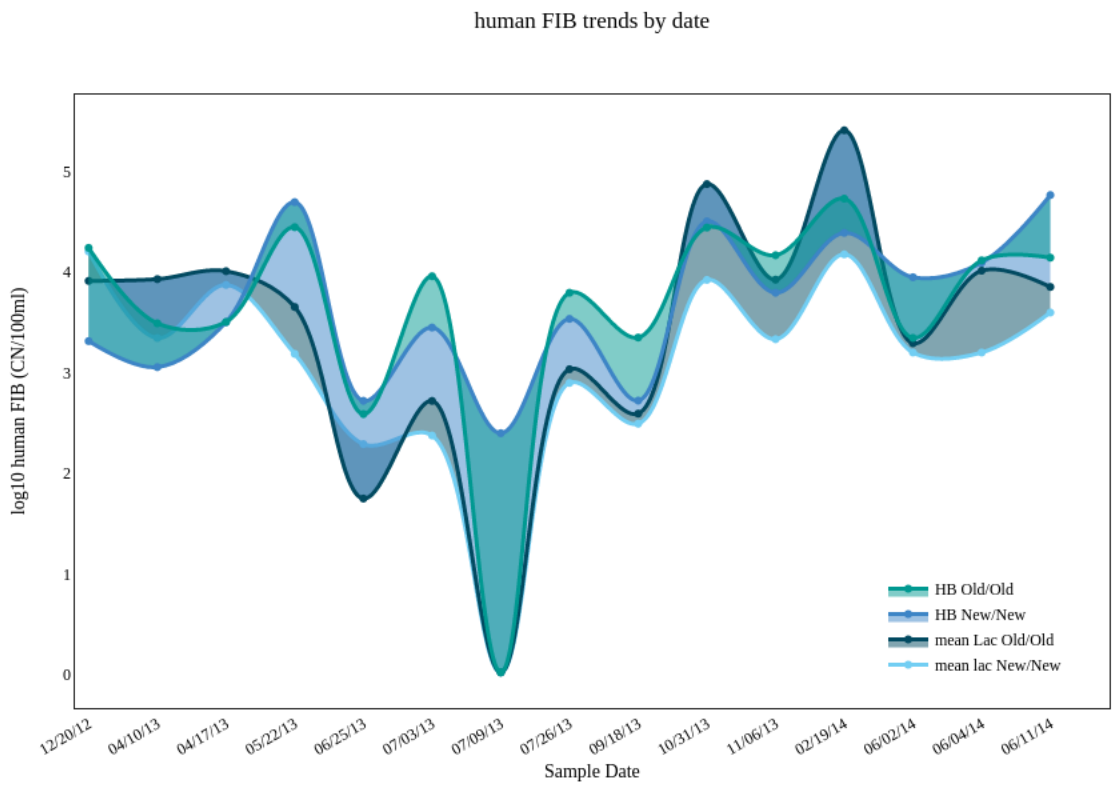

Figure 8. Depiction of human Bac by date and age of pipe. Levels throughout the system are driven by conditions on each sample date, more so than infrastructure conditions within the system.

Because rainfall is a large driver of human fecal indicator concentrations at all sites, paired t-tests by date provide the best indication of whether or not a real difference in concentration is occurring between the age of pipe categories. We performed several separate paired sample t-tests in Excel® to evaluate the difference in mean concentration of human FIB by date for New/New versus New/Old, New/New versus Old/Old and New/Old versus Old/Old. A summary of the sample date precipitation statistics and human FIB concentrations by date is depicted below in Table 10.

			human *Bacteroides* (CN/100ml)			human *Lachnospiraceae* (CN/100ml)		
Sample Date	Rainfall Amount (in)	24hr Antecedent Conditions (in)	Mean New/New	Mean New/Old	Mean Old/Old	Mean New/New	Mean New/Old	Mean Old/Old
12/20/12	1.14	1.21	1,947	9,671	16,411	15,101	22,194	7,710
04/10/13	0.34	1.38	1,074	45,041	2,923	2,091	7,819	8,028
04/17/13	0.49	0.49	3,001	25,876	3,016	7,037	26,024	9,632
05/22/13	0.38	0.5	46,720	2,205	26,333	1,455	3,479	4,247
06/25/13	1.45	1.45	497	225	367	185	113	53
07/03/13	0.16	0.16	2,655	1,991	8,578	225	369	496
07/09/13	0.35	0.66	237	4,040	1	1	354	1
07/26/13	0.16	0.16	3,237	1,952	5,879	748	754	1,024
09/18/13	0.12	0.12	500	949	2,112	295	559	371
10/31/13	0.15	0.15	30,054	41,823	26,108	7,894	47,183	70,520
11/06/13	0.29	0.29	5,877	10,129	13,870	2,035	3,585	7,931
02/19/14	snowmelt	snowmelt	23,128	4,656	50,563	14,167	3,267	241,350
06/02/14	0.1	0.9	8,383	-	2,092	1,500	-	1,854
06/04/14	0.1	0.1	11,706	4,902	12,335	1,500	1,500	9,746
06/11/14	0.55	0.6	54,901	79,519	13,118	3,742	6,163	6,716
Mean for Age Category			12,928	16,641	12,247	3,865	8,812	24,645

Table 10. Summary Table of Age of Pipe by Date

The results of the paired sample t-tests (Table 11 below) indicate that the concentrations observed in each age category by date for human *Bac* were not significantly different from each other (p-value >0.1), but a clear trend was observed. The differences in mean human *Lachno* concentrations between the New/New to New/Old and the New/New to Old/Old categories did exhibit significant p-values (0.04 and <0.01, respectively). The results of this analysis are consistent with the findings stated previously, comparing the entire population of New/New to Old/Old for human *Lachno* (<0.05).

Paired t-test p-value's for Age of Pipe by Date	human *Bacteroides* Log_{10} Mean (CN/100ml)		human *Lachno* Log_{10} Mean (CN/100ml)	
	New/New	New/Old	New/New	New/Old
New/Old	0.23	-	**0.04**	-
Old/Old	0.43	0.32	**< 0.01**	0.46

Table 11. Paired t-test results for age of pipe by date

The statistics presented above do not provide enough conclusive evidence to suggest that age of pipe alone acts to influence presence or absence of human FIB. Although we see differences in human *Lachno*, concentrations of human *Bac* appear to be relatively consistent. As stated in our original hypothesis, we believe a number of factors act together to mobilize human FIB to adjacent stormwater pipes; as such, external environmental variables were evaluated for the potential to act accordance with age to influence FIB presence in the system. The difference in the human *Bac* and human *Lachno* also appear seasonal. There are several explanations as to why the human *Bac* did not show significant differences, but human *Lachno* was significantly reduced. For example, human *Lachno* may survive longer, particularly during winter months, or the localized human inputs may contain more human *Lachno* than human *Bac* since human microbiomes are highly variable (Newton et al. 2011). Further analysis could be directed toward more segregated portions of the system that hold some of these variable constant.

Although the results of the Age of Pipe analysis do not directly support our hypothesis that the age of pipe is a factor in the transport of human FIB to surface waters, the knowledge gained from this investigation highlights the importance of this type of study to be conducted. Details and further analysis of the environmental factors evaluated for this study and their influence on human FIB is presented in Section 3.2.

Section 3.1.3 – Physical Infrastructure Conditions

During the onset of this study, we set out to explore the physical characteristics (depth of pipe, diameter, proximity to laterals and/or sanitary) of separated sanitary systems with the hypothesis that a breach in the system coupled with one or more of these variables, would have a systemic effect on the water quality of stormwater conveyed to the river. Although the potential for cross-connections exist in every system, our hypothesis is that the more substantial source of FIB contamination is due in part to the extremely large number of older laterals that have the potential to be intermittently or continuously contaminating adjacent stormwater lines. This hypothesis is supported by the diffuse nature of contamination throughout the system as indicated by 64% of samples positive for human *Bac* across. This portion of the research aimed to identify the physical characteristics of the system as a whole that act to mobilize FIB contamination from surrounding laterals and unfit sanitary lines to the stormwater pipeline.

Infrastructure conditions such as depth of stormwater pipe, sanitary and storm pipe diameter, whether the stormwater manhole was located in a mainline street (i.e. within the main corridor of Wright Street, Meinecke Avenue or 90th Street) or within an artery side street were evaluated in this section. The purpose of this exercise, as discussed above, was to capture the potential for laterals to be contributing to the stormwater lines. As anticipated, we observed higher geomean concentration of human *Bac* in the smaller artery street manholes (2,619 CN/100 ml) when compared to the larger diameter and larger flow manholes (1,273 CN/100 ml) within the main corridors (Table 12). A one-tailed t-test performed in Excel® confirmed this observation with a p-value of 0.03. This result provides evidence for the potential that laterals draining to the adjacent feeder street, are contaminating the stormwater system nearby. It would appear that once the stormwater is conveyed to the main pipeline (none of which have laterals within proximity to the stormwater pipes), the concentration of human FIB decreases, either through dilution or lack of additional inputs.

In addition to the findings above, we found that deeper pipes (greater than 10 feet bgs) had higher concentrations of human FIB than the shallow manholes (less than 10 feet bgs) that were investigated, where the geomean for human *Bac* in deeper pipes (3,007 CN/100 ml) is more than double when compared to the geomean of the shallow pipes (1,455 CN/100 ml). A one-tailed t-test of unequal variance comparing mean concentrations of human *Bac* in shallow pipes to mean concentrations of human *Bac* in deep pipes yielded a p-value of 0.05 for human *Bac*. One thought for this occurrence, which is consistent with our initial hypothesis, is that the deeper pipes are in closer proximity to the lower sanitary lines. It may be that the concentrations observed in these manholes are more representative of sanitary exfiltration or a breach in the system than leaking laterals. Table 12 below summarizes the findings of the physical location of the stormwater pipelines.

Infrastructure Condition	Artery	Mainline	Shallow	Deep
Sample count (n)	65	132	166	30
human *Bacteroides*				
Geomean [h Bac]	2,619	1,273	1,445	3,007
T-test	**p-value 0.03**		**p-value 0.05**	
human *Lachnospiraceae*				
Geomean [h Lac2]	972	535	696	460
T-test	*p-value <0.1*		p-value > 0.1	

Table 12. Summary of infrastructure conditions for human markers *Bac* and human *Lachno*.

To understand the response of the system as a whole, we took a step back and broadened our evaluation of the network as a drainage system, using a mass balance model. The mass balance would help identify sub-drainage areas that contributed more than others.

Section 3.1.4 – Mass Balance Mixing Model

In our conceptual mass balance model (described in Section 2.3), "mixing" refers to the addition of stormwater inputs at each branch point as it flows through the conveyance system. Some of these inputs may be co-mingled with external non-storm related flows, such as groundwater and/or sanitary wastewater that have entered the stormwater system via infiltration or illicit connections. Municipal stormwater systems are generally designed to mimic the topography of the area for which it is servicing. In terms of land area, stormwater basins vary in size; the pipeline network that underlies the service area can range in complexity from a single catch basin to an elaborate network channeling stormwater from thousands of homes and impervious surfaces that often exceed several hundred acres.

The M09020N1 storm basin services a highly residential urban area exceeding 170 acres and begins draining stormwater near 80th and Clark Street, the highest topographical elevation within the network at 753 feet mean sea level. Stormwater entering the system from this point flows and drains by gravity, accumulating more and more stormwater as it flows towards the terminal outfall at the Menomonee River. In a closed system, the volume of stormwater increases with each new influx of overland flow and assumes no exfiltration or infiltration of foreign material.

Prior to the initiation of this study, wet-weather up-the-pipe and corresponding terminal outfall sampling was not extensively covered. In the presence of rain, we hypothesize that concentrations of human FIB present in upper branches of the system will be diluted as the system accumulates stormwater. The principal question we wanted to answer is: Are there increasing amounts of human indicators added as flow travels toward the terminal outfall, or are inputs diluted? This study evaluated wet-weather samples collected from within the stormwater corridor in an effort to compare human FIB concentrations within the system, as paired with the downgradient manhole and/or terminal outfall outcome. If additional FIB is added along the way, as either more concentrated or less concentrated, the new concentration at the downgradient manhole will reflect a ratio of what was previously inputted to the volume of water drained to that section of pipe.

Based on the model described below, we used a mixing calculation to estimate the contribution of human *Bac* to each downgradient segment in the conveyance system, as measured by the samples collected from the select stormwater manholes. We calculated the square meters of impervious surface drainage area (DA) of each subsection of stormwater piping as an estimate of the contribution of runoff entering the conveyance system from the stormwater basin (refer to methods for detailed description). Table 13 summarizes the statistics and physical characteristics for each mainline street.

Mainline Street Name	Sample Count	MH DA's (m^2)	Mean human *Bac* per Street	Standard Error	Pollutant Load (human *Bac*/m^2)	Age of Pipe
Wright St	40	15,497	17,718	4,994	1.143	New/Old
Meinecke	83	32,984	6,852	1,498	0.208	New/New
90th	29	46,072	10,547	3,219	0.229	New/New

Table 13. Summary of human FIB concentrations and drainage areas by mainline.

For each major street, a well-defined increasing or decreasing trend was observed in the model for human *Bac* geomean when calculated from an upgradient manhole to a downgradient manhole. Due to the layout of the selected study site manholes, each manhole had various drainage area measurements and therefore provided different mixing contributions of human *Bac*.

Wright Street

The results of the Wright Street mixing analysis demonstrate decreasing trends in both human *Bac* concentration and mixing contribution of human *Bac*. Manhole sites within Wright Street (WA18, WA17 and WA16) are designed to flow from Wright Street at 80th Street to Wright and 85th Street where the stormwater pipes deliver stormwater south along 85th to the Meinecke Avenue pipeline. Table 14 below summarizes the mass balance model calculations for select sample dates: July 26, 2013, September 18, 2013 and October 31, 2013 (Table 14).

Wright Ave Manhole ID	Drainage Area(m^2)	**07/26/13 human *Bac* (CN/100ml)**	Mixing Calculation	**09/18/13 human *Bac* (CN/100ml)**	Mixing Calculation	**10/31/13 human *Bac* (CN/100ml)**	Mixing Calculation
WA18	5,383	6,597	6,597	2,172	2,172	60,951	60,951
WA17	4,114	4,100	833	1,304	168	51,900	40,057
WA16	6,000	2,530	45	800	2	31,825	50

Table 14. Wright Street mass balance summary for select sample dates. The human *Bac* concentrations observed for each date (07/26/13, 09/18/13 and 10/31/13) and the mass balance calculation for each date is depicted in the table above. The mass balance calculation for each sample date decreases in concentration from the upgradient manhole WA18 to the downgradient manhole WA16.

Table 14 above depicts the human *Bac* concentrations observed during a specific sample date. A trend that we continue to see throughout this dataset is that despite the differences in concentration from date to date, there is an obvious decreasing trend in concentration from WA18 to WA16 for each sample date.

The conceptual diagram below (Figure 9), depicts the human *Bac* geomean concentrations within the Wright Street pipeline. This diagram was prepared to illustrate the dilution effect of increased stormwater load to this section of the pipeline; as such, the volume of stormwater in the contributions calculated for each study site manhole along Wright Street are proportional to the drainage area it receives water from. It is evident from this diagram that as drainage volume increases, human *Bac* contributions decrease suggesting that dilution is occurring in the system.

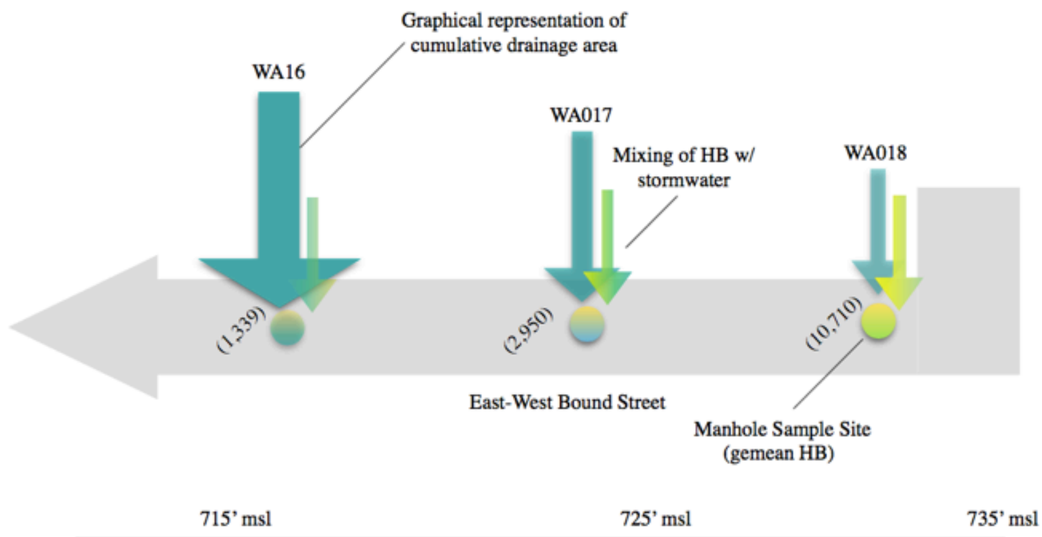

Figure 9. Wright Street Conceptual Model. The manhole sample ID is depicted above each arrow and the geomean for each manhole sample site is depicted in parentheses next the circle (manhole location). The green arrows represent the cumulative drainage area per manhole site. The yellow arrow at WA18 represents a high concentration of HB observed at this location. The yellow arrow changes as the stormwater moves downgradient because the concentrations at these sites are lower.

The results of the mixing calculation performed for Wright Street suggest that a dilution effect is taking place, which is indicated by the observed downward trend in concentration from WA18 (10,710 CN/100 ml) to the end of the stormbasin along Wright Street at WA16 (1,339 CN/100 ml). One possible explanation for this dilution effect may be that WA18 consists of an old sanitary and old stormline ("Old/Old" category) that may be receiving sanitary water through cracks and loose joints, therefore higher concentrations would be observed at this location.

90th Street

The results of the 90th Street mixing analysis mimic the decreasing trends observed at Wright Street for both human *Bac* concentration and mixing contribution of human *Bac*. The manhole sites within 90th Street (WA09, WA03 and terminal outfall WA01) are designed to flow from 90th

Street at Meinecke south to the terminal outfall (WA01). Table 15 below summarizes the results of the mass balance analysis performed for the 90th Street manholes on sample date June 11, 2014.

90th Street Manhole ID	**06/11/14 human *Bac* (CN/100ml)**	Mixing Calculation
WA09	54,900	54,900
WA03	60,741	62,848
WA01	54,900	9

Table 15. Mass balance model for 90th Street manhole samples collected on 06/11/14.

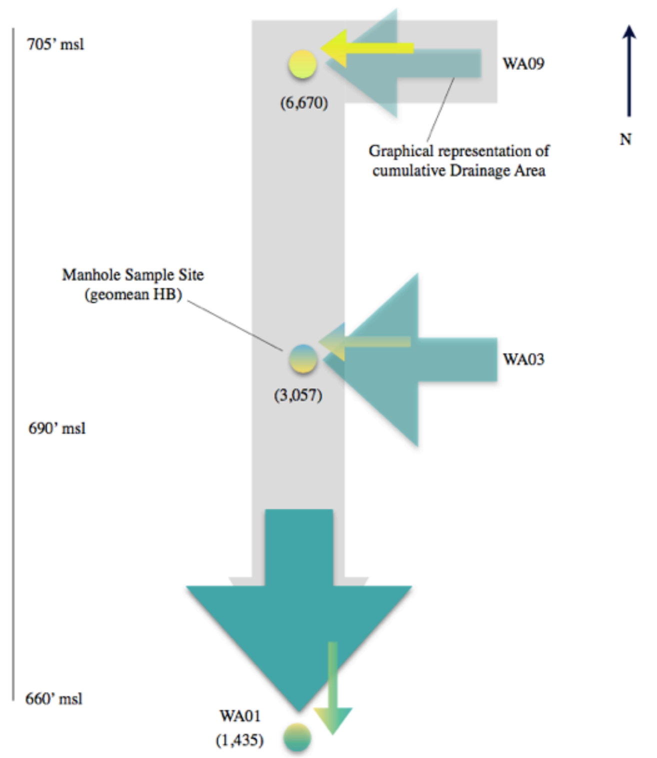

Figure 10. 90th Street Conceptual Model. The manhole sample ID is depicted near each manhole location and the geomean per manhole sample site is depicted in parentheses below the site ID. The site elevation (in feet msl) for each manhole is depicted to the left, along the black y-axis. In this model, a yellow arrow, indicating a high concentration of HB enters the mainline at WA09, the yellow arrow changes to green as the stormwater accumulates.

Figure 10 illustrates the 90th Street conceptual mass balance model where a decreasing trend in geomean human *Bac* is observed from WA09 (6,670 CN/100 ml) to the terminal outfall at WA01

(1,435 CN/100 ml). This decreasing trend can be explained based on the infrastructure characteristics of the pipeline which consists of new sanitary and new storm, which minimizes exfiltration of sanitary sewage. It is also noteworthy that lateral replacements were also provided for a stretch of 90^{th} between North Avenue and Meinecke. The stormwater line is approximately 12 feet in diameter and moves a significant volume of water during heavy rain events. Additionally, the mainline along 90^{th} is not in direct proximity to the laterals located at 90^{th}.

Meinecke

The Meinecke Avenue mixing analysis generally demonstrates an increasing trend in both human *Bac* concentration and mixing contribution of human *Bac* from the east end of the storm watershed at Meinecke and 80^{th} (WA15) to the west end of Meinecke (WA09) where the stormwater system changes flow south, along 90^{th} Street. The human *Bac* geomean mixing analysis observed within the Meinecke Avenue pipeline indicates that human *Bac* concentrations are somewhat intensified with increasing volumes of water draining to the downgradient manholes. In Table 16 below, the geomean human *Bac* concentrations seem to bounce around the threshold value (1000 CN/100 ml) from WA13 to WA10; when WA09 adds of stormwater drainage, the contribution is large. From the results of the mixing model for the geomean human *Bac* at Meinecke, it is evident that there is an issue somewhere between WA10 and WA09, where the mixing contribution increases from 894 CN/100 ml to 26,880 CN/100 ml with an added drainage volume of 9,030 m^2. This may indicate an improper lateral hook-up near WA09 or a breach in the sanitary system itself.

Meinecke Ave Manhole Sample ID	Geomean human *Bac* CN/100ml	Standard Error (mean)	Drainage Area(m^2)	Cumulative Drainage Area (m^2)	Mixing Contribution human *Bac*
WA15	139	702	2,683	2,683	139
WA14	527	2,645	936	3,619	1,639
WA13	1,319	3,458	4,396	8,015	1,971
WA12	1,301	2,723	4,966	12,981	1,272
WA11*	2,319	5,779	18,856	31,837	3,020
WA10	2,044	8,491	7,614	39,451	894
WA09	6,670	10,611	9,030	48,481	26,880

Table 16 – Meinecke Avenue mass balance mixing model summary
*WA11 represents combined drainage area from Wright St manholes (WA18, WA17 and WA16)

In the schematic conceptual model below, an increasing trend of human *Bac* concentrations are depicted with decreasing elevation. The concentrations identified in the figure below (Figure 11) represent the observed geomean for each manhole sample location. The larger green arrows represent the cumulative drainage area calculated per manhole, which is obviously increasing with decreasing elevation. The smaller yellow/green arrows, portrayed as a concentration gradient, demonstrate an increasing concentration of human *Bac* with decreasing elevation and increasing drainage area.

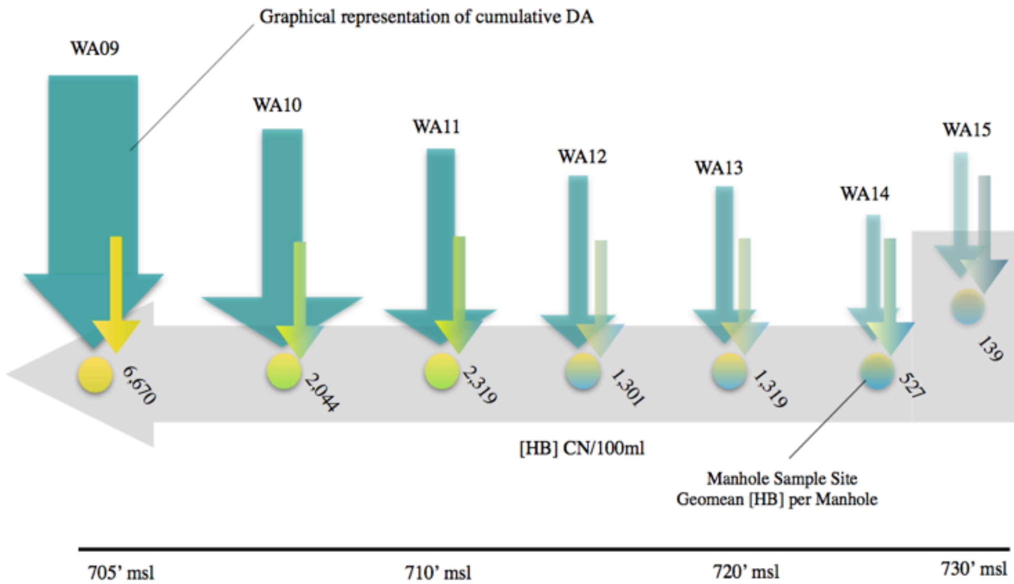

Figure 11. Meinecke Avenue Conceptual Model for geomean human *Bac*. The manhole sample ID is depicted above each arrow and the geomean for each manhole sample site is depicted in parentheses next the circle (manhole location). The green arrows represent the cumulative drainage area per manhole site. The yellow arrow at WA09 represents a high concentration of HB observed at this location. The yellow arrow changes as the stormwater moves upgradient because the concentrations at these sites are lower, suggesting HB increases along the pipeline.

The mass balance calculations performed for individual sampling events for the manholes along Meinecke also demonstrate an increasing trend in mixing concentrations from upgradient manhole site WA15 to downgradient manhole sample site WA09. Table 17 summarizes the mass balance calculations for samples collected on October 31, 2013 and on June 4, 2014.). On July 26, 2013, the highest value observed for the mixing analysis was 11,252 CN/100 ml at WA14. The peak at WA14 on July 26 is most likely attributable to the relatively smaller drainage area of WA14 (936 m^2) and the low concentrations of human *Bac* at WA15 (where 1 = no detect). An increase in mixing concentrations was observed for the sample events in October and July, where WA10 and WA09 demonstrate the highest values (73,911 and 58,175 CN/100 ml, respectively).

Meinecke Ave Manhole ID	Drainage Area(m^2)	**10/31/13 human *Bac* (CN/100ml)**	Mixing Calculation 10/31/13	**06/04/14 human *Bac* (CN/100ml)**	Mixing Calculation 06/04/14
WA15	2,683	1,252	1,252	2,207	2,207
WA14	936	950	84	4,306	10,323
WA13	4,396	3,768	6,088	-	-
WA12	4,966	12,454	26,473	10,072	12,301
WA11*	18,856	-	-	11,196	11,970
WA10	7,614	53,260	73,271	-	-
WA09	9,030	54,416	59,466	21,789	42,027

Table 17. Meinecke Avenue mass balance mixing table for select dates: 10/31/13 and 06/04/14. The mass balance calculations for Meinecke Avenue appear to be increasing for 10/31/13 and 06/04/14. Cells with missing values indicate that no sample was collected on that date from that manhole site. The asterisk on WA11 indicates that the drainage area for WA11 represents the accumulated drainage from the Wright Street pipeline.

The results of the mixing analyses for Meinecke suggest that the human *Bac* concentrations observed in each manhole are increasing from the beginning of the stormwater pipeline at WA15 to the end of the pipeline at WA09, these results also highlight problem areas along the Meinecke Avenue pipeline between WA12 and WA09 where a breach in the system may exist.

A one-way ANOVA was performed in R to evaluate the differences in mean concentration of human *Bac* and *Lachno* by Street. Given that each street has its own unique set of characteristics (i.e. age of pipe, elevation, residential density and lateral connections, etc), it was not surprising to find that the results of the ANOVA by pipeline indicated differences in means for both human *Bac* and human *Lachno* of <0.01. Tukey's *post hoc* pairwise comparison revealed that the differences observed in the mean concentrations of FIB were attributable to the differences between Wright Street (mean human Bac 17,718 CN/100 ml) and Meinecke (mean human Bac 6,852 CN/100 ml) with a p-value of 0.02 (human *Bac*) and ≤ 0.01 (human *Lachno*). Mean human *Lachno* concentrations for Wright Street were 23,115 CN/100 ml and Meinecke Avenue mean values were 3,114 CN/100 ml. Table 13, presented earlier summarizes the drainage areas, human *Bac* mean and geomean and estimated pollutant load per major pipeline.

The observed differences in mean concentrations from this analysis are consistent with our findings from the mixing calculations described above, where Meinecke Avenue demonstrates lower concentrations of human *Bac* at the beginning of the pipeline and increases somewhere between WA11 and WA09, which is where Wright Street enters the system. The Wright Street mixing model demonstrates an influx of highly concentrated human *Bac* at WA18 (an old/old category manhole site). The results of the mixing model provide information as to where the contaminant mass may be entering along the system at Meinecke Avenue, and the ANOVA analysis by pipeline and pollutant load calculations provide evidence that suggests that the influx of contaminantion observed between WA11 and WA09 is likely coming from the Wright Street pipeline. Average concentrations of human FIB for Wright Street are significantly higher than those at Meinecke (17,718 > 6,852 CN/100 ml (human *Bac*) and 23,115 > 3,114 CN/100 ml (human *Lachno*). Furthermore, the stormwater system from Wright Street to Meinecke is designed to flow from Wright Street south to Meinecke along 85th Street, the intersection where WA11 is located. The results of this analysis supports our hypothesis that Old/Old pipes (in this case WA18) contribute a significant load to the system.

Section 3.1.5 Other Parameters Investigated

The Storm/Sanitary Reconstruction Assessment examined many potential mechanisms that were thought to aid in the transport of human FIB to adjacent stormwater systems however, several analyses investigated were either not significant or not directly relevant to the central hypotheses. This is likely that there is a combination of factors affecting human FIB levels, and/or multiple mechanisms that each occur under different conditions.

Separate factorial ANOVA analyses were performed in R to evaluate whether age of pipe combined with another categorical variable(s) have an effect on the outcomes of human *Bac* and human *Lachno*. The results for the ANOVA analyses for age of pipe and the following categories: season, rainfall type, rainfall event duration, 24-hr antecedent conditions and site elevation did not demonstrate a significant effect on the concentration of human *Bac* (p-value >0.1) or human *Lachno* (p-value >0.1).

Based on the ANOVA analyses discussed above, age of pipe does not appear to be a primary factor in the transport of human FIB to nearby surface waters when evaluated against season, rainfall type, antecedent conditions or site elevation. The ANOVA analysis did not reveal any significant differences in the mean concentrations of human FIB between age of pipe and these variables, and the differences observed are driven by the event, as indicated by the paired summary results in Table 10 and the paired t-test results in Table 11, where concentrations of human FIB are increasing and decreasing by sample date.

Pipe diameters were evaluated for each manhole sample site using logistic regression analysis. Due to the large sampling variability in concentration, human *Bac* and human *Lachno* were binned into binary form and categorized as either positive (receiving a 1 value) or negative (receiving a 0 value) for exceeding the threshold for sewage contamination. The results of the logistic regression indicate that neither storm or sanitary pipe diameter influence the presence of human FIB in the stormwater system (p-value >0.1).

Site elevation was explored with the intention of evaluating whether or not concentrations of human FIB increased with decreasing elevation, either due to accumulation throughout the system or in relation to proximity to groundwater. The results of the logistic regression for site elevation and human FIB presence indicate that site elevation does not have an effect on human FIB (p-value >0.1) for either human marker. A summary of the logistic regression analysis results is provided in Table 18.

Logistic Regression Values for Pipe Diameter & Elevation	log Odds (coef)	Standard Error	p-value
human *Bac* (pos/neg)			
Sanitary pipe diameter (in)	0.02	0.02	0.41
Storm pipe diameter (in)	-0.01	0.01	0.22
Site elevation (ft msl)	0.0002	0.01	0.97
human *Lachno* (pos/neg)			
Sanitary pipe diameter (in)	-0.004	0.02	0.85
Storm pipe diameter (in)	0.001	0.01	0.93
Site elevation (ft msl)	-0.003	0.01	0.57

Table 18. Logistic regression analysis of human FIB and pipe diameter

Although the logistic regression analysis did not provide supportive evidence that site elevation was influencing human FIB presence, the mass balance model provides a much more detailed approach for evaluating the effect of accumulated pipe flow and human FIB concentrations. Initially, we explored the idea of improper sanitary pipe diameter as a factor in the increased potential for sanitary exfiltration, however, the many of the sanitary pipes in this system were replaced with newer (and larger diameter) pipes, so it is likely that the pipes analyzed in this portion of the study were of adequate size and unlikely to reach capacity. If pipe diameter is explored in another stormbasin, pipe age and construction should be consistent as other variables are explored.

Section 3.2 – Hydrogeological Mechanisms

An aging system such as this is anticipated to have an unknown number of cracks, illicit connections, failures and others problems due to the ever changing stormwater environment, therefore the system was not considered to be a "closed-loop" as its design originally intended. It was anticipated that contamination would travel from the highest elevation at the start of the stormwater basin and accumulate as stormwater traveled downgradient. However, as discussed in the sections below, FIB was detected at each site under various conditions and concentration of human FIB was influenced by several factors including rainfall duration, drainage area and pipeline street channel.

Section 3.2.1 – Seasonal Analysis

The presence of microbial fecal indicator bacteria (*E. coli*, fecal coliforms, enterococci) in stormwater has been described in the literature to be correlated with seasonal variability in several studies (Selvakumar & Borst 2006; Pan & Jones 2012) where highest concentrations of FIB were observed in the summer months and a 1968 study conducted by Geldreich et al. where summer and fall demonstrated the highest concentrations of FIB in stormwater samples collected from street gutters during rainfall (Geldreich et al. 1968) The issue of relying on microbial indicators in correlation with external factors is that they are not human specific; therefore, the high concentrations observed in the summer months from previous studies could be attributable to the increased traffic of urban and domestic animals such as raccoons, dogs and even birds. Prior to the initiation of this study, seasonal analysis using molecular markers had not been explored. The association between human specific FIB concentrations in stormwater and season was evaluated for the samples collected during the 19-month sampling timeframe. Table 19 below summarizes the results of the human FIB concentrations by season during the sampling time period.

Season	Fall	Spring	Summer	Winter
Sample count (n)	44	96	66	11
Mean [HB]	15,248	14,193	1,958	19,982
Geomean [HB]	2,832	2,474	410	12,460
Mean [Lachno2]	16,343	5,920	363	54,671
Geomean [Lachno2]	2,104	1,922	44	15,274

Table 19. Summary of human FIB concentration by season

One-way ANOVA analyses

Separate one-way ANOVA's were performed in R to evaluate differences in mean concentration of human *Bac* and *Lachno* by season. Based on the results of the ANOVA, concentrations of human *Bac* and human *Lachno* in summer are significantly different than the concentrations observed in fall, winter and spring with a pairwise p-value of <0.001 for each category. The human *Lachno* samples also demonstrated differences in means among the groups winter and fall (p-value 0.07) and winter and spring (p-value 0.03). Table 20 below summarizes the results of the ANOVA analyses for each seasonal category.

Seasonal ANOVA Analyses		Standard error	Pairwise p-value
Log10 human Bac (ANOVA p <0.0001)			
Spring	Fall	0.20	0.957
Summer	Fall	0.22	**<0.001**
Winter	Fall	0.38	0.38
Summer	Spring	0.18	**<0.001**
Winter	Spring	0.36	0.201
Winter	Summer	0.37	**<0.001**
Log10 human *Lachno* (ANOVA p <0.0001)			
Spring	Fall	0.19	0.9909
Summer	Fall	0.21	**<0.001**
Winter	Fall	0.36	*0.0793*
Summer	Spring	0.17	**<0.001**
Winter	Spring	0.34	**0.0369**
Winter	Summer	0.35	**<0.001**

Table 20. Seasonal ANOVA summary statistics

Figure 12 below depicts a box plot of human *Bac* and *Lachno* concentrations by season. As you can see, winter appears to have the highest mean concentrations of human *Bac* and *Lachno* than the other seasons, while summer is the lowest. The results of this analysis suggest that a relationship is present between season and concentration of human FIB. It is important to note here, however that the winter sample population (N:11) is predominantly represented by snowmelt (N:10), rather than a rain event (N:1). It is possible that snowmelt acts as a mechanism to flush the stormwater system of residual FIB in the absence of rain, but more sample events during the winter time period would be needed to justify this as a real occurrence.

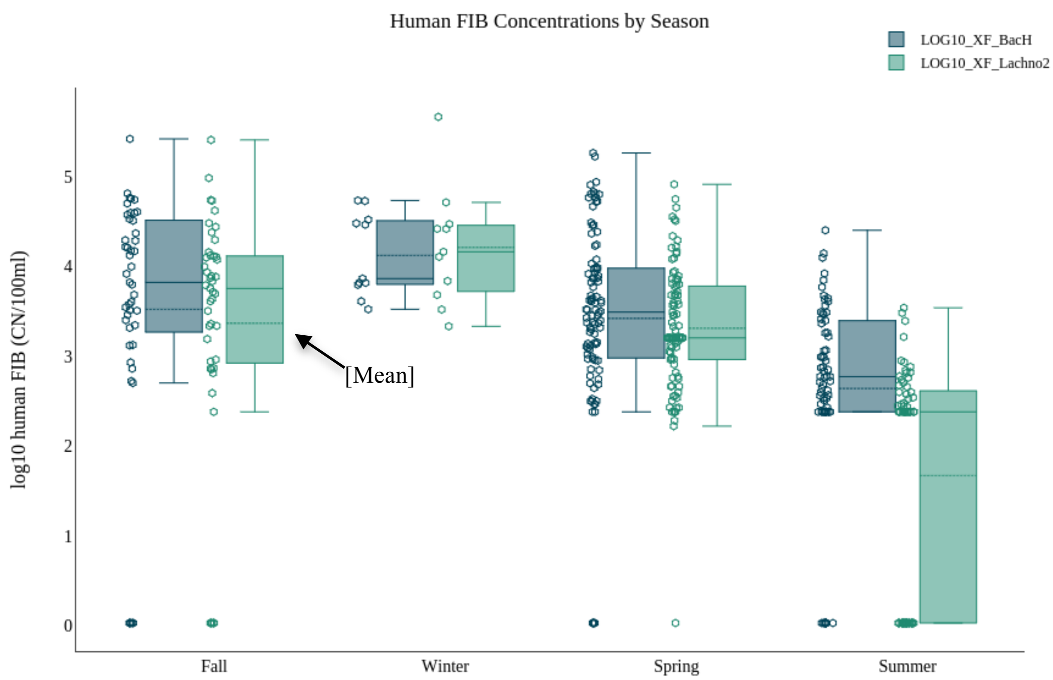

Figure 12. Boxplot of human *Bac* by season. The dotted lines on each box represent the mean value

Section 3.2.2 – Precipitation and Hydrogeological Interactions

The question raised by the seasonal analysis brings us back to our overall hypothesis: that rain is a driving factor in presence/absence of FIB indicators. We evaluated the human FIB concentrations against precipitation characteristics such as; rainfall amount, rainfall event duration, antecedent conditions and rainfall intensity. The potential for hydrogeological interactions to occur within the pipe system as a result of rainfall was also evaluated by determining the relative peak and average discharge rates of rainfall overland-flow draining from the stormwater basin, represented as Q and Qmax in cubic feet per second (cfs). Peak streamflow calculations were also evaluated as a proxy for groundwater elevation to determine if we could observe trends in streamflow highs and presence of human FIB in the manholes.

Rainfall type, defined as: heavy, medium, low and snowmelt was determined for each sampling date and the mean and geomean concentrations of human *Bac* and *Lachno* for each rain type was calculated (Table 21). A one-way ANOVA was performed on log transformed data and based on the results of the Tukey *post hoc* pairwise comparison, concentrations of both human markers were significantly higher in the samples collected from snowmelt events compared to the samples collected from heavy rain events (p-value 0.03 (*Bac*) and <0.001 (*Lachno*)). Snowmelt samples also had significantly different mean concentrations when compared to low rainfall event samples (p-value <0.05 (*Bac*) and <0.01 (*Lachno*)). None of the other rain types indicated differences among means for human *Bac*.

Rainfall Type	Heavy (>1.00")	Medium (0.50 - 1.00")	Low (0.10 - 0.49")	Snowmelt
Sample count (n)	29	40	138	10
Mean [h Bac]	4,232	21,900	8,479	21,365
Geomean [h Bac]	883	2,913	1,304	13,371
Percent pos [h Bac]	0.41	0.70	0.65	1.0
Mean [h Lac2]	5,467	5,801	6,921	57,361
Geomean [h Lac2]	112	2,288	583	14,388

Table 21. Mean and geomean human *Bac* concentrations by rainfall type

Reviewing the ANOVA results discussed above and the summary statistics in Table 22, it appears that the category identified as having low rainfall, is also lower in geomean human *Bac* and human *Lachno* when compared to the snowmelt concentrations and the medium type concentrations. The lowest concentrations observed in this data set are found in the heavy rainfall type category, with geomean human *Bac* and *Lachno* below their respective threshold values. This observation could be due to heavy rains acting to 'flush' the system, diluting and/or removing contaminants with the increase in volume and flow. However, if that were the case then it would be anticipated that the low rainfall type would demonstrate higher concentrations than what we observed. Although, it may be possible that lower rain events do not have enough volume to mobilize the FIB that has collected in the system. Another possible explanation is infiltration; referring back to Figure 1 and the report prepared by Bradbury et al.(2012), it may be possible that during periods of heavy rainfall, the subsurface reaches saturated conditions and the stormwater pipes are submerged, which may result in infiltration of rainwater, thus diluting the concentrations present in the system.

Rainfall event duration was also analyzed using ANOVA and we found that human FIB concentrations are effected by rainfall duration, specifically when comparing concentrations of long rainfall events (those exceeding 24 hours) to shorter rainfall events (less than 5 hours), p-value <0.001 for both human markers. The long rainfall events have a higher mean value of human *Bac* (16,551 CN/100 ml) compared to the medium (11,985 CN/100 ml) and short (3,445 CN/100 ml) categories. The boxplot depicted in Figure 13 illustrates the human *Bac* concentrations observed during short, medium and long rainfall events. Longer duration of rainfall would indicate increased saturation of the subsurface, which may result in the mobility of human FIB in the vadose zone to the adjacent stormwater pipes.

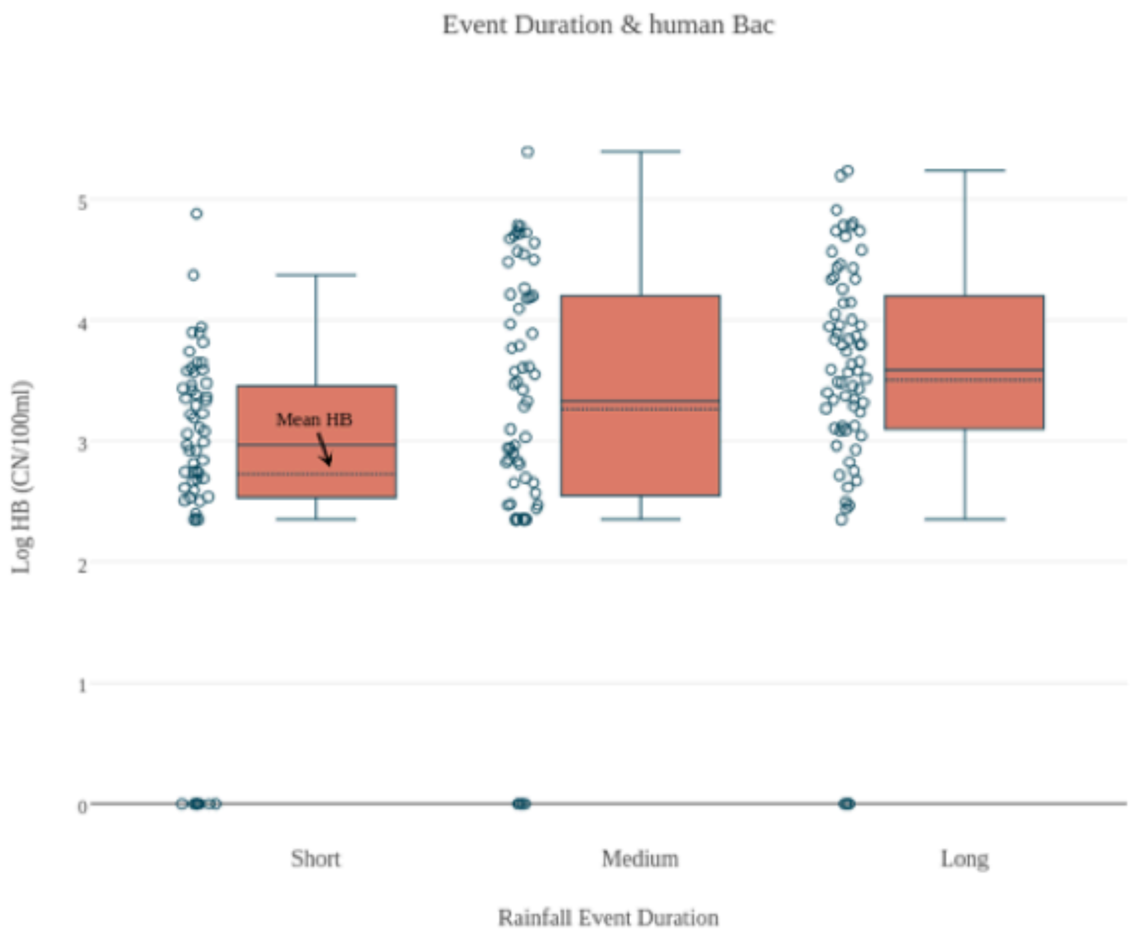

Figure 13. Boxplot depicting human Bac concentrations observed for rainfall duration.

These findings lead us back to one of the initial thoughts when first analyzing the results of this study; the environmental variability in this data set may be too broad to define the mechanisms that result in human FIB transport. The day to day variability seems to have an influence on the system as a whole. In the paired t-test analysis presented in Figure 8 (Section 3.1.2), we evaluated the trends of human FIB by date and age of pipe. Concentrations of human *Bac* and *Lachno* appeared to increase and decrease by date, regardless of age. When we incorporate rainfall volume and antecedent conditions, a relationship to mean FIB is evident. Figure 8 was modified to illustrate the relationship that appears with the human *Bac* concentrations grouped by age of pipe and sample date and rainfall amount (Figure 19). The secondary y-axis to the right of Figure 14 has been reversed to show this relationship. Logistic regression analysis was performed to evaluate whether or not this is a quantifiable occurrence.

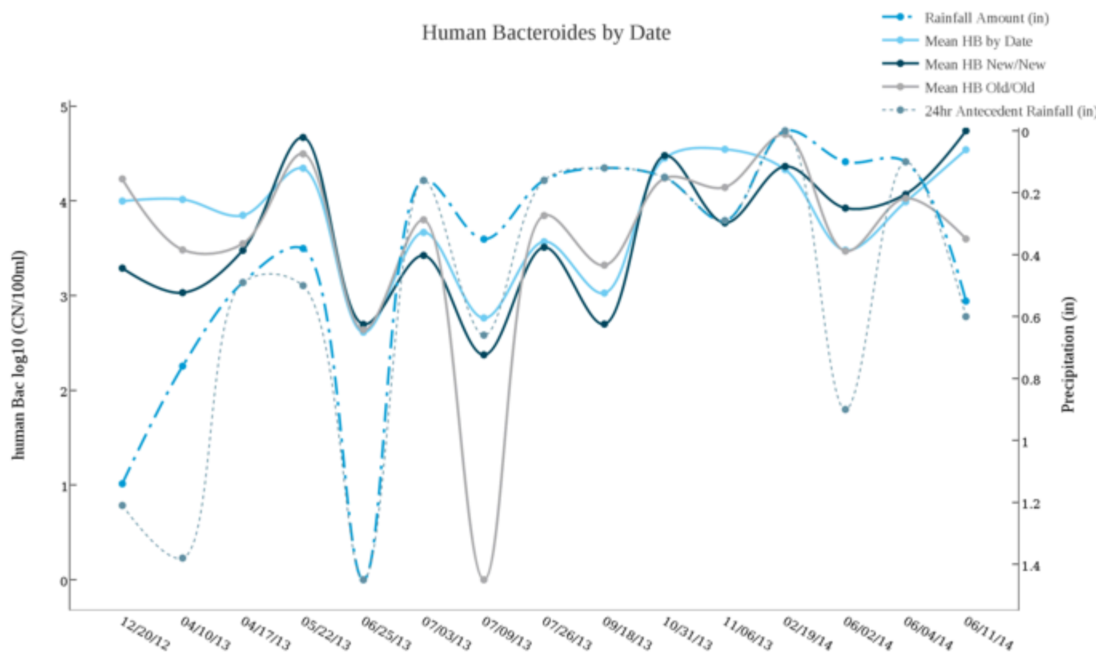

Figure 14, Trend in concentration of human *Bac* by age of pipe and date. Simultaneous fluctuations of mean human *Bac* in the old and new pipes appear to be related to precipitation.

Logistic Regression

Logistic regression analysis was performed in R to evaluate the influence of rainfall characteristics and their relative influence on presence/absence of human FIB. The continuous variables analyzed in this section were: rainfall event duration (hours), 24-hour antecedent conditions (inches), mean rainfall event intensity (in/hour), peak rainfall event intensity (in/hour), peak streamflow (cfs), discharge (Q) of water due to overland flow from the basin (cfs) and sample event rainfall amount (inches). A Wald Goodness of Fit test was performed for each of the analyses run and significance against the null hypothesis is represented by a p-value. Values exceeding the 95% confidence interval are not highlighted. The variables and statistics highlighted in orange in Table 22 below, denote the models that met the criteria for significance and goodness of fit.

Response Variable (pos/neg marker)	Independent Variable	log odds (coef B	B>0 inc B<0 dec	odds ratio EXP(B)	Probability [(1/(1+1/OR)]	Standard Error	p-value	Confidence 95% Interval (Probability) [1/(1+1/(EXP(CI))]	
Pos/Neg HB	Intercept	0.082		1.08534	0.520	0.216	7.05E-01		
Rainfall Event duration (hrs)		0.021	inc	1.02072	0.505	0.008	1.37E-02	50.1%	50.9%
Pos/Neg Lachno	Intercept	-0.693		0.49994	0.333	0.220	1.63E-03		
Rainfall Event duration (hrs)		0.031	inc	1.03165	0.508	0.008	1.35E-04	50.4%	51.2%
Pos/Neg HB	Intercept	1.089		2.97071	0.748	0.213	3.20E-07		
24hr Antecedent Precip (in)		1.128	inc	3.08978	0.755	0.288	8.94E-05	15%	36%
Pos/Neg Lachno	Intercept	-0.693				0.220	1.63E-03		
24hr Antecedent Precip (in)		0.031	inc	1.03165	0.508	0.008	1.35E-04	50%	51%
Pos/Neg HB	Intercept	1.278		3.58945	0.782	0.280	4.86E-06		
Event Intensity (in/hr)		-10.017	dec	0.00004	0.00004	2.947	6.80E-04	0.00001%	1.3%
Pos/Neg Lachno	Intercept	0.972		2.64243	0.725	0.274	3.89E-04		
Event Intensity (in/hr)		-13.920	dec	0.000001	0.000001	3.229	1.62E-05	0.000000%	0.040016%
Pos/Neg HB	Intercept	1.722		5.59347	0.848	0.359	1.64E-06		
Peak Rainfall Intensity (in/hr)		-5.623	dec	0.00362	0.004	1.462	1.20E-04	0.02%	5.59%
Pos/Neg Lachno	Intercept	0.881		2.41259	0.707	0.331	7.00E-03		
Peak Rainfall Intensity (in/hr)		-4.457	dec	0.01159	0.011	1.427	1.79E-03	0.07%	15.24%
Pos/Neg HB	Intercept	0.796		2.21695	0.689	0.184	1.55E-05		
Peak Streamflow		-0.001	dec	0.99948	0.500	0.000	8.66E-03	49.98%	50.00%
Pos/Neg Lachno	Intercept	-0.693		0.49994	0.333	0.220	1.63E-03		
Peak Streamflow		0.031	dec	1.03165	0.508	0.008	1.35E-04	50%	51%
Pos/Neg HB	Intercept	1.745		5.72544	0.851	0.360	1.24E-06		
Q Discharge over basin(cfs)		-0.085	dec	0.91890	0.479	0.022	9.28E-05	47%	49%
Pos/Neg Lachno	Intercept	0.884		2.41972	0.708	0.330	7.41E-03		
Q Discharge over basin(cfs)		-0.066	dec	0.93609	0.483	0.021	1.67E-03	47%	49%
Pos/Neg HB	Intercept	1.066		2.90258	0.744	0.214	7.67E-07		
Sample event precip (in)		-1.070	dec	0.34311	0.255	0.343	1.83E-03	15%	40%
Pos/Neg Lachno	Intercept	0.227		1.25458	0.556	0.197	2.49E-01		
Sample event precip (in)		-0.643	dec	0.52566	0.345	0.339	5.76E-02	21%	50%

Table 22. Summary of successful logistic regression analysis for human FIB markers based on a positive or negative result.

Based on the results of the logistic regression analyses, the only variables correlated with a positive result for human FIB markers were rainfall event duration and 24-hour antecedent conditions. The regression indicates that increasing rainfall duration results in a 50% higher probability of both human markers testing positively. This is also true for human *Bac* and 24-hour antecedent conditions, where increasing 24-hour antecedent precipitation results in a 75% probability of obtaining a positive result for human *Bac*. The confidence intervals were calculated based on percent probability and fall well within the range of certainty (50 – 51% for duration and antecedent rainfall for *Lachno* and 15 – 36% for antecedent rainfall for human *Bac*).

The remaining logistic analyses suggest decreasing trends in both human *Bac* and *Lachno* in the event of increasing the independent variable. The likelihood/probability of a positive human FIB result decreases very slightly with increasing rainfall intensity; so increasing rainfall in a short period of time decreases the odds of getting a positive human marker. We can back-calculate the probability of a positive FIB result with a given intensity measurement, for example: let's say it rained 0.2 in one hour. In this scenario, we know that the Odds Ratio for human *Bac* and rainfall intensity is 0.004 (log odds coefficient = -5.62, EXP(-5.62) = 0.004); for a 0.2 in/hr rainfall intensity, we see a 33% ($OR^{\wedge 0.2}$ = 0.33) reduction in the likelihood of a positive human *Bac* result.

In general, the results of the logistic regression analysis indicate that longer rains and increased rainfall prior to a sampling event (antecedent conditions) result in a positive correlation with FIB presence. On the contrary, increasing in rainfall intensity, precipitation volume and overland flow discharge seem to lessen the likelihood of a positive result. Figure 15 illustrates the logistic regression results indicating a likelihood of decreasing concentrations of human *Bac* with increased rainfall intensity and overland flow discharge volume (represented as Qmax). The graph depicted in Figure 15 is not a direct interpretation of the logistic model, rather it is to be used as an interpretative illustration of the model results.

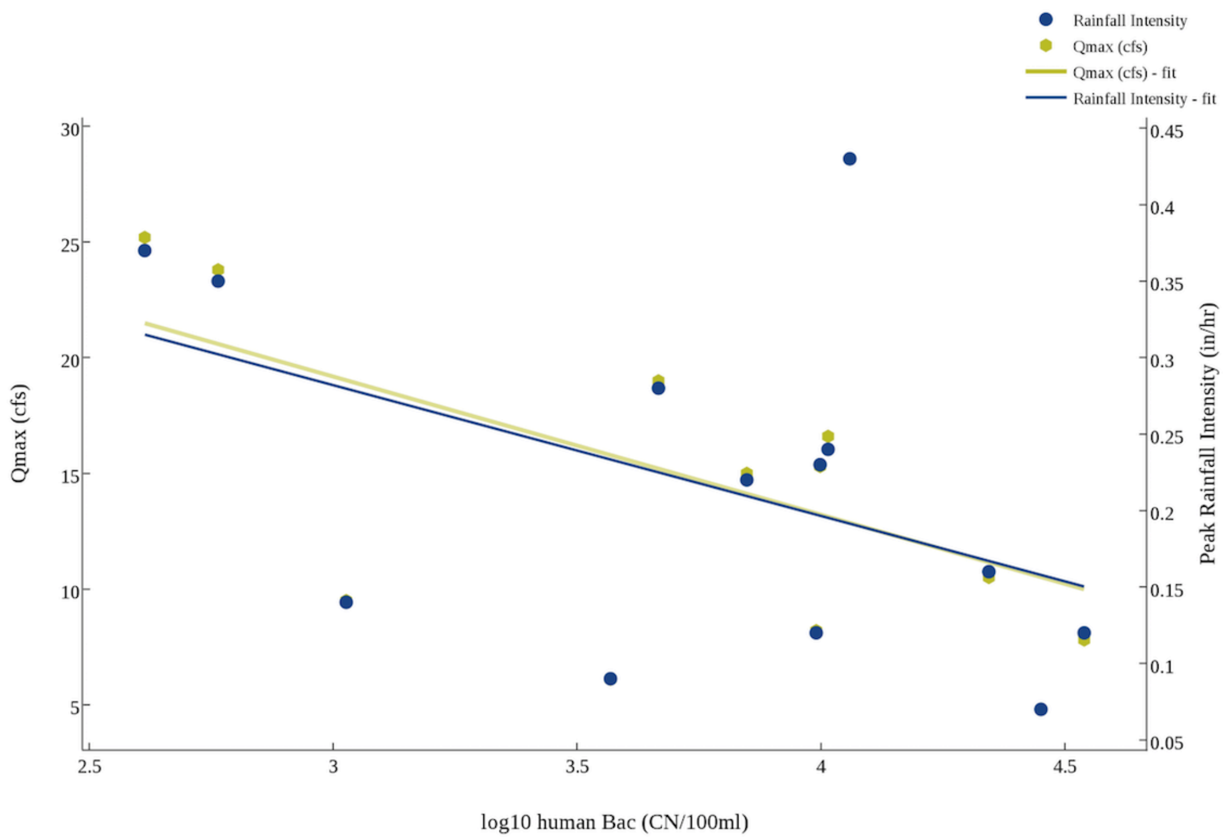

Figure 15. Illustration depicting log10 transformed mean human *Bac* decreasing concentrations with increased rainfall intensity and overland flow discharge volume (represented as Qmax).

The results of the logistic regression model for rainfall event duration are consistent with our findings from the ANOVA analyses, where longer duration rainfall events demonstrated higher mean FIB concentrations. The logistic model results evaluating sample event precipitation volume also supports our ANOVA results for the rainfall type category, where heavier rains demonstrated lower concentrations of both human *Bac* and *Lachno*. The logistic model results summarized in Table 24, also suggests that rainfall intensity, peak discharge of overland flow and peak streamflow are correlated with decreasing probability of a positive human FIB results. From the negatively correlated logistic results, we can deduce that intense heavy flow rains dilute the system. In contrast, steady longer medium flow events seem to be correlated with increasing likelihood of a positive human FIB result.

Chapter 3.3 – Groundwater Mechanisms

Our hypothesis for the groundwater portion of this research suggests that contamination from leaking laterals and sanitary lines is able to travel through the subsurface, via groundwater flow and transport especially where "preferential pathways" are present. We hypothesize that the sand and gravel backfill surrounding the stormsewer pipes acts as a preferential pathway corridor for contaminated groundwater and results in the infiltration of this water to the stormwater lines. These preferential pathway corridors typically exhibit certain characteristics, including elevated potential for hydraulic conductivity, which differentiate them from the undisturbed native soils that may prohibit or lessen contaminant flow and transport due to their presumed lower hydraulic conductivity. The goal of this objective was to assess the physical and hydrogeographical components of the stormwater system in relation to FIB in the groundwater. The City of Wauwatosa approved of the installation of 3 temporary monitoring wells (WAMW01 – WAMW03) to be installed within the utility corridor of select construction zones. The wells were designed to capture subsurface flow within the utility corridor in an effort to evaluate the presence or absence of FIB in the newly constructed utility corridor.

Section 3.3.1 – Monitoring Well Data

Typical groundwater elevations within the study area were identified as being approximately 9-11 feet bgs (WDNR File Review, January 2013), and according to Maggie Anderson, Wauwatosa City Engineer, groundwater elevations can, at times be in contact with sanitary and stormwater pipes.

We installed three (3) groundwater monitoring wells within the gravel backfill of the newly installed sanitary pipe along Wright Street at Wright Street and 83rd (WAMW01 and WAMW02) and Wright Street and 86th (WAMW03). Groundwater elevation measurements were collected from each well and if sufficient water was present, samples were collected and analyzed for molecular and microbiological parameters. Groundwater samples were analyzed for traditional culture-based FIB and human markers human *Bac* and human *Lachno* in each of the samples collected.

During sample collection from the existing monitoring wells installed, groundwater elevations were measured to be on average between 9 feet bgs (WAMW01 and WAMW02) to 16 feet bgs (WAMW03). Groundwater samples were collected from the wells on 10 to 22 different occasions, when groundwater was present in the sample collection chamber. Monitoring well WAMW02 was unable to be sampled from on many occasions as the PVC was cracked during installation making it difficult to collect a groundwater sample.

The results of the groundwater sample analysis indicate that concentrations of human *Bac* were detected in each of the three wells on multiple sampling occasions. Monitoring well WAMW01 exhibited an average human *Bac* concentration of 1,969 CN/100 ml which is above the threshold of 1000 CN/100 ml. However, human FIB presence in groundwater is not considered to be persistent as only 33% of the samples collected from WAMW01 were identified as positive (above threshold). Table 23, below provides a summary of the statistics for each of the monitoring wells installed within the study area.

Monitoring Well ID	Mean human *Bac* (CN/100ml)	# of events	% Positive	Well Depth (ft)	Site Elevation (ft msl)	Well Depth (ft msl)	Average gw Elevation (ft)	Adjusted gw elevation (msl)	Record high gw elevation (ft msl)
WAMW01	1,969	15	33%	11.8	717	705.2	9.7	707.3	709.4
WAMW02	561	10	20%	11.0	717	706.0	9.3	707.7	709.0
WAMW03	427	22	9%	16.3	713	696.7	16.3	696.7	699.0

Table 23. Summary of groundwater monitoring well data. Mean human Bac for WAMW01 was above threshold and 33% positive out of 15 samples. Monitoring wells WAMW02 and WAMW03 had concentrations of human Bac below threshold and were only 20% and 9% positive, respectively.

One explanation for the lack of adequate sample volume in WAMW02 and WAMW03 could be attributable to the fact that groundwater elevations in these area were too low to reach the well screen. As stated previously, average groundwater elevations in the area were recorded to be around 9 to 11 feet bgs and during the sampling timeframe of this study, lower than average precipitation values were observed. We would have expected to see similar concentrations of human *Bac* in the nested wells WAMW01 and WAMW02, however these results are likely skewed due to the possible broken PVC screen at WAMW02 and lower than average groundwater elevations.

Monitoring well WAMW03, located downgradient at 86th and Wright was able to provide water consistently (N:22), despite the lower groundwater elevations observed at this location. Groundwater does not appear to be impacted within the vicinity of this well, which is consistent with the mass balance calculations performed for Wright Street which demonstrated decreasing contaminant trends with decreasing elevation and increasing drainage. Figure 16 below provides a cross-section of the wells installed on Wright Street. The green bars represent the mean concentration of human *Bac* observed at each location, and the blue dashed line represents the estimated subsurface groundwater elevation at the time of sample collection.

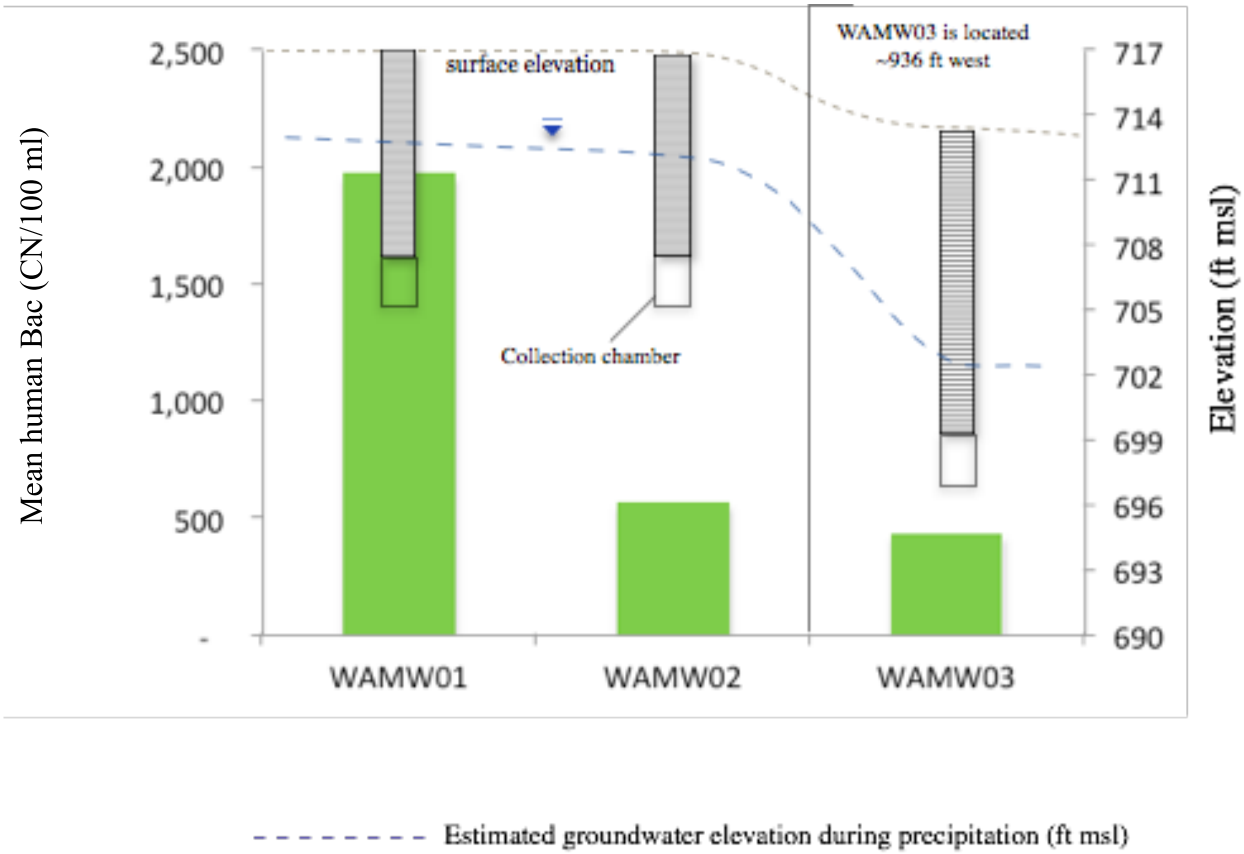

Figure 16. Cross-section diagram depicting the average human *Bac* concentrations observed for each monitoring well. Lower than average groundwater elevations are assumed for this sampling period. Due to lower than average rainfall volumes recorded for 2012-2014, samples were collected from the wells during or shortly after rainfall when the water table was considered to be at its peak.

Only seven of the 22 total monitoring well sampling events had samples collected from all three wells. On May 29, 2013 nested wells WAMW01 and WAMW02 had concentrations of human *Bac* exceeding threshold at 1,796 and 2,031 CN/100 ml, respectively, but the sample collected from downgradient well WAMW03 indicated a concentration below level of detection (BLD). The manhole samples collected on that event exhibited an average concentration of human *Bac* below threshold (857 CN/100 ml), suggesting that the event was not a significant contributor of human FIB to the Menomonee River. Although the precipitation measured for May 29, 2013 was considered baseline, the 24-hour antecedent volume was over 1.5 inches of rain.

The only other date demonstrating increased concentrations of human *Bac* in all three wells was in March 2014, during a snowmelt event. Monitoring well WAMW01 exhibited the highest concentration of human Bac at 11,712 CN/100 ml and WAMW02 had a concentration of 2,342 CN/100 ml. Downgradient well WAMW03 exhibited a concentration of HB at 3,856 CN/100 ml. The only stormwater sample collected on March 19, 2014 was from terminal outfall WA01, which demonstrated a human *Bac* concentration of 3,838 CN/100 ml.

Although only one sample was able to be collected during the monitoring well sampling event on January 29, 2013, the concentration of human Bac observed at WAMW01 for that date was 10,494 CN/100 ml, and the precipitation volume recorded for that date was 1.28 inches. However, not all heavy rainfall events demonstrated this trend. On June 25, 2013 a heavy rainfall of 1.45 was recorded for that date and none of the groundwater samples collected (N:3) exhibited concentrations above threshold. The results of the groundwater investigation for this project remain inconclusive as water was not able to be collected from each well on every sample date, however there appears to be a association between human FIB presence and event precipitation and/or 24-hour antecedent conditions. The results of this study suggest that saturated subsurface conditions yield high concentrations of human *Bac.* Additional research is needed to investigate this phenomena further.

Section 3.4 – Conclusions

The results of the before and after analysis for stormwater basin M09020N1 demonstrate a significant improvement in the quality of stormwater runoff entering the Menomonee River, with a 10-fold reduction in average human FIB concentrations observed in the post-construction samples collected. The age of pipe analyses did not show correlation in driving overall FIB concentrations. Rather, levels at all the sites fluctuated in conjunction with sample day. Age of pipe did appear to contribute a significant load of human FIB to the system as demonstrated by the mass balance mixing calculations and subsequent ANOVA analyses, where a single old/old category manhole (WA18) appears to be the primary contributor of human FIB to the pipeline along Wright Street and to Meinecke at WA11 through WA09. Further work is needed to delineate the weather or hydrological drivers across different events.

The t-tests performed on the physical categories: artery and mainline, and depth of pipe demonstrated conflicting, but perhaps insightful results. Smaller diameter artery pipes located along the ‘feeder’ streets had higher mean concentrations of human FIB than the larger mainline pipes located within the main corridor. We hypothesize that this occurs as a result of leaking laterals that are within close proximity to the smaller stormwater lines. Conversely, deeper pipes (located at WA03 and WA09 through WA11) exhibited higher concentrations of human FIB than their shallower counterparts. The results of the mass balance model and subsequent ANOVA analyses provide some insight as to why we observed higher concentrations of human FIB in the deeper pipes when compared to the shallow pipes. The Meinecke Avenue mass balance model indicates a high contribution of human FIB between WA11 and WA09, where increasing concentrations and peak mixing ratios were observed. Meanwhile, the Wright Street mass balance model indicates decreasing concentrations of human FIB from upgradient manhole WA18 to downgradient manhole WA16, where the storm system changes flow south along 85th Street and is delivered to Meinecke where WA11 is located. The ANOVA analysis comparing each street's mean human FIB value to each other supports the mass balance model findings that

Wright Street is most likely contributing the human FIB concentrations to Meinecke Avenue (Wright (mean human *Bac* 17,718 CN/100 ml) to Meinecke (mean human *Bac* 6,852 CN/100 ml) with a pairwise p-value <0.01 (both human markers). WA18 is categorized as an Old/Old manhole site with an average human Bac concentration of 18,957 CN/100 ml. These results may be caused by two types of sanitary infiltration: the first being more direct inputs of sanitary due to breaches in the system and/or the presence of an illicit connection(s) and the second being diffuse contamination from the many presumed leaking laterals located along the older feeder streets and getting into the old stormwater pipeline near WA18.

Human *Bac* concentrations along Wright Street and 90th Street decreased in proportion to the accumulated drainage of each downgradient manhole. The mixing model for these pipelines indicate that although the pipeline may start with more concentrated FIB inputs, the cumulative effect of drainage and lack of additional inputs essentially dilutes the concentration as more water is added. It would appear that once the stormwater is conveyed to the main pipeline (none of which have laterals within proximity to the stormwater pipes), the concentration of human FIB is diluted with increasing stormwater volume added.

Seasonal one-way ANOVA analysis indicated differences amongst seasons, with summer demonstrating the lowest concentrations of FIB and winter snowmelt representing the highest mean concentrations. The sampling time period from December 2012 to June 2014 had below average precipitation volumes for each month with annual rainfall volumes less than 60% of the annual average total rainfall (Table 2). The extremely low rainfall amounts mean lower groundwater tables and less saturated conditions in the subsurface for this study area.

We also found that longer and steady duration rainfall events tended to have higher concentrations of human *Bac* than shorter and intense rainfall events. Peak rainfall intensity (in/hr), sample event precipitation volume (in), peak streamflow (cfs) and peak discharge of stormwater overland flow (cfs) were negatively correlated with presence of human *Bac*, suggesting that fast intense rains with high volumes may be diluting the system. This occurrence is illustrated in Figure 14 (Section 3.2.2) where an inverse relationship of human *Bac* is observed with rainfall volume and antecedent conditions, regardless of the age of pipe. The results of the ANOVA and logistic models for rainfall duration suggest increasing presence of human FIB with longer duration rainfall. We believe this to be occurring in part, due to saturated subsurface conditions acting to mobilize exfiltrated sanitary waste from adjacent laterals into the stormwater system.

Human *Bacteroides* in groundwater appears to be correlated with heavy rainfall events. One explanation for this finding is that broken laterals near the shallow subsurface exfiltrate to the nearby soils and during periods of prolonged or heavy rains, the soils become saturated and the exfiltrated bacteria is mobilized via subsurface flow. This finding may support our hypothesis that fluctuating groundwater elevation mobilizes human FIB to adjacent stormwater systems.

The findings presented in this study offer some insight as to the physical characteristics that influence human FIB concentration, but the results were not consistent for each sampling event. The environmental variability during our sampling collection period provides a snap-shot of what these systems endure annually and elucidates the variable nature of their physical environment. These observations summarize only a fraction of the system and its irregularities but there are overarching trends in the dataset that provide promising results. We observed a 40% decrease in percent positive human *Bac* concentrations in the samples collected from the post-construction timeframe, compared to the pre-construction sample results. Mass balance calculations provided insight as to what is happening within an ever changing system that contains multiple infrastructure conditions, where newer systems are more likely to demonstrate decreasing contaminant trends with increasing drainage area flow. Within those systems, contributions of human FIB appear to be inevitable but hot-spots can be traced, as evidenced by our findings from the mass balance model for Meinecke and Wright Street highlighting old/old site WA18 as a likely source. The primary mechanism observed to have the greatest influence on the presence and/or absence of human FIB in the system appears to be driven by date, with precipitation characteristics acting as the driving factor.

Section 3.4.1 – Additional Research and Recommendations

The results of this study highlight the need for a more extensive and robust sampling regimen to be conducted. If these multiple parameters are to be explored further, a power analysis should be run to evaluate the adequate sample size needed to explore select categorical and continuous variables response. First flush samples and concurrent (2^{nd} and 3^{rd} flush) samples should be collected and evaluated within each sample date for all sites. If possible, automated inline 24-hour composite stormwater samplers (such as an Isco Flowlink® device) should be installed within select manholes to evaluate concentrations of human FIB throughout a storm event. The use of an automated sampler will aid in reducing sampling error and will allow for stronger statistical power analysis due to the ability to collect samples from each location over a 24-hour time period.

Sampling of an adjacent stormwater basin and terminal outfall should also be conducted on a smaller scale to evaluate day to day variability within the system as a whole. Interactions among the explanatory variables should be explored further. Simple linear regression models were not suitable for this study as we observed immense variability among sample concentrations by site and date.

Groundwater could not be fully analyzed to meet the needs of this study as liability, access and expense were all extenuating circumstances. Groundwater monitoring should be evaluated further as each of the three wells installed for this study exhibited concentrations of human FIB above threshold. If groundwater monitoring is considered, proper installation of semi-permanent NR 141 groundwater monitoring wells should be installed within older systems at several suspect locations in both upgradient and downgradient positions to evaluate groundwater elevations and the potential for contaminant transport via groundwater.

The primary findings of this study provide evidence in support of infrastructure repair as a means to reduce human fecal load to nearby surface waters. However, the results demonstrate that costly infrastructure improvements do not eliminate the problem entirely. Detection of human FIB correlated with age of pipe, seasonality, rainfall duration and volume, antecedent conditions and select infrastructure conditions. The mechanisms of transport quantified in this study appear to be associated with infiltration of contaminated material from broken laterals and intermittent delivery inputs through specific weak points in the stormwater conveyance system. As such, the results of this study should prove useful to municipalities, water resource managers, urban planners, and policy makers who are seeking to rectify aging and failing sanitary lines in their attempts to reduce human fecal load to nearby surface waters.

REFERENCES

Ahmed, W. et al., 2009. Evaluation of multiple sewage-associated Bacteroides PCR markers for sewage pollution tracking. *Water Research*, 43(19), pp.4872–4877.

Ariaratnam, S.T., El-Assaly, A. & Yang, Y., 2001. Assessment of Infrastructure Inspection Needs Using Logistic Models. *Journal of Infrastructure Systems*, 7(4), pp.160–165.

Arnone, R.D. & Walling, J.P., 2007. Waterborne pathogens in urban watersheds. *Journal of Water and Health*, 5(1), p.149.

Bailey, J. et al., 2012. *Lake Superior Lakewide Management Plan: 1990-2010 Crital Chemical Reduction Milestones*,

Bannerman, R.T. et al., 1993. SOURCES OF POLLUTANTS IN WISCONSIN STORMWATER. *Water Science & Technology*, 23(3), pp.241–259.

Bernhard, A.E. & Field, K.G., 2000. Identification of Nonpoint Sources of Fecal Pollution in Coastal Waters by Using Host-Specific 16S Ribosomal DNA Genetic Markers from Fecal Anaerobes. *Applied and Environmental Microbiology*, 66(4), pp.1587–1594.

Blackwood, D.J. et al., 2005. Factors influencing exfiltration processes in sewers. *Water Science and Technology*, 51(2), pp.147–154.

Bower, P.A. et al., 2005. Detection of genetic markers of fecal indicator bacteria in Lake Michigan and determination of their relationship to Escherichia coli densities using standard microbiological methods. *Applied and environmental microbiology*, 71(12), pp.8305–13.

Bradbury, K., Borchardt, M. & Gotkowitz, M., 2012. *Evaluation of Sanitary Sewers as a Source of Pathogen Contamination of Municipal Water Supply Wells*, Madison.

Dick, L.K. & Field, K.G., 2004. Rapid estimation of numbers of fecal Bacteroidetes by use of a quantitative PCR assay for 16S rRNA genes. *Applied and environmental microbiology*, 70(9), p.5695e5697.

Ellis, J.B. et al., 2003. Experimental studies of sewer exfiltration. *Water Science and Technology*, 47(4), pp.61–67.

Faraone, A.M. et al., 2013. *A REGIONAL WATER QUALITY MANAGEMENT PLAN UPDATE FOR THE GREATER MILWAUKEE*, Waukesha.

Gaffield, S.J. et al., 2003. Public Health Effects of Inadequately Managed Stormwater Runoff. *American Journal of Public Health*, 93(9), pp.1527–1533.

Geldreich, E. et al., 1968. The Bacteriological Aspects of Stormwater Pollution. *Water Pollution Control Federation*, 40(11), pp.1861–1872.

Google Earth, 2015. Wauwatosa, Wisconsin 43° 3’21.86“N, 88° 1’31.24”W.

Harwood, V.J. et al., 2014. Microbial source tracking markers for detection of fecal contamination in environmental waters: relationships between pathogens and human health outcomes. *FEMS microbiology reviews*, 38(1), pp.1–40.

Hunt, R.J. et al., 2010. Assessment of sewer source contamination of drinking water wells using tracers and human enteric viruses. *Environmental science & technology*, 44(20), pp.7956–63.

Kildare, B.J. et al., 2007. 16S rRNA-based assays for quantitative detection of universal, human-, cow-, and dog-specific fecal Bacteroidales: a Bayesian approach. *Water research*, 41(16), pp.3701–15.

Kummu, M. et al., 2011. How close do we live to water? A global analysis of population distance to freshwater bodies. *PloS one*, 6(6), p.e20578.

Mclellan, S.L. et al., 2007. Distribution and Fate of Escherichia coli in Lake Michigan Following Contamination with Urban Stormwater and Combined Sewer Overflows. *Journal of Great Lakes Research*, 33(3), p.566.

Mclellan, S.L. et al., 2010. Diversity and population structure of sewage-derived microorganisms in wastewater treatment plant influent. *Environmental microbiology*, 12(2), pp.378–92.

Mclellan, S.L. & Dila, D.K., 2013. *Greater Milwaukee Watersheds Pathogen Source Identification*, Milwaukee.

Mclellan, S.L. & Sauer, E.P., 2009. *Greater Milwaukee Watersheds Pathogen Source Identification Executive Summary Greater Milwaukee Watersheds Pathogen Source Identification Executive Summary*, Milwaukee.

Mickleson, D.. et al., 1984. *Pleistocene stratigraphy Units of WI, Wi Geological and Natural History Survey*.,

Milwaukee River Keepers, 2012. Milwaukee River Report Card Milwaukee River Basin Grade : What Do the Grades Mean? , pp.3–5. Available at: http://www.milwaukeeriverkeepers.org.

National Oceanic & Atmospheric Administration (NOAA), Average rainfall for Milwaukee Mt. Mary College (43.0719°, -88.0294°). Available at: http://www.ncdc.noaa.gov/cdo-web/datatools/normals [Accessed January 1, 2015].

Newton, R.J. et al., 2013. A microbial signature approach to identify fecal pollution in the waters off an urbanized coast of Lake Michigan. *Microbial ecology*, 65(4), pp.1011–23.

Newton, R.J. et al., 2011. Lachnospiraceae and Bacteroidales alternative fecal indicators reveal chronic human sewage contamination in an urban harbor. *Applied and environmental microbiology*, 77(19), pp.6972–81.

O'shea, M.L. & Field, R., 1992. Detection and disinfection of pathogens in storm-generated flows. *Canadian Journal of Microbiology*, 38(4), pp.267–276.

Pan, X. & Jones, K., 2012. Seasonal variation of fecal indicator bacteria in storm events within the US stormwater database. *Water Science & Technology*, 65(6), pp.1076–1080.

Rutsch, M. et al., 2008. Towards a better understanding of sewer exfiltration. *Water Research*, 42(10-11), pp.2385–2394.

Salmore, A.K., Hollis, E.J. & Mclellan, S.L., 2006. Delineation of a chemical and biological signature for stormwater pollution in an urban river. *Journal of Water and Health*, 4, pp.247–262.

Sauer, E.P. et al., 2011. Detection of the human specific Bacteroides genetic marker provides evidence of widespread sewage contamination of stormwater in the urban environment. *Water research*, 45(14), pp.4081–91.

Selvakumar, A. & Borst, M., 2006. Variation of microorganism concentrations in urban stormwater runoff with land use and seasons. *Journal of Water and Health*, 4, pp.109–124.

Sercu, B. et al., 2011. Sewage exfiltration as a source of storm drain contamination during dry weather in urban watersheds. *Environmental science & technology*, 45(17), pp.7151–7.

Shoblom, A., 2014. Hydraulic Design Manual Appendix F - Rational Method. In E. and A. M. U. G.-E. Section, ed. *Hydraulic Design Manual*. Oregon Department of Transportation, p. Appendix F – Rational Method.

Sidhu, J.P.S. et al., 2013. Sewage pollution in urban stormwater runoff as evident from the widespread presence of multiple microbial and chemical source tracking markers. *The Science of the total environment*, 463-464, pp.488–96.

USEPA, 2006. Method 1600: Enterococci in Water by Membrane Filtration Using Membrane-Enterococus Indoxyl-B-D- Glucoside Agar (mEI).

USEPA, 2002. Method 1603: Escherichia coli (E. coli) in Water by Membrane Filtration Using Modified Membrane- thermotolerant Escherichia coli Agar (Modified mTEC).

Wisconsin Department of Natural Resources, 2010. *Menomonee Watershed Plan*,