

Greater Milwaukee Watersheds Pathogen Source Identification

Executive Summary

MMSD Contract No. M03016P02



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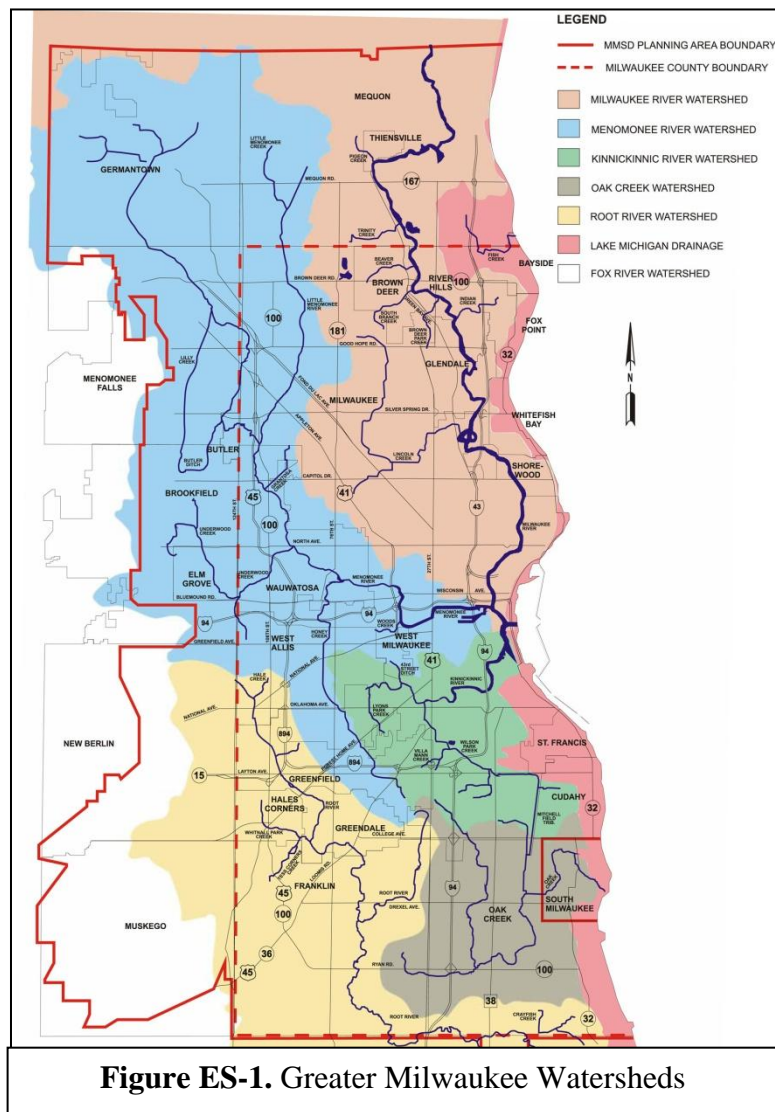


EXECUTIVE SUMMARY

Background and Significance

The overall goal of this project was to determine sources of high fecal indicator bacteria in stormwater discharges within the Milwaukee Metropolitan Sewerage District (MMSD) service area. This research addresses a high priority area for the MMSD, whose mission is to protect public health and the environment and improve water quality. Prior analysis during the MMSD's 2020 *Facilities Planning* effort, which also included work by the Southeastern Wisconsin Regional Planning Commission (SEWRPC) to update the *Regional Water Quality Management Plan (RWQMP)*, and the McLellan Bacterial Genetics Laboratory (functioning as part of the University of Milwaukee's Great Lakes WATER Institute) had identified stormwater inputs as a major source of water quality impairment in the rivers of the Greater Milwaukee Watersheds (**Figure ES-1**) and subsequently Lake Michigan. High levels of fecal coliforms and *Escherichia coli* (*E. coli*) have been routinely detected in the absence of reported sanitary or combined sewage overflows; however the source of this bacterial contamination is currently unknown or unrecognized.

The water quality goals for the region include reducing the number of days surface waters exceed the State of Wisconsin's water quality standard for fecal coliforms. According to SEWRPC's 2009 *Regional Water Quality Management Plan*, Milwaukee Area rivers often exceed recreational standards. In 1998 to 2001, fecal coliform levels in both the Menomonee and Kinnickinnic Rivers often exceeded the water quality standard of 1,000 colony forming units (CFUs) per 100 ml of sample (water), which is a special variance standard for limited recreational use. For example, the Menomonee River station located between 25th Street and North 70th Street exceeded this standard in 38% of samples and the Kinnickinnic River station located between South 7th Street and South 27th Street exceeded the standard in 49% of samples (SEWRPC, 2008). Sites along the Milwaukee River that are



designated as limited recreational use were usually in compliance (~75% of samples in compliance). However, Milwaukee River sites that are designated full recreational use are expected to meet a more stringent standard of 200 CFUs/100 ml; these sites often exceeded the limit (SEWRPC, 2008).

In order to meet water quality goals for swimmable, fishable waters, it is critical to determine what the major sources of pollution are so that remediation strategies can be formulated and implemented. While upstream rural sources account for a portion of the fecal indicator bacteria, a larger portion is derived from stormwater runoff in the urbanized areas (SEWRPC, 2008). It should be noted that water quality standards and monitoring are based upon an indicator bacteria that is only a general proxy for the presence of fecal pollution and disease-causing organisms (e.g. pathogens). Fecal pollution from different sources will carry different pathogens; however fecal pollution from sanitary sewage generally constitutes a more serious public health risk because multiple human pathogens including bacteria, viruses, and protozoan can be present in high concentrations. Additionally, agricultural/rural waste may also pose a public health risk due to pathogens such as *E. coli* O157:H7, *Giardia*, or *Cryptosporidium parvum*. The numbers and types of pathogens associated with stormwater (non-point) runoff (pet and wildlife waste) are not well known. Domestic pets and wildlife may carry some organisms that are pathogenic to humans such as *Salmonella* or *Campylobacter*, but overall, pathogen occurrence is expected to be much less than what is found in sanitary sewage. This means that even small inputs of sanitary sewage that results in only a modest increase in fecal indicator bacteria may be more significant in terms of health risk than large fecal indicator inputs from surface runoff that is free of sanitary sewage contamination.

Results and Discussion

The McLellan Bacterial Genetics Laboratory has developed DNA based methodology for detecting human sources of fecal pollution. This approach is based upon detecting a certain bacteria type that is specifically found in humans, a species of *Bacteroides*, which was first described by Field and co-workers (Bernhard & Field, 2000). This bacterium is found and is present in almost all humans, but not other animals. The McLellan Laboratory and others have found that the human *Bacteroides* genetic marker (e.g. the specific 16S rRNA gene sequence that identifies this organism) is a sensitive and specific indicator of sanitary sewage contamination (Stewart, *et al.*, 2003, Bower, *et al.*, 2005, Santoro & Boehm, 2007). Detection is based upon amplification of the 16S rRNA gene using polymerase chain reaction (PCR). If the human *Bacteroides* is present in a water sample, the genetic marker for this organism can be amplified and thereby be measured as to its relative strength.

The McLellan Bacterial Genetics Laboratory analyzed more than 1,000 stormwater samples (including inline stormwater and grab samples) from 62 municipal stormwater discharge locations over a three-year period for the presence of the human *Bacteroides* genetic marker (**Figure ES-2**). Three stormwater outfalls along Lake Michigan had positive results in more than 70% of the samples tested. Stormwater outfalls along the Menomonee River were positive in 73% of the samples tested (**Table ES-1**).

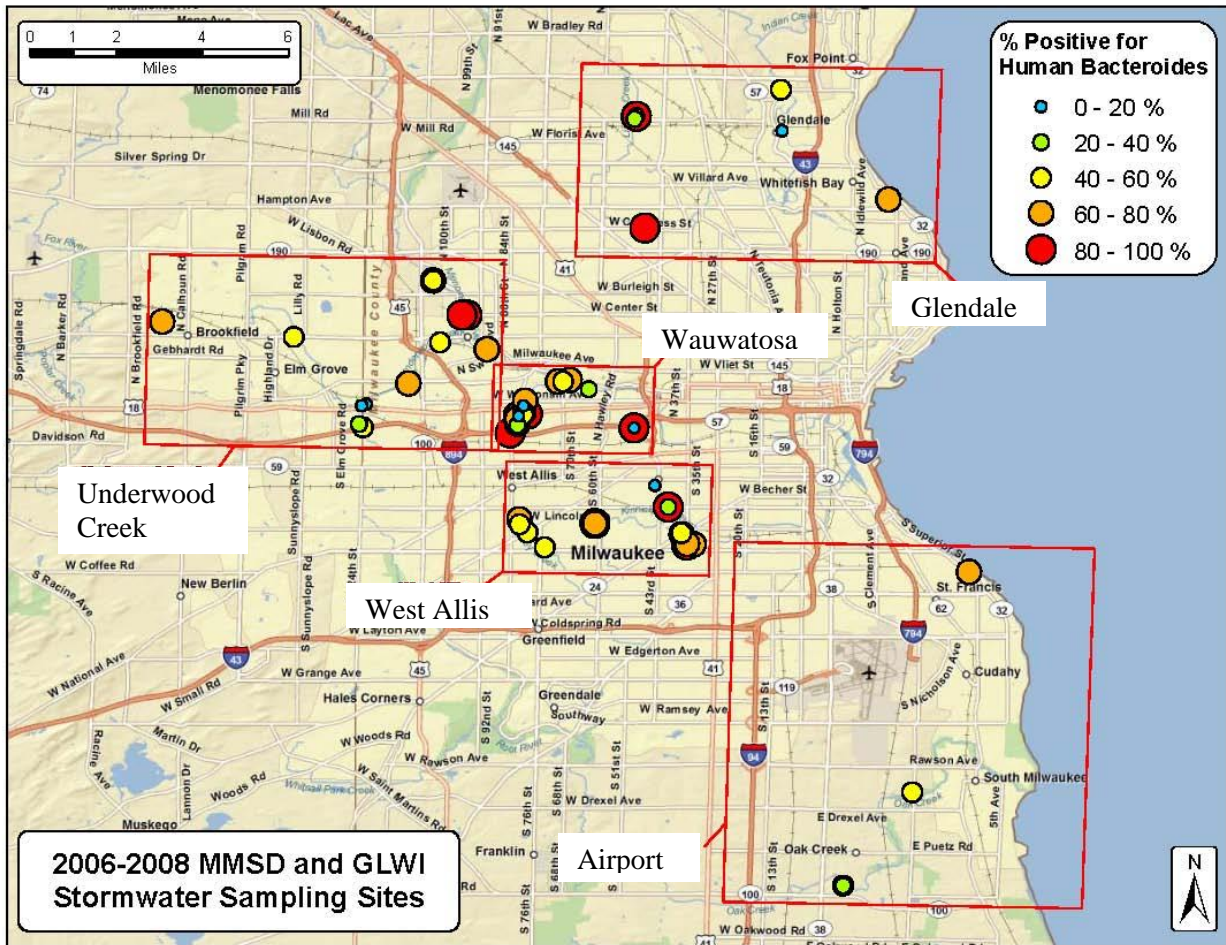


Figure ES-2: Percent of samples positive for the human *Bacteroides* genetic marker at MMSD and McLellan Laboratory stormwater site locations.

Similarly, stormwater outfalls along the Kinnickinnic River and Lincoln Creek were found to have a persistent signal of human *Bacteroides*, with 60% and 65% of the samples testing positive, respectively. The other four receiving waters, Honey Creek, Underwood Creek, Oak Creek, and the Milwaukee River, also demonstrated evidence of sanitary sewage contamination of stormwater. Overall, these results indicate that sanitary sewage contamination of stormwater is a serious concern throughout the Greater Milwaukee Watersheds, and locally owned stormwater collection & conveyance systems in specific areas should be given high priority for further investigation: those discharging directly to Lake Michigan, and those within the Menomonee, Lincoln Creek, and Kinnickinnic watersheds.

One stormwater outfall along Lincoln Creek was tested for the presence of human viruses since samples from this location demonstrated a persistent signal of human *Bacteroides* marker. Virus detection was performed by Dr. Mark Borchardt's laboratory at Marshfield Clinic (Marshfield, WI). High concentrations of three human viruses were found, including adenovirus, enterovirus, and G1 norovirus. These concentrations are similar to what is found in sewage influent and confirms the presence of human sewage contamination in this stormwater outfall. This level of human virus contamination in stormwater constitutes a potential public health risk.

Table ES-1: Summary of *E. coli*, Enterococcus and human *Bacteroides* genetic marker data compiled by receiving waters for stormwater samples collected by MMSD and GLWI during the 2006-2008 sampling seasons.

Receiving Waters	Number of Samples 2006-2008	% Positive for human <i>Bacteroides</i>	<i>E. coli</i>		Enterococcus	
			Average (CFUs/100ml)	Geometric Mean (CFUs/100ml)	Average (CFUs/100ml)	Geometric Mean (CFUs/100ml)
Milwaukee River	12	33%	6,550	4,040	6,020	4,250
Oak Creek	93	39%	358,000	9,020	153,000	11,000
Underwood Creek	74	42%	113,000	12,600	158,000	13,500
Honey Creek	203	51%	40,300	5,850	43,400	6,990
Kinnickinnic River	201	60%	329,000	19,700	175,000	21,600
Lincoln Creek	108	65%	51,100	14,600	60,500	9,770
Menomonee River*	209	73%	382,000	24,300	111,000	21,100
Lake Michigan	95	71%	364,000	36,400	211,000	29,400

*two representative samples from each SWWA13 pollutograph were included in this analysis. Special sampling at Miller Park, SWMI18 is not included in this analysis.

** Russell Ave outfall is not included because it is a submerged outfall; indicator levels may not accurately reflect fecal pollution levels.

The findings of this research showed no correlation between the presence of the human specific *Bacteroides* marker and culturable *E. coli* and enterococci. This demonstrates that human sources are only one contributor (of many) to fecal indicator bacteria. However, even a low amount of human sources may carry pathogens and therefore could pose a significant health risk, regardless of the contribution of fecal indicator bacteria. The relationship between rainfall amounts and the numbers of human marker positives were evaluated to explore possible connections between, for example, high rainfall amounts and subsequent ground saturation favoring infiltration/exfiltration of the sanitary system. No relationship between rainfall and positive results were found. Certain dates had higher numbers of stormwater outfalls positive across the five watersheds, suggesting that the dynamics of a storm event may contribute to the process by which sanitary sewage enters the stormwater collection & conveyance systems and different conditions can vary by community and soil types. Identifying the environmental and climate variables that are linked with the presence of sanitary sewage contamination in the stormwater collection & conveyance systems warrants further research.

A major challenge in advancing this research is to develop a quantitative assay for the human specific marker. The McLellan Bacterial Genetics Laboratory has developed a qPCR (quantitative PCR) assay for three fecal indicators (*E. coli*, enterococci, and total *Bacteroides* spp.) and the human *Bacteroides* genetic marker. This allows for the comparison of “total fecal pollution” to the “human specific” portion using the same methodology (e.g. by DNA based methods). A high ratio of human to total fecal pollution would indicate the major source is

sanitary sewage. Another major challenge in this work is to recover and amplify bacterial DNA from environmental samples that contain compounds that inhibits the PCR reaction. This is a problem when working with any environmental sample (soil, water, etc.), but stormwater appears to be particularly problematic in this regard due to high amounts of other confounding contaminants in the sample. To address this issue an inhibitor assay was developed in this study to determine if the PCR reaction is impaired, and to what degree. This assay was utilized in conjunction with the quantitative assay to accurately quantify the human *Bacteroides* genetic marker, and in traditional PCR to determine if there are potentially false negative results, e.g. PCR completely impaired due to inhibition. The quantitative assay requires validation before it can be employed for investigative studies, however it was used in this study to give a general sense of the level of sanitary sewage contamination.

Successes

The human *Bacteroides* genetic marker has been used successfully in a number of spin-off investigations that originated from early results of this study. Working with MMSD monitoring crews, an outfall near Miller Park in Milwaukee was found to have positive results for the human *Bacteroides* genetic marker in nearly 100% of the samples. In March of 2007, dye testing was conducted to investigate the potential of illicit connections (**Figures ES-3 and ES-4**). It was found that some of the stadium's sanitary sewage lines (Luxury Box level) were mistakenly connected to a stormwater line. The problem was immediately remedied and the outfall has since tested negative for sanitary waste.

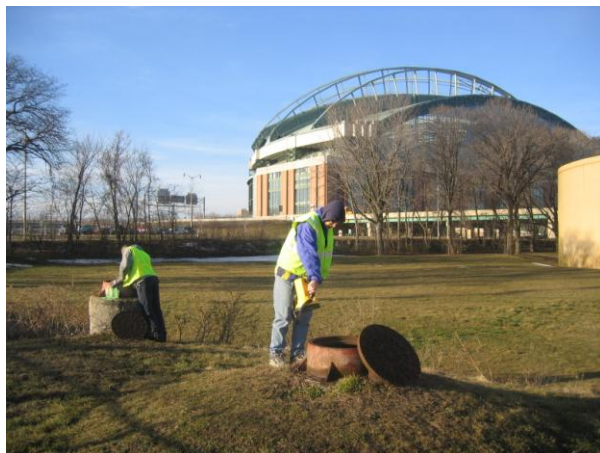


Figure ES-3. Dye testing of sanitary sewer lines near Miller Park. Testing for the human *Bacteroides* genetic marker was positive in a nearby stormwater outfall.



Figure ES-4. Fluorescein dye, which was introduced into sanitary facilities within Miller Park, was released through a stormwater outfall on the Menomonee River.

Stormwater outfalls along Honey Creek north of I-94 were tested for the presence of the human *Bacteroides* marker because of consistently high bacteria levels reported by MMSD's Water Quality monitoring group for this section of Honey Creek. Based upon these results (consistent detection of the human *Bacteroides* genetic marker) a separate investigation was conducted by the City of Milwaukee, in the area near 72nd Street and Mt Vernon Avenue. Smoke and dye

testing was conducted and in this case it was found that the sanitary sewer line was directly on top of the stormwater line, both which had multiple fractures/cracks. The lines were televised during dye testing and showed infiltration into the stormwater lines at several points. The sanitary sewer pipes have since been lined and the area is currently being retested.

Communication and Outreach

One of the McLellan Bacterial Genetics Laboratory's primary goals is to educate the public and integrate this information into the decision-making process. As citizens understand the impacts of urban stormwater on rivers and Lake Michigan, they may be more willing to support investments in infrastructure and expect their communities to address serious pollution inputs such as illicit cross connections or failing sewer lines. The McLellan Bacterial Genetics Laboratory staff has made numerous presentations in the past three years conveying research findings and illustrating the impact of urban stormwater on water quality.

The McLellan Bacterial Genetics Laboratory has also worked with nonprofit groups to cooperate with citizen monitoring efforts and also continues to maintain a green roof (**Figure ES-5**) that was funded through MMSD in 2003 as a model for others to implement their own stormwater runoff reduction/retention strategies. The McLellan Bacterial Genetics Laboratory provides the public with tours of the green roof, which also serves as an outdoor classroom for GLWI and UWM. In addition Dr. McLellan has served on two key committees, SEWRPC Water Quality Modeling Subcommittee and the Southeastern Wisconsin Watersheds Trust Science Council (SWWT), and has highlighted research findings from this work during discussions.



Figure ES-5. Green roof at the GLWI; a demonstration project to highlight strategies for mitigating stormwater runoff.

Conclusions and Recommendations

Overall, this research demonstrates that unknown or unrecognized sanitary sewage inputs into the local municipal stormwater collection & conveyance system are a major source of fecal pollution in area waterways and Lake Michigan. This poses a potential, yet serious public health risk due to the likelihood of other pathogen occurrence. The following recommendations are made from this research:

(1) Investigate sanitary and storm sewers infrastructure integrity by community using traditional engineering approaches (dye testing, smoke testing, etc.) in areas of the stormwater collection & conveyance system where stormwater has shown evidence of chronic sanitary sewage discharges. This project has identified approximately 41 high priority stormwater outfalls of the 62 investigated. Stormwater outfalls that tested positive for the human *Bacteroides* genetic

marker most frequently should be a high priority for follow-up investigation by the locally responsible communities. Additional stormwater outfalls in these high priority areas should also be tested (see recommendation #2). A concentrated investigative effort in the stormwater system may provide valuable insight into the nature of infrastructure failures (sanitary sewers and storm sewers) in the urban environment.

(2) Broaden the investigation in areas of concern using quantitative methods to identify the stormwater outfalls and locations within the stormwater collection & conveyance system that have significant sanitary sewage contamination. There are specific areas of concern; Lake Michigan, Lincoln Creek, Menomonee and Kinnickinnic watersheds. This study has only investigated a very small fraction of the stormwater systems within these areas. Stormwater outfalls should be prioritized using quantitative results for the human *Bacteroides* genetic marker by qPCR. A comprehensive investigative effort in the stormwater collection & conveyance systems that discharge to these receiving waters should be conducted to guide formulation of watershed management plans and improvement/restoration goals.

(3) Perform human virus testing in conjunction with human *Bacteroides* genetic marker sampling. The McLellan Bacterial Genetics Laboratory found that conducting virus testing on a small volume of stormwater is feasible; it was found that the same concentration of human derived viruses found in sewage was also present in one stormwater sample. Quantification of human viruses will provide the basis for assessment of public health risks associated with sanitary sewage discharges caused either by illicit connections or failing integrity of the associated infrastructure.

(4) Identify hydrological, physical, and meteorological parameters that correlate with high levels of sanitary sewage contamination in stormwater outfalls to better understand the conditions that favor contamination processes. Findings from this research demonstrate that the human *Bacteroides* signal is intermittent; suggesting that exfiltration of sewage from sanitary sewer systems and subsequent migration into stormwater collection & conveyance systems is the mechanism in which contamination occurs. Mapping infrastructure age using geographical information systems (GIS) in conjunction with sanitary sewage detection in stormwater collection & conveyance systems may provide insight into relationships between sanitary sewer system age and lack of integrity, which would help prioritize capital improvement investments.

(5) Quantify the overall human contribution to fecal pollution in Lincoln Creek, Menomonee River and Kinnickinnic River and correlate levels with hydrological and climate parameters. Determining the contribution of human sources will allow a direct comparison of stormwater inputs to combined sewer overflows in terms of health risk. The real time monitoring stations operated by MMSD and USGS and the sampling capabilities at these stations offer an investigative platform to accomplish this goal. A quantitative estimate of sanitary sewage contamination in rivers can serve as a benchmark that can be used to evaluate the effectiveness of management strategies that are formulated and implemented through stakeholder groups like Watershed Action Teams (WATs) of the Southeastern Wisconsin Watersheds Trust (SWWT).

(6) Incorporate estimates of human sources to supplement fecal coliform levels in future modeling efforts. Considerable effort has been invested in defining fecal coliform loads in

relation to specific assessment points contained within the Greater Milwaukee Watersheds. Information as to the percentage of sanitary sewage contamination, in relation to total fecal pollution, can be incorporated into modeling efforts as a higher tier of information.

(7) Assimilate water quality data (e.g. *E. coli*) and physical parameters (e.g. age of infrastructure and diameter of pipe) from the GLWI, MMSD, and Milwaukee Riverkeepers into a GIS database. The GIS database would be able to (1) perform initial analysis; (2) serve as a central repository for geographic data; and (3) produce visually intuitive map figures that supplement study findings.

(8) Continue public education and outreach. The McLellan Bacterial Genetics Laboratory works with the Milwaukee Riverkeepers, the Urban Ecology Center, and other grassroots organizations to help educate the public and collaborate on citizen monitoring programs.

These recommendations provide the framework to progress to the next level of achieving better water quality in the Greater Milwaukee Metropolitan area. Quantification of human sources of bacteria in stormwater discharges and surface waters will provide a benchmark from which the improvements due to investments in removing illicit connections and repairing/replacing sanitary or storm sewer lines (or privately owned laterals) may be measured. Such repairs are expensive, and the benefits need to be evaluated on an ongoing basis. The research contained herein may offer new tools for more comprehensive assessments that include fecal pollution source identification, to enable better preservation, planning, and management of our water resources.