DISSERTATION

ADDRESSING BARRIERS TO THE WIDE-SCALE IMPLEMENTATION OF ROOF RUNOFF AND STORMWATER COLLECTION AND USE PROJECTS FOR NON-POTABLE END USES IN THE U.S.

Submitted by

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ABSTRACT

ADDRESSING BARRIERS TO THE WIDE-SCALE IMPLEMENTATION OF ROOF RUNOFF AND STORMWATER COLLECTION AND USE PROJECTS FOR NON-POTABLE END USES IN THE U.S.

Roof runoff and stormwater have the potential to serve as important local water sources and diversify the water budget portfolio in regions with dwindling water supplies and increasing populations. Due to the lack of guidance regulating the use of roof runoff for non-potable end uses, characterizing its microbial quality is necessary to promote roof runoff use across the U.S. Similarly, the degree of stormwater microbial contamination is still not well understood, and uncertainty about the required treatment is a barrier for the implementation of stormwater capture and use (SCU) projects. Stormwater runoff could become contaminated with human fecal matter in areas with aging infrastructure where raw wastewater exfiltrate from sewer networks to stormwater collection networks, areas with homeless encampments, or areas with sanitary sewer overflows (SSOs). Stormwater practitioners wanting to benefit from stormwater to augment the available water resources struggle with the selection and design of efficient stormwater treatment trains that are protective of public health for the designated end use. Knowledge of the degree to which stormwater is contaminated with human fecal matter, termed here as the human fecal contamination analog (HFCA), is critical for the design process and estimating the required pathogen log reduction targets (LRTs).

To address the barrier to wide-scale implementations of roof runoff collection and use projects, a 2-year research study was designed to examine roof runoff microbial quality in four

U.S. cities: Fort Collins, CO; Tucson, AZ; Baltimore, MD; and Miami, FL. Sample collection was conducted as part of a citizen science approach. The occurrence and concentrations of indicator organisms (*E. coli* and enterococci) and potentially human-infectious pathogens (PHIPs) including *Salmonella* spp., *Campylobacter* spp., *Giardia duodenalis*, and *Cryptosporidium parvum* in roof runoff were determined using culture methods and digital droplet polymerase chain reaction (ddPCR), respectively. *E. coli* and enterococci were detected in 73.4% and 96.2% of the analyzed samples, respectively. Concentrations of both *E. coli* and enterococci ranged from <0 $\log_{10}$ to >3.38 $\log_{10}$ MPN/100 mL. *Salmonella* spp. *invA*, *Campylobacter* spp. *ceuE*, and *G. duodenalis β – giardin* gene targets were detected in 8.9%, 2.5%, and 5.1% of the analyzed samples, respectively. *Campylobacter* spp. *mapA* and *C. parvum* 18S rRNA gene targets were not detected in any of the analyzed samples. This dataset represents the largest-scale study to date of enteric pathogens in U.S. roof runoff collections and will inform treatment targets for different non-potable end uses for roof runoff.

To address barriers to the wide-scale implementation of SCU projects for non-potable end uses, stormwater microbial contamination originating from human fecal matter was examined using the detection frequencies and concentrations of human microbial source tracking (MST) markers and PHIPs observed in stormwater. Measurements of human MST markers in wet weather flows, dry weather flows, and influent wastewater in addition to measurements of viral and protozoan pathogens in wet weather flows and influent wastewater were compiled through a systematic review. Human MST marker and PHIP datasets were statistically analyzed and used to estimate HFCAs based on relative concentrations of microbial contaminants in stormwater compared to municipal wastewater. Analytical statistical distributions of the original data, unpaired Monte Carlo simulation, and paired Monte Carlo simulation were applied for the

estimates of HFCAs in wet and dry weather flows. Estimates of human MST-based HFCAs are more reliable than PHIP-based HFCAs because the current PHIP datasets are limited by detection limits and the range of data observed within the statistical distributions. Unpaired Monte Carlo simulations and analytical statistical distributions were found to be the best methods for the estimation of human MST-based HFCAs in wet and dry weather flows which ranged from $<10^{-7.0}$ to $10^{-1.5}$ and 10^{-12} to $10^{-2.6}$, respectively.

Pathogen LRTs were determined in this study using HFCAHuman MST Markers and previously published quantitative microbial risk assessments (QMRAs) to guide the selection of stormwater treatment process trains based on the intended end use (e.g., unrestricted irrigation or indoor use) of stormwater. Combinations of stormwater treatment trains at varying HFCA levels were evaluated based on complexity and reliability of the suggested trains. To use stormwater safely for unrestricted irrigation and indoor uses, treatment trains containing both filtration and disinfection unit processes are required. The HFCA threshold beyond which the complexity of stormwater trains becomes considerably higher is 10^{-4}. Performance evaluation of the suggested stormwater treatment trains revealed that trains consisting of membrane filtration and at least two disinfection unit treatment processes, specifically ultraviolet (UV) and ozone (O_3) or UV and chloramine are recommended at HFCA values of 10^{-3}, 10^{-2}, and 10^{-1}. At HFCA value of 10^{-4}, a treatment train consisting of membrane filtration and O_3 or chloramine is recommended. The use of free chlorination at all HFCA levels is not recommended due to the high continuous monitoring requirements associated with the use of free Cl_2.

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DEDICATION

I would like to dedicate my dissertation to my parents, Hamdi Alja'fari and Thawaba Abdeljawad, and my uncle, Hani Abdeljawad.

My parents have encouraged me throughout the years to pursue science, and they helped me gain confidence. I could not have done this without their love and support. They believed in me more than I believed in myself.

Uncle Hani and I are both civil engineers. He has been invested in my success, and he would be glad to learn that I successfully defended my dissertation if it were not for his deteriorating health.

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CHAPTER 1: INTRODUCTION

1.1 Problem Statement

Roof runoff, i.e., roof-harvested rainwater; stormwater, including both wet and dry weather flows; graywater, i.e., water collected from dishwashers, washing machines, showers, and bathroom sinks; and wastewater are sustainable, alternative water resources that can help cities acclimate to the increasing stress on conventional water resources caused by climate change and population growth. The National Water Reuse Action Plan developed by the USEPA in 2019 underlines the necessity of addressing water scarcity challenges in many U.S. regions (Arden et al., 2020). Furthermore, under the current water management paradigm where drinking water, wastewater, and stormwater are managed separately, drinking water and wastewater treatment facilities count among the largest energy consumers in municipalities (Olsson, 2015). Even though raw wastewater undergoes extensive treatment at wastewater treatment plants (WWTPs), treated effluents remain significant contributors to coastal and riverine eutrophication (USEPA, 2013).

Changing the current water management paradigm to one where roof runoff, stormwater, greywater, wastewater, and drinking water are managed together can help reduce energy consumption, attenuate eutrophication (Howe et al., 2013), and reduce the pressure on freshwater resources. Reuse of these alternative water resources shows promise as a low energy option to augment the available water supply (Tow et al., 2021). Water to be reused is available locally, therefore making long-distance transport unnecessary (Schaum et al., 2015). Furthermore, adjusting the applied treatment technology to the intended end use, i.e., fit-for-purpose, minimizes energy consumption (Schaum et al., 2015).

Water reuse for agricultural irrigation, landscape irrigation, toilet flushing, air conditioning, cooling water, streamflow augmentation, and groundwater recharge where people

have reduced exposure to water and as a result the source water does not require treatment to potable water standards offers an attractive opportunity to reduce the amount of energy allocated to water treatment. As for eutrophication, reuse of treated wastewater for agricultural and landscape irrigation diverts water rich in plant nutrients, nitrogen and phosphorus, from streams and receiving water bodies, thereby reducing potential eutrophication (Howe et al., 2013).

Demonstration projects across the U.S. show that the use of these alternate water sources is both publicly acceptable and technologically achievable (NBRC, 2018; Kehoe and Chang, 2019; SFPUC, 2018; WJW, 2018). Many barriers, however, hinder the widespread adoption of alternative water resources, specifically roof runoff and stormwater, despite the advantages of incorporating these resources into an integrated urban water management (IUWM) paradigm.

For roof runoff, the lack of proper characterization of microbial quality across the U.S is a barrier to the wide-scale implementation of roof-runoff collection and use projects. As a result, regulations on water quality targets for its use are highly variable, indicating the absence of knowledge on appropriate treatment of roof runoff for potable and non-potable use (Sharvelle et al., 2017). The lack of data on roof runoff microbial quality to support a quantitative microbial risk assessment (QMRA) limited developing U.S data-based pathogen log reduction targets (LRTs) to inform the selection of treatment processes based on roof runoff end use (Sharvelle et al., 2017).

The risk-based guidance developed so far for the use of roof runoff for non-potable purposes did not include protozoa LRTs due to the lack of data (Sharvelle et al., 2017, Table 1.1). In this guidance, bacteria LRTs are based on the animal feces approach using seagulls and the density of fecal indicator bacteria in stored Australian roof-runoff (Chapman et al., 2008; Sharvelle et al., 2017, Table 1.1). Therefore, more data should be collected on the concentrations and detection frequency of bacterial and protozoan pathogens in roof runoff across the U.S.

For stormwater, guidance frameworks regulating the use of this alternative water resource are scarce, which limits the implementation of neighborhood-scale and municipal stormwater capture and use (SCU) projects (Luthy et al., 2019). As a result, guidance on the required treatment of stormwater for potable and non-potable end uses is essential to ensure public health protection. Using a quantitative risk methodology, Sharvelle et al. (2017) developed a risk-based framework for the use of stormwater for non-potable purposes. In the risk-based framework, Sharvelle et al. (2017) established pathogen, i.e., virus, protozoa, and bacteria, LRTs based on 10^{-1} and 10^{-3} sewage dilutions in stormwater (Table 1.1). The pathogen LRTs are specific to the potential infection risk (10^{-2} or 10^{-4} / person / year) and stormwater end use (unrestricted irrigation and indoor use) (Table 1.1).

Even though the 2017 stormwater risk-based framework addressed an important gap in guiding the non-potable uses of stormwater, utilities and public health departments hesitated to apply the sewage dilution-based LRTs (Lackey et al., 2019). This is because utilities and public health departments were uncertain regarding where their local stormwater lied within the 10^{-1} to 10^{-3} sewage dilution range. Therefore, more datasets on stormwater microbial contamination are needed to tackle the ambiguity associated with pathogen LRT selection based on sewage dilutions in stormwater.

Table 1.1. Ninety Fifth Percentile Pathogen LRTs to Meet 10^{-4} or 10^{-2} Infection Risk per Person per Year Benchmarks for Healthy Adults[a, b]

Water Use Scenario	Log_{10} Reduction Targets for 10^{-4} (10^{-2}) / person / year Benchmarks		
	Enteric Viruses	Parasitic Protozoa	Enteric Bacteria
Roof Runoff			
Unrestricted Irrigation	NA	No data	3.5 (1.5)
Indoor Use	NA	No data	3.5 (1.5)
Stormwater 10^{-1} Dilution			
Unrestricted Irrigation	5.0 (3.0)	4.5 (2.5)	4.0 (2.0)
Indoor Use	5.5 (3.5)	5.5 (3.5)	5.0 (3.0)
Stormwater 10^{-3} Dilution			
Unrestricted Irrigation	3.0 (1.0)	2.5 (0.5)	2.0 (0.0)

Indoor Use	3.5 (1.5)	3.5 (1.5)	3.0 (1.0)

a: The pathogen LRTs were obtained based on quantitative microbial risk assessment, b: Sharvelle et al. (2017)

The broader goal of this research effort is addressing some of the barriers to the adoption of roof runoff and stormwater as alternate water sources by characterizing the microbial quality of roof runoff across different land uses and climate regions in the U.S. and providing guidance on the use of stormwater for non-potable purposes. The first barrier, the lack of data on roof runoff microbial quality, was addressed by a 2 – year study characterizing the microbial quality of roof runoff in four U.S. cities: Fort Collins, CO; Tucson, AZ; Baltimore, MD; and Miami, FL (Alja'fari et al., 2022a).

The second barrier, the scarcity of regulatory frameworks promoting the use of stormwater, is addressed through the provision of guidance on the safe use of stormwater for non-potable purposes. The basic elements for this guidance are the synthesis of data on stormwater microbial quality in the U.S., estimation of sewage dilution in stormwater (hereafter termed as the human fecal contamination analog (HFCA)), informing the selection of pathogen LRTs required for the safe use of stormwater based on HFCA estimates, and evaluation of the effects of LRT choices on the selection of treatment process trains for a specific HFCA and end use. Multiple stormwater treatment trains are suggested for each HFCA estimate, and the treatment trains are evaluated based on the degree of complexity and reliability to help water managers make informed decisions upon selecting stormwater treatment trains for SCU projects in their watersheds. The guidance on the safe use of stormwater builds upon literature synthesis of stormwater microbial quality for SCU projects. The literature synthesis was prepared for the Water Research Foundation (Alja'fari et al., 2022b).

1.2 Research Questions

The questions addressed in this research are as follows:

1. What is the range for the detection frequency and concentrations of FIB and potentially human-infectious pathogens in roof runoff across different U.S cities?
2. Does roof runoff microbial quality vary across different U.S regions, and do physicochemical parameters of roof runoff, storm properties, the percent of impervious cover surrounding each sampling location, and seasonality affect roof runoff microbial quality?
3. What is the range of estimated presence of human fecal contamination in stormwater based on concentrations of pathogens and human MST markers observed in stormwater?
4. How do the estimates of HFCA in stormwater based on human MST markers and pathogens compare with the ones suggested by Sharvelle et al. (2017), which were based on a more limited set of stormwater pathogen data?
5. How does the selection of pathogen LRTs impact the choice of treatment process trains protective of public health for the designated stormwater end use?

1.3 Research Objectives and Hypotheses

This research attempts to provide a large dataset describing roof runoff microbial quality across different U.S cities and establish a framework to guide the best practices for system design of stormwater capture, treatment, and use projects. The objectives described below are intended to achieve the research goals.

O.1 Investigate the occurrence and concentrations ranges of FIB and potentially human-infectious pathogens in roof runoff across different U.S cities with varying climate and land use characteristics.

H.1 Fecal indicator bacteria will be detected at higher frequencies and concentrations than potentially human-infectious pathogens in all study cities

O.2 Examine the effect of geographic location, storm properties, sampling season, presence of overhanging trees, the percent of impervious cover surrounding each sampling location, and the physicochemical parameters of roof runoff on its microbial quality.

H.2 Sampling season, geographic location, overhanging trees, percent of impervious cover, roof runoff physicochemical parameters, and storm properties will have an impact on roof runoff microbial quality.

O.3 Assess HFCA ranges in stormwater based on concentrations of pathogens and human MST markers observed in stormwater

H.3 HFCA in stormwater based on human MST markers will differ from those estimated based on potentially human-infectious pathogens.

O.4 Explore how the estimates of HFCA in stormwater based on human MST markers and pathogens compare with the estimates developed by Schoen et al. (2017) and adopted by Sharvelle et al. (2017), which were based on a more limited set of stormwater pathogen data.

H.4 Estimates of HFCA in stormwater based on the newer, more comprehensive dataset of stormwater human MST marker and pathogen data will slightly differ from the ones estimated Schoen et al. (2017), which were based on a limited set of stormwater pathogen data.

O.5 Investigate how the selection of pathogen LRTs and HFCAs impacts the choice of treatment process trains protective of public health for the designated end use of stormwater.

H.5 A threshold for pathogen LRTs/HFCAs exists beyond which the complexity of treatment process trains significantly changes based on metrics such as monitoring and dosing requirements.

1.4 Dissertation Structure

This dissertation is organized in three parts such that each part is considered separately. Chapters two through four are research papers addressing the objectives described above.

- Characterization of roof runoff microbial quality in four U.S. cities with varying climates and land use characteristics (Objectives 1 and 2)
- Assessment of human-source microbial contamination of stormwater in the U.S. (Objectives 3 and 4)
- Evaluation of the impact of pathogen reduction targets selection on treatment process trains for the safe use of stormwater (Objective 5)

CHAPTER 2: CHARACTERIZATION OF ROOF RUNOFF MICROBIAL QUALITY IN FOUR U.S. CITIES WITH VARYING CLIMATE AND LAND USE CHARACTERISTICS [1]

2.1 Abstract

Roof runoff has the potential to serve as an important local water source in regions with growing populations and limited water supply. Given the scarcity of guidance regulating the use of roof runoff, a need exists to characterize the microbial quality of roof runoff. The objective of this 2-year research effort was to examine roof runoff microbial quality in four U.S. cities: Fort Collins, CO; Tucson, AZ; Baltimore, MD; and Miami, FL. Seven participants, i.e., homeowners and schools, were recruited in each city to collect roof runoff samples across 13 precipitation events. Sample collection was done as part of a citizen science approach. The presence and concentrations of indicator organisms and potentially human-infectious pathogens in roof runoff were determined using culture methods and digital droplet polymerase chain reaction (ddPCR), respectively. The analyzed pathogens included *Salmonella* spp., *Campylobacter* spp., *Giardia duodenalis*, and *Cryptosporidium parvum*. Several factors were evaluated to study their influence on the presence of potentially human-infectious pathogens including the physicochemical characteristics (total suspended solids, volatile suspended solids, total dissolved solids, chemical oxygen demand, and turbidity) of roof runoff, concentrations of indicator organisms, presence/absence of trees, storm properties (rainfall depth and antecedent dry period), percent of impervious cover surrounding each sampling location, seasonality, and geographical location. *E. coli* and enterococci were detected in 73.4% and 96.2% of the analyzed samples, respectively. Concentrations of both *E. coli* and enterococci ranged from <0 $\log_{10}$ to >3.38 $\log_{10}$ MPN/100 mL.

[1] This chapter is adapted from: Alja'fari, J., Sharvelle, S., Brinkman, N. E., Jahne, M., Keely, S., Wheaton, E. A., Garland, J., Welty, C., Sukop, M. C., and Meixner T. (2022). "Characterization of Roof Runoff Microbial Quality in Four U.S. Cities with Varying Climate and Land Use Characteristics". Water Research Volume 225: 119123

Salmonella spp. *invA*, *Campylobacter* spp. *ceuE*, and *G. duodenalis β – giardin* gene targets were detected in 8.9%, 2.5%, and 5.1% of the analyzed samples, respectively. *Campylobacter* spp. *mapA* and *C. parvum* 18S rRNA gene targets were not detected in any of the analyzed samples. The detection of *Salmonella* spp. *invA* was influenced by the geographical location of the sampling site (Chi-square p-value < 0.001) as well as the number of antecedent dry days prior to a rain event (p-value = 0.002, negative correlation). The antecedent dry period was negatively correlated with the occurrence of *Campylobacter* spp. *ceuE* as well (p-value = 0.07). On the other hand, the presence of *G. duodenalis β–giardin* in roof runoff was positively correlated with rainfall depth (p-value = 0.05). While physicochemical parameters and impervious area were not found to be correlated with the presence/absence of potentially human-infectious pathogens, significant correlations were found between meteorological parameters and the presence/absence of potentially human-infectious pathogens. Additionally, a weak, yet significant positive correlation, was found only between the concentrations of *E. coli* and those of *Giardia duodenalis β-giardin*. This dataset represents the largest-scale study to date of enteric pathogens in U.S. roof runoff collections and will inform treatment targets for different non-potable end uses for roof runoff. However, the dataset is limited by the low percent detection of bacterial and protozoan pathogens, an issue that is likely to persist challenging the characterization of roof runoff microbial quality given sampling limitations related to the volume and number of samples.

2.2 Introduction

Roof runoff, also known as roof-harvested rainwater, refers to precipitation collected directly from roof surfaces. The use of roof runoff for potable or non-potable purposes offers an attractive opportunity to reduce water use at the building scale and creates awareness of water conservation. Additionally, the collection and use of roof runoff is an efficient stormwater

management practice that reduces the urban runoff and pollutant load to receiving water bodies (Ahmed et al., 2011a; Askarizadeh et al., 2015; Sharvelle et al., 2017). Regulations on water quality targets for the use of roof runoff are highly variable, resulting in ambiguity and a lack of guidance on appropriate treatment of roof runoff for non-potable use (Sharvelle et al., 2017). The lack of data on the microbial quality of roof runoff to support a quantitative microbial risk assessment (QMRA) has limited development of pathogen log reduction targets to guide treatment selection based on the end use, particularly for protozoan pathogens (Sharvelle et al., 2017). More data on microbial quality of roof runoff is needed to inform treatment requirements for safe use of roof runoff. Consequently, roof runoff remains underutilized as an alternate water resource in the United States (Hamilton et al., 2018).

Previous studies assessed the occurrence and concentrations of fecal indicator organisms and potentially human-infectious pathogens in roof runoff. Most of these studies were conducted in Australia (Thomas and Greene, 1993; Verrinder and Keleher, 2001; Spinks et al., 2006; Chapman et al., 2008; Evans et al., 2006; Ahmed et al., 2008; Ahmed et al., 2010a; Ahmed et al., 2010b; Ahmed et al., 2011b), with fewer studies in the U.S. (Isquith and Winters, 1987; Kim et al., 2016; Kirs et al., 2017; Hamilton et al., 2018). Fecal indicator bacteria are usually abundant in roof runoff due to the access of small mammals and birds to roof surfaces (Bae et al., 2019; Ahmed et al., 2011b). However, correlations between potentially human-infectious pathogens and fecal indicator bacteria in roof runoff are generally weak to non-existent (Horman et al., 2004; Ahmed et al., 2008), which invalidates the prediction of the occurrence of potentially human-infectious pathogens in roof runoff using fecal indicator organisms.

Both opportunistic pathogens and human enteric pathogens have been reported to exist in roof runoff (Crabtree et al., 1996; Kim et al., 2016; Kirs et al., 2017; Hamilton et al., 2018; Bae

et al., 2019). Studies conducted in the U.S. focused mostly (Hamilton et al., 2018) or entirely (Kim et al., 2016) on the detection and quantification of opportunistic pathogens. Studies investigating the occurrence of human enteric pathogens in the U.S. are few, and these studies examined only 1 to 3 human enteric pathogens at a time, e.g., *Salmonella* (Isquith and Winters, 1987), *Cryptosporidium spp*. and *Giardia spp.* (Crabtree et al., 1996; Kirs et al., 2017), *Campylobacter jejuni* (Hamilton et al., 2018), and *E. coli O157*, *E.coli stx1*, and *E.coli stx2* (Bae et al., 2019). Additionally, research quantifying the presence of human enteric pathogens in the U.S. is limited in geographic scope and number of samples (14 – 36), (Isquith and Winters, 1987; Crabtree et al., 1996; Kim et al., 2016; Kirs et al., 2017; Hamilton et al., 2018; Bae et al., 2019); Each of these studies focused on samples collected in only one US city. All of the studies analyzed stored roof runoff, with the exception of the study conducted by Bae et al. (2019) where fresh roof runoff samples were analyzed. Pathogen*s* undergo decay mechanisms under storage conditions in tanks/barrels (Ahmed et al., 2012), which influences the measured microbial quality of roof runoff. The collection of fresh samples reflects roof runoff's raw quality more accurately.

An integrated approach to investigate a suite of parameters affecting the microbial quality of roof runoff across different geographic regions and seasons has not been reported previously. This absence may be largely due to the complexities of a nationwide sampling campaign spanning multiple seasons. Collection of roof runoff samples across different regions requires a high level of human capital due to the time-consuming nature of sample collection and comes with logistical challenges. Seasonality could be an important element of the characterization of roof runoff microbial quality due to the potential influence of wildlife (e.g., bird migration patterns), vegetation, and temperature. The effect of these seasonal factors on the microbial quality is currently not well understood. This study aims to characterize the microbial quality of freshly

collected roof runoff with special emphasis on an array of four human enteric pathogens, *Salmonella* spp. *invA*, *Campylobacter* spp. *ceuE* and *mapA*, *Giardia duodenalis β-giardin*, and *Cryptosporidium parvum* 18S rRNA, across different regions and sampling seasons within the U.S. The study also investigates the influence of a suite of parameters on roof runoff microbial quality including roof runoff physicochemical parameters (total suspended solids (TSS), volatile suspended solids (VSS), total dissolved solids (TDS), turbidity, and chemical oxygen demand (COD)), percent of impervious cover surrounding each sampling location, meteorological parameters (i.e., rainfall depth and antecedent dry period), the presence/absence of overhanging trees on roofs, seasonality, and geographical location. The potential for these factors to serve as predictors of potentially human-infectious pathogens was evaluated. The microbial quality of roof runoff was assessed in four U.S cities: Ft. Collins, CO; Tucson, AZ; Baltimore, MD; and Miami, FL. A citizen science approach was used to enable the collection of a large number of samples after each precipitation event. The study cities were selected to enable a representative data analysis reflecting geographic diversity in addition to different climatic conditions, city sizes, and population density.

2.3 Materials and Methods

This project engaged citizen scientists to collect roof runoff from buildings in the four study cities. Seven participants were recruited in each city to collect roof runoff samples. Study participants were provided with rain barrels, sampling equipment, and rain gauges. Collaborating university staff in each city collected samples from participants, processed them in their laboratories, and then shipped them to either Colorado State University (CSU) in Fort Collins, CO or the U.S. Environmental Protection Agency Office of Research and Development laboratory (EPA-ORD) in Cincinnati, Ohio for analysis of chemical and microbial quality.

2.3.1 Sample Collection

In each study city, Ft. Collins, Tucson, Baltimore, and Miami, seven participants, including homeowners and schoolteachers, were selected. A citizen science approach was used for sample collection. Sampled buildings are located in different areas of each city and reflect a range of roof age, roof material, and gutter cleaning frequency to enable a representative data analysis. The presence/absence of overhanging trees was a factor in roof selection as well. Roof surface areas ranged from 800 to 6000 ft^2, and roof ages ranged from less than 5 years to greater than 80 years. Roof material included composite or asphalt shingles, clay tiles, metal standing seams, concrete barrel tiles, rolled roofing, and slate roofing (**Table**). While microbial contamination could rise from the atmosphere in addition to the roof surface, the main interest of this study is the FIB and pathogenic content of roof runoff regardless of the source.

The participants cleaned their gutters at frequencies ranging from never to once per month, and they were not instructed to change these behaviors. Each participant was provided with two, 55-gallon rain barrels (Uline barrels for Miami and Tucson, Aquabarrel for Baltimore, and Colorado Rain Catcher for Fort Collins), a 5-inch capacity rain gauge, and a kit to divert roof runoff from downspouts to the barrels (Colorado Rain Catcher). Additionally, participants were provided with documents and videos detailing the barrel installation procedure and sample collection process. A web portal titled "Off the Roof" was established on CitSci (www.citsci.org) for each city. The webpage was designed for this study and included the training documents and videos. Study participants used the portal to indicate the date and time of sample collection, rainfall depth, approximate volume of water collected in each barrel, and any observations they had prior to or during rainfall events.

Table 2.1: Roof Material in Each City and Number of Roofs for Each Material

City	Roof Type	Number of Roofs	Sampled Events
Fort Collins	Composite or Asphalt Shingles	7	spring (May 28th, 2018) summer (August 14th, 2018) fall (October 7th and 8th, 2018) winter (March 13th, 2019)
Tucson	Composite or Asphalt Shingles	3	summer (August 22nd, 2018) summer (August 10th, 2019) winter (February 3rd, 2019)
	Rolled Roofing	4	
Baltimore	Slate Roof	3	summer (Sept 7th, 2018) fall (October 26th and 27th, 2018) fall (October 16th, 2019)
	Composite or Asphalt Shingles	4	
Miami	Composite or Asphalt Shingles	3	summer (September 7th and 8th, 2018) fall (December 15th, 2018) winter (March 19th, 2019)
	Clay Tile	1	
	Metal Standing Seam	1	
	Concrete Barrel Tiles	1	
	Rolled Roofing	1	

When possible, the sampled precipitation events were selected to reflect seasonality (**Table**) with 31 samples for summer, 24 samples for fall, and 17 samples for winter. This was considered important to capture the potential influence of wildlife (e.g., bird migration patterns), vegetation, and temperature. A minimum rainfall depth of 0.1–0.13 inch including the first flush, i.e., enough to fill half of the two 55–gallon barrels per building, was the criterion for the selection of sampled events. The samples included the first flush because not all roof runoff collection systems include diversion of first flush. Before each sampling event, study participants were directed to drain their barrels and rinse them with a hose to prevent contamination from a previous event. Each participant was provided with one 1-L sterile bottle, three 1-gallon sterile polypropylene plastic bottles, gloves, a stirrer, and a rain gauge. Participants were instructed to mix the contents of their barrels and fill each bottle with equal aliquots from each barrel. To ensure that participants followed the sample collection protocol, they were asked the following questions: (1) Did you drain the barrels and rinse them in preparation for the sampling event; (2) Did you wear gloves during sample collection; (3) Did you mix the contents of the barrel using the provided stirrer prior to sample collection; and (4) Did you fill each container from both barrels? Participants

indicated that the correct protocol was followed for each event. Participants placed their samples on ice packs in coolers at their front porch for collection by partner university research staff.

2.3.2 Sample Handling

Samples were kept on ice in coolers until picked up by the project team and stored at 5°C in the laboratory. Samples were analyzed for physicochemical parameters and indicator organisms at CSU. Roof runoff samples (1 L) collected outside Fort Collins were shipped overnight on ice in coolers to CSU for analysis. For pathogen analysis, roof runoff samples from all cities were filtered at CSU labs, University of Arizona labs, University of Maryland, Baltimore County labs, and Florida International University labs (see below). The filters were shipped overnight on ice in coolers to EPA-ORD lab in Cincinnati, OH to conduct potentially human-infectious pathogen analyses. When possible, the time between sample collection and analysis was kept under 72 hours.

2.3.3 Sample Analysis

2.3.3.1 Physicochemical Parameters

The 1-L samples were analyzed for TSS (Standard Methods 2540 D., APHA, 2012), VSS (Standard Methods 2540 E., APHA, 2012), TDS (Standard Methods 2540 C., APHA, 2012), turbidity (Hach Turbidimeter 2100P), and COD (Hach DRB 200 Reactor and DR 3900™ Benchtop Spectrophotometer).

2.3.3.2 Fecal Indicator Organisms

The 1-L samples were also analyzed for *E. coli* using method 9223 B–2004 (USEPA, 2017): the Colilert Method Quanti-Tray/2000 (defined substrate technologies; IDEXX, Westbrook, Maine) and for enterococci using the Enterolert Method (USEPA, 2007): Quanti-Tray/2000 (defined substrate technologies; IDEXX, Westbrook, Maine). The lower limit of quantification for both *E. coli* and enterococci was 1 MPN/100 mL, and the upper limit of

quantification was 2419.6 MPN/100 mL. In the Quanti-Tray, there are 48 large wells, 48 small wells, and 1 overflow well. The large wells were not always completely filled when the sample was poured into the tray (i.e., 100 ml of roof runoff were added to the trays, but the added volume sometimes filled half of the large wells with the remainder going to the overflow well). Two half wells were counted as one which led to the upper limit of quantification being 1011.2 MPN/100 mL. To adjust the upper limit of quantification to the original method upper limit of quantification, maximum concentrations, i.e., 1011.2 MPN/100 mL, were changed to 2419.6 MPN/100 mL since 2419.6 MPN/100 mL is the next higher concentration after 1011.2 MPN/100 mL.

2.3.3.3 Enteric Bacterial and Protozoan Pathogens

Sample Filtration

The three 1-gallon samples from each building were concentrated locally in each city by passing the sample through Rexeed 15S ultrafilters (Dial Medical Supply, Chester Springs, PA). A Masterflex peristaltic pump (Cole-Parmer, Vernon Hills, IL) was used to filter the sample at a speed of 50 – 300 rpm. A Wika pressure gauge (Cole-Parmer) was used to monitor the trans-membrane pressure to avoid exceeding 15 psi. The ultrafilters were shipped overnight on ice to the EPA-ORD lab for pathogens analysis. A field blank consisting of 1 liter of molecular grade water was included with every rain event and filtered using the same protocol as the roof runoff samples.

Sample Elution and Secondary Concentration

The ultrafilters were eluted by circulating 200 mL of a filter-sterilized solution composed of 0.01% sodium polyphosphate, 0.01% Tween-80, and 0.001% Antifoam Y-30 emulsion for 1 min each in clockwise and counterclockwise directions for a total of 4 min. The 200 ml elute was centrifuged at 3000 × g for 15 min at 4°C to pellet large particles. The supernatant was then split

and filtered through 2- 0.45 µm mixed cellulose ester membrane filters in a sterile, disposable MicroFunnel unit (PALL, Port Washington, NY), followed by addition of the pellet onto the membrane filter. The membrane filters were then aseptically rolled and transferred to a PowerWater DNA bead tube (Qiagen, Valencia, CA) and stored at -20 ºC. Microbes genomic DNA in concentrated roof runoff samples was extracted from membrane filters using the DNeasy PowerWater Kit (Qiagen). Each membrane filter was processed independently, eluted in 50 µl of the kit supplied Solution EB and then combined for a total of 100 µl for each sample. To assess proficiency during DNA extraction, quality control samples were included with every batch of samples. Negative quality control samples consisted of Solution EB, and positive quality controls were composed of predetermined concentrations of *P. syringae* cells in glycerol. Quality control samples were evaluated immediately after extraction by assessing DNA yield using Qubit dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA) and the Qubit 2.0 Fluorometer (Invitrogen).

Quantification of bacterial and protozoan pathogens using droplet digital PCR (ddPCR)

The QX200 Droplet Digital PCR System (BioRad Laboratories, Inc., Hercules, CA) was employed to quantify specific genes for bacterial and protozoan pathogens. Each 25 µl ddPCR reaction was prepared by combining ddPCR Supermix for Probes (no dUTP, BioRad Laboratories, Inc.), forward and reverse primers (900 nM each; Appendix A, **Table A.1**), probe (250 nM; Appendix A, **Table A.1)** nuclease free water, and 5µl of sample. Droplet generation was carried out in the QX200 Auto DG (BioRad Laboratories, Inc.) as instructed by the manufacturer. The PCR thermal cycling conditions were 95°C for 5 min, followed by 50 cycles of 95°C for 30 sec and 55°C (60°C for the Internal Amplification Control (IAC) assay) for 1 min. A final 98°C incubation occurred for 10 minutes. All PCR reactions were performed in a C1000 Touch Thermal Cycler with a 96-Deep Well Reaction Module (BioRad Laboratories, Inc.). PCR amplification was

assessed by measuring fluorescence in each droplet using the QX200 Droplet Reader (BioRad Laboratories, Inc.). PCR inhibition was evaluated using an internal amplification control (IAC) as described by (Jahne et al., 2020). The IAC was added to the ddPCR master mix to deliver approximately 2×10^4 molecules in each reaction. Ratios of IAC concentrations in roof runoff samples to sample-free reactions were determined and considered inhibited below 0.75. The ddPCR assays for *C. parvum* and the IAC were monoplex assays, but the remaining assays were duplexed: *invA, ceuE*, *mapA, β-giardin*. Quality control reactions were run for each assay on every ddPCR plate. Negative quality control reactions (no template controls) consisted of nuclease free water, and positive quality control reactions contained approximately 10^4 molecules of genomic DNA or synthetic DNA construct (Appendix A, **Table A.1**). QuantaSoft Analysis Pro (v1.0.596; BioRad Laboratories, Inc.) was used to quantify the target gene in each sample. For most assays, clustering droplets into negative or positive bins was performed by selecting all sample and control reactions on each plate and utilizing the Auto Analyze with Combined Wells function. However, the Multi-Well Auto Analysis function failed for *C. parvum* and IAC assays. For these assays, the mean and 10 standard deviations of the fluorescence values of the No Template Control (NTC) droplets were determined and used as the threshold for clustering droplets. Gene quantities were calculated from total and positive droplet counts using a Bayesian model as previously described (Jahne et al., 2020). The Minimum Information for Publication of Quantitative Digital PCR Experiments (dMIQE2020) checklist (The dMIQE Group and Huggett, 2020) (Appendix A, **Table A.2**) is included to provide sufficient information about the ddPCR assays and adherence to the checklist.

The lower limit of quantification (LLOQ) was determined from the upper 95% credible interval of the respective PCR assay NTCs. Molecules/liter were converted to cell, cysts, or oocyst

equivalents based on the reported number of gene copies per typical cell, cyst, or oocyst: 1 copy *invA* per cell of *Salmonella* spp. (Fey et al., 2004), 1 copy of *ceuE* (Gonzalez et al., 1997) and *mapA* (Inglis and Kalischuk, 2004) per *Campylobacter* spp., 16 copies *β-giardin* per cyst of *G. duodenalis* (Guy et al., 2003), and 5 copies 18S rRNA per oocyst of *C. parvum* (Le Blancq et al., 1997).

The recovery efficiency of the microbes targeted in this study was not assessed. However, previous studies indicate that recovery efficiencies could result in underestimates of no more than 2.5-fold for the parasites (Francy et al., 2013). Although data on ultrafiltration recovery efficiency of *Campylobacter* and *Salmonella* from water is non-existent, in general, ultrafiltration tends to recover the majority of fecal indicator bacteria (Hill et al., 2007), suggesting minimal impact of loss of these organisms during processing.

2.3.4 Meteorological, Seasonal, and Sample Location Characteristics

Meteorological parameters, i.e., rainfall depth and the antecedent dry period, presence of overhanging trees, sampling season, and percent impervious cover were investigated as factors that could potentially influence the concentrations of indicator organisms and the presence of human infectious pathogens in roof runoff. The objective of sampling during different seasons was to capture the potential impacts of ambient temperature, wildlife presence, and falling of debris on roof surfaces (leaf droppings and dust). The effects of site-specific hydrologic conditions on roof runoff microbial quality were captured through measurements of rainfall depth and recording the number of antecedent dry days prior to each event. Study participants received rain gauges with 5-inch capacity, and they recorded the rainfall depth for each event. Afterwards, participants uploaded the rainfall depth to “Off the Roof” webpage. Participants also used the webpage to indicate whether their roofs had overhanging trees, and they included photos in many cases.

For rain events when the participants could not record the rainfall depth, the Community Collaborative Rain, Hail, and Snow (CoCoRaHS) network was used to obtain rainfall depth from rain stations closest to the location of missing readings. The antecedent dry period was also obtained for each sampling location and sampling event from CoCoRaHS. The percent of impervious cover surrounding each sampling location was obtained from the 2016 National Land Cover Database (Homer et al., 2020).

2.3.5 Statistical Analysis

Minitab 18 was used to conduct statistical tests. The non-parametric Kruskal-Wallis and Mann-Whitney tests were used to examine differences in physicochemical parameters among the study cities and seasons. Statistical analyses on the indicator organisms were conducted using censored data analysis techniques. To employ these techniques in Minitab 18, indicator organism concentrations were coded to account for left censoring at the lower limit of quantification as (0, 1) and for right censoring at the upper limit of quantification as (2419.6, *). All values between the limits of quantification were used and expressed as intervals. For instance, a concentration of 20.2 MPN/100 mL was expressed as (20.2, 20.2). Originally, regression with life data was used to determine if one or more predictors influence the failure time of a certain product (Minitab 18 Support, 2022). Regression with life data, unlike other analyses, accepts censored data and employs different distributions to fit data (Minitab 18 Support, 2022). Regression with life data can also be used to find other percentiles besides the 50^{th} (Minitab 18 Support, 2022). This method can be adapted to environmental data, i.e., concentrations of chemicals, microorganisms, etc., to include samples below and/or above the analytical limit of detection in the statistical analysis (Helsel, 2012).

The statistical distribution, significant differences in indicator organisms counts among study seasons, cities, and locations with and without trees, and correlation analyses were performed using the Reliability/Survival package in Minitab 18 and the Maximum Likelihood Estimation (MLE) Method as described by (Helsel, 2012). This is because the number of collected samples was greater than 50 and the percent of censored data (i.e., data below and above the limit of detection) for different groupings of *E. coli* and enterococci (by city, season, etc.) ranged from less than 50% to over 50%. Helsel (2012) recommends the use of the MLE method for more than 50 data points and for a censoring range of 50 – 80%.

For the significant difference analyses, an overall Log likelihood (L_{groups}) was calculated using MLE life-regression process in Minitab to determine if the means of three or more groups were significantly different. Another Log likelihood for a case of no grouping was then calculated (L_{null}). The test statistic for a significant difference (-2 Log Likelihood) was calculated using:

$$-2Log\ Likelihood = -2 \times \left(L_{null} - L_{groups}\right) \quad \text{..... (2.1)}$$

The test statistic was compared to a Chi-square distribution with n-1 degrees of freedom, where n is the number of data groups.

For paired comparisons, i.e., significant difference between 2 groups, a censored regression process included in the Reliability/Survival package in Minitab employing the MLE method was used. Roof material was considered as a factor that might influence roof runoff microbial quality, but the frequency distribution of roof materials (**Table**) did not allow for a meaningful analysis. However, the goal was to select different roof materials to enable a representative analysis. Censored indicator organisms were correlated with physicochemical parameters, meteorological parameters, and percent of impervious cover surrounding each

sampling location. To conduct the correlation, regression with life data was used to produce a Log likelihood statistic. This statistic is calculated for the regression of censored indicator organisms versus one other variable, e.g., COD, using the MLE method. The Log likelihood statistic is called *L(β)*. Another Log likelihood statistic was calculated using no other variables, *L(0)*. Then, the test statistic G_o^2 was calculated, in a manner similar to equation 1, using:

$$G_o^2 = 2 \times (L(\beta) - L(0)) \quad \text{..... (2.2)}$$

A Chi-square p-value for the test statistic G_o^2 was found to determine the significance of the correlation for 1 degree of freedom, i.e., the number of explanatory variables. The *likelihood-r*, similar to the coefficient of determination (r^2), was determined using:

$$likelihood\ r = regression\ slope\ sign \times \sqrt{1 - e^{-G_o^2/m}} \quad \text{..... (2.3)}$$

Where *m* is the total number of data points.

Significant differences in the occurrence of pathogens among study cities and sampling seasons were estimated using Chi-square and cross tabulation analysis in Minitab. Binary logistic regression was used to assess the correlation between the presence/absence of potentially human-infectious pathogens and the presence/absence of overhanging trees, physicochemical parameters, meteorological parameters, and percent of impervious cover. Concentrations of indicator organisms were correlated with concentrations of potentially human-infectious pathogens using the censored regression procedure in the Reliability/Survival package in Minitab as described previously in Equations 2 and 3. The significance of all statistical differences, correlations, and regressions was assumed at the 90% confidence level (α-value = 0.1). This confidence level was selected due to the low detection frequency of pathogens and the uncertainty associated with

microbiological measurements in general. Boxplots of *E. coli* and enterococci were generated using Microsoft Excel.

2.4 Results and Discussion

2.4.1 Physicochemical Parameters

Roof runoff samples were collected from Fort Collins, CO; Tucson, AZ; Baltimore, MD; and Miami, FL during the spring, summer, fall, and winter seasons. Freshly collected samples were collected over a period of approximately 17 months, spanning 13 rain events. The levels of TDS, TSS, VSS, turbidity, and COD in roof runoff samples for all cities exhibited high variability and ranged from 0-376 mg/L, <1-293 mg/L, <1-147 mg/L, 1-168.5 NTU, and <3-608 mg/L, respectively. There won't always be organic matter among the dust and debris accumulated on roof surfaces prior to a storm. COD vials with low range (3 – 150 mg/L) and high range (20 – 1500 mg/L) were used to measure COD in roof runoff. As for TSS and VSS, the lowest amount that could be detected was 1 mg/L using SM 2540 D. and 2540 E., respectively. For TDS, the lowest amount that could be detected was 2 mg/L using SM 2540. **Table A.3** in Appendix A summarizes the physicochemical parameters of all roof runoff samples collected over the study period.

Roof runoff physicochemical parameters varied greatly across study cities and across the sampled events within each city (

Table *2.2*). The non-parametric Kruskal-Wallis test was used to determine whether the median concentrations of each physicochemical parameter differed significantly among the study cities, and the non-parametric Mann-Whitney test was used to determine city pairings with significantly different median concentrations for each physicochemical parameter. Among the four

cities, Fort Collins had the highest TDS, COD, TSS, and VSS concentrations with the median COD concentration in Fort Collins being significantly higher than that in the other cities (Fort Collins–Tucson, Mann-Whitney's p-value = 0.004; Fort Collins–Baltimore, Mann-Whitney's p-value < 0.001 and Fort Collins–Miami, Mann-Whitney's p-value = 0.001). Factors such as the presence of overhanging trees and long antecedent dry periods were explored as possible explanatory variables for high physicochemical parameter values in Fort Collins. However, the number of houses with overhanging trees and the length of antecedent dry period were not notably different from other cities to justify the elevated values of physicochemical parameters. Rainfall depths, on the other hand, were on average the lowest in Fort Collins, which could potentially result in more concentrated values of the physicochemical parameters.

The highest turbidity values were observed in Tucson followed by Fort Collins. However, statistically significant differences in median turbidity were only noted for the following city pairings: Fort Collins–Baltimore, Tucson–Baltimore, and Miami–Baltimore (Mann-Whitney's p–values were <0.001, 0.001, and 0.002, respectively). One possible explanation for the high turbidity values in Tucson is the desert nature of the city with dust blowing frequently and accumulating on the roof surfaces during extended antecedent dry periods. On average, Tucson had the highest antecedent dry period of all the study cities. Miami and Baltimore had the lowest TDS, COD, and turbidity values. A possible explanation for the low values of these parameters in Miami could be the high average rainfall depth, which might have resulted in more diluted roof runoff samples. Additionally, Miami had on average the lowest antecedent dry period of all cities. Similarly, Baltimore had the highest average rainfall depth among the cities, which could explain the low concentrations of TDS, COD, and turbidity.

Table 2.2: Median Values and Ranges for Roof Runoff Physicochemical Parameters in Each City*

City	TDS (mg/L)	COD (mg/L)	TSS (mg/L)	VSS (mg/L)	Turbidity (NTU)
Fort Collins	81 (22 – 376)[a]	83.5 (19.5 – 608)[a]	13.5 (0 – 293)[a]	12.5 (0 – 147)[a]	12.5 (1 – 168.5)[a]
Tucson	72 (18 – 214)[a]	37.5 (1 – 215)[b]	4 (0 – 36)[b,c]	4 (0 – 32)[b]	16 (1 – 36)[a]
Baltimore	27 (0 – 49)[b]	16 (2 – 156)[b]	0 (0 – 75)[b]	0 (0 – 71)[b]	2.5 (1 – 25)[b]
Miami	38 (14 – 100)[c]	36 (0 – 170)[b]	4 (0 – 102)[a,c]	4 (0 – 70)[a,b]	11 (1 – 85.5)[a]

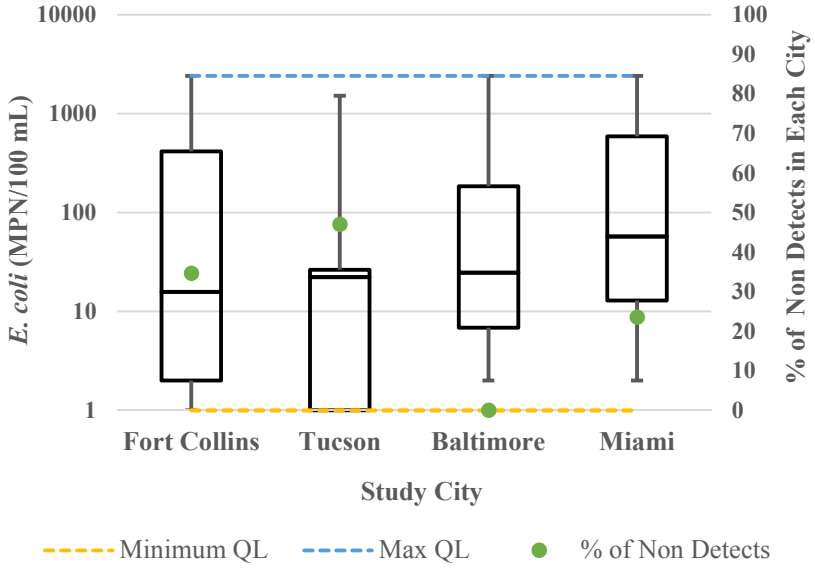
*Ranges are between parentheses; Letters a through c are used to designate whether city pairings are statistically different or the same: different letters indicate that the cities are statistically different with respect to (w.r.t) a given parameter. If a letter is repeated, then cities in the pairing are statistically the same w.r.t a given parameter.

TDS concentrations ranged from 0 to 376 mg/L; this range is consistent with the TDS range reported by Hamilton et al. (2018) of 18.6 to 780 mg/L. However, combined turbidity values for all study cities (1-168.5 NTU) are higher than those reported by Mendez et al. (2011), Hamilton et al. (2018), Crabtree et al. (1996), Vialle et al. (2011), and Schets et al. (2010). The concentrations of TSS in this study are similar to those found by Mendez et al. (2011) but higher than those found by Gikas and Tsihrintzis (2012). Overall, the turbidity values in this study are higher than what has been reported in the literature. This could be due to the wider range of sampled regions in this study reflecting different roof conditions and storm properties as well as the larger number of collected samples.

2.4.2 Fecal Indicator Organisms

To evaluate the influence of geographical location and climate region on the concentration of indicator organisms in roof runoff samples, analysis results were grouped by study city (**Figure 2.1**). Median concentrations of *E. coli* in positive roof runoff samples collected from Fort Collins, Tucson, Baltimore, and Miami were 1.20 $\log_{10}$, 1.35 $\log_{10}$, 1.39 $\log_{10}$, and 1.76 $\log_{10}$ MPN/100 mL, respectively (**Figure 2.1**). *E. coli* concentrations for all observations (including both left-and right-censored) differed significantly for at least one study city (Chi-square p–value

= 0.02). City pairings with significantly different *E. coli* concentrations were Baltimore-Fort Collins, Baltimore-Tucson, and Miami-Tucson (p-values = 0.03, 0.002, and 0.05, respectively). Median concentrations of enterococci in positive roof runoff samples collected from Fort Collins, Tucson, Baltimore, and Miami were 2.73 $\log_{10}$, 3.23 $\log_{10}$, 2.11 $\log_{10}$, and 2.94 $\log_{10}$ MPN/100 mL, respectively (**Figure 2.1**). Enterococci concentrations for all observations did not differ significantly among cities (Chi-square p-value = 0.16). Overall, there are not consistent trends to note w.r.t indicator organisms in roof runoff collected in the different cities.



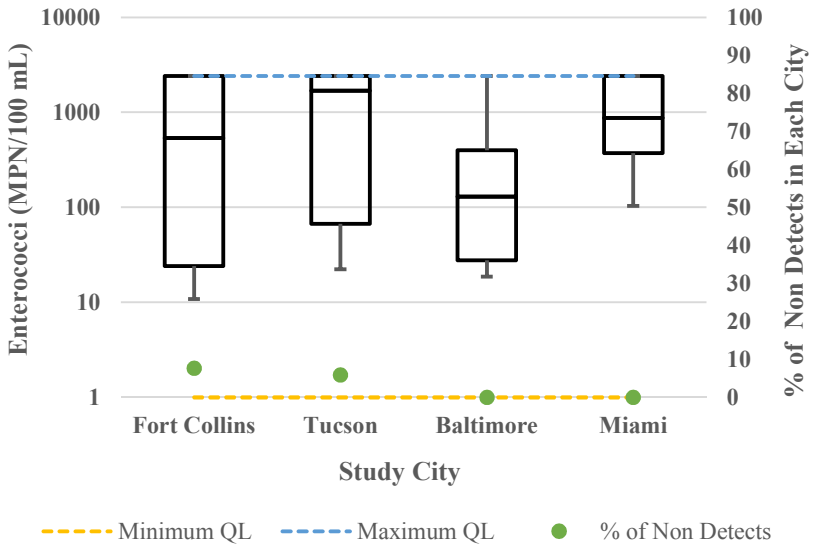


Figure 2.1: Boxplots of *E. coli* (a) and enterococci (b) concentrations in positive samples for each city. The total number of samples = 79 including both detects and non-detects. The number of samples collected from Fort Collins, Tucson, Baltimore, and Miami is 26, 17, 19, and 17, respectively. The middle line represents the median; the first and third lines represent the 25th and 75th percentiles, respectively. The vertical lines extend to the 5th and 95th percentiles. Dashed lines indicate the upper and lower limits of quantification. Circles represent the percentage of samples with non-detects.

To evaluate the influence of sampling season on the concentration of indicator organisms in roof runoff samples, the analysis results were grouped by season regardless of the study location, and boxplots of *E. coli* and enterococci concentrations in positive samples were constructed (**Figure 2**). Enterococci were more frequently detected than *E. coli* for all seasons. For both indicator organisms, more non detects occurred during winter (**Figure 2**). Using the MLE method for both left- and right- censored observations, *E. coli* concentrations were significantly different for at least one season (Chi-square p-value = 0.03). Season pairings with significantly different *E. coli* counts were summer-winter and fall-winter (p-values = 0.05 and 0.01, respectively). Enterococci concentrations for all observations were not significantly different among the sampling seasons (Chi-square p-value = 0.23). The highest occurrences of *E. coli* were recorded during the summer and fall sampling seasons. Similar to results comparing analyses among cities, there are no consistent trends to note w.r.t indicator organisms in roof runoff collected across seasons. The

influence of seasonality on roof runoff microbial quality was previously investigated by Vialle et al. (2011) who assessed the concentrations of both *E. coli* and enterococci in roof runoff in southwestern France and found that the highest fecal contamination occurred in the summer.

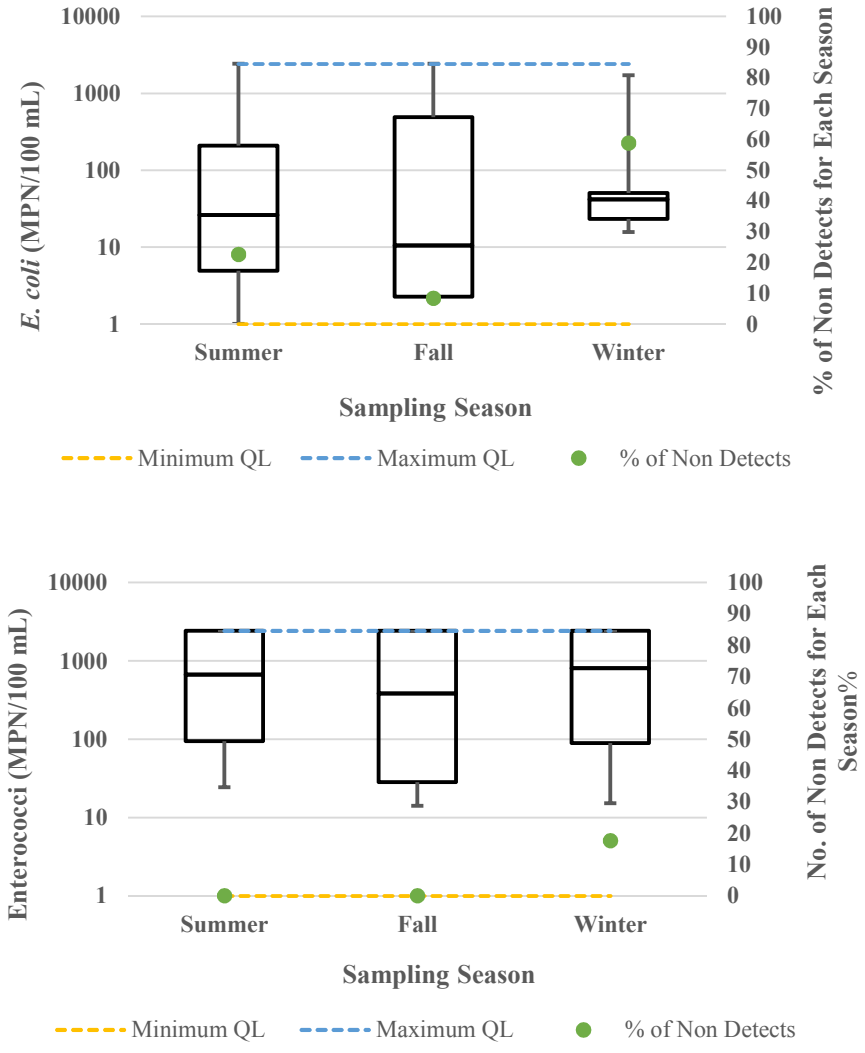


Figure 2.2: Boxplots of *E. coli* and enterococci concentrations in positive samples for each sampling season. The total number of samples = 72 including both detects and non-detects. The number of samples collected during the summer, fall, and winter sampling seasons is 31, 24, and 17, respectively. The middle line represents the median; the first and third lines represent the 25th and 75th percentiles, respectively. The vertical lines extend to the 5th and 95th percentiles. Dashed lines indicate the upper and lower limits of quantification. Circles represent the percentage of samples with non-detects.

Both *E. coli* and enterococci were detected at concentrations ranging from <0 $\log_{10}$ to >3.38 $\log_{10}$ MPN/100 mL. These concentrations are consistent with the *E. coli* and enterococci

concentrations in roof runoff samples collected in urban and peri-urban Philadelphia (Hamilton et al., 2018), a rural village in southwestern France (Vialle et al., 2011), and the Netherlands (Schets et al., 2010). However, *E. coli* values in this study are higher than those in roof runoff samples collected in American Samoa (Kirs et al., 2017).

2.4.3 Potential Human Infectious Pathogens

Roof runoff samples were analyzed for *Salmonella* spp., *Campylobacter* spp., *G. duodenalis*, and *C. parvum*. The analyzed pathogens were detected in 16.5% of the roof runoff samples (**Table 2.3**). *Salmonella* spp. *invA*, *Campylobacter* spp. *ceuE*, and *G. duodenalis β-giardin* were detected in 8.9%, 2.5%, and 5.1% of the analyzed samples, respectively. *Campylobacter* spp. *mapA* and *C. parvum* 18S rRNA genes were not detected in any of the analyzed samples. Roof runoff samples collected from Tucson did not test positive for any of the analyzed pathogens. Roof runoff samples collected from Baltimore were only positive for *G. duodenalis β-giardin* which was detected in 10.5% of Baltimore's samples. Fort Collins was the only city where *Campylobacter* spp. *ceuE* was detected; *Salmonella* spp. *invA*, *Campylobacter* spp. *ceuE*, and *G. duodenalis β-giardin* were found in 3.8%, 7.7%, and 7.7% of Fort Collins samples, respectively.

Roof runoff samples collected from Miami were only positive for *Salmonella* spp. *invA* which was detected in 35.3% of Miami's samples. The detection of only *Salmonella* spp. *invA* in Miami is notable and might be explained by higher temperatures throughout the year, which could result in a more consistent animal activity. Additionally, the rates of pathogen detection for three of the four cities in the study (Fort Collins, Tucson, and Baltimore; almost 80% of total samples), is considerably lower than the overall pathogen detection rate for the study.

Table 2.3: Detection Frequency of the Analyzed Pathogens in Roof Runoff Samples

Location[a]	*Salmonella* spp. *invA* (0.3983 cells/L)[b]		*Campylobacter* spp. *ceuE* (0.3906 cells/L)[b]		*Campylobacter* spp. *mapA* (0.5789 cells/L)[b]		*G. duodenalis β-giardin* (0.03616 cyst/L)[b]		*C. parvum* 18S (0.07412 oocyst/L)[b]		Total	
	Positive Samples	% Positive	Positive Samples	% Positive	Positive Samples	% Positive	Positive Samples	% Positive	Positive Samples	% Positive	Positive Samples	% Positive
Ft. Collins	1	3.8	2	7.7	0	0	2	7.7	0	0	5	19.2
Tucson	0	0	0	0	0	0	0	0	0	0	0	0
Miami	6	35.3	0	0	0	0	0	0	0	0	6	35.3
Baltimore	0	0	0	0	0	0	2	10.5	0	0	2	10.5
Total	7	8.9	2	2.5	0	0	4	5.1	0	0	13	16.5

a: Total number of collected samples that were analyzed for pathogens = 79 as follows: 26 from Fort Collins, 18 from Tucson, 17 from Miami, and 19 from Baltimore; b: lower limit of quantification is between parentheses.

The concentrations *of Salmonella* spp. *invA* and *Campylobacter* spp. *ceuE* ranged from 0-252.6 and 0-3.8 cells/L, respectively (**Figure 2.3**). The concentration of *G. duodenalis β-giardin* ranged from 0-15 cysts/L (**Figure 2.3**). The highest concentrations of *Salmonella* spp. *invA* were found in roof runoff samples collected from Miami while the highest concentration of *G. duodenalis β-giardin* was found in a roof runoff sample collected from Baltimore. *Campylobacter* spp. *ceuE* was detected at low concentrations ($\leq$ 3.8 cells/L) in samples collected from Fort Collins only. The influence of geographical location on the presence of potential human-infectious pathogens was only explored for *Salmonella* spp. *invA* because it had a sufficient detection rate to run the Chi-square Cross Tabulation test. The geographical location affected the occurrence of *Salmonella* spp. *invA* (Chi-square p-value <0.001) with the most frequent detections occurring in Miami followed by Fort Collins. The influence of sampling season on the occurrence of pathogens was also investigated. Again, only *Salmonella* spp. had sufficient detection rates to conduct the Chi-square Cross Tabulation test. No relationship was found between the occurrence of *Salmonella* spp. *invA* and sampling season (Chi-square p-value = 0.53).

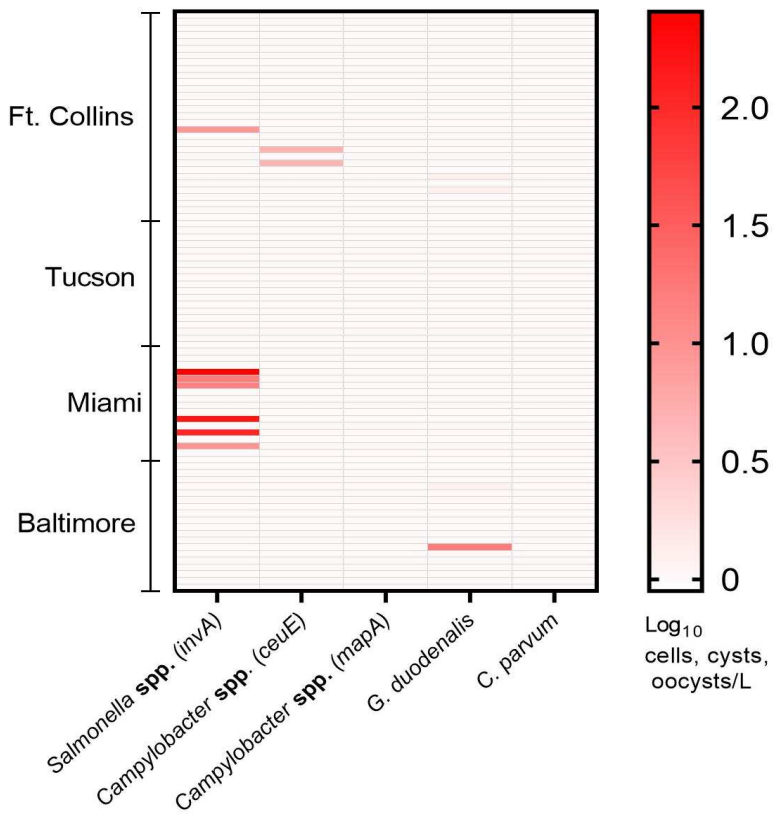


Figure 2.3: Concentrations of potentially pathogenic microorganisms in roof runoff samples collected from each study city; concentrations are expressed in cell equivalents/L for *Salmonella* spp. *(invA)* and *Campylobacter* spp. *(ceuE, mapA),* cyst equivalents/L for *Giardia duodenalis*, and oocysts for *Cryptosporidium parvum* (3 of the bars indicating *Giardia duodenalis* concentrations in Baltimore and Fort Collins are shown in light red to reflect how low these concentrations are).

Comparing the occurrences and concentrations of pathogens among studies is complicated by the different methods of measurement (Schets et al., 2010), reporting units, and targeted genes used in studies. The percent detection and concentration range for Salmonella spp. invA are similar to those reported for roof runoff samples collected in Southeast Queensland, Australia (Ahmed et al., 2010b; **Table 2.4**). However, in a study conducted on stored roof runoff microbial quality in the U.S. Virgin Islands, Isquith and Winters (1987) reported total Salmonella spp. counts of 6×10^2 – 8×10^6 cells/L which are 2 to 4 orders of magnitude higher than the Salmonella counts reported in this study (**Table 2.4**). This could be because this study only quantified Salmonella spp. invA genes and/or because more birds and wildlife had access to the

sampled roofs in the U.S. Virgin Islands study. Another potential explanation is that Isquith and Winters (1987) studied large underground storage cisterns, rather than fresh collections following rainfall events, noting extensive biological contamination and poor maintenance conditions in these systems.

In a more recent study conducted by health officials in the U.S. Virgin Islands, Salmonella was detected in 32% (15 out of 47 households) of roof runoff cistern water (The Virgin Islands Consortium, 2020). Despite the difference in Salmonella spp. concentrations between this study and those reported by Isquith and Winters (1987), a similar trend can be noted between Miami's concentrations and the U.S. Virgin Islands'. Concentrations of Salmonella spp. invA in half of the positive Miami samples (values not shown) are of the same order of magnitude as the minimum Salmonella spp. concentrations in the U.S. Virgin Islands. This could be explained by the tropical climate in both places. For Salmonella spp. invA in Miami, three houses tested positive during the summer event (houses 5, 6, and 7), 1 house tested positive during the fall event (house 7), and 2 houses tested positive during the winter event (houses 3 and 5). Therefore, Salmonella detections suggest a potential regional trend in Miami. Similar to the U.S. Virgin Islands, Miami is located near the ocean where more birds are concentrated. Compared to Tucson, Fort Collins, and Baltimore, a higher population of lizards could result in a higher wildlife activity in Miami. In contrast, Schets et al. (2010) did not detect Salmonella spp. in any of the analyzed roof runoff samples in a study conducted in the Netherlands (**Table 2.4**).

Table 2.4: Percent Detection and Concentration Ranges of Potential Human-Infectious Pathogens in National and International Studies

Study/Potential Pathogen	U.S. Studies					International Studies	
	This Study	Isquith and Winters (1987)	Crabtree et al. (1996)	Kirs et al. (2017)	Hamilton et al. (2018)	Schets et al. (2010)	Ahmed et al. (2010b)
Salmonella (cells/L)	8.9% (0 – 252.6)[a]	88% ($6*10^2$ – $8*10^6$)[b]	-	-	-	0%[c]	10.7% ($6.5*10^1$ – $3.8*10^2$)[a]
Campylobacter (cells/L)	2.5% (0 – 3.8)[d]	-	-	-	2.6% ($2.82*10^2$)[e]	12.5% (26 - >240)[f]	0.4% (not quantified)[g]
Giardia (cysts/L)	5.1% (0 – 15)[h]	-	26% (<0.01 – 0.038)[i]	0%[j]	-	4.2% (1)[k]	9.8% ($0.6*10^0$ – $3.6*10^0$)[h]
Cryptosporidium (oocysts/L)	0%[l]	-	48% (<0.01 – 0.703)[m]	86% (<D.L – 250)[n]	-	4.2% (1)[k]	0%[o]

a: *Salmonella* spp. *invA*, b: Total *Salmonella* spp. count (cfu)/L. Samples were collected over 3 sampling rounds; however, the concentrations were only reported for rounds 2 and 3 since round 1 samples were only analyzed for presence/absence of total *Salmonella* spp., c: total *Salmonella* spp., d: *Campylobacter* spp. *ceuE,* e: *Campylobacter jejuni* (single sample detection). The reporting unit is gene copies/L, f: Overall 40 samples were collected with five samples testing positive for *Campylobacter*. Four of the five samples were identified as *Campylobacter jejuni* with concentrations ranging from 26 to >240 MPN/L, g: *Campylobacter jejuni mapA*, h: *Giardia duodenalis* (*Giardia lamblia β-giardin*), i: *Giardia* cysts, j: *Giardia* spp., k: detection in one sample, l: *Cryptosporidium parvum* 18S rRNA gene, m: *Cryptosporidium* cysts, n: *Cryptosporidium* spp., o: *Cryptosporidium parvum* oocyst wall protein.

Concentrations of *Campylobacter* spp. *ceuE* observed in this study are lower than the concentrations of *Campylobacter jejuni* reported for roof runoff samples collected from urban and peri-urban Philadelphia and the Netherlands (Hamilton et al., 2018; Schets et al., 2010). However, the concentrations of *G. duodenalis β-giardin* in this study are higher than the concentrations of *Giardia spp.* in roof runoff samples collected from the U.S. Virgin Islands and the Netherlands (Crabtree et al., 1996; Schets et al., 2010); they are also higher than the concentrations of *G. duodenalis β-giardin* in roof runoff samples collected from Southeast Queensland, Australia (Ahmed et al., 2010b).

Cryptosporidium parvum 18S rRNA genes were not detected in any of the samples collected in this study. On the other hand, *Cryptosporidium spp.* were detected in 48%, 86%, and 4.2% of the roof runoff samples collected from the U.S. Virgin Islands, American Samoa, and

Netherlands (Crabtree et al., 1996; Kirs et al., 2017; Schets et al., 2010). These comparisons reveal that the occurrence and concentrations of pathogens in roof runoff are highly variable from one location to the other. The variations could be attributed to the study location, analytical methods (molecular versus culture methods), sample type (stored versus fresh), and the analytical method detection limit or the analyzed sample volume.

2.4.3 Analysis of Correlations between Physicochemical Parameters, Indicator Organisms, and Potentially Human-Infectious Pathogens

E. coli concentrations did not have any significant correlations with the physicochemical parameters, meteorological parameters, or percentage of impervious cover (

Table 2.5). Enterococci concentrations showed significant weak positive correlations with COD, TDS, TSS, and VSS (

Table 2.5). Enterococci concentrations did not correlate with turbidity, meteorological parameters, or percentage of impervious cover. However, Schets et al. (2010) found an association between the concentration of fecal indicator bacteria and rainfall intensity, and Vialle et al. (2011) found that the presence of *E. coli* and enterococci was associated with precipitation.

Table 2.5: Maximum Likelihood Correlation of Fecal Indicator Organisms with Physicochemical Parameters, Meteorological Parameters, and % of Impervious Cover

	E. coli (MPN/100 mL)		Enterococci (MPN/100 mL)	
	Likelihood–r	P–value*	Likelihood–r	P–value*
COD (mg/L)	0.05	0.66	0.24	**0.03**
TDS (mg/L)	0.05	0.65	0.30	**0.01**
TSS (mg/L)	-0.01	0.95	0.23	**0.04**
VSS (mg/L)	0.07	0.56	0.29	**0.008**
Turbidity (NTU)	-0.09	0.42	0.17	0.13
Rainfall Depth (inch)	0.09	0.45	-0.11	0.31
Ant. Dry Period (day)	-0.16	0.16	-0.12	0.27
Impervious Cover (%)	-0.18	0.12	0.16	0.16

*Bold faced p–values are significant at the 90% confidence level.

Overhanging trees might be conducive to more wildlife activity on roof tops and contribute to potentially higher concentrations of fecal indicator bacteria. The presence of overhanging trees provides a habitat and an easy access for animals to roof surfaces. Thus, the effect of overhanging trees on the concentrations of indicator organisms in roof runoff was also investigated. No significant difference was found in the concentrations of *E. coli* between sampling locations with and without overhanging trees (p-value = 0.17). A significant difference in enterococci concentrations was found between locations with and without trees (p-value = 0.08). Locations with overhanging trees had higher concentrations of enterococci.

To better understand the factors that affect the occurrence of pathogens in roof runoff samples, binary logistic regression was used to investigate the correlation between the presence/absence of pathogens and the physicochemical parameters of roof runoff samples, the meteorological parameters, the percentage of impervious cover, and the presence of overhanging trees. The MLE method was also used to correlate the concentrations of potential human-infectious pathogens with the concentrations of indicator organisms. However, the low percent of pathogen detection (**Table 2.3**) could potentially limit findings driven by the correlation between the presence/absence of pathogens and geographic location, storm properties, physicochemical parameters, overhanging trees, and % impervious cover. Further research is recommended to capture the effects of location and surrounding conditions on roof runoff microbial quality.

The concentration of *G. duodenalis β-giardin* correlated weakly, but significantly, with *E. coli* concentrations (Chi-square p-value = 0.005, positive correlation). No other indicator organisms showed a significant correlation with potential human pathogens (**Table**). This is consistent to what has been reported in the literature regarding the weak to non-existent correlations between fecal indicator bacteria and potentially human-infectious pathogens (Ahmed

et al., 2008). Fecal indicator organisms do not appear to be well suited to assess human health risk associated with roof runoff. Physicochemical parameters also do not appear to be good predictors of the presence of potential human pathogens (**Table 2.6**).

An observation worth noting is that enterococci were detected in 96% of samples but pathogens in only 16%. Nonetheless, it is of some interest that 100% of bacterial pathogen detections and 85% of all pathogen detections, occurred in samples with enterococci counts >350 MPN/100mL. This would suggest that samples with enterococci counts below this level (almost 50% of samples overall) could represent low pathogen risk.

The presence of *Salmonella* spp. *invA* and *Campylobacter* spp. *ceuE* had a significant weak negative correlation with the number of antecedent dry days (**Table 2.6**, p-values = 0.002 and 0.07, respectively). The presence of *G. duodenalis β-giardin* had a significant weak positive correlation with rainfall depth (p-value = 0.05). Because antecedent dry period was significantly correlated to *Salmonella* spp. *invA* and *Campylobacter* spp. *ceuE*, and rainfall depth was significantly correlated to *G. duodenalis β-giardin*, characteristics of precipitation events may impact the presence of pathogens.

Longer antecedent dry periods may result in reduced buildup of pathogenic organisms. This is possibly due to the desiccation undergone by pathogens during extended dry periods (Spickler, 2012). With longer antecedent dry periods, more cells could enter pore spaces within the roof surface and become more bound to the surface; therefore, the cells might be less easily washed off into rain barrels. Another potential explanation for the reduced buildup of *Campylobacter* spp. *ceuE* and *Salmonella* spp. *invA* with longer antecedent dry period is the influence of UV radiation. Yang et al. (2020) demonstrated using qPCR that UV induced DNA damage in longer gene amplicons.

Higher rainfall depths resulted in more frequent occurrence of *G. duodenalis β-giardin*. This is somewhat counterintuitive because one may expect lower concentrations when roof runoff is more diluted with precipitation from larger depth events. However, more cysts could be possibly washed from the roof surface into the barrels with higher rainfall depths which cover a larger surface of the roof, thereby releasing more cysts. Despite the study's intended goal to provide a comprehensive evaluation of pathogen risk and the large geographical scope of the study, the final dataset remains limited, and the rates of pathogen detection too low, to definitively identify strong relationships between pathogen occurrence and site-associated or seasonal factors. Low rates of pathogen detection in roof runoff is an issue that will continue to challenge robust characterization of microbial quality of roof runoff due to pragmatic considerations with number and volume of collected samples.

All data collected to date suggest that potentially human pathogenic bacteria and protozoan occur with low frequency in roof runoff and that concentrations are notable when detected. Because predictors of pathogen occurrence are difficult to assess in roof runoff, treatment for uses with human exposure should consider the presence of human infectious bacteria and protozoan pathogens to meet health targets. It should also be noted that human enteric pathogen measurements obtained using ddPCR reflect both viable and non-viable pathogens which may or may not be infectious to humans. As result, any risk assessments based on ddPCR measurements could potentially overestimate the risks from exposure to environmental waters.

Table 2.6: Binary Logistic Regression between Pathogens Occurrence and Collected Metadata

	Salmonella spp. invA		*Campylobacter spp. ceuE*		*Giardia duodenalis β–giardin*	
	p-value	Deviance [a] R^2 (%) or Likelihood r[b]	p-value	Deviance [a] R^2 (%) or Likelihood r[b]	p-value	Deviance [a] R^2 (%) or Likelihood r[b]
E. coli	0.15 (+)	0.16	0.95 (+)	0.01	**0.005[c] (+)**	0.31
Enterococci	0.11 (+)	0.18	0.35 (+)	0.10	0.13 (+)	0.17
Trees	0.19 (+)	0.04	0.67 (-)	0.01	0.65 (+)	0.007
TDS	0.47 (-)	0.01	0.91 (-)	0.001	0.90 (-)	0.001
COD	0.74 (-)	0.002	0.63 (+)	0.01	0.95 (-)	0.0001
TSS	0.70 (+)	0.003	0.25 (+)	0.07	0.42 (-)	0.02
VSS	0.28 (+)	0.03	0.31 (+)	0.06	0.56 (-)	0.01
Turbidity	0.61 (-)	0.006	0.29 (+)	0.06	0.23 (-)	0.05
Rainfall Depth	0.10 (+)	0.06	0.14 (-)	-0.12	**0.05[c] (+)**	0.12
Antecedent Dry Period	**0.002[c] (-)**	0.20	**0.07[c] (-)**	0.18	0.62 (-)	0.008
Impervious Cover (%)	0.90 (-)	0.0003	0.77 (+)	0.004	0.48 (-)	0.02

a: Deviance R^2 is calculated for the correlation between the presence of potential human-infectious pathogens with the presence of trees, roof runoff physicochemical parameters, meteorological parameters, and % of impervious cover, b: Likelihood r is calculated for the correlation of indicator organisms' concentrations with the concentrations of potential human-infectious pathogens, c: Bold-faced p – values indicate significant correlation at the 90% confidence level, and the parentheses include the correlation sign.

2.5 Conclusions

- Physicochemical parameters of roof runoff (COD, TDS, TSS, and VSS) were associated with the concentrations of enterococci.
- Fecal indicator bacteria were not significantly correlated to the concentrations of potentially human-infectious pathogens (with the exception of a weak, yet significant, positive correlation between *Giardia duodenalis β-giardin* and *E. coli*) consistent with findings from other studies. Physicochemical parameters and impervious area were not found to be important factors for determination of the presence/absence of potentially human-infectious pathogens.
- Consistent trends between microbial data and geographical location were not found despite noting some differences among study cities when *E. coli* concentrations and *Salmonella spp.* presence in roof runoff were compared.

- There might be an association between meteorological parameters and the presence/absence of potentially human-infectious pathogens based on identified correlations.
- The characterization of the presence and concentrations of potentially human-infectious pathogens obtained from this study can be used as inputs to a QMRA to develop pathogen log reduction targets and select treatment processes compatible with roof runoff end use. This will advance the status of roof runoff as an alternative water resource, thereby reducing the demand on conventional freshwater resources.

CHAPTER 3: ASSESSING HUMAN-SOURCE MICROBIAL CONTAMINATION OF STORMWATER IN THE U.S[2]

3.1 Abstract

Stormwater capture and use (SCU) projects have the potential to provide a significant portion of municipal water demand. However, uncertainty about the degree of stormwater microbial contamination and the required treatment is a barrier for the implementation of SCU projects. Stormwater runoff could become contaminated with human fecal matter in areas with deteriorating infrastructure where raw wastewater exfiltrate from sewer networks to stormwater collection networks, areas with homeless encampments, or areas with sanitary sewer overflows (SSOs). Estimation of human fecal contamination can inform selection of stormwater treatment targets. This study investigates stormwater microbial contamination originating from human fecal matter using the concentration and percent detection of human microbial source tracking (MST) markers and potentially human-infectious pathogens (PHIPs) observed in stormwater. First, a systematic review complied measurements of human MST markers in wet weather flows, dry weather flows, and influent wastewater, and measurements of viral pathogens, e.g., adenoviruses, norovirus GI+GII, and enteroviruses, and protozoan pathogens, e.g., *Giardia lamblia* and *Cryptosporidium parvum*, in wet weather flows and influent wastewater. Human MST marker and PHIP data were statistically analyzed and applied to estimate the human fecal contamination analog (HFCA) which is an estimate of the amount of human fecal matter in wet and dry weather flows based on relative concentrations of microbial contaminants in stormwater compared to municipal wastewater. Analytical statistical distributions of the original data, unpaired Monte

[2] This chapter is adapted from a prepared journal article to be submitted for publication: Alja'fari, J., Sharvelle, S., Branch, A., Jahne, M., Pecson, B., Olivieri, A., and Garland, J. (2023). "Assessing Human-Source Microbial Contamination of Stormwater in the U.S".

Carlo simulation, and paired Monte Carlo simulation were applied for the estimates of HFCAs in wet and dry weather flows. Unpaired Monte Carlo simulation and analytical statistical distributions were found to be the best methods for the estimation of human MST-based HFCAs in wet and dry weather flows which ranged from $<10^{-7.0}$ to $10^{-1.5}$ and 10^{-12} to $10^{-2.6}$, respectively. Unpaired Monte Carlo simulation was found to be the best method to estimate PHIP-based HFCAs in wet weather flows which ranged from $\sim10^{-8}$ to $10^{-0.5}$ based on adenoviruses, $10^{-3.7}$ to $10^{-0.14}$ based on norovirus GI+GII, $<10^{-8}$ to $10^{-0.7}$ based on enteroviruses, $10^{-6.7}$ to $10^{-3.3}$ based on *Giardia lamblia*, and $10^{-4.4}$ to $10^{-0.5}$ based on *Cryptosporidium parvum*. Estimates of human MST-based HFCAs are more reliable than PHIP-based HFCAs because the current PHIP datasets are limited by detection limits and the range of data observed within the statistical distributions. Human MST-based HFCAs can be employed to guide the selection of stormwater treatment process trains based on the intended end use of stormwater (wet and/or dry weather flows).

3.2 Introduction

Locally collected water sources including roof runoff, stormwater, graywater, and wastewater emerge as sustainable options to improve the adaptability of municipalities to the rising pressure on freshwater resources due to population growth and climate change impacts. Demonstration projects across the U.S. show that the use of stormwater is both publicly acceptable and technologically achievable (NBRC, 2018; Kehoe and Chang, 2019; SFPUC, 2018; WJW, 2018; Luthy et al., 2019). However, despite the benefits of incorporating stormwater as part of an integrated urban water management (IUWM) paradigm, many barriers prevent widespread adoption of stormwater capture and use (SCU) projects. The scarcity of regulatory frameworks promoting stormwater use is among the major hindrances to SCU projects (Luthy et al., 2019).

Therefore, guidance on the required treatment of stormwater for potable and non-potable purposes is needed to ensure the protection of public health.

Evidence of stormwater contamination with human fecal matter in areas where stormwater collection systems are separate from wastewater collection systems has been documented across the U.S through the detection of human MST markers and/or PHIPs. Examples include the Northeast/MidAtlantic (Bambic et al., 2011), the Southeast (Bambic et al., 2011; Gonzalez et al., 2020), the Midwest (Sauer et al., 2011), the South (Bambic et al., 2011), and the West (Sercu et al., 2011; Bambic et al, 2011; the City of Santa Barbra, 2012; Environmental Incentives and EcoNorthwest, 2017; Steele et al., 2018; the California Regional Water Quality Control Board, San Diego Region, 2019). The California Regional Water Quality Control Board, San Diego Region (2019) has found that the intrusion of human fecal matter to stormwater collection networks originates from different sources including sanitary sewer overflows (SSOs) from publicly-owned wastewater collection systems, sewage spills from lateral sewer lines which are privately-owned, the exfiltration of wastewater from both publicly- and privately-owned sanitary sewer lines, faulty onsite wastewater treatment systems, e.g., septic tanks, illicit discharges and illegal connections to MS4s, and the indirect or direct contribution from homeless encampments.

While contamination of stormwater with human fecal matter is well documented, guidance on pathogen reduction for safe use of stormwater is scarce and ambiguous. Sharvelle et al. (2017) developed a risk – based framework for the use of stormwater for non-potable purposes. In their work, Sharvelle et al. (2017) recommended pathogen $\log_{10}$ reduction targets (LRTs) specific to stormwater end use and infection risk (i.e., 10^{-2} or 10^{-4} / person / year) based on quantitative microbial risk assessment (QMRA) conducted by Schoen et al. (2017). The LRTs

applied sewage dilutions of 10^{-1} or 10^{-3} in stormwater (Chapter 1, Table 1.1) with the rationale to provide flexibility for selection of LRT based on potential for fecal contamination in stormwater. Sewage dilution refers to the fraction of municipal wastewater present in stormwater and serves as a surrogate for human fecal contamination.

The 10^{-3} to 10^{-1} sewage dilution range is based on measurements of pathogens in stormwater (Schoen et al., 2017; Bambic et al., 2011). Recommended LRTs estimated from sewage dilutions (Sharvelle et al., 2017) are compared to LRTs based on pathogen concentrations measured in stormwater (Figure 3.1). Comparisons between the different LRTs reflect that recommended LRTs based on sewage dilution generally fall within similar ranges as LRTs based on measurements of pathogens in stormwater. LRTs for bacteria and protozoa estimated using sewage dilution are lower than those estimated using measured data (Figure 3.1). This is because viral pathogens were the driver for selection of sewage dilutions due to relatively high concentrations of viruses measured in stormwater compared to bacteria and protozoa (McBride et al., 2013).

Utilities and public health departments were hesitant to apply these LRTs based on sewage dilutions for stormwater use due to the ambiguity in selection of appropriate LRTs within the recommended ranges and a lack of understanding for the basis for selection of 10^{-1} and 10^{-3} sewage dilutions (Lackey et al., 2019). Furthermore, there was confusion pertaining to the exact meaning and application of the term sewage dilution among users of the risk-based framework. Consequently, more data on human fecal contamination in stormwater is needed to address the ambiguity associated with the selection of pathogen LRTs.

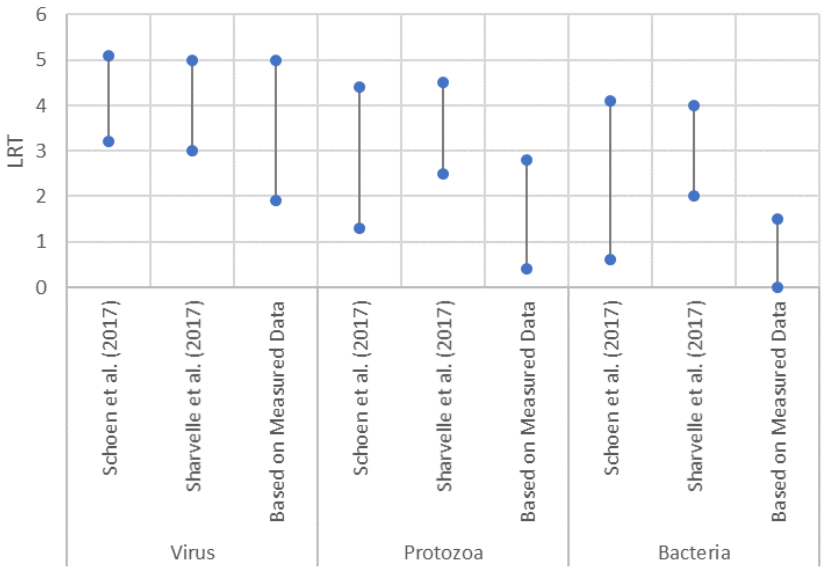


Figure 3.4. Range of LRTs (where dots indicate minimum and maximum) for unrestricted irrigation using stormwater based on 10^{-3} through 10^{-3} dilutions of sewage in stormwater as estimated by Schoen et al. 2017 (includes range for all pathogen organisms for which LRTs were estimated) that served as basis for selection of LRTs for each pathogen group in guidance by Sharvelle et al. (2017). LRTs estimated based on measured data were reported by Schoen et al. (2017) using data reported for different land uses by Bambic et al. (2011) and McBride et al. (2013)

One approach to estimate human fecal contamination in stormwater could be to consider factors that may impact presence and concentration of human fecal contamination. Alja'fari et al., (2022b) assessed a combination of factors including land use, rainfall depth, antecedent dry period, runoff volume, climate region, area of the collection system, and infrastructure conditions were evaluated based on the data synthesized from the literature to determine if any of these factors can serve as predictors of stormwater microbial quality. Consistent trends for human MST markers and/or PHIPs could not be found among the different land uses, storm properties (rainfall depth, antecedent dry period, and runoff volume), and area of the collection system. However, infrastructure conditions were found to impact stormwater contamination with human fecal matter as measured by human MST markers. Some differences were noted in human MST markers and PHIPs concentrations among different U.S climate regions.

The stormwater guidance developed by Sharvelle et al. (2017) and supplemented by Olivieri et al. (2021) overcame a major limitation to the use of stormwater as an alternate water source, namely the lack of risk-based guidelines for the use of stormwater across a range of applications (NRC, 2016). However, the pathogen data driving the sewage dilutions and ultimately the pathogen LRTs were derived from a single study, i.e., Bambic et al. (2011). Given the documented presence of human fecal contamination in stormwater in different locations across the U.S., the limited availability of frameworks regulating the use of stormwater, and the reluctance to adopt currently available risk-based guidance for the use of stormwater for non-potable purposes (Sharvelle et al., 2017; Olivieri et al., 2021), a need exists to estimate ranges for human fecal contamination in stormwater across the U.S. based on more recent, comprehensive datasets.

This study aims to synthesize data available on the microbial quality of stormwater in the U.S. in an effort to assess human fecal contamination in stormwater using concentrations of pathogens and human MST markers observed in stormwater and wastewater. The potential to apply these measurements to estimate human fecal contamination of stormwater and guide selection of appropriate pathogen LRTs is assessed. This study also aims to explore how the estimates of human fecal contamination in stormwater based on human MST markers and pathogens compare with the estimates developed by Schoen et al. (2017) and adopted by Sharvelle et al. (2017), which were based on a limited dataset of stormwater pathogen data.

3.3 Methods

The scope of the study is limited to urban areas where stormwater collection is separate from wastewater collection. Data on stormwater microbial quality in areas with combined sewer overflows is irrelevant for the identification of the required stormwater treatment because stormwater is collected and treated along with wastewater at wastewater treatment plants.

Stormwater microbial quality for wet and dry weather flows is estimated by occurrence (% detection) and concentrations of human MST markers and PHIPs.

Stormwater collection networks convey flows during both wet and dry weather conditions. Wet weather flows refer to precipitation runoff from rain events or snowmelt which flows over pervious and/or impervious surfaces such as parking lots, roof tops, and streets (Sharvelle et al., 2017). Dry weather flows refer to runoff that enters the separate stormwater network from several activities such as over-irrigation, illegal discharges, lawn watering, groundwater seepage, and car washing (Engelhorn and Krish, 2018).

3.3.1 Synthesis of Stormwater Microbial Quality Data for Wet and Dry Weather Flows

3.3.1.1 Screening of Stormwater Microbial Quality Data

An extensive review of peer-reviewed and gray literature reporting stormwater microbial quality (human MST markers and PHIPs) for both wet and dry weather flows was conducted. Initially, stormwater microbial quality data was screened to ensure the following:

- The collected samples were obtained from stormwater outfalls, manholes, pipes, channels, or ditches. Samples collected from stormwater-impacted waters were not considered for data synthesis because human MST markers and pathogens would be diluted in the receiving water body.
- The sampling conditions were clearly defined as either wet or dry.

Data reporting human MST markers and potentially human-infectious pathogens in wet and dry weather flows found in the peer-reviewed and gray literature is summarized in a review; (Alja'fari et al., 2022b). Based on initial screening, datasets from Parker et al., 2010; Staley et al., 2012; and Steele et al., 2017 and the San Diego River dataset from Steele et al. (2018) were

excluded because they reflect the microbial quality of stormwater-impacted waters, or the collection source of samples was not clearly defined. Microbial quality data from Rajal et al., 2007; Feng et al., 2018; and Paar et al., 2015 was excluded due to ambiguous sampling conditions, i.e., wet versus dry, were not reported, or dry weather data was grouped with wet weather data.

3.3.1.2 QA/QC Criteria for the Inclusion of Stormwater Microbial Quality Measurements in the Data Synthesis

Following the initial screening of stormwater microbial quality data collected during the literature review phase, Quality Assurance (QA) / Quality Control (QC) were defined for the remaining datasets (undiluted stormwater collected under defined sampling conditions). The inclusion criteria for data on human MST markers and potentially human-infectious pathogens in wet and dry weather flows are as follows:

- The measured source tracking markers must be human rather than animal or general source tracking markers.
- The target DNA recovery method, recovery rate, and limit of detection and quantification should be defined (Schoen et al., 2017).
- For each human MST marker and PHIP, the percentage of detection, concentration, and number of analyzed samples should be defined.
- Fresh rather than stored samples should be analyzed (Schoen et al., 2017).
- The collected samples should be composite, or event mean concentrations should be reported.
- Monitoring should be conducted at multiple locations over time (Schoen et al., 2017).

Each QA/QC criterion was assigned high, medium, or low priority based on the importance of the criterion in question to the data synthesis process (Appendix B, Tables B.2 and

B.3). Ideally, all low, medium, and high priority criteria should be met to include the data. However, given the limited number of studies on stormwater microbial quality, meeting only high priority criteria is deemed suitable to include the studies for practical purposes. Human MST marker data in dry weather flows was included in the analysis. However, only 8 data points were found on PHIPs in dry weather flow in one study location, i.e., California (Bambic et al., 2011). These data points are not included in the synthesis as they are deemed insufficient to estimate the presence of human fecal contamination in dry weather flows using pathogen concentrations.

3.3.2 Statistical Analyses of Microbial Quality Data in Wet and Dry Weather Flows

Human Microbial Source Tracking Markers Data

Data on the detection and concentration of human MST markers (HF183 and BacHum) in wet weather flows were obtained for sampling locations in the Mid-Atlantic and the Southeastern regions of the U.S., Texas (Bambic et al., 2011), California (Cao et al, 2017; Steele et al., 2018), and Southeastern Virginia (Gonzalez, 2022; personal communication). Data on human MST markers (HF183 and BacHum) in dry weather flows were obtained for sampling locations in California (Bambic et al., 2011; Cao et al, 2017; Steele et al., 2018), and Southeastern Virginia (Gonzalez, 2022; personal communication).

Adenoviruses, Noroviruses, and Enteroviruses Data

Data on adenoviruses, norovirus GI+GII, and enteroviruses in wet weather flows were obtained for sampling locations in the Mid-Atlantic and Southeastern regions of the U.S., Texas (Bambic et al., 2011), Milwaukee, WI (Sauer et al., 2011) and California (Steele et al., 2018). Not enough data was available on adenoviruses, noroviruses, and enteroviruses in dry weather flows, e.g., eight data points for each virus from sampling locations in California only (Bambic et al.,

2011). A review of the available literature on the presence and concentration of adenoviruses in wet weather flows revealed some inconsistencies in the way that researchers report their findings. For instance, Bambic et al. (2011) measured adenovirus A, B, C, and 40/41. Sauer et al. (2011) measured adenovirus serotypes 1, 2, 5, 6, 40, and 41. On the other hand, Steele et al. (2018) reported measurements of adenovirus without specifying the species or the serotypes.

Upon closer examination of the literature on adenoviruses in stormwater, adenovirus species were matched with their serotypes, e.g., adenovirus species C has the serotypes 1, 2, 5, and 6, and adenovirus species F has the serotypes 40 and 41 (Turnell, 2008). Consequently, adenoviruses C and F from Bambic et al. (2011) were added, and the sum was referred to as total adenoviruses. Adenoviruses A and B were not detected in any sample (Bambic et al., 2011).

Similarly, adenovirus serotypes 1, 2, 5, and 6, i.e., species C, and adenoviruses serotypes 40 and 41, i.e., species F, from Sauer et al. (2011) were also added, and the resulting sum was also referred to as total adenoviruses. It was assumed that human adenovirus measured by Steele et al. (2018) encompasses serval species and serotypes, among which the ones measured by Bambic et al. (2011) and Sauer et al. (2011) could be found.

Giardia lamblia and Cryptosporidium parvum Data

Data on the concentration of *Giardia lamblia* and *Cryptosporidium parvum* in wet weather flows could only be obtained from a single study (Bambic et al., 2011). Nonetheless, the sampling efforts in this study covered different locations such as the Mid-Atlantic and Southeastern regions of the U.S., and Texas (Bambic et al., 2011). Not enough data was available on protozoan pathogens in dry weather flows, e.g., 8 data points for each protozoan pathogen from sampling locations in California only (Bambic et al., 2011).

Statistical Analyses

Descriptive statistics for human MST markers and potentially human-infectious pathogens were obtained using the statistical software Minitab 18. The summary statistics include the 5th percentile, 25th percentile, median, 75th percentile, and 95th percentile. Summary statistics were obtained using censored data analysis techniques. The term censored data refers to observations that are not quantified and are known only to be less or more than a threshold value (Helsel, 2012). Since the concentrations of human MST markers (in up to 46% of samples) and potentially human-infectious pathogens (in 26% to 93% of samples) fall below the analytical limit(s) of detection for stormwater samples, censored data analysis techniques were used.

Gilliom and Helsel (1986) found that substituting a fraction ranging from 0 to 0.99 times the analytical limit of detection is a poor method for calculating descriptive statistics. Justifying substitution rarely consider changing reporting limits (Helsel, 2012), which in the case of stormwater change not only from one study to another but also from one sample to another within the same study. Substitution of values that are reliant on changing limits of detection introduces an external signal into microorganisms' concentrations, which is not originally present in the sampled stormwater (Helsel 2012). A consequence of substitution is that microbial contamination could go unnoticed, treatment might be deemed unnecessary, and public health might be unknowingly compromised (Helsel, 2012).

Multiple censored data techniques were considered for the estimation of summary statistics including the maximum likelihood estimation (MLE) method, imputation methods, Kaplan-Meier/Turnbull, robust regression on order statistics (ROS), and robust MLE following the recommendations of Helsel (2012) for the analysis of censored environmental data.

The MLE method is most suitable for the estimation of descriptive statistics when a large number of data points (n > 50) is available, and 50 – 80% of the observations are censored (Helsel, 2012). For cases with different censoring conditions and number of data points, other analysis methods should be considered (Table 3.1; Helsel, 2012). The MLE method was selected for the current analysis because almost 60% of the data from individual and combined land uses and grouped studies met the MLE method conditions. Furthermore, maintaining consistency in the analysis methods among all data points was deemed important. Summary statistics were not obtained for any data grouping with more than 80% censored observations to avoid unreliable estimates (Helsel, 2012).

Table 3.1 Recommended analysis methods for estimation of descriptive statistics (Helsel, 2012)

Percent of Censored Observations	Number of Observations	
	<50	**>50**
<50%	KM/Turnbull or Imputation	KM/Turnbull or Imputation
50 – 80%	Robust ROS, Robust MLE, or Multiple Imputation	Multiple Imputation or MLE

To employ the MLE method in Minitab 18, concentrations of human MST markers and potentially human-infectious pathogens were coded to account for left censoring at the lower limit of detection as follows:

- A sample with human MST marker or pathogen concentration less than a detection limit of, for example, 3 GC/100 mL was expressed as (0, 3).
- A positive sample with a pathogen concentration of 493 GC/100 mL was expressed as (493, 493).

The descriptive statistics were found using the Reliability/Survival package in Minitab 18. A lognormal distribution was fitted to the different groupings of microbial data based on:

- The Anderson-Darling (AD) value for datasets including censored data and the p-value for datasets with 100% positive detections.
- Visual inspection of the distribution fit to the dataset in cases where the p-value and/or the AD value are not conclusive or suitable to make a judgement.

For the case when human MST data from combined studies were analyzed (Bambic et al., 2011; Sauer et al., 2011; Cao et al., 2017; Steele et al., 2018; Gonzalez, 2022: personal communication; Clary et al., 2020), the genetic markers of human fecal contamination, BacHum and HF183, were grouped together since both are 16S rRNA human marker genes derived from the same taxa, *Bacteroidales* and the same genus *Bacteroides* (Ahmed et al., 2016).

The detection unit GC/100 mL was used for human MST markers, norovirus, adenovirus, and enterovirus following convention in the reviewed literature (e.g., Sauer et al., 2011 and Steele et al., 2018). Sauer et al. (2011) expressed the concentrations of viruses in genomic equivalents whereas Bambic et al. (2011) and Steele et al. (2018) expressed the concentrations of viruses in genomic copies. For purposes of this analysis, genomic equivalents were assumed to be the same as genomic copies (WHO & WHO Expert Committee on Biological Standardization, 2015). The detection unit (oo)cyst/L was used for protozoan pathogens since most dose-response relationships used in QMRAs use this unit (e.g., Schoen et al., 2017). A log normal distribution was fit to human MST marker data and potentially human-infectious pathogen data.

3.3.3 Estimation of Human-Source Microbial Contamination in Wet and Dry Weather Flows

The term Human Fecal Contamination Analog (HFCA) is used in this study to describe the amount of human fecal contamination in wet and dry weather flows as a function of either human MST markers ($HFCA_{human\ MST\ markers}$) or PHIPs ($HFCA_{PHIPs}$). Estimating HFCA requires

knowledge of the occurrence and concentrations of human MST markers or PHIPs in wet and dry weather flows and in raw wastewater.

3.3.3.1 Synthesis of HF183 Data for Sewage (Raw Wastewater)

Concentrations of HF183 in influent wastewater were obtained for 49 wastewater treatment facilities (WWTFs) across the United States. Schoen et al. (2020) reported these concentrations based on earlier investigation conducted by Shanks et al. (2010). This data was supplemented by monthly measurements of HF183 in influent wastewater from 2016 to 2017 across 4 WWTPs in Virginia (Gonzalez, 2022; personal communication). A censored data analysis was not applied to HF183 measurements in influent wastewater since all samples were positive for HF183. A lognormal distribution was fit to the entire HF183 dataset (p-value = 0.36), including influent concentrations obtained from Shanks et al. (2010) and Gonzalez (2022; personal communication).

3.3.3.2 Synthesis of Pathogen Data for Sewage (Raw Wastewater)

Eight studies with pathogen concentrations in influent wastewater in different locations across the U.S., including California (Pecson et al., 2021), Arizona (Kitajima et al., 2014 a and b; Schmitz et al., 2016), Michigan (Fong et al., 2010; Simmons et al., 2011; Simmons and Xagoraraki, 2011), and Ohio (Francy et al., 2012), were identified. The selection of high-quality wastewater pathogen dataset(s) to estimate HFCA in stormwater was based on the following criteria established by Schoen et al. (2017) and Pecson et al. (2021):

- Pathogen or spike recovery is measured and reported for a minimum of one influent wastewater sample
- The sample limit of detection is clearly specified for each pathogen
- The measured recovery is equal to or greater than 5%

- The percentage of samples with pathogen concentrations greater than the limit of detection is at least 50%
- The percent detection, concentration, and number of collected samples are reported
- Fresh, rather than stored samples, are analyzed
- Composite samples are collected; and multiple locations are monitored over time

The eight identified studies measuring the concentrations of potentially human-infectious pathogens in raw wastewater were classified based on the degree to which they adhere to the aforementioned criteria (high adherence: study meets all 7 criteria; medium adherence: study meets 6 out of the 7 criteria; low adherence: study meets less than 6 criteria; Appendix B, Table B.4). The study conducted by Pecson et al. (2021) satisfies all the high-quality data criteria.

While the data collected by Kitajima et al. (2014a and b) and Schmitz et al. (2016) also meet all the high-quality criteria, the complete dataset was not available to the author, and thus descriptive statistics could not be completed. The ranges of the concentrations observed by Pecson et al. (2021) do not vary substantially from the ranges observed by Kitajima et al. (2014a and b) and Schmitz et al. (2016) (Figure 3.2). The dataset from Francy et al. (2012) meets most of the high-quality data criteria (6 out of 7).

Datasets obtained from Simmons et al. (2011), Simmons and Xagoraraki (2011), and Fong et al. (2010) are excluded from the analysis because they meet less than 6 of the 7 criteria . Considering that the complete datasets from Kitajima et al. (2014a and b) and Schmitz et al. (2016) are not available and that Francy et al. (2012) did not provide the detection limit for norovirus GII, the datasets provided by Pecson et al. (2021) emerge as the only suitable dataset for estimating HFCAs. Additionally, the pathogen concentrations in California, Arizona, Ohio, and Michigan are not substantially different (Figure 3.2).

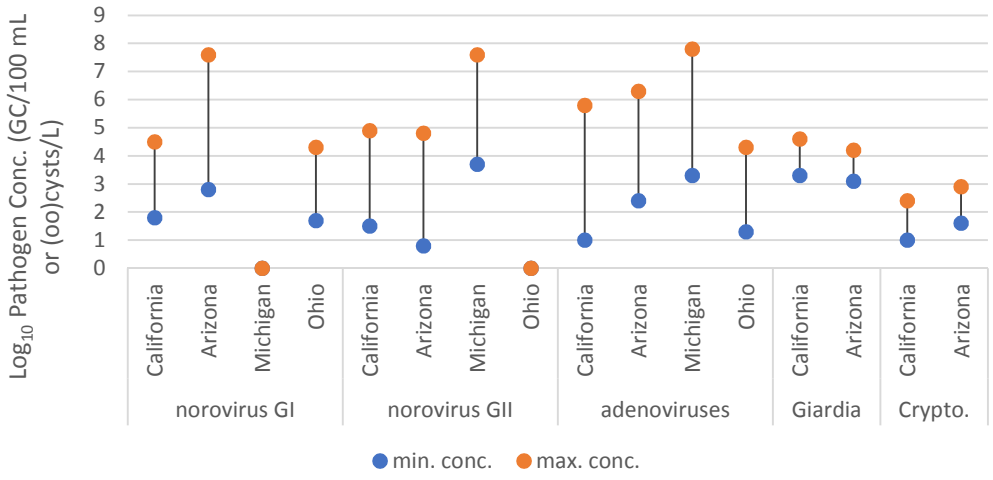


Figure 3.5. Concentration Ranges of PHIPs in Raw Wastewater in Different U.S States (Virus concentrations are expressed in GC/100 mL and protozoan concentrations are expressed in (oo)cysts/L; orange circles on the horizontal axis without a range represent concentrations below the detection limit)

Measurements of adenovirus, norovirus GI+GII, enterovirus, *Giardia lamblia*, and *Cryptosporidium parvum* were obtained from a comprehensive study of pathogens in raw wastewater across 5 WWTPs in California over a monitoring period of 14 months (Pecson et al., 2021). Unlike HF183, adenoviruses, noroviruses GI+GII, enteroviruses, and *Cryptosporidium parvum* were not detected in all influent wastewater samples. Therefore, the pathogen data in influent wastewater was analyzed in the same manner applied for wet weather flows, i.e., censored data analysis (Section 3.3.2). A log normal probability distribution was fit to adenovirus F, norovirus GI+GII, enteroviruses, and *Cryptosporidium parvum* in influent wastewater. All wastewater samples were positive for *Giardia lamblia*. A lognormal distribution was fit to influent *Giardia lamblia* concentrations in influent wastewater.

3.3.4 Human Fecal Contamination Analog in Wet and Dry Weather Flows

To calculate HFCAs in wet and dry weather flows, a Monte Carlo simulation is used for unpaired and paired analysis of data. Unpaired analysis is used because the stormwater measurements were not obtained from the same locations where the wastewater measurements

were obtained. Paired analysis is used for comparison. Analytical distributions (no simulated data) are also used for unpaired analysis of data to generate HFCA values.

Monte Carlo Unpaired Simulations for Human Microbial Source Tracking Markers

Based on human MST markers distribution parameters (location and scale), 10^3 data points following a lognormal distribution were generated (wet weather distribution or dry weather distribution). Using the distribution parameters (location and scale) of HF183 in wastewater, 10^3 data points following the lognormal distribution were also generated (wastewater distribution). The HFCA in wet weather flows was calculated by dividing each human MST marker data point in the wet weather distribution by each HF183 data point in the wastewater distribution (Equation 3.1). A lognormal distribution was fitted to the HFCA values for both wet and dry weather flows (p-value = 0.14 and 0.13, respectively).

$$HFCA_{Human\ MST\ Marker} = C_{Human\ MST\ Marker\ i} / C_{Human\ MST\ Marker\ j} \quad \text{(3.1)}$$

Where:

$HFCA_{Human\ MST\ Marker}$: Human fecal contamination analog in wet or dry weather flow based on human MST markers.

$C_{Human\ MST\ Marker\ i}$: concentration of human MST marker in wet or dry weather flow (GC/100 mL).

$C_{Human\ MST\ Marker\ j}$: concentration of human MST marker in influent wastewater (GC/100 mL).

Monte Carlo Paired Simulations for Human Microbial Source Tracking Markers

Based on human MST markers distribution parameters (location and scale), 10^3 data points following a lognormal distribution were generated (wet weather distribution or dry weather distribution). Using the distribution parameters (location and scale) of HF183 in wastewater, 10^3 data points following the lognormal distribution were also generated (wastewater distribution).

The 10^3 data points for both wet/dry weather flows and wastewater were ordered from lowest to highest (paired analysis) such that the lowest concentrations of human MST markers in wet/dry weather flows are paired with the lowest concentrations of HF183 in raw wastewater.

A lognormal distribution was fitted to the HFCA values for wet and dry weather flows (p-value = 0.01, AD value = 1.03). Even though the p-value is less than 0.05, the lognormal distribution provides the best fit for HFCAs of all the assessed statistical distributions (normal, lognormal, Weibull, and Gamma). Similarly, a lognormal distribution was fitted to the HFCA values for dry weather flows (p-value = 0.98).

Monte Carlo Unpaired Simulations for Adenoviruses, Noroviruses, and Enteroviruses

HFCAs in wet weather flows based on total adenoviruses, norovirus GI+GII, and enteroviruses were obtained using a Monte Carlo simulation for unpaired and paired analysis of data. However, out of the 1000 generated HFCA scenarios for the unpaired analysis, 25.3%, 22.1%, and 12.2% were unrealistic scenarios for total adenoviruses, norovirus GI+GII, and enteroviruses, respectively. An unrealistic scenario is a simulation where the fraction of wastewater (human fecal matter) in stormwater is greater than 100%. This means that 253 total adenoviruses – HFCA estimates, 221 norovirus GI+GII – HFCA estimates, and 122 enterovirus – HFCA estimate indicated that stormwater is comprised of more than 100% wastewater. This is not unprecedented, especially considering the sparse detection of pathogens at high concentrations in stormwater, e.g., 16% for total adenoviruses, and the long persistence of viruses in environmental water matrices (Ahmed et al., 2014). A similar result has been observed in surface water measurements (Boehm et al., 2018). Boehm et al. (2018) also excluded combinations that were considered unrealistic from their analysis. A lognormal and Weibull distributions were fit to the

remaining HFCAs for each viral pathogen. Both distributions are evaluated for their suitability to fit the datasets.

Monte Carlo Paired Simulations for Adenoviruses, Noroviruses, and Enteroviruses

The 1000 paired Monte Carlo simulations of adenoviruses and enteroviruses did not result in any unrealistic scenarios, i.e., stormwater having more than 100% of raw wastewater. On the other hand, 8.3% of the norovirus GI+GII simulations were unrealistic and were discarded. A lognormal distribution was fit to the adenovirus-, norovirus, and enterovirus-based HFCAs.

Monte Carlo Unpaired Simulations for Giardia lamblia and Cryptosporidium parvum

Unlike viral pathogens, protozoan pathogens did not result in unrealistic HFCA estimates. All protozoan – based dilution estimates indicated a stormwater matrix with less than 100% sewage. A lognormal distribution was fit to the HFCA estimates based on *Giardia lamblia* and *Cryptosporidium parvum*.

Monte Carlo Paired Simulations for Giardia lamblia and Cryptosporidium parvum

Similar to the unpaired simulations for protozoan pathogens, the 1000 paired simulations for *Giardia lamblia* and *Cryptosporidium parvum* all resulted in stormwater matrices with less than 100% sewage. A lognormal distribution was also fit to the HFCA estimates based on *Giardia lamblia* and *Cryptosporidium parvum*.

Analytical Distribution Method for Estimation of HFCAs Based on Human MST Markers and PHIPs

HFCAs can be estimated using the analytical distributions for the original datasets (human MST markers and PHIPs in wet weather and dry weather flows) instead of using Monte

Carlo simulations to generate the distributions. However, this method is only applicable for unpaired data analyses. Using the distribution parameters for human MST markers and PHIPs (e.g., location and scale), the location and scale parameters for HFCAs can be generated as follows assuming a lognormal distributional fit for human MST markers, PHIPs, and HFCAs:

$$Location_{HFCA} = Location_{x_i} - Location_{x_j} \quad \text{(3.2)}$$

$$Scale_{HFCA} = \sqrt{Scale_{x_i}^{\ 2} + Scale_{x_j}^{\ 2}} \quad \text{(3.3)}$$

Where:

$Location_{HFCA}$: the location parameter of the lognormal distribution of HFCA based on either human MST markers or PHIPs

$Location_{xi}$: the location parameter of the lognormal distribution of human MST markers or PHIPs in wet or dry weather flows

$Location_{xj}$: the location parameter of the lognormal distribution of human MST markers or PHIPs in influent wastewater

The descriptive statistics for HFCA based on human MST markers or PHIPs in wet or dry weather flows can be found using the following equations:

$$5th\ percentile_{HFCA} = Location_{HFCA} - (1.645 \times Scale_{HFCA}) \quad \text{(3.4)}$$

$$25th\ percentile_{HFCA} = Location_{HFCA} - (0.675 \times Scale_{HFCA}) \quad \text{(3.5)}$$

$$Median_{HFCA} = Location_{HFCA} \quad \text{(3.6)}$$

$$75th\ percentile_{HFCA} = Location_{HFCA} + (0.675 \times Scale_{HFCA}) \quad \text{(3.7)}$$

$$95th\ percentile_{HFCA} = Location_{HFCA} + (1.645 \times Scale_{HFCA}) \quad \text{..... (3.8)}$$

3.3.5 Selection of the Ideal Dataset for Estimation of Human Microbial Contamination in Stormwater

Human MST markers, viral pathogens (adenoviruses, norovirus GI+GII, and enteroviruses), and protozoan pathogens (*Giardia lamblia* and *Cryptosporidium parvum*) can be used to estimate human fecal contamination in stormwater. Different markers and PHIPs will results in different estimates of HFCAs. The following criteria will be used to select the ideal dataset/microorganism to estimate human fecal contamination in stormwater:

- The microorganism dataset should be well described by the statistical distribution (e.g., lognormal or Weibull). This can be judged by visual inspection, p-value (greater than 0.05), and/or the Anderson-Darling (AD) value.
- The selected distribution for the microorganism should cover a wide range of the microorganism dataset (from low to high percentiles) taking into account the percentage of data points that fall below the limit of detection.

3.4 Results and Discussion

3.4.1 Data Synthesis of Human MST Markers in Wet and Dry Weather Flows

Based on the data obtained from the literature review, human MST markers were detected in 65.5% of wet weather flows at a median concentration of $10^{2.9}$ GC/100 mL (Figure 3.3, Table 3.3). This confirms the contamination of stormwater with human fecal matter, which could arise from different mechanisms including sewage exfiltration from wastewater collection pipes to stormwater pipes, SSOs, and/or direct or indirect contributions from homeless encampments.

In contrast to wet weather flows, human MST markers are detected less frequently in dry weather flows, i.e., 25.3% (Table 3.3) at a median concentration of $10^{-0.2}$ GC/100 mL, approximately three orders of magnitude less than the median concentration of human MST markers in wet weather flows. This is likely because storm events flush sewage/human fecal matter that has accumulated in soil during the antecedent dry period. Runoff during dry periods (dry weather flow) is not sufficient to wash off sewage/human fecal contamination. In contrast, Sercu et al. (2011) reported HF183 concentrations in storm drains in three watersheds located in Santa Barbara, CA ranging from non-detects to $10^{6.2}$ copies/100mL during dry weather periods. However, two of the investigated storm drains were known to be contaminated with sewage, with one of them receiving wastewater exfiltrating from a sanitary sewer subject to surcharge conditions at regular intervals.

The median concentration of human MST markers in influent wastewater ($10^{7.4}$ GC/100 mL) is about 4 orders of magnitude higher than that in wet weather flows, whereas the median concentration of human MST markers in influent wastewater is about 8 orders of magnitude higher than that in dry weather flows (Table 3.3). A lognormal probability distribution provided a good fit for the concentrations of human MST markers in wet weather flows (AD-value = 2.4), dry weather flows (AD-value = 27.1), and influent wastewater (p-value = 0.36 and AD-value = 0.4). The lognormal probability plot covers a wider percentile range for wet weather flows than dry weather flows (Table 3.3).

3.4.2 Data Synthesis of PHIPs in Wet Weather Flows

Adenoviruses, Noroviruses, and Enteroviruses

Adenoviruses and enteroviruses were detected in wet weather flows at lower frequency, i.e., 41.4% and 38.6%, respectively, compared to norovirus GI+GII, which was detected at a

frequency of 74.3% (Table 3.3). Median norovirus GI+GII concentration is approximately two orders of magnitude higher than median adenoviruses and enteroviruses concentrations in wet weather flows (Table 3.3). Adenoviruses and enteroviruses have much lower median concentrations in wet weather flows when compared to influent wastewater (Table 3.3 and Figure 3.4). However, norovirus GI+GII median concentration in wet weather flows is only 1 order of magnitude less than that in influent wastewater (Table 3.3 and Figure 3.4).

The high concentrations of norovirus GI+GII indicate that noroviruses could drive the risk associated with SCU projects, and thus, treatment process trains selected for stormwater treatment should include unit processes that remove noroviruses effectively. In general, viral pathogens, e.g., human adenoviruses, have long survival times in water matrices (Ahmed et al., 2014). As such, the bacterial human MST markers would be more suitable for the identification of recent human fecal contamination events, while human viruses, e.g., adenoviruses, enteroviruses, and norovirus GI+GII, could be utilized as additional viral markers for the assessment of potential health risks related to longer-term human fecal contamination of environmental waters (Ahmed et al., 2014).

Considering the high concentrations of adenoviruses, norovirus GI+GII, and enteroviruses in stormwater at the 95th percentile (Table 3.3) and their closeness to their corresponding values in wastewater, the host-species of these viruses in stormwater samples were investigated (Table 3.4) to find whether the measured viral concentrations originated from animal and human hosts or human hosts only.

For most of the measured viruses in stormwater (adenoviruses, norovirus GI+GII, and enteroviruses), the host species was human based on the reported methods (Table 3.4). However, it is possible that some of the measured viruses were zoonotic, specifically, adenovirus 40/41 since

Bambic et al. (2011) reported the measurement method as obtained through personal communication without specifying the host species. As for norovirus GI+GII values reported by Bambic et al. (2011), the norovirus GII assay did not distinguish between porcine strains and human ones (Wolf et al., 2009).

The lognormal probability distribution provides a good fit for viral pathogens in both wet weather flows and influent wastewater based on the AD value (Table 3.3). However, for wet weather flows, the lognormal probability distribution covers a wider percentile range for norovirus GI+GII compared to adenoviruses and enteroviruses (Table 3.3).

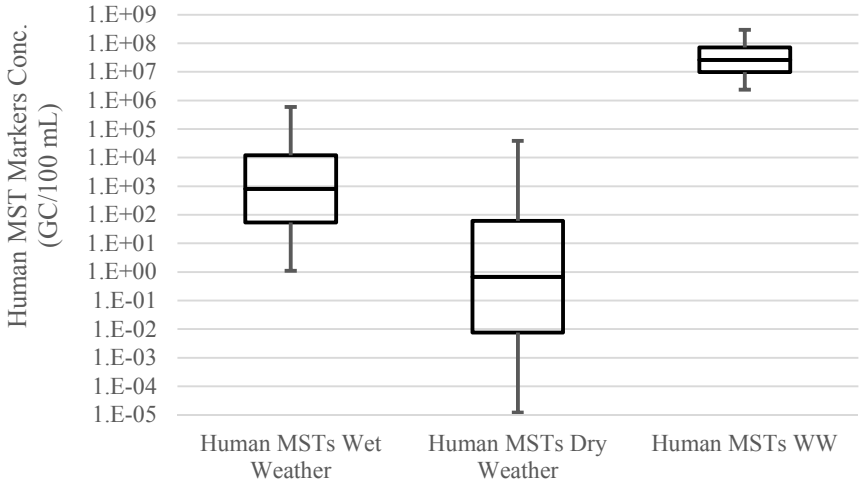


Figure 3.3 Boxplot of Human MST Markers in Wet Weather Flows and Influent Wastewater (WW) where $n_{huamn\ MST\ markers\ wet\ weather} = 116$, $n_{huamn\ MST\ markers\ dry\ weather} = 383$, and $n_{huamn\ MST\ markers\ WW} = 91$. The middle line represents the median; the first and third lines represent the 25th and 75th percentiles, respectively. The vertical lines extend to the 5th and 95th percentiles.

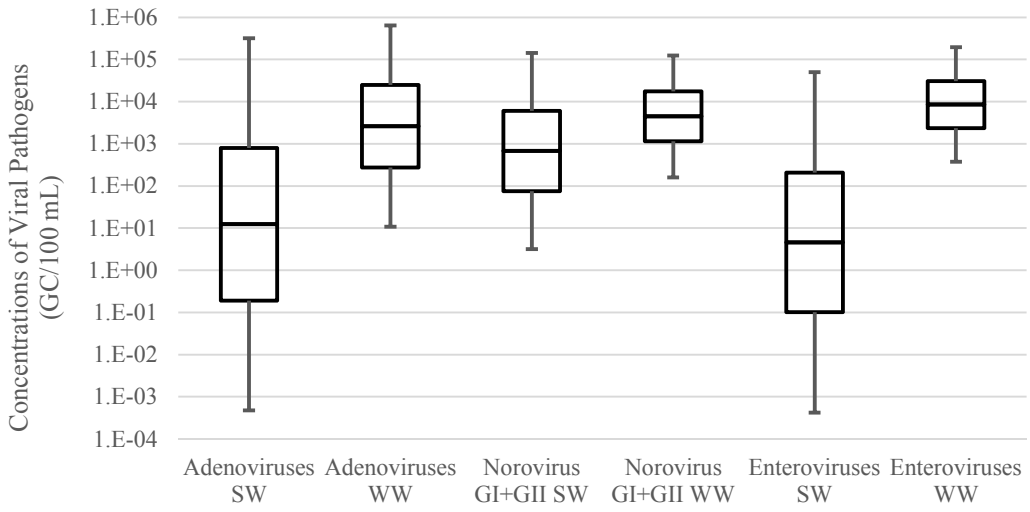


Figure 3.6. Boxplot of Viral Pathogens in Wet Weather Flows (SW) and Influent Wastewater (WW) where $n_{adenoviruses\ SW} = 70$, $n_{adenoviruses\ WW} = 122$, $n_{norovirus\ GI+GII\ SW} = 70$, $n_{norovirus\ GI+GII\ WW} = 122$, $n_{enteroviruses\ SW} = 70$, $n_{enteroviruses\ WW} = 122$. The middle line represents the median; the first and third lines represent the 25th and 75th percentiles, respectively. The vertical lines extend to the 5th and 95th percentiles.

Table 3.3 Summary of Data Sets, Descriptive Statistics, and Goodness of Fit for the Concentrations of Human MST Markers (HF183 and BacHum) in Wet Weather, Dry Weather, and Influent Wastewater (WW)

Stormwater Microbial Quality / Descriptive Statistics	Human MST Markers			Potentially Human-Infectious Pathogens									
	National Dataset			Adenoviruses		Norovirus GI+GII		Enteroviruses		*Giardia*		*Cryptosporidium*	
	Wet Weather	Dry Weather	WW	Wet Weather	WW	Wet Weather	WW	Wet Weather	WW	Wet Weather	WW	Wet Weather	WW
No. of Samples	116	383	91	70	122	70	122	70	122	48	120	48	120
% Non-detects	34.5	74.7	0%	58.6	31	25.7	23	61.4	28	50	0	43.8	2
LOD in GC/100 mL or (oo)cysts/L	$10^{1.7}$ - $10^{3.5}$	$10^{1.3}$ - $10^{3.5}$	NA	$10^{0.5}$ - $10^{3.3}$	$10^{2.8}$	$10^{0.78}$ - $10^{3.53}$	$10^{2.8}$ - $10^{2.9}$	$10^{0.48}$ - $10^{2.58}$	$10^{3.5}$	$10^{-1.0}$ - $10^{-0.52}$	$10^{0.6}$	$10^{-1.0}$ - $10^{-0.52}$	$10^{0.6}$
LOQ in GC/100 mL or (oo)cysts/L	NS[a]	NS[a]	500	NS[a]	$10^{3.3}$	NS[a]	$10^{3.2}$ - $10^{3.6}$	NS[a]	$10^{3.9}$	NS[a]	$10^{0.6}$	NS[a]	$10^{0.6}$
5th Percentile in GC/100 mL or (oo)cysts/L	$10^{0.04}$	$10^{-4.9}$	$10^{6.4}$	$10^{-3.3}$	$10^{1.0}$	$10^{0.50}$	$10^{2.21}$	$10^{-3.38}$	$10^{2.57}$	$10^{-2.6}$	$10^{3.3}$	$10^{-2.7}$	$10^{1.0}$
25th Percentile in GC/100 mL or (oo)cysts/L	$10^{1.7}$	$10^{-2.1}$	$10^{7.0}$	$10^{-0.7}$	$10^{2.4}$	$10^{1.88}$	$10^{3.06}$	$10^{-1.00}$	$10^{3.37}$	$10^{-1.7}$	$10^{3.7}$	$10^{-1.6}$	$10^{1.4}$
Median in GC/100 mL or (oo)cysts/L	$10^{2.9}$	$10^{-0.2}$	$10^{7.4}$	$10^{1.1}$	$10^{3.4}$	$10^{2.83}$	$10^{3.65}$	$10^{0.66}$	$10^{3.93}$	$10^{-1.1}$	$10^{4.0}$	$10^{-0.8}$	$10^{1.7}$
75th Percentile in GC/100 mL or (oo)cysts/L	$10^{4.1}$	$10^{1.8}$	$10^{7.9}$	$10^{2.9}$	$10^{4.4}$	$10^{3.78}$	$10^{4.24}$	$10^{2.32}$	$10^{4.49}$	$10^{-0.4}$	$10^{4.2}$	$10^{-0.1}$	$10^{2.0}$
95th Percentile in GC/100 mL or (oo)cysts/L	$10^{5.8}$	$10^{4.6}$	$10^{8.5}$	$10^{5.5}$	$10^{5.8}$	$10^{5.16}$	$10^{5.09}$	$10^{4.70}$	$10^{5.29}$	$10^{0.5}$	$10^{4.6}$	$10^{1.0}$	$10^{2.4}$
Data Distribution	LN[b]	LN[b]	LN[b]	LN[b]	LN[b]	LN[b]	LN[b]	LN[b]	LN[b]	LN[b]	LN[b]	LN[b]	LN[b]
Goodness of Fit													
Percentiles Range Bound by the Distribution	25th - 99th	70th - 99.8th	1st - 99th	50th - 99th	30th - 99th	10th - 99th	50th - 99th	60th - 97th	10th - 99th	50th - 98th	1st - 99th	40th - 98th	5th - 99th
p-value (greater than 0.05)	NA[c]	NA[c]	0.36	NA[c]	NA[c]	NA[c]	NA[c]	NA[c]	NA[c]	NA[c]	0.08	NA[c]	NA[c]
AD value	2.4	27.1	0.4	3.5	3.9	0.93	4.17	4.98	1.34	2.82	0.68	2.65	0.64
Criteria met for Suitability for HFCA Estimation	✓		✓		✓		✓		✓		✓		✓

a: not specified, b: lognormal probability distribution, c: not applicable

Table 3.4 Host Species of the Viruses Measured in Stormwater Samples

Study	Virus	Method	Source: human/zoonotic
Bambic et al. (2011)	Adenovirus A	Leruez-Ville et al. (2004)	Human (44 immunocompromised patients)
	Adenovirus B		
	Adenovirus C		
	Adenovirus 40/41	Leutenger (personal communication)	Unclear
	Enterovirus	Fuhrman et al. (2005)	Human
	Norovirus GI+GII	Wolf et al. (2007)	Norovirus GII assay does not distinguish between porcine strains and human ones (Wolf et al., 2009).
Steele et al. (2018)	Adenoviruses	Jothikumar et al. (2005) and Cao et al. (2015)	Human
	Enteroviruses	Gregory et al. (2006)	Human fecal contamination
	Norovirus GI+GII	Da Silva et al. (2007)	Human
Sauer et al. (2011)	Adenoviruses serotypes 1, 2, 5, 6, 40, and 41	Lambertini et al. (2008)	Human
	Enteroviruses	Borchardt et al. (2004), Lambertini et al. (2008)	Human
	Norovirus GI+GII	Borchardt et al. (2004), Lambertini et al. (2008)	Human

Giardia lamblia and Cryptosporidium parvum

Giardia lamblia and *Cryptosporidium parvum* are detected in almost half of the wet weather flow measurements at lower median concentrations compared to viral pathogens (Table 3.3 and Figure 3.5). *Giardia lamblia* median concentration in influent wastewater is 5 orders of magnitude higher than that in wet weather flows, while *Cryptosporidium parvum* median concentration in influent wastewater is approximately 3 orders of magnitude higher than that in wet weather flows. Therefore, *Giardia lamblia* seems to drive the risk arising from protozoan pathogens.

The lognormal probability distribution provides a good fit for protozoan pathogens in both wet weather flows and influent wastewater as evidenced by the p-values and AD values (Table 3.3). However, compared to influent wastewater the lognormal probability distribution covers a narrow percentile range for protozoan pathogens in wet weather flows (Table 3.3).

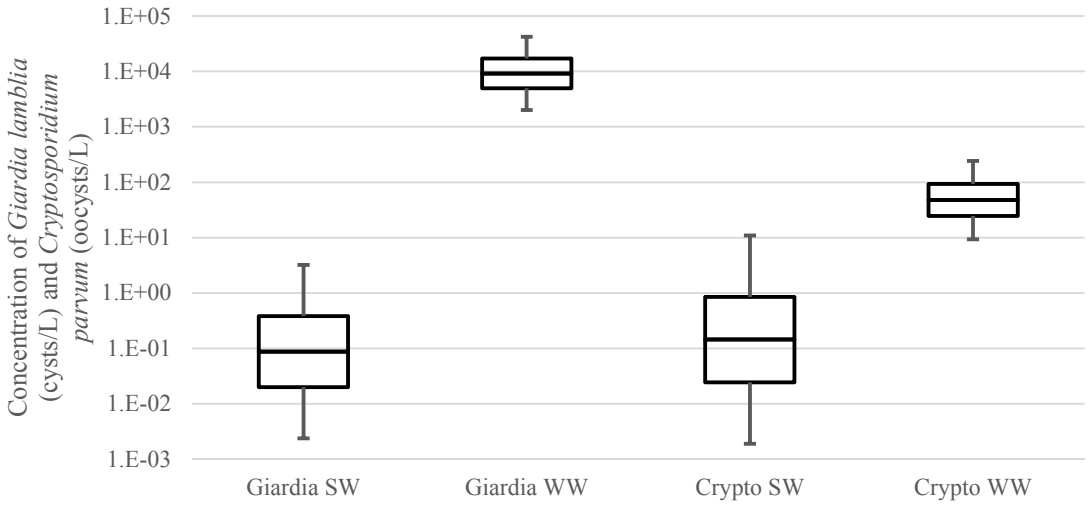


Figure 3.7 Boxplot of Viral Pathogens Wet Weather Flows (SW) and Influent Wastewater (WW) where $n_{Giardia\ SW} = 48$, $n_{Giardia\ WW} = 120$, $n_{Cryptosporidium\ SW} = 48$, and $n_{Cryptosporidium\ WW} = 120$. The middle line represents the median; the first and third lines represent the 25th and 75th percentiles, respectively. The vertical lines extend to the 5th and 95th percentiles.

3.4.3 Human Fecal Contamination Analog

Simulations for Human Microbial Source Tracking Markers in Wet and Dry Weather Flows

Using human MST markers as a surrogate to estimate HFCAs in wet weather and dry weather flows (n = 1000 iterations each, Table 3.5) provides a wide range of estimates based on the descriptive statistics obtained using unpaired Monte Carlo simulations: 10^{-7} – $10^{-1.5}$ and 10^{-12} to $10^{-2.6}$, respectively. This variation, for both wet and dry weather flows, could be attributed to differences among the sampled locations in terms of the age of infrastructure, the extent and distribution of homeless encampments, and stormwater and wastewater pipe construction material.

Table 3.5 HFCAs Based on Human MST Markers for Wet and Dry Weather Flows Using Paired Monte Carlo Simulations (n = 1000 iterations), Unpaired Monte Carlo Simulations (n = 1000 iterations), and the Analytical Distribution Method

Percentile	Wet Weather Flows			Dry Weather Flows		
	Paired Monte Carlo	Unpaired Monte Carlo	Analytical distribution	Paired Monte Carlo	Unpaired Monte Carlo	Analytical distribution
5th	$10^{-6.3}$	$<10^{-7.0}$	$10^{-7.6}$	$\sim10^{-11}$	$10^{-12.0}$	$10^{-12.5}$
25th	$10^{-5.3}$	$10^{-5.8}$	$10^{-5.8}$	$\sim10^{-9.0}$	$10^{-9.0}$	$10^{-9.6}$
Median	$10^{-4.6}$	$10^{-4.5}$	$10^{-4.5}$	$\sim10^{-8.0}$	$10^{-7.0}$	$10^{-7.6}$
75th	$10^{-3.9}$	$10^{-3.3}$	$10^{-3.3}$	$10^{-6.1}$	$10^{-5.4}$	$10^{-5.6}$
95th	$10^{-2.9}$	$10^{-1.5}$	$10^{-1.5}$	$10^{-3.8}$	$10^{-2.6}$	$10^{-2.7}$

Estimates of human MST-based HFCAs in dry weather flows using unpaired Monte Carlo simulations suggest that dry weather flows are less contaminated with human fecal matter compared to wet weather flows (Table 3.5). The median human MST-based HFCA for dry weather flows is 2.5 orders of magnitude less than that for wet weather flows, and the 95th percentile for dry weather flows is approximately 1 order of magnitude less than that for wet weather flows (Table 3.5). Based on unpaired Monte Carlo analysis, an HFCA estimate of 10^{-2} can be a conservative estimate to inform selection of LRTs and treatment requirements for dry weather flows.

For wet weather flows, the human MST-based HFCAs estimated using paired Monte Carlo analysis (Table 3.5, n = 1000 iterations) are similar to those estimated using unpaired Monte Carlo analysis except at the 75th and 95th percentiles, where the paired analyses result in lower HFCA estimates. Values of HFCAs based on human MST markers in dry weather flows using paired Monte Carlo analysis (n=1000 iterations, Table 3.5) are lower than those obtained using the unpaired analysis except at the 5th percentile where the estimated HFCA is one order of magnitude higher than that for the unpaired analysis. At the 25th percentile, the HFCAs for paired and unpaired analyses are the same.

Past studies on the microbial contamination of stormwater have not provided an estimate of human fecal contamination in dry weather flows based on human MST markers. For areas with limited precipitation considering the collection, treatment, and use of stormwater, combining dry weather and wet weather flows could increase the quantity of collected water, thereby making SCU projects more feasible for these areas. Knowledge of the amount of human fecal contamination in wet and dry weather flows facilitates estimates of the combined contamination and required LRTs for more targeted treatment process trains.

In general, using an unpaired analysis based on the currently available wet weather and wastewater datasets provides more reliable estimates than a paired analysis. This is because a paired Monte Carlo analysis assumes that wet weather samples and influent wastewater samples were collected together from the same areas, which is not the case for the datasets used in this analysis. For future targeted studies examining the integrity of stormwater and wastewater infrastructure or the extent of microbial contamination arising from homeless encampments where wet/dry weather samples and influent wastewater samples are collected from the same watershed/sewershed, using a paired Monte Carlo analysis is recommended for the estimation of HFCAs.

In addition to using Monte Carlo simulations to generate distributions for human MST markers, HFCAs can be estimated using the distribution parameters (location and scale) of the original data points (analytical distribution method). However, this solution is only applicable for unpaired data points. The descriptive statistics for human MST-based HFCAs in wet weather flows using the analytical distribution method are almost identical to those obtained using the unpaired Monte Carlo simulation method (Table 3.5). The same trend is noticed for dry weather flows. This

indicates the suitability of using human MST markers as surrogates for the estimation of human fecal contamination in stormwater.

The 95th percentile HFCA based on human MST markers in wet weather flows is equal to $10^{-1.5}$. Using unpaired Monte Carlo analysis and the analytical distribution method, an HFCA of 10^{-1} for wet weather flows remains a conservative estimate. The human MST based-HFCAs are consistent with recommendations from Schoen et al. (2017) to apply a pathogen-based sewage dilution (HFCA) of $10^{-1} – 10^{-3}$ which is the same range that informed guidance recommendations by Sharvelle et al. (2017).

Schoen et al. (2017) and Sharvelle et al. (2017) did not provide sewage dilutions (HFCAs) based on human MST markers, and the pathogen data on which the sewage dilution calculations were based came from a single study on wet weather flows (Bambic et al., 2011). In contrast, the human MST-based HFCAs provided in this study are based on a more recent, robust dataset from several studies across different locations within the U.S (Bambic et al., 2011; Sauer et al., 2011; Cao et al., 2017; Staley et al., 2018; Gonzalez, 2022: personal communication).

Simulations for PHIPs in Wet Weather Flows

For unpaired Monte Carlo simulations, both Weibull and lognormal distributions were used to fit HFCAs obtained based on viral pathogens as surrogates for human fecal contamination in wet weather flows. Eliminating scenarios where the simulated concentrations of viral pathogens in wet weather flows are greater than those in influent wastewater still resulted in more than 100% sewage in wet weather at the 95th percentile using the lognormal distribution. However, fitting the Weibull distribution to the remaining scenarios resulted in realistic HFCAs in wet weather flows at all percentiles. The pathogens used to estimate HFCA including norovirus GI+GII,

adenoviruses, enteroviruses, *Giardia lamblia*, and *Cryptosporidium parvum* are zoonotic pathogens.

Estimates of HFCA using adenoviruses and enteroviruses as surrogates for human fecal contamination in wet weather flows are similar (Table 3.6), but both $HFCA_{\text{wet weather adenoviruses}}$ and $HFCA_{\text{wet weather enteroviruses}}$ are much lower than $HFCA_{\text{wet weather norovirus GI+GII}}$. *Cryptosporidium*-based HFCAs are higher than *Giardia*-based HFCAs in wet weather flows. While $HFCA_{\text{wet weather } \textit{Cryptosporidium}}$ values are higher than $HFCA_{\text{wet weather adenoviruses}}$ and $HFCA_{\text{wet weather enteroviruses}}$, except at the 95th percentile, $HFCA_{\text{wet weather } \textit{Cryptosporidium}}$ values remain lower than $HFCA_{\text{wet weather norovirus GI+GII}}$ values (Table 3.6). Consequently, noroviruses seem to drive the risk from SCU projects, and thus, the selected treatment process trains should be effective for the removal of noroviruses. The viral-based HFCA results for wet weather flows are in agreement with the data synthesis results for the concentrations of viral pathogens in wet weather flows (Section 3.4.2).

Table 3.6 HFCAs Based on PHIPs as Surrogates for Stormwater Human Fecal Contamination Using Paired and Unpaired Monte Carlo Simulations

Percentile	HFCA norovirus GI+GII		HFCA adenoviruses		HFCA enteroviruses		HFCA *Giardia*		HFCA *Cryptosporidium*	
	Paired Monte Carlo (LN)[a]	Unpaired Monte Carlo (LN[a]/Weibull)[b]	Paired Monte Carlo (LN)[a]	Unpaired Monte Carlo (LN[a]/Weibull)[c]	Paired Monte Carlo (LN)[a]	Unpaired Monte Carlo (LN[a]/Weibull)[d]	Paired Monte Carlo (LN)[a]	Unpaired Monte Carlo (LN)[a]	Paired Monte Carlo (LN)[a]	Unpaired Monte Carlo (LN)[a]
5th	$10^{-1.6}$	$10^{-3.5}$ / $10^{-3.7}$	$10^{-4.5}$	$<10^{-7.0}$/ ~10^{-8}	$10^{-6.0}$	$<10^{-7.0}$/$<10^{-8.0}$	$10^{-6.0}$	$10^{-6.7}$	$10^{-3.7}$	$10^{-4.4}$
25th	$10^{-1.1}$	$10^{-2.4}$ / $10^{-2.2}$	$10^{-3.4}$	$10^{-5.2}$ / $10^{-4.8}$	$10^{-4.3}$	$10^{-5.4}$/ $10^{-5.1}$	$10^{-5.4}$	$10^{-5.7}$	$10^{-3.0}$	$10^{-3.2}$
Median	$10^{-0.8}$	$10^{-1.6}$ / $10^{-1.4}$	$10^{-2.5}$	$10^{-3.6}$ / $10^{-3.2}$	$10^{-3.2}$	$10^{-3.9}$ / $10^{-3.5}$	$10^{-5.0}$	$10^{-5.0}$	$10^{-2.5}$	$10^{-2.4}$
75th	$10^{-0.5}$	$10^{-0.9}$ / $10^{-0.8}$	$10^{-1.6}$	$10^{-2.0}$ / $10^{-1.9}$	$10^{-2.1}$	$10^{-2.3}$ / $10^{-2.2}$	$10^{-4.6}$	$10^{-4.3}$	$10^{-2.0}$	$10^{-1.7}$
95th	$10^{-0.1}$	$10^{0.2}$ / $10^{-0.1}$	$10^{-0.4}$	$10^{+0.3}$ / $10^{-0.5}$	$10^{-0.5}$	$10^{-0.2}$ / $10^{-0.7}$	$10^{-4.0}$	$10^{-3.3}$	$10^{-1.3}$	$10^{-0.5}$

a: lognormal distribution with 1000 iterations, b: no. of iterations = 677 , c: no. of iterations = 747 , d: no. of iterations = 878

For paired Monte Carlo simulations, only the lognormal distribution was fitted to PHIPs-based HFCAs. Adenoviruses-based HFCAs using paired Monte Carlo analysis (n = 1000) are greater than those using unpaired Monte Carlo analysis and lognormal distribution (n = 747 iterations, Table 3.6). Despite the fact that norovirus GI+GII concentrations were paired (arranged in ascending order) for both wet weather and influent wastewater, 8.3% of the simulated HFCA values resulted in unrealistic scenarios (wet weather flows are made up of more than 100% sewage). After the unrealistic scenarios were eliminated (n = 917, Table 3.6), norovirus GI+GII-based HFCAs using paired Monte Carlo analysis were higher than those obtained using the unpaired analysis and lognormal distribution, (n = 677) except at the 95th percentile.

Enteroviruses based HFCAs using paired analysis (n = 1000) are also higher than those obtained using unpaired analysis and lognormal distribution (n = 878 iterations, Table 3.6), except at the 95th percentile. For both *Giardia lamblia* and *Cryptosporidium parvum*, the HFCAs obtained using unpaired analysis are similar to those obtained using the paired analysis except at the 95th percentile as well where the protozoan based HFCAs obtained using the unpaired Monte Carlo analysis are higher than those obtained using the paired Monte Carlo analysis (Table 3.6).

PHIPs-based HFCAs obtained using unpaired Monte Carlo analysis for the available datasets are more reliable than the ones obtained using paired Monte Carlo analysis since the pathogen data in wet weather flows and influent wastewater were not obtained from the same watersheds/sewersheds. Studies in which the pathogen measurements for wet weather flows and influent wastewater take place in the same watershed/sewershed paired Monte Carlo analyses are more reliable for the determination of HFCAs. However, as stated for the unpaired Monte Carlo analysis results, human MST-based HFCAs remain a better estimate for human fecal contamination in wet weather flows than PHIP-based HFCAs.

The analytical distribution method is not suitable for estimating PHIP-based HFCAs in wet weather flows. None of the investigated pathogens result in a realistic estimation of the amount of human fecal contamination in wet weather flows without the deletion of a considerable number of the available data points. Unlike Monte Carlo simulations, where 1000 data points are generated, the original data points cannot be deleted without biasing the human fecal contamination estimate. Furthermore, while past studies (e.g., Boehm et al., 2018) eliminated a small percentage of simulated data points, no precedent could be found where the actual data points (measured concentrations from the field) were eliminated.

For unpaired analyses, viral-based HFCAs are higher than human MST-based HFCAs for wet weather flows (Tables 3.5 and Table 3.6). Specifically, norovirus GI+GII-based HFCAs are 1 to 3 orders of magnitude higher than human MST-based HFCA for wet weather flows. In contrast to viral pathogens, *Giardia*-based HFCAs are similar to human MST-based HFCAs at the 5^{th}, 25^{th}, and 50^{th} percentiles (Tables 3.5 and Table 3.6). However, at the 75^{th} and 95^{th} percentiles, *Giardia*-based HFCAs are 1 and 2 orders of magnitude lower than those for human MST-based HFCAs, respectively. *Cryptosporidium*-based HFCAs in wet weather flows are 1 to 3 orders of magnitude higher than human MST-based HFCAs.

Human MST-based HFCAs provide a more reliable estimate of the amount of human fecal contamination in wet weather flows compared to viral and protozoan pathogens. This is because viral pathogens concentrations in stormwater are highly variable compared to their corresponding concentrations in influent wastewater. While viral pathogens are found less frequently and at lower median concentrations in stormwater than wastewater, they can be detected in stormwater at concentrations almost as high as those in wastewater at the 95^{th} percentile resulting in unrealistic Monte Carlo simulation scenarios where wet weather flows are made up of

more than 100% sewage. As for protozoan pathogens, the number of original data points available for wet weather flows (n = 48) are lower than those available for human MST markers (n = 116), which makes HFCA estimates based on protozoan pathogens in wet weather flows less reliable that those based on human MST markers.

3.4.4 Selection of the Ideal Dataset for Estimation of Human Microbial Contamination in Stormwater

The only microorganism meeting criteria for estimation of HFCA in stormwater was human MST markers (Section 3.3.5, Table 3.3). Human MST markers in wet weather flows are well described by the lognormal distribution which covers a wide range of human MST datasets from the 25th percentile to the 99th percentile (Figure 3.9, Table 3.3, AD-value = 2.4; Appendix B, Figure B.1). Even though, some PHIP datasets, e.g., *Cryptosporidium parvum* (Figure 3.10; Appendix B, Figure B.7), appear to satisfy HFCA estimation criteria, the limited number of protozoan data points (n = 48) compared to human MST markers (n=116) makes protozoan pathogens unsuitable to estimate HFCAs. Most PHIP datasets were limited by detection limits and subsequently the range of data observed within the distributions (Table 3.3, Figure 3.11; Appendix B, Figures B.3, B.4, B.5, and B.6).

While the human MST marker concentrations in wet weather and influent wastewater result in more confident estimates of HFCA, HFCAs were still estimated for potentially human-infectious pathogens to serve as a comparison to verify the approach for use of MSTs to estimate HFCA in stormwater and provide multiple analyses to inform guidance on the range of HFCA observed and associated treatment requirements.

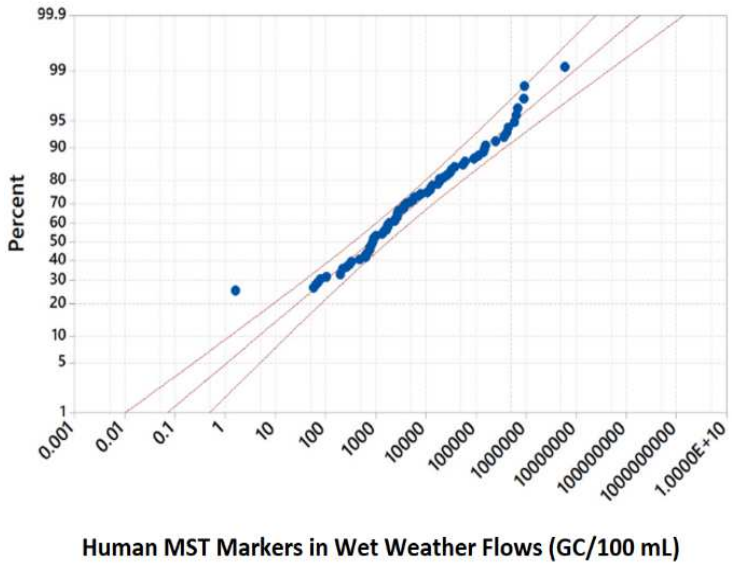


Figure 3.9 Lognormal Statistical Distribution Fitted to Human MST Marker Data in Wet Weather Flows (n = 116 observations, the upper and lower red lines represent the 95th percentile confidence interval)

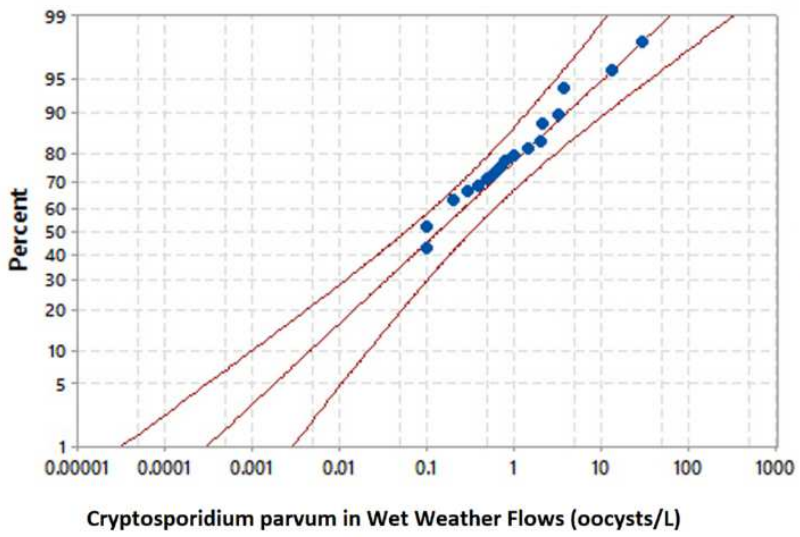


Figure 3.10 Lognormal Statistical Distribution Fitted to *Cryptosporidium parvum* Data in Wet Weather Flows (n = 48 observations, the upper and lower red lines represent the 95th percentile confidence interval)

In this study, the 95th range of PHIP-based HFCA in wet weather flows, $10^{-3.3}$ (based on *Giardia lamblia*) to $10^{-0.1}$ (based on norovirus GI+GII), is similar to the pathogen-based sewage dilutions provided by Schoen et al. (2017) at the low end, 10^{-3} (based on *Giardia lamblia*) to10^{-1}

(based on norovirus GII). The dissimilarity at the high end could be due to the fact that the current study is based on a more recent, comprehensive dataset and both norovirus GI+GII were used in this study whereas Schoen et al. (2017) only used norovirus GII. Schoen et al. (2017) remarked that using only norovirus GII could result in lower treatment requirements compared to a combined norovirus GI+GII characterization.

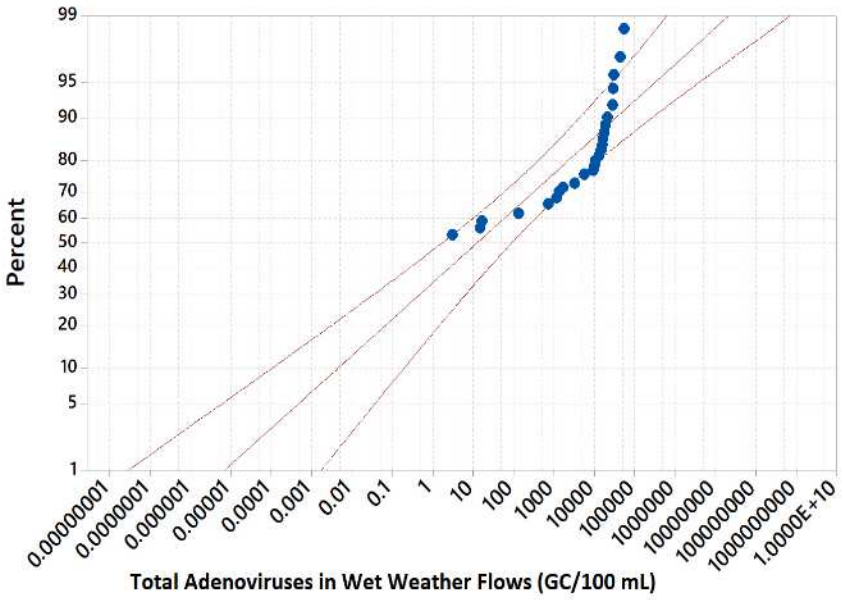


Figure 3.11 Lognormal Probability Plot Fitted to Total Adenoviruses Data in Wet Weather Flows (n = 70 observations, the upper and lower red lines represent the 95th percentile confidence interval)

The 95th percentile HFCA based on human MST markers for wet weather flows in this study ($10^{-1.5}$) falls within the 95th range of sewage dilutions calculated by Schoen et al. (2017), i.e., 10^{-1} to 10^{-3}. Despite the advantages that human MST marker data have over PHIP data to estimate human fecal contamination in stormwater, the literature is inconclusive on correlations between human MST markers and PHIPs (Bambic et al., 2011; Staley et al., 2012). More research is needed to determine if human MSTs can be used to estimate concentrations of human-infectious pathogens. However, the detection of stormwater contamination with human fecal matter is often

achieved through the measurement of human MST markers (e.g., HF183) in lieu of using pathogens due to the challenges associated with measuring pathogens in stormwater.

Pathogens are often present in stormwater at low concentrations (Rajal et al., 2007). Large volumes of stormwater are required for detection of pathogens which could potentially limit the sampling technique to grab sampling. The complex chemical and physical characteristics of stormwater cause the performance of filtration and concentration techniques for pathogen recovery to be ineffective or highly variable (Loge et al., 2002). The available methods for nucleic acid extraction and purification from natural waters are limited to small starting volumes (Fuhrman et al., 2005). Unlike pathogens, the human – source tracking marker HF183 can be detected in sample volumes as small as 500 mL. It should also be noted that while qPCR measures both viable and non-viable RNA (Ju et al., 2016), viral RNA is unlikely to survive long outside its capsid once the capsid is destroyed. Therefore, the measured virus concentrations may not be an overestimate.

3.5 Conclusions

- Human MST markers are detected in wet weather flows at higher percentages and concentrations (% detection = 65.5%, median concentration = $10^{2.9}$ GC/100 mL) compared to dry weather flows (% detection = 25.3%, median concentration = $10^{-0.2}$ GC/100 mL).
- Adenoviruses, norovirus GI+GII, and enteroviruses are detected in 41.4%, 74.3%, and 38.6% of the reviewed wet weather flow measurements across the U.S at median concentrations of $10^{1.1}$, $10^{2.83}$, and $10^{0.66}$ GC/100 mL, respectively. *Giardia lamblia* and *Cryptosporidium parvum* are detected in 50% and 56.2% of the reviewed wet weather flow measurements, respectively, at much lower median concentrations compared to viral pathogens ($10^{-1.1}$ cysts/L median concentration for *Giardia lamblia* and $10^{-0.8}$ oocysts/L median concentration for *Cryptosporidium parvum*).

- Viral pathogens, specifically norovirus GI+GII, will drive the risk associated with the use of stormwater. Therefore, unit treatment processes in stormwater treatment trains should be selected and designed for the effective removal of noroviruses.
- The human MST markers dataset is better than the viral and protozoan datasets for the estimation of stormwater HFCA because viral and protozoan datasets are limited by the limit of detection and the range of data observed within the selected statistical distributions.
- Unpaired Monte Carlo simulation is the best method to estimate stormwater HFCA when the stormwater microbial quality data is collected from a different place than wastewater quality data. In cases where both stormwater and wastewater microbial quality data are collected within the same watershed/sewershed, a paired Monte Carlo simulation should be used to estimate HFCA values.
- Using unpaired Monte Carlo simulations, human MST-based estimates of HFCA range from $<10^{-7.0}$ to $10^{-1.5}$ (median = $10^{-4.5}$) in wet weather flows and from 10^{-12} to $10^{-2.6}$ (median = 10^{-7}) in dry weather flows. Estimates of human MST-based HFCAs from collected stormwater samples could guide the selection and design of unit treatment processes in stormwater treatment trains according to the required virus, protozoa, and bacteria LRTs.
- The 95th percentile human MST-based HFCA estimated in this study ($10^{-1.5}$) is within the 95th percentile sewage dilution range estimated by Schoen et al. (2017) and adopted by Sharvelle et al. (2017) despite using a different method of analysis. Schoen et al. (2017) sewage dilution range is 10^{-3} to 10^{-1}, where 10^{-1} is 95th percentile sewage dilution based on norovirus GII and 10^{-3} is the 95th percentile sewage dilution based on *Giardia*.

CHAPTER 4: THE IMPACT OF PATHOGEN REDUCTION TARGETS SELECTION ON TREATMENT PROCESS TRAINS FOR THE SAFE USE OF STORMWATER[3]

4.1 Abstract

Stormwater practitioners wanting to benefit from stormwater to augment the available water resources struggle with the selection and design of efficient stormwater treatment trains that are protective of public health for the designated end use. In areas with aging stormwater and wastewater collection networks, sanitary sewer overflows (SSOs), and homeless encampments, stormwater could be potentially contaminated with human fecal matter. Knowledge of the degree to which stormwater is contaminated with human fecal matter, termed here as the human fecal contamination analog (HFCA), is critical for the design process and estimating the required pathogen log reduction targets (LRTs). To ensure the safe use of stormwater for non-potable purposes, pathogen LRTs were estimated in this study based on the level of human fecal contamination and previously published quantitative microbial risk assessments (QMRAs). Combinations of stormwater treatment trains at different HFCAs were evaluated based on complexity and reliability of the suggested trains. To use stormwater safely for unrestricted irrigation and indoor uses, treatment trains containing both filtration and disinfection unit processes are required. The HFCA threshold beyond which the complexity of stormwater trains becomes considerably higher is 10^{-4}. Based on the results of the performance evaluation, treatment trains consisting of membrane filtration and at least two disinfection unit treatment processes, specifically ultraviolet (UV) and ozone (O_3) or UV and chloramine are recommended at HFCA values of 10^{-3}, 10^{-2}, and 10^{-1}. At HFCA value of 10^{-4}, a treatment train consisting of membrane

[3] This chapter is adapted from a prepared journal article to be submitted for publication: Alja'fari, A., Sharvelle, S., Branch, A., Jahne, M., Pecson, B., Olivieri, A., and Garland, J. (2022). "The Impact of Pathogen Reduction Targets Selection on Treatment Process Trains for the Safe Use of Stormwater".

filtration and O_3 or chloramine is recommended. The use of chlorination at both low and high HFCA levels is not recommended due to the high continuous monitoring requirements associated with the use of free Cl_2.

4.2 Introduction

The collection of stormwater, including both wet and dry weather flows, for non-potable end uses could reduce reliance on freshwater supplies treated to comply with drinking water standards. Stormwater capture and use (SCU) projects also enable the mitigation of flood risks and limit the migration of associated contaminants to receiving water bodies in light of the increase in paved, impermeable surfaces (Wong, 2006; Wong and Brown, 2009; Feng et al., 2022). However, human fecal contamination of stormwater has been detected across the U.S in areas where wastewater collection systems are separate from stormwater collection systems, e.g., Bambic et al. (2011), Sauer et al. (2011), Sercu et al. (2011), The City of Santa Barbara (2012), Steele et al. (2018), and Gonzalez et al. (2020).

Human fecal contamination of stormwater in the U.S as evidenced through the detection of human microbial source tracking (MST) markers and potentially human-infectious pathogens (PHIPs) necessitates treatment of stormwater based on the level of microbial contamination and end use, i.e., fit-for-purpose treatment (Chapter 3). A review conducted for the Water Research Foundation (WRF #5034) identified 46 SCU projects in the U.S with the majority located in the upper Midwest (Alja'fari et al., 2022b). Projects reporting the use of stormwater treatment for a specific end use applied green infrastructure (GI) practices, engineering treatment technologies, or a combination of both (NRC, 2016; Santa Ana River Watermaster, 2014; Patterson et al., 2021; IEUA, 2014; Chino Basin Watermaster, 2001; Higgins and Roth, 2005; Minnesota Department of

Public Health, 2016 (personal communication); Alja'fari et al., 2022b; Philadelphia Water Department, 2021 (personal communication); MWMO, 2021).

In general, stormwater GI practices, e.g., bioretention basins, de/retention basins, swales, and constructed wetlands, provide either limited or no pathogen reduction (Chandrasena et al., 2016; Patterson et al., 2021; Meng et al., 2018; Chandrasena et al., 2017; Minnesota Department of Health, 2018) and their performance is often measured using fecal indicator bacteria (FIB) (Clary et al., 2020) whose concentrations do not correlate with those of PHIPs (Ahmed et al., 2008). On the other hand, engineered treatment technologies including a combination of filtration and disinfection process trains could achieve pathogen removal (Sharvelle et al., 2017; Olivieri et al., 2021).

In the U.S., only about half of the identified SCU projects applying some degree of treatment include monitoring of treatment performance parameters, with only 23% of these projects monitoring human MST markers and/or PHIPs (Alja'fari et al., 2022b). The most common treatment practices applied for SCU projects where the treated water is used for irrigation are de/retention basins, filtration, and settling (Figure 4.1). Very few SCU projects using the treated water for irrigation implement disinfection (Figure 4.1). Considering the reported human fecal contamination of stormwater along with the inefficiency of stormwater GI practices for pathogen removal, current and future SCU projects require guidance for the selection of treatment process trains based on the expected level of microbial contamination and end use.

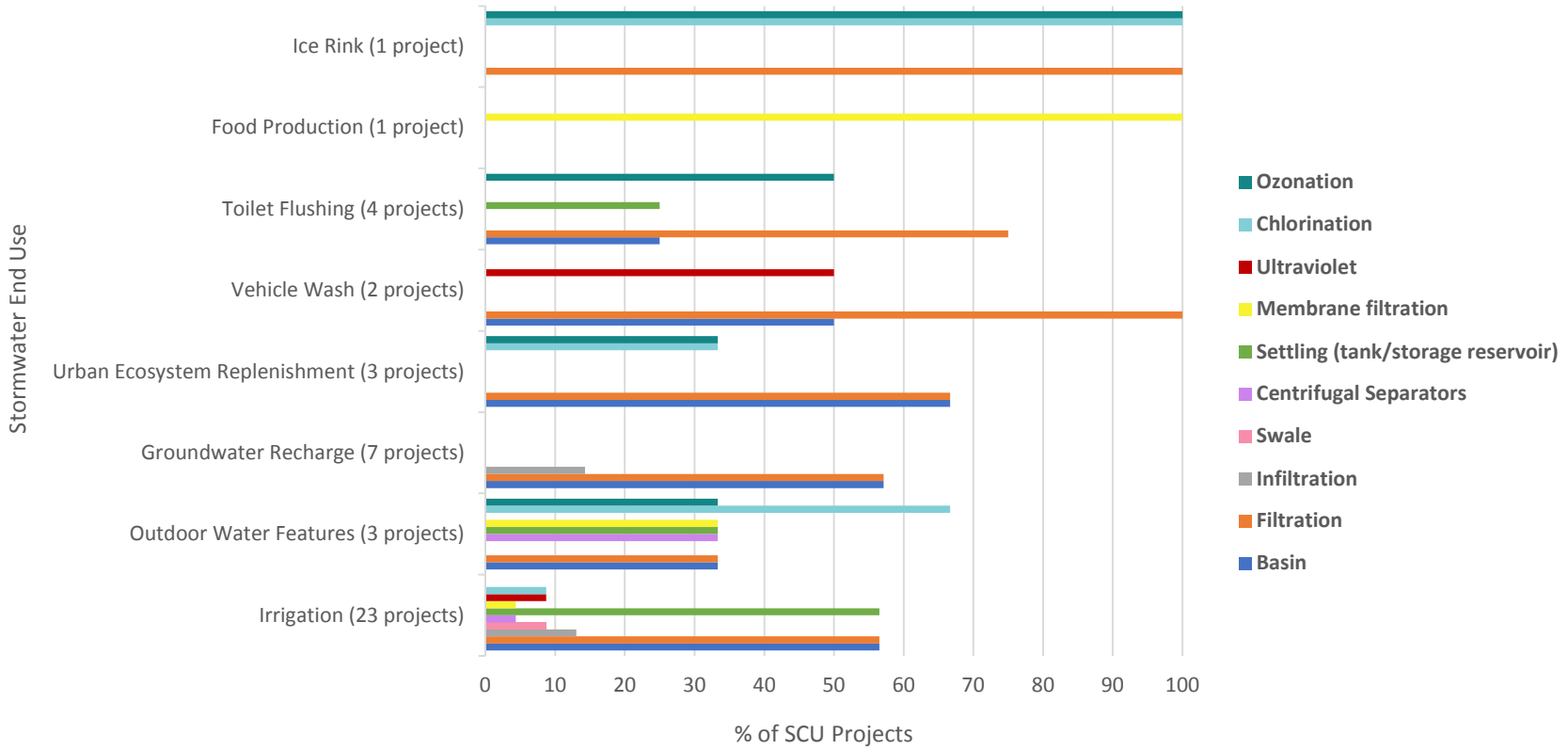


Figure 4.1 Frequency Distribution of Stormwater Treatment Practices Implemented in SCU Projects in the U.S. Separated by Stormwater End Uses

Few guidance frameworks provide recommendations on the selection of stormwater treatment process trains in the U.S based on the level of human fecal contamination and intended end use (e.g., Sharvelle et al., 2017; Olivieri et al., 2021; Luthy et al., 2019). The guidance developed by Sharvelle et al. (2017) and Olivieri et al. (2021) employs quantitative microbial risk assessment (QMRA) to estimate pathogen $\log_{10}$ reduction targets (LRTs) according to the level of human fecal contamination in stormwater (termed sewage dilution in both frameworks) and the intended end use (irrigation or indoor use). Sharvelle et al. (2017) and Olivieri et al. (2021) use the same sewage dilutions of 10^{-1} and 10^{-3} estimated by Schoen et al. (2017). However, these sewage dilutions and pathogen LRTs are derived from a single study quantifying PHIPs in stormwater (Bambic et al., 2011). Additionally, sufficient guidance on the selection of the appropriate sewage dilution (i.e., between 10^{-3} and 10^{-1}) was not provided. This is largely because the contamination of urban stormwater with sewage is highly variable and poorly defined (Nshimyimana et al., 2014; Chong et al., 2013). Therefore, public health departments and stormwater utilities were reluctant to apply the sewage dilution – based estimates of LRTs (Lackey et al., 2019) due to the uncertainty about where local stormwater lies within the 10^{-3} to 10^{-1} range of sewage dilution and a lack of understanding of the rationale for applying these sewage dilutions.

Pathogen LRT approaches are now being adopted into regulatory frameworks for stormwater capture and use (U.S Water Alliance, WE&RF, and WRF, 2017), but guidance on treatment process selection is still needed. Unlike water and wastewater engineers, stormwater professionals use design manuals and require prescriptive methods to guide the design process (e.g., Colorado UDFCD, 2010; Minnesota Stormwater Manual, 2017; City of Portland, 2016). However, these manuals only address stormwater GI practices which provide little to no removal

of PHIPs. Therefore, a set of treatment process trains meeting pathogen LRTs should be recommended to help practitioners choose the appropriate treatment systems for SCU projects.

Pathogen crediting frameworks in the US include virus and protozoa bacteria log reduction values (LRVs) are not included in existing US regulatory frameworks. LRV is an observed $\log_{10}$ pathogen reduction performance reported for a unit treatment process (Sharvelle et al., 2017). The LRV can be found as the difference in concentration of an indigenous or added surrogate or pathogen between paired influent and effluent samples (Sharvelle et al., 2017). In contrast, pathogen LRT is a $\log_{10}$ reduction requirement for a certain pathogenic group, i.e., bacteria, protozoa, or viruses, to achieve an agreed-upon level of risk to the exposed population, e.g., 10^{-4} infection/year or 10^{-6} disability adjusted life years per person, per year (Sharvelle et al., 2017).

The guidance developed by Sharvelle et al. (2017) is limited by assigning pathogen LRV credits to treatment processes, which have not been based on regulatory frameworks. Log_{10} reduction credit (LRC) is a pathogen or surrogate $\log_{10}$ reduction value that is given/credited to a unit treatment process according to validation test results of the process. Typically, LRCs are conditional on process operation within limits of one or a number of performance monitoring surrogate parameters, e.g., maintaining filter effluent turbidity less than 0.15 NTU or maintaining a minimum ultraviolet (UV) dose delivery and remaining under a maximum flow for a validated UV system (US Water Alliance, WE&RF, and WRF, 2017).

A more extensive review of pathogen LRV credits that can be achieved by different treatment processes should be conducted to examine the ranges of LRV credits provided in the peer-reviewed literature, challenge testing studies, official reports, and crediting frameworks.

Furthermore, the currently available frameworks do not provide guidance on the selection of treatment process trains based on the level of human fecal contamination.

In light of the random manner in which stormwater treatment is applied in SCU projects and lack of guidance on stormwater treatment and use in the U.S, this study aims to estimate pathogen LRTs based on stormwater end use and the level of human fecal contamination in stormwater using more recent datasets. The study also aims to investigate how the selection of pathogen LRTs based on the level of human fecal contamination impacts the choice of treatment process trains that are protective of public health for the designated end use of stormwater. An evaluation of the suggested treatment process trains based on the degree of complexity and reliability is also provided. This evaluation is intended to facilitate the decision-making process for stormwater utilities and public health departments in charge of selecting stormwater treatment trains.

4.2 Materials and Methods

4.2.1 Estimation of pathogen LRTs based on the level of human fecal contamination and stormwater end use

Recent datasets on the percent detection and concentrations of human MST markers and PHIPs in stormwater were synthesized and evaluated for their use to estimate the level of human fecal contamination in stormwater (Alja'fari et al., 2022b). The human MST markers datasets were found to be more suitable for the estimation of human fecal contamination in stormwater termed the human fecal contamination analog (HFCA) (Equation 4.1). This is because PHIPs datasets were limited by low percent detections resulting in a narrow range of observed data within the statistical distributions. The range of human MST marker based HFCA in stormwater is 10^{-6} – 10^{-1} (Alja'fari et al., 2022b).

$$HFCA_{Human\ MST\ Marker} = C_{Human\ MST\ Marker\ i} / C_{Human\ MST\ Marker\ j} \quad \text{(4.1)}$$

Where:

$HFCA_{Human\ MST\ Marker}$: Human fecal contamination analog based on human MST markers.

$C_{Human\ MST\ Marker\ i}$: concentration of human MST marker in stormwater in GC/100 mL.

$C_{Human\ MST\ Marker\ j}$: concentration of human MST marker in influent wastewater in GC/100 mL.

Quantitative microbial risk assessments (QMRAs) developed by Schoen et al. (2017) informed the use pathogen LRTs adopted by Sharvelle et al. (2017) and Olivieri et al. (2021). These targets were estimated at 10^{-1} and 10^{-3} sewage dilutions (termed here HFCA) (Table 4.1). To ensure protection of public health, the maximum values of pathogen LRTs from Table 4.1 are selected to calculate new pathogen LRTs for the HFCAs obtained from human MST markers estimated by Alja'fari et al. (2022b) (i.e., 10^{-2}, 10^{-4}, 10^{-5}, and 10^{-6}).

In their guidance, Sharvelle et al. (2017) included bacteria LRTs as part of the guidance. Olivieri et al. (2021) recommended that guidance does not include bacteria LRTs due to the lack of crediting frameworks including bacteria. In addition, Olivieri et al. (2021) noted that meeting bacteria and virus LRTs would be protective of public health when residual chlorine is required. Achieving viral and protozoa LRTs has been considered acceptable for Direct Potable Reuse regulatory frameworks (Trussell et al., 2013).

Pathogen LRTs at HFCAs of 10^{-2}, 10^{-4}, 10^{-5}, and 10^{-6} are calculated assuming a linear relationship between LRTs and 10^{-1} & 10^{-3} HFCAs such that each 10^{-1} change in HFCA results in 1 $\log_{10}$ change. For a given exposure to stormwater, a set of pathogen concentrations (dose) exists that results in acceptable risk informing estimation of LRTs. Because HFCAs and LRTs are on a log scale, a linear increase in log HFCA results in a linear increase in the required LRT.

Table 4.1 Stormwater Pathogen LRTs for Nonpotable Uses and a HFCA Dilution Range of 10^{-1} to 10^{-6} Selected to Develop Treatment Process Trains (10^{-4} ppy Infection Risk)

HFCA Scenario	Bacteria[a]	Protozoa[b]	Virus[b]
10^{-1} [c]			
Unrestricted Irrigation	4	4.5	6.5
Indoor Use	5	5.5	7.0
10^{-3} [c]			
Unrestricted Irrigation	2	2.5	4.5
Indoor Use	3	3.5	5.0

a: Bacterial LRT is obtained from Sharvelle et al. (2017);b: protozoan and viral LRTs are obtained from Olivieri et al. (2021); c: Bacterial LRTs (Sharvelle et al., 2017) and viral & protozoan LRTs (Olivieri et al., 2021) are estimated based on QMRA

4.2.2 Selection of pathogen LRCs for filtration and disinfection processes

Treatment processes considered in this study include filtration (e.g., cartridge filters, bag filters, sand filtration, ultrafiltration, and microfiltration) and disinfection (e.g., ultraviolet disinfection, chlorination using free chlorine, chlorination using chloramines, and ozonation). A combination of filtration and disinfection processes have been recommended for fit-for-purpose and cost-effective stormwater treatment (Feng et al., 2022).

To assign LRVs to each process in treatment process trains, a comprehensive review of the literature, e.g., peer-reviewed literature, challenge testing studies, official reports, and crediting frameworks, is conducted. The intent of the review is to provide a summary of the ranges of the LRVs that could be achieved and potentially credited to different unit treatment processes. The assigned LRV credits to treatment technologies are obtained from U.S crediting frameworks where available. Treatment processes for which U.S LRT credits were not provided were assigned credits based on international crediting frameworks, e.g., Australian crediting frameworks.

Filtration unit treatment processes

For slow sand filtration and cartridge filtration, a range of studies, reports, factsheets, and guidelines were reviewed to select LRV credits (Sharvelle et al., 2017 based on NRMMC, EPHC, and NHMRC, 2008; USEPA, 2005; and Harrington et al. 2001; USEPA, 2019; Manitoba

Sustainable Development, 2017; WHO, 2004; WHO, 2017; Rose et al., 2004 as reported by Oakley and Mihelcic, 2019; Health Canada, 2012; California Surface Water Treatment Rule, 2018) (Appendix C, Table C.1). Rapid sand filtration is not included in this study because it is not a common technology for stormwater treatment. Cartridge filters, on the other hand, are commonly used in stormwater treatment and come in many types including the StormFilter which contains filter cartridges filled with a mixture of perlite, zeolite, and granular activated carbon (NSF, 2004). The mixture is designed to remove metals, sediments, and stormwater contaminants from wet weather flow (NSF, 2004).

Pathogen LRVs achieved by ultrafilters are reported in multiple sources (Sharvelle et al., 2017 based on USEPA 2005, NRMMC, EPHC, and NHMRC, 2008, and Harrington et al., 2001; Soller et al., 2017 based on Kachelesky and Masterson, 1995; Jacangelo et al., 1997; USEPA, 2001; and Qiu et al., 2015; Soller et al., 2019 as reported by Olivieri et al., 2016; Dwyer et al., 1995; Trussell et al., 1998; and Kruithof et al., 1999 as reported by USEPA, 2005; USEPA, 2019; Fu et al., 2010 as reported by Oakley and Mihelcic, 2019; WHO, 2017; Manitoba Sustainable Development, 2017; Jacangelo et al., 1995 and Jacangelo et al., 1991 as reported in WHO, 2004) (Table C.1). Similar to slow sand filtration, these sources were reviewed to select LRV credits.

Pathogen LRVs achieved by microfilters have been extensively examined and reviewed (California Surface Water Treatment Rule, 2018; Sharvelle et al., 2017 based on USEPA 2005, NRMMC, EPHC, and NHMRC, 2008, and Harrington et al., 2001; Francy et al., 2012; Soller et al., 2017 based on Reardon et al., 2005; Soller et al., 2019 as reported by Olivieri et al., 2016; Jacangelo et al., 1997; Wilinghan et al., 1993; Schneider et al., 1999; and Trussell et al., 1998 as reported by USEPA, 2005; USEPA, 2019; WHO, 2017; Manitoba Sustainable Development,

2017; Jacangelo et al., 1995; Karimi et al., 1999; Parker et al.,1999; and Yoo et al., 1995 as reported in WHO, 2004) (Appendix C, Table C.1).

Disinfection Unit Treatment Processes

The use of stormwater for unrestricted irrigation or indoor use, e.g., toilet flushing necessitates some form of disinfection using ultraviolet (UV) radiation, chlorination, chloramination, and/or ozonation. Guidelines, reports, and studies were reviewed to select UV doses required to achieve different LRV credits (Sharvelle et al., 2017 based on USEPA, 2006 a; USEPA, 2020a; Soller et al., 2017 based on USEPA, 2006 a; USEPA, 2003; and Gerba et al., 2002; Olivieri et al., 2016; Hijnen et al., 2006; Olivieri et al., 2021; Malayeri et al., 2016; SFPUC, 2020; WaterSecure, 2017a) (Appendix C, Table C.2).

Pathogen LRV credits achieved using different free chlorine and chloramine doses are selected based on a review of multiple reports and studies (Sharvelle et al., 2017 based on Tchobanoglous et al., 2014; USEPA, 1999; Soller et al., 2019; WaterSecure, 2017b; USEPA, 2020b; Keegan et al., 2012; Soller et al., 2017 based on USEPA, 1991; and Nasser, 2016) (Appendix C, Table C.2). Several studies and reports were also reviewed to obtain pathogen LRV credits achieved by ozonation (Sharvelle et al., 2017 based on Tchobanoglous et al., 2014; USEPA, 1999; USEPA, 2020b; Soller et al., 2017 based on Wickramanayake et al., 1985 and USEPA, 1991; WaterSecure, 2017c; Soller et al., 2017; Olivieri et al., 2016) (Appendix C, Table C.2).

4.2.3 Selection and evaluation of stormwater treatment process trains based on the level of human fecal contamination

Selection of stormwater treatment process trains

For each level of HFCA, i.e., 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}, two to five options of treatment process trains are selected. Each treatment process train includes filtration and

disinfection processes operated at doses that achieve LRCs obtained as outlined in Section 4.2.2 for each pathogen category, i.e., viruses, protozoa, and bacteria. The sum of pathogen LRCs for the unit treatment processes included in each treatment process train should be equal to or exceed pathogen LRTs determined in Section 4.2.1 for each level of HFCA.

Evaluation of stormwater treatment process trains complexity and reliability

Treatment train complexity and reliability are used as criteria to evaluate treatment process trains for different levels of HFCA (Table 4.2) and provide recommendations for the selection of stormwater treatment trains. Economic and environmental performance are not included as part of the evaluation methodology. Cost differences among the different components of each treatment process train (disinfection and filtration components) are minimal. Even though the environmental impact of implementing treatment is important, this study is driven by the importance of understanding the trade-offs between complexity and reliability rather than conducting a life cycle assessment. Treatment train complexity is estimated based on monitoring and dosing requirements for each unit process in the treatment process train (Table 4.2). Treatment train reliability is estimated based on performance redundancy and the risk associated with the unit treatment processes within the treatment train (Table 4.2).

For treatment train complexity, the number of continuously monitored control points for each unit treatment process within the treatment train is used as a sub-criterion to estimate monitoring requirements (Table 4.2). The number of continuously monitored control points for each process was obtained from the USEPA Manual for Compliance with the Surface Water Treatment Rules (USEPA, 2020c), USEPA Membrane Filtration Guidance Manual (USEPA, 2005), WaterSecure (2017a), USEPA Ultraviolet Disinfection Guidance Manual (USEPA, 2006 a), WaterSecure (2017b), USEPA Disinfection Profiling and Benchmarking Technical Guidance

Manual (USEPA, 2020b), Keegan et al. (2012), and WaterSecure (2017c) (Appendix C, Table C.3).

Dosing impacted by stormwater chemical quality, specifically ammonia content, is used as a sub-criterion to measure dosing requirements (Table 4.2). This is because achieving free chorine disinfection in stormwater is complicated by the presence of ammonia and subsequent formation of chloramines. Either breakpoint chlorination should be achieved, and ideally free chlorine residual monitored or monochloramine formation monitored to appropriately credit disinfection due to chloramine residual.

Due to variable concentrations of ammonia in stormwater (0 – 11.9 mg/L with a median of 0.3 mg/L; NSQD, 2018), chorine dosing may vary and requires continuous monitoring to ensure appropriate dosing to achieve pathogen LRTs. If one or more unit treatment processes in the treatment train is/are impacted by the ammonia content is stormwater, the treatment train is assigned a value of 1. If the ammonia content in influent stormwater does not impact the dosing, the treatment process train is assigned a value of 0. Another sub-criterion to measure dosing requirements is CT (the dose or concentration of disinfectant multiplied by the contact time). It is desired to minimize CT as this will lower the reactor (tank) volume and the amount of added disinfectant. Treatment processes should be selected to minimize complexity.

For treatment train reliability, performance redundancy is measured using sub-criteria accounting for the number of treatment units that could go offline while still maintaining viral, protozoan, and bacterial LRTs estimated using the method outlined in Section 4.2.1 (Table 4.2). Risk is measured by a sub-criterion accounting for the number of virus LRV credits that rely on one unit treatment process (Table 4.2). Ideally, unit treatment processes should be selected to maximize redundancy and minimize risk so that the system reliability is maximized.

The stormwater treatment trains at HFCA values ranging from 10^{-6} to 10^{-1} are evaluated using an evaluation matrix based on the aforementioned criteria and sub-criteria (Table 4.2). A weighted average method is applied to calculate an overall score for each treatment train at each HFCA value. Using numerical values for each sub-criterion and the weighted average method (Equation 4.2; Triantaphyllou, 2000), an overall score is calculated for each treatment train at each HFCA value with rankings ranging from 1 to 5. The treatment train with the highest overall score is the best performing option and ranked at number 1. The treatment train with the lowest overall score is the worst performing option and ranked at number 5.

$$A_{WAM-score} = \sum_{j=1}^{n} a_{ij} \times w_j, \text{ for i} = 1, 2, 3, \ldots, \text{m} \quad (4.2)$$

Where: $A_{WAM-score}$ is the overall score for a treatment train in the decision matrix with m options (the number of options for treatment trains at each HFCA) and n criteria

a_{ij} is the value of the i^{th} option in terms of the j^{th} criterion

w_j is the weight of the importance for the j^{th} criterion

Table 4.2 Criteria and Sub-Criteria Used to Evaluate the Performance of Different Stormwater Treatment Trains

Stormwater Treatment Train Evaluation Criteria	**Evaluation Sub-Criteria**
System Complexity	Monitoring requirements: - No. of continuously monitored parameters - No. of routinely monitored parameters
	Dosing Requirements: - Dosing impacted by influent chemical quality (ammonia, 0/1) - CT: concentration or dose of disinfectant × contact time
System Reliability	Redundancy: - No. of units that can go offline while maintaining virus LRTs - No. of units that can go offline while maintaining protozoa LRTs - No. of units that can go offline while maintaining bacteria LRTs
	Risk: - No. of virus LRVs relying on 1 unit treatment process

a: if one or more unit treatment processes in the treatment train is/are affected by the ammonia content of influent stormwater, the treatment train is assigned a value of 1.

4.4 Results and Discussion

4.4.1 Pathogen LRTs Based on HFCA Levels and Stormwater End Use

Bacterial, protozoan, and viral LRTs were determined based on the level of human fecal contamination in stormwater (HFCA) for two end use scenarios: unrestricted irrigation and indoor use (Table 4.3). The estimated LRTs for HFCAs of 10^{-2}, 10^{-4}, 10^{-5}, and 10^{-6} (as determined by Alja'fari et al., 2022b) should achieve public health protection at an infection risk of 10^{-4} ppy. An increase in HFCA results in a linear increase in the required LRTs for each pathogen category to meet the health targets. Viruses drive the risk associated with the use of stormwater for non-potable purposes as evidenced by the high number of LRTs required to ensure public health protection (Table 4.3).

Table 4.3 Stormwater Pathogen LRTs for Nonpotable Uses and HFCA Range of 10^{-6} to 10^{-1} Used to Select Treatment Process Trains (10^{-4} ppy Infection Risk)

HFCA Scenario	Bacteria[a]	Protozoa[b]	Virus[b]
10^{-1} [c]			
Unrestricted Irrigation	4	4.5	6.5
Indoor Use	5	5.5	7.0
10^{-3} [c]			
Unrestricted Irrigation	2	2.5	4.5
Indoor Use	3	3.5	5.0
10^{-2} [d]			
Unrestricted Irrigation	3	3.5	5.5
Indoor Use	4	4.5	6.0
10^{-4} [d]			
Unrestricted Irrigation	1	1.5	3.5
Indoor Use	2	2.5	4.0
10^{-5} [d]			
Unrestricted Irrigation	0	0.5	2.5
Indoor Use	1	1.5	3.0
10^{-6} [d]			
Unrestricted Irrigation	0	0.0	1.5
Indoor Use	0	0.5	2.0

a: Bacterial LRT is obtained from Sharvelle et al. (2017); b: protozoan and viral LRTs are obtained from Olivieri et al. (2021); c: Bacterial LRTs (Sharvelle et al., 2017) and viral & protozoan LRTs (Pecson et al., 2021) are estimated based on QMRA; d: LRTs are estimated based on the assumption of a linear relationship between HFCAs and LRTs.

4.4.2 Pathogen LRCs for Filtration and Disinfection Processes

Filtration unit treatment processes

Pathogen crediting frameworks that were used to assign LRT credits to filtration unit treatment processes include the USEPA Surface Water Treatment Rule Fact Sheet (USEPA, 2019), California Surface Water Treatment Rule, Alternative Filtration Technology – Membrane Filtration (California SWTR, 2018), and the USEPA Membrane Filtration Guidance Manual (USEPA, 2005).

For protozoan and viral LRVs achieved by slow sand filtration, values reported by the USEPA (2019) were selected because they were among the most recent and conservative values reported for slow sand filtration (Table 4.4). Bacterial LRVs were absent from nearly all the reviewed literature on slow sand filtration in the U.S. The WHO (2017) Guidelines for Drinking Water Quality assigned 2 to 6 log reduction credits for the removal of bacterial pathogens in slow sand filters. A conservative value of 2 LRVs was selected for the removal of bacterial pathogens in slow sand filters (Table 4.4).

Similar to sand filtration, the removal credits of viruses and protozoa assigned by the USEPA (2019) and California SWTR (2018), respectively, to ultrafilters were selected as they reflect recent and conservative estimates (Table 4.4). Fewer values of bacterial LRVs achieved by ultrafilters are reported in the literature, i.e., Sharvelle et al. (2017), WHO (2017), and Soller et al. (2019). Soller et al. (2019) compared allowable bacterial LRV credits for different unit treatment processes currently used in U.S. states to LRVs in the peer-reviewed literature. In their assessment of microbial risk, Soller et al. (2019) analyzed treatment process trains in direct potable reuse projects using allowable LRV credits in lieu of literature review values. As such, the selected bacterial LRV for ultrafilters was 3 which was also used for the selection of appropriate treatment process trains in this study (Table 4.4).

Table 4.4. Selected LRV Credits for Filtration Unit Processes

Filtration Technology	Log Reduction Value			Crediting Framework or Pathogen Removal Studies
	Bacteria	Protozoa	Viruses	
Pretreatment plus bag or cartridge filter[a,b]	NA	2	0	USEPA (2019) – U.S. Crediting Framework
Slow sand filtration[b, c]	2[d]	2	2	
Ultrafiltration[e]	3	4	0	Bacteria: Soller et al. (2019) – Peer Reviewed Literature Protozoa: California SWTR (2018) – U.S. Crediting Framework Virus: USEPA (2019) – U.S. Crediting Framework
Microfiltration[e]	3	4	0	

a: Prefiltration requirements for membrane cartridge filtration are strainers and/or bag filters with size ranging from 300 to 3000 μm, **b:** combined filter effluent turbidity (95% monthly/max) = 1/5 in NTU, max logs of credit (USEPA, 2019), **c:** filter performance depends on presence of schmutzdecke, grain size, flow rate, operating conditions (mainly temperature, pH) (WHO, 2017), **d:** WHO (2017), **e:** combined filter effluent turbidity (95% monthly/max) = 0.1/0.5 in NTU.

Virus and protozoa log reduction credits assigned by the USEPA (2019) and California SWTR (2018), respectively, to microfilters were used for the selection of appropriate treatment process trains here (Table 4.4) because they are also among the most conservative and recent estimates reported for microfilters. Following the methodology used by Soller et al. (2019) to assess microbial risk from direct potable reuse projects, a bacterial LRV of 3 for microfilters was selected (Table 4.4).

Removal credits for sand filtration, ultrafiltration, and microfiltration assigned by the USEPA (2019) are based on filtration systems conforming to the operational and design requirements in the Surface Water Treatment Rule (SWTR) policy No. 2. The filtration systems should also consistently meet the specified individual and combined filter effluent turbidity requirements (Table 4.4). The SWTR approach is considered appropriate for stormwater treatment because LRVs were developed for surface water, where most stormwater is anticipated to be captured.

Within the context of MF vs UF there may be differences in virus removal, with higher virus removal achieved by the tighter nominal pore-sized UF. However, there is no direct integrity test (DIT) that can verify virus sized defects in either MF or UF (USEPA, 2005). To that end, following the requirements of the USEPA MFGM, MF or UF are typically not credited with virus removal. Similarly, Pecson et al. (2021) did not assign any virus LRCs to membrane filtration processes for a technical guidance on stormwater treatment for non-potable end uses. Any potential virus credit is often granted to the use of coagulation before MF and UF per the monitoring and control requirements in the USEPA Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) (USEPA, 2010).

Protozoa removal by either MF or UF can be verified daily using pressure decay testing as a DIT, and it is typically possible to demonstrate more than 4-log protozoa removal. However, in the guidance developed by Pecson et al. (2021), no protozoa LRCs were assigned to membrane filtration processes because monitoring to confirm membrane integrity was not considered a requirement for the model treatment train that Pecson et al. (2021) suggested to treat stormwater with HFCA of 10^{-1}. Protozoa LRCs achieved by membrane filters, with the proper monitoring, could increase confidence in the stormwater treatment trains reliability, and these credits should be accounted for to confirm compliance to future regulatory frameworks.

Disinfection Treatment Processes: Ultraviolet Disinfection

The USEPA developed a comprehensive guidance manual for UV disinfection for the final Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) (USEPA, 2006 a) in addition to Innovative Approaches for Validation of Ultraviolet Disinfection Reactors for Drinking Water Systems (USEPA, 2020a). These guidance manuals were selected for protozoan and viral LRV credits that can be achieved using different UV doses (Table 4.5) because they provide the

most detailed guidelines among the reviewed documents. Pecson et al. (2021) used a UV dose of 276 mJ/cm^2 to achieve 6 LRCs for both viruses and protozoa. This dose is the same as the one recommended in Table 4.5 to gain 6 LRCs for virus removal (viruses will control the selected UV dose).

Due to the lack of bacteria crediting frameworks in the US, bacterial LRV credits were absent from most of the reviewed literature on UV disinfection, e.g., USEPA (2006 a), Olivieri et al. (2016), and Pecson et al. (2021). However, an Australian framework (WaterVal^{TM}) providing national consistency in treatment technologies validation for the water industry (WaterSecure, 2017a) reported on a study analyzing the inactivation kinetics of pathogens using UV (Hijnen et al., 2006). In their study, Hijnen et al. (2006) calculated bacterial LRVs for *Campylobacter jejuni*, *E. coli O157*, and *Salmonella typhi*. Literature review data was used to calculate the inactivation rate constant k and find the UV dose required to achieve a certain LRV.

Ultraviolet disinfection doses found by Hijnen et al. (2006) were selected herein (Table 4.5) to assign 1 to 4 LRV credits to UV disinfection in stormwater treatment process trains (Tables 7 and 8). It is assumed that the dose achieving 5 and 6 virus LRV credits are capable of achieving more than 4 (> 4) bacteria LRV credit.

Disinfection unit treatment processes: Chlorination and Chloramination

Pathogen LRV credits achieved using free chlorine in this study were selected based on the values provided by WaterSecure (2017b) (Table 4.5). The WaterSecure (2017b) protocol approach is consistent with the USEPA (1999) manual: Disinfection Profiling and Benchmarking Guidance, that is concentration x time (CT) is used to correlate disinfectant dose requirements with LRVs. However, WaterSecure (2017b) outlines specific CT values that are more conservative (i.e.,

require higher CTs) when compared to USEPA (1999) values based on more recent studies conducted using coxsackievirus B5. Furthermore, chloramine values recommended by WaterSecure (2017b) are associated with a range of potential water matrix turbidities (0.2 – 20 NTU).

Virus inactivation was the main focus of WaterSecure (2017b) protocol. This is because enteric viruses have a stronger resistance to free chlorine compared to enteric bacteria (WaterSecure, 2017b). As such, the same free chlorine CT can be used for bacterial and viral LRVs (WaterSecure, 2017b). Chorine is not effective for *Cryptosporidium* inactivation; therefore, no attempt was made to claim *Cryptosporidium* LRV credits by chlorination under WaterSecure (2017b) protocol. *Giardia* is resistant to chlorination, but CTs are provided by USEPA (1999) that should be suitable to claim a *Giardia* LRV. Pathogen LRV credits achieved by free chlorine are a function of water temperature, pH, and turbidity (WaterSecure, 2017b). Bacterial and viral LRVs achieved under different CT values (Table 4.5) are for pH ≤ 8 (NSQD, 2018).

The Cl_2 dose required to achieve 2 virus LRCs is 10 mg.min/L (Table 4.5). In contrast, Pecson et al. (2021) assigned 2 virus LRCs to a Cl_2 dose of 7 mg.min/L. This is because Pecson et al. (2021) assumed higher stormwater temperature (≥15°C) and lower stormwater turbidity (≤0.5 NTU). In this study, a turbidity of turbidity ≤ 2 NTU and temperature of 10°C are assumed for stormwater based on national averages (NSQD, 2005; WaterSecure, 2017b) as opposed to the values assumed by Pecson et al. (2021), which reflect stormwater characteristics in California.

Table 4.5. Selected LRV Credits Achieved Using Different Disinfection Doses

LRV Credit	Bacteria	Protozoa	Viruses	Crediting Framework or Pathogen Removal Studies
	Ultraviolet Disinfection Dose (mJ/cm^2)			
1 log_{10} reduction	3 - 6	2.5	58	Bacteria: Hijnen et al. (2006) – Peer Reviewed Literature Protozoa and Viruses: USEPA (2006 a) – US Crediting Framework
2 log_{10} reduction	7 - 12	5.8	100	
3 log_{10} reduction	10 - 17	12	143	
4 log_{10} reduction	14 - 51	22	186	
5 log_{10} reduction	NA	45	231	
6 log_{10} reduction	NA	85	276	
	Free Chlorine CT Dose (mg.min/L)[a]			
1 log_{10} reduction	7	LRV cannot be claimed under WaterSecure (2017b) protocol	7	WaterSecure (2017b) – Australian Crediting Framework
2 log_{10} reduction	10[b]		10	
3 log_{10} reduction	13		13	
4 log_{10} reduction	16		16	
	Chloramine CT Dose (mg.min/L)[c]			
1 log_{10} reduction	NA	615[d]	1482	Protozoa: USEPA (1999) – U.S. Crediting Framework Virus: Keegan et al. (2012) – Peer Reviewed Literature
2 log_{10} reduction	NA	1230[d]	2326	
3 log_{10} reduction	NA	1850[d]	3160	
4 log_{10} reduction	NA	NA	3949	
	Ozone CT Dose (mg.min/L)[e]			
1 log_{10} reduction	0.85	9.9[f]	0.85	WaterSecure (2017c) - Australian Crediting Framework
2 log_{10} reduction	1.23[g]	20[f]	1.23	
3 log_{10} reduction	1.63	30[f]	1.63	
4 log_{10} reduction	2.01	NA	2.01	

a: bacterial and viral LRVs are for pH ≤ 8, turbidity ≤ 2 NTU, and temperature = 10°C, **b**: Hoff (1986) recommended a free chlorine CT dose of 0.034 to 0.05 (mg.min)/L to achieve 2 bacterial LRVs based on *E. coli* as the test microorganism, pH of 6 to 7, and a temperature of 5°C, **c**: the chloramine CT is for pH = 6-9, temp. = 10°C , **d:** These credits are for *Giardia*, **e:** CT values required to achieve pathogen LRV credits using ozone are obtained from WaterSecure (2017c) validation protocol at a temperature of 10°C, **f**: for protozoa, the CT values in WaterSecure (2017c) are based on USEPA (2006 b) guidance, **g**: Hoff (1986) recommended an ozone CT value of 0.02 (mg.min)/L to achieve 2 LRV for bacteria using *E. coli* as the test microorganism, pH of 6 to 7, and a temperature of 5°C.

Disinfection Treatment Processes: Ozonation

Several studies and reports were reviewed to obtain pathogen LRV credits achieved by ozonation (Table 4.5). WaterSecure (2017c) validation protocol provides comprehensive guidance for validating the disinfection of bacteria, protozoa, and viruses using ozone. Thus, pathogen LRV credits achieved using ozone selected herein (Table 4.5) are based on WaterSecure (2017c). The protocol established by WaterSecure (2017c) is largely based on the USEPA (2006 b) guidance and on research by Melbourne Water for the validation of ozone treatment processes.

WaterSecure (2017c) validation protocol recommends the same values of CT for the removal of bacteria and viruses using ozone (Table 4.5). CT values for pathogens (Table 4.5) are for a temperature of 10°C (NSQD, 2005; WaterSecure, 2017b). It should be noted that bacterial and viral LRVs were developed for wastewater.

4.4.3 Selection and Evaluation of Stormwater Treatment Trains

Selection of Stormwater Treatment Trains

Different treatment process train combinations were selected for unrestricted irrigation (Table 4.6) and indoor use (Table 4.7) for HFCA values of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. Treatment trains for HFCAs of 10^{-5} and 10^{-6} are not included since these contamination levels require minimum treatment that does not significantly differ from treatment required at HFCA of 10^{-4}. Pecson et al. (2021) suggested a treatment train for an HFCA of 10^{-1} consisting of membrane filtration (without specifying whether it was UF or MF), UV, free Cl_2, and secondary disinfection to treat stormwater for indoor use. The secondary disinfection consists of adding ammonium sulfate to convert residual Cl_2 to chloramine and boosting sodium hypochlorite to provide operational flexibility and increase chloramine residual. No LRCs were assigned to secondary disinfection.

In this study, one of the treatment process trains suggested for indoor use at HFCA of 10^{-1} (Table 4.7) is similar to the train suggested by Pecson et al. (2021). Both trains include membrane filtration, UV disinfection, and chlorination. However, a lower UV dose (186 mJ/cm^2) and a higher Cl_2 dose (13 mg.min/L) are selected in this study unlike the doses selected by Pecson et al. (2021), i.e., UV dose of 276 mJ/cm^2 and Cl_2 dose of 7 mg.min/L. These are minor differences attributed to designer's choice and the difference in the virus LRC values assigned to chlorination (Section 4.4.2). The treatment process train suggested here does not include secondary disinfection since the train meets or exceeds the required pathogen LRTs. Furthermore, the addition of more chemicals, e.g., ammonium sulfate, requires additional chemical transport and expenses.

Table 4.6 Suggested Treatment Process Trains Required to Achieve LRTs for Unrestricted Irrigation

Treatment Process	LRV		
	Virus	Protozoa	Bacteria
10^{-1} HFCA (Options 1 and 2)			
Microfiltration	0	4	3
UV (186 mJ/cm^2)	4	6	>4
Free Cl_2 (13 mg.min/L) (Chloramine 3160 mg.min/L)[a]	3 3	0	3 (NA)
Total LRV	**7 (7)[a]**	**10 (10)[a]**	**>10 (>7)[a]**
Required LRT	**6.5**	**4.5**	**4**
10^{-1} HFCA (Option 3)			
Microfiltration	0	4	3
UV (186 mJ/cm^2)	4	6	>4
O_3 (1.63 mg.min/L)	3	0	3
Total LRV	**7**	**10 (10)**	**>10**
Required LRT	**6.5**	**4.5**	**4**
10^{-1} HFCA (Option 4)			
Microfiltration	0	4	3
UV (276 mJ/cm^2)	6	6	>4
Chloramine 2326 mg.min/L	2	0	NA
Total LRV	**8**	**10**	**>7**
Required LRT	**6.5**	**4.5**	**4**
10^{-1} HFCA (Option 5)			
MF	0	4	3
UV1 (143 mJ/cm^2)	3	6	>4
UV2 (186 mJ/cm^2)	4	6	>4
Total LRV	**7**	**16**	**>11**
Required LRT	**6.5**	**4.5**	**4**
10^{-2} HFCA (Options 1 and 2)			

Microfiltration	0	4	3
UV (186 mJ/cm^2)	4	6	>4
Free Cl_2 (10 mg.min/L) (Chloramine 2326 mg.min/L)[a]	2 (2)	0 (0)	3 (NA)
Total LRV	**6 (6)[a]**	**10 (10)[a]**	**>10 >(7)[a]**
Required LRT	**5.5**	**3.5**	**3**
10^{-2} HFCA (Option 3)			
Microfiltration	0	4	3
UV (186 mJ/cm^2)	4	6	>4
O_3 (1.23 mg.min/L)	2	0	3
Total LRV	**6**	**10**	**>10**
Required LRT	**5.5**	**3.5**	**3**
10^{-2} HFCA (Option 4)			
Microfiltration	0	4	3
UV (276 mJ/cm^2)	6	6	>4
Total LRV	**6**	**10**	**>7**
Required LRT	**5.5**	**3.5**	**3**
10^{-3} HFCA (Options 1 and 2)			
Microfiltration	0	4	3
UV (186 mJ/cm^2)	4	6	>4
Free Cl_2 (7 mg.min/L) (Chloramine 1482 mg.min/L)[a]	1 (1)	0 (0)	1 (NA)
Total LRV	**5 (6)[a]**	**10 (10)[a]**	**>8 (>7)[a]**
Required LRT	**4.5**	**2.5**	**2**
10^{-3} HFCA (Option 3)			
Microfiltration	0	4	3
UV (186 mJ/cm^2)	4	6	>4
O_3 (0.85 mg.min/L)	1	0	1
Total LRV	**5**	**10**	**>8**
Required LRT	**4.5**	**2.5**	**2**
10^{-3} HFCA (Option 4)			
Microfiltration	0	4	3
UV (231 mJ/cm^2)	5	6	>4
Total LRV	**5**	**10**	**>7**
Required LRT	**4.5**	**2.5**	**2**
10^{-4} HFCA (Option 1)			
Microfiltration	0	4	3
UV (186 mJ/cm^2)	4	6	>4
Total LRV	**4**	**10**	**>7**
Required LRT	**3.5**	**1.5**	**1**
10^{-4} HFCA (Option 2)			
Microfiltration	0	4	3
O_3 (9.9 mg.min/L)	4	1	4
Total LRV	**4**	**5**	**7**
Required LRT	**3.5**	**1.5**	**1**
10^{-4} HFCA (Option 3)			
Microfiltration	0	4	3
Free Cl_2 (16 mg.min/L)	4	0	4
Total LRV	**4**	**4**	**7**
Required LRT	**3.5**	**1.5**	**1**
10^{-4} HFCA (Option 3)			
Microfiltration	0	4	3

Chloramine (3949 mg.min/L)	4	2	NA
Total LRV	**4**	**6**	**3**
Required LRT	**3.5**	**1.5**	**1**

a: The LRVs in parentheses are for when chloramine is used rather than free chlorine

Table 4.7 Suggested Treatment Process Trains Required to Achieve LRTs for Indoor Use

Treatment Process	**LRV**		
	Virus	**Protozoa**	**Bacteria**
10^{-1} HFCA (Options 1 and 2)			
Microfiltration	0	4	3
UV (186 mJ/cm^2)	4	6	>4
Free Cl_2 (13 mg.min/L) (Chloramine 3160 mg.min/L)[a]	3 (3)	0 (0)	3 (NA)
Total LRV	**7 (7)[a]**	**10 (10)[a]**	**>10 (>7)[a]**
Required LRT	**7.0**	**5.5**	**5**
10^{-1} HFCA (Option 3)			
Microfiltration	0	4	3
UV (186 mJ/cm^2)	4	6	>4
O_3 (1.63 mg.min/L)	3	0	3
Total LRV	**7**	**10**	**>10**
Required LRT	**7.0**	**5.5**	**5**
10^{-1} HFCA (Option 4)			
Microfiltration	0	4	3
UV (276 mJ/cm^2)	6	6	>4
Chloramine 2326 mg.min/L	2	0	NA
Total LRV	**8**	**10**	**>7**
Required LRT	**7.0**	**5.5**	**5**
10^{-1} HFCA (Option 5)			
Microfiltration	0	4	3
UV (143 mJ/cm^2)	3	6	>4
UV (186 mJ/cm^2)	4	6	>4
Total LRV	**7**	**16**	**>11**
Required LRT	**7.0**	**5.5**	**5**
10^{-2} HFCA (Options 1 and 2)			
Microfiltration	0	4	3
UV (186 mJ/cm^2)	4	6	>4
Free Cl_2 (10 mg.min/L) (Chloramine 2326 mg.min/L)[a]	2 (2)	0 (0)	3 (NA)
Total LRV	**6 (6)[a]**	**10 (10)[a]**	**>10 (>7)[a]**
Required LRT	**6**	**4.5**	**4**
10^{-2} HFCA (Option 3)			
Microfiltration	0	4	3
UV (186 mJ/cm^2)	4	6	>4
O_3 (1.23 mg.min/L)	2	0	3
Total LRV	**6**	**10**	**>10**
Required LRT	**6.0**	**4.5**	**4**
10^{-2} HFCA (Option 4)			
Microfiltration	0	4	3
UV (276 mJ/cm^2)	6	6	>4

Total LRV	**6**	**10**	**>7**
Required LRT	**6**	**4.5**	**4**
10^{-3} HFCA (Option 1 and 2)			
Microfiltration	0	4	3
UV (186 mJ/cm^2)	4	6	>4
Free Cl_2 (7 mg.min/L) (Chloramine 1482 mg.min/L)[a]	1 (2)	0 (0)	1 (NA)
Total LRV	**5 (6)[a]**	**10 (10)[a]**	**>8 (>7)[a]**
Required LRT	**5**	**3.5**	**3**
10^{-3} HFCA (Option 3)			
Microfiltration	0	4	3
UV (186 mJ/cm^2)	4	6	>4
O_3 (0.85 mg.min/L)	1	0	1
Total LRV	**5**	**10**	**>8**
Required LRT	**5.0**	**3.5**	**3.0**
10^{-3} HFCA (Option 4)			
Microfiltration	0	4	3
UV (231 mJ/cm^2)	5	6	>4
Total LRV	**5**	**10**	**>7**
Required LRT	**5.0**	**3.5**	**3.0**
10^{-4} HFCA (Option 1)			
Microfiltration	0	4	3
UV (186 mJ/cm^2)	4	6	>4
Total LRV	**4.0**	**10**	**>7**
Required LRT	**4.0**	**2.5**	**2.0**
10^{-4} HFCA (Option 2)			
Microfiltration	0	4	3
O_3 (9.9 mg.min/L)	4	1	4
Total LRV	**4**	**5**	**7**
Required LRT	**4.0**	**2.5**	**2.0**

a: The LRVs in parentheses are for when chloramine is used rather than free chlorine

It is important to note that the HFCA value on which Pecson et al. (2021) based their design is pathogen-derived and, it was obtained from older and fewer stormwater datasets. On the other hand, the different HFCA values in this study are derived from large and recent human MST marker datasets. Aside from the single treatment train suggested by Pecson et al. (2021) for one value of HFCA (10^{-1}) and one end use application (indoor use), no other guidance documents or studies attempted to provide selections of stormwater treatment trains with recommended disinfectant doses based on human fecal contamination levels and end uses.

Feng et al. (2022) proposed stormwater treatment trains consisting of GI practices (e.g., biofilters or constructed wetlands) and disinfection unit processes (e.g., chlorination, UV

disinfection, solar radiation, and ozonation). However, Feng et al. (2022) did not consider human fecal contamination of stormwater, nor did they recommend disinfectant doses. The treatment train recommendations were descriptive, i.e., pathogen LRCs achieved by unit treatment processes and the required pathogen LRTs were not quantified.

Evaluation of Stormwater Treatment Trains

Treatment trains for the use of stormwater for unrestricted irrigation (Table 4.6) are evaluated with respect to trains complexity and reliability at HFCA levels of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. The performance evaluation results are only shown for unrestricted irrigation due to the small difference in pathogen LRTs between unrestricted irrigation and indoor use (Tables 4.6 and 4.7). Inputs to the performance evaluation matrix (Appendix C; Table C.4) to calculate complexity and reliability scores are obtained from regulatory frameworks used to select LRCs (Appendix C; Table C.2) and Table 4.6, respectively.

It is important to note that the performance evaluation scores only reflect the performance of treatment trains at a single HFCA, i.e., 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. These scores cannot be used to compare treatment trains among different HFCAs due to the method used for performance evaluation (Section 4.2.3). The top performing treatment trains at HFCA values of 10^{-1}, 10^{-2}, at 10^{-3} are MF+UV+chloramine and MF+UV+O_3 (Table 4.8; Appendix C, Tables C.5, C.6, and C.7). While these stormwater treatment trains are as reliable as the one including MF+UV+Cl_2, they outperform the MF+UV+Cl_2 treatment train with their complexity score because MF+UV+chloramine and MF+UV+O_3 have less continuous monitoring requirements (Figure 4.2). Additionally, the disinfection components in the MF+UV+chloramine and MF+UV+O_3 treatment trains are not affected by the ammonia content of influent stormwater (Section 4.2.3; Figure 4.2). Stormwater content of ammonia is variable (0 -11.9 mg/L; NSQD, 2018) and has a Cl_2 demand

for the oxidation of ammonia to nitrate. This demand impacts the amount of both the available and residual Cl_2 needed for disinfection.

At HFCA levels of 10^{-1}, 10^{-2}, and 10^{-3}, the treatment trains with the lowest overall performance are MF+UV1+UV2, MF+UV, and MF+UV, respectively (Table 4.8; Appendix C, Tables C.5, C.6, C.7, and C.8). While these trains have the least continuous monitoring requirements (Figure 4.2), they are also the least reliable compared to other treatment trains (Table 4.8). This is because these treatment trains have the highest risk of failure since all the required virus LRTs are achieved by only one unit treatment process (i.e., UV; Figure 4.2). If the UV unit(s) went offline, then the stormwater would be out-of-compliance. In comparison, if the UV process went offline in the treatment trains including MF+UV+(chloramine or O_3), stormwater can still be in compliance by increasing O_3 or chloramine doses to achieve the required virus LRTs. Note that virus LRTs were selected to as sub-criteria for reliability (Section 4.2.3) because viruses were found to drive the exposure risk (Section 4.4.1).

At HFCA of 10^{-4}, the treatment trains with the highest overall performance is MF+UV. Since all the suggested treatment trains have equal reliability (Table 4.8; Appendix C, Tables C.5, C.6, C.7, and C.8), the treatment performance is driven by complexity. Compared to MF+O_3, with the second highest performance, MF+UV has less continuous monitoring requirements (Figure 4.2). It should be noted that the top performing treatment train at 10^{-4} (MF+UV) is protective of public health.

Table 4.8 Complexity, Reliability, and Overall Scores of Stormwater Treatment Train Options at HFCA Levels of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} for the Use of Treated Stormwater for Unrestricted Irrigation

Stormwater Treatment Trains	Complexity Score	Reliability Score	Overall Score
HFCA = 10^{-1}			
MF, UV (186 mJ/cm^2), Cl_2 (13 mg.min/L)	2.3	5.0	3.7
MF, UV (186 mJ/cm^2), chloramine (3160 mg.min/L)	3.2	5.0	4.1
MF, UV (186 mJ/cm^2), O_3 (1.63 mg.min/L)	3.7	5.0	4.3
MF, UV (276 mJ/cm^2), chloramine (232 mg.min/L)	4.5	5.0	4.7
MF, UV1 (143 mJ/cm^2), UV2 (186 mJ/cm^2)	5.0	1.0	3.0
HFCA = 10^{-2}			
MF, UV (186 mJ/cm^2), Cl_2 (10 mg.min/L)	2.3	5.0	3.7
MF, UV (186 mJ/cm^2), chloramine (2326 mg.min/L)	2.7	5.0	3.9
MF, UV (186 mJ/cm^2), O_3 (1.23 mg.min/L)	3.7	5.0	4.3
MF, UV (276 mJ/cm^2)	5.0	2.0	3.5
HFCA = 10^{-3}			
MF, UV (186 mJ/cm^2), Cl_2 (7 mg.min/L)	2.3	5.0	3.7
MF, UV (186 mJ/cm^2), chloramine (1482 mg.min/L)	2.7	5.0	3.9
MF, UV (186 mJ/cm^2), O_3 (0.85 mg.min/L)	4.1	5.0	4.5
MF, UV (231mJ/cm^2)	5.0	2.0	3.5
HFCA = 10^{-4}			
MF, UV (186 mJ/cm^2)	5.0	5.0	5.0
MF, O_3 (9.9 mg.min/L)	4.6	5.0	4.8
MF, Cl_2 (16 mg.min/L)	2.3	5.0	3.7
MF, chloramine (3949 mg.min/L)	3.2	5.0	4.1

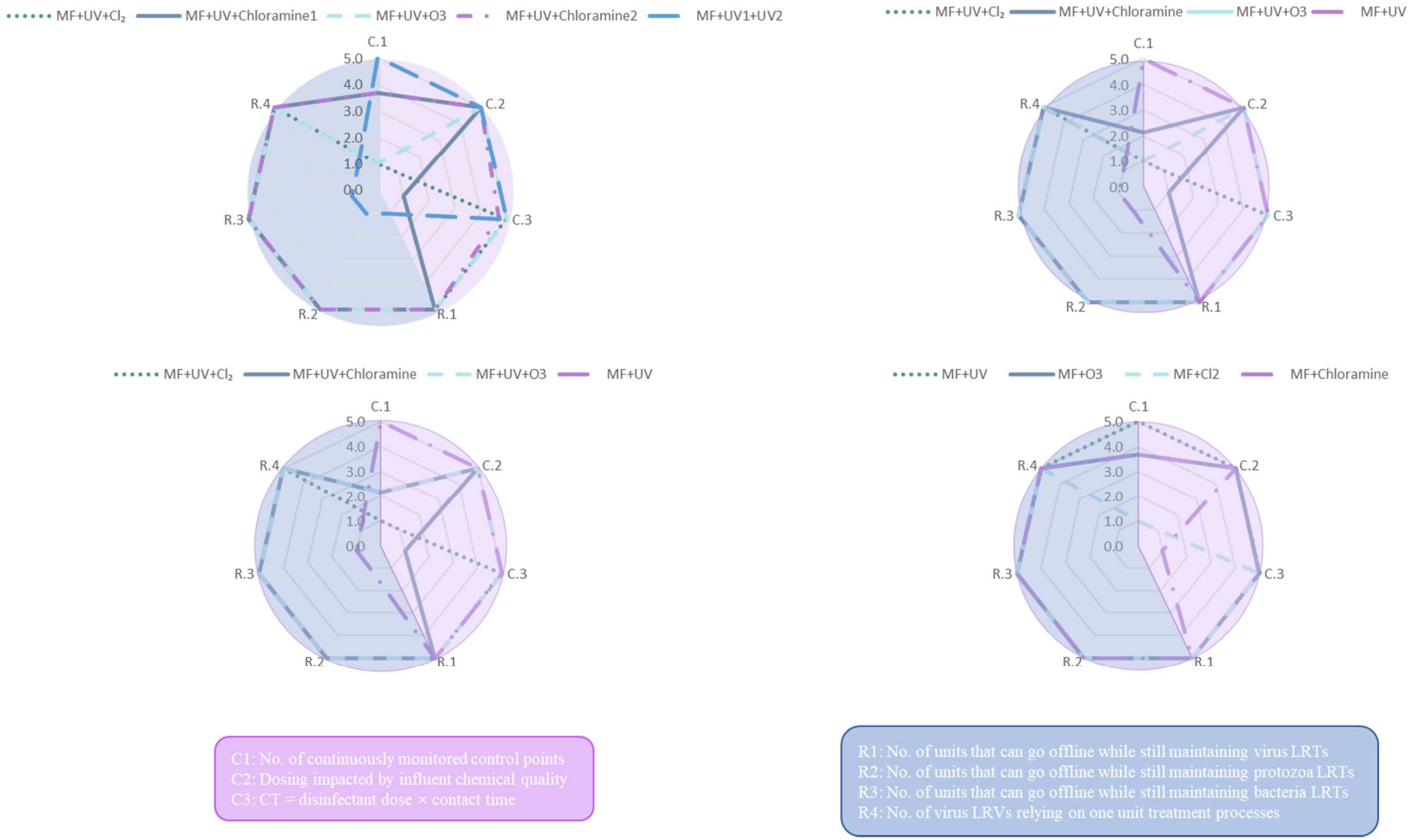


Figure 4.2 Complexity and Reliability Sub-criteria Scores for Each Stormwater Treatment Train option Suggested for HFCA Values of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}

The treatment train with the lowest performance at HFCA of 10^{-4} is MF+Cl_2 (Table 4.8; Appendix C, Tables C.5, C.6, C.7, and C.8). Even though MF+Cl_2 has the same continuous monitoring requirements as MF+UV (Figure 4.2), MF+UV outperforms MF+Cl_2 because UV disinfection is not affected by the ammonia content of influent stormwater.

During a workshop carried out to inform stormwater treatment requirements for the Water Research Foundation (WRF) project WRF#5034, stormwater practitioners were mostly in favor of using a single stormwater treatment train comprised of only MF+UV regardless of the microbial contamination level. This is because practitioners wanted to simplify the treatment trains (lower operational complexity). The reasoning for this preference was the belief that pathogen LRTs could still be achieved by increasing the UV dose for higher microbial contamination levels. However, this analysis shows that this decision is unwise because relying on one disinfection process to achieve the required pathogen LRTs increases the risk of being out-of-compliance in case the UV treatment unit goes offline, especially at the higher contamination levels (10^{-1}, 10^{-2}, and 10^{-3}).

In this study, at least two disinfection units in addition to membrane filtration are recommended for HFCA levels of 10^{-1}, 10^{-2}, and 10^{-3}, where virus LRTs are greater than 4 (Table 4.3). Guidelines for Validating Treatment Processes for Pathogen Reduction developed by Victoria's Department of Health recommend adopting a multiple-barrier approach which is risk-based (State of Victoria Department of Health, 2013). In this risk-based approach, no more than 4 LRVs can be attributed to a single treatment process. However, at HFCA of 10^{-4}, the highest performing treatment train has only one disinfection process (UV, Table 4.8) because the virus LRT is less than 4, i.e., 3.5 (Table 4.3). Thus, as the HFCA value increases from 10^{-4} to 10^{-3}, 10^{-2}, and 10^{-1}, the complexity of stormwater treatment trains increases with the overall performance

score decreasing from 5.0 at 10^{-4} to 3.73 at 10^{-3}, 10^{-2}, and 10^{-1} (Table 4.3; Figure 4.2, and Figure 4.3).

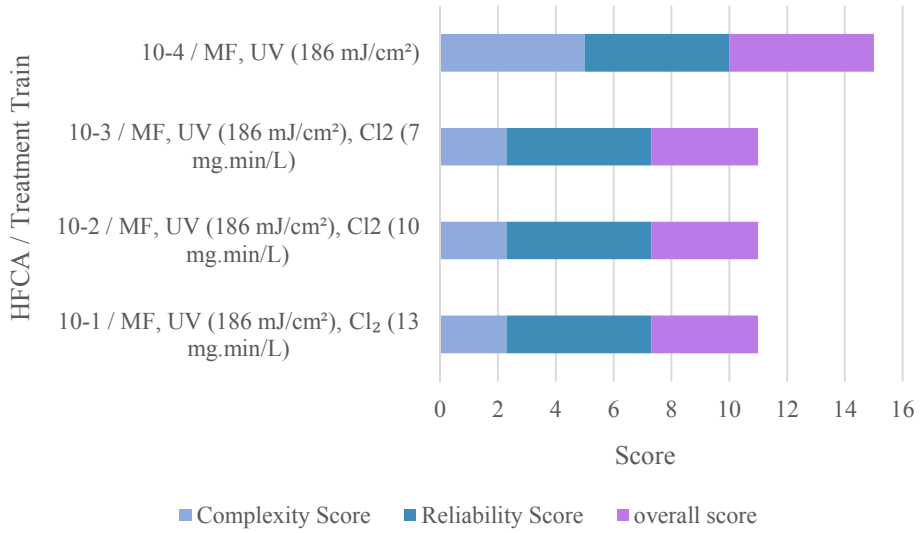


Figure 4.3 Complexity, Reliability, and Overall Scores for one of the Suggested Stormwater Treatment Trains across Different HFCA Values

4.5 Conclusions

- Using LRCs published in regulatory frameworks and a performance evaluation method based on weighted averages, the highest performing stormwater treatment train with

respect to treatment complexity and reliability at high HFCA values (e.g., 10^{-1}) consists of membrane filtration, UV disinfection (276 mJ/cm^2), and chloramine (232 mg.min/L).

- Reliance on UV alone to achieve disinfection at high microbial contamination levels is not recommended in order to avoid producing out-of-compliance effluent in the event of process failure.
- At low HFCA values, a treatment train consisting of membrane filtration and only one disinfection unit treatment process, specifically UV or O_3 is recommended because virus LRT decreases with decreasing HFCA values.
- The use of chlorination at both low and high HFCA levels is not recommended due to the high continuous monitoring requirements associated with the use of free Cl_2.
- The HFCA threshold above which the complexity of stormwater treatment trains significantly increases is 10^{-4}. The treatment trains suggested at an HFCA of 10^{-4} are still protective of public health.

CHAPTER 5: CONCLUSIONS

5.1 Roof Runoff and Stormwater Capture and Use Projects

The research presented here attempted to address barriers to the wide-scale implementation of roof runoff and stormwater capture and use projects for non-potable end uses across the U.S through investigating roof runoff microbial quality in different U.S cities with varying climate and land use characteristics; examining suitability of potential factors to predict roof runoff microbial quality, e.g., geographic location, storm properties, and sampling season; assessing ranges of human fecal contamination (HFCA) in stormwater based on concentrations of potentially human-infectious pathogens (PHIPs) and human microbial source tracking (MST) markers; exploring how these HFCAs compare with estimates developed by Schoen et al. (2017) and adopted by Sharvelle et al. (2017); and investigating how the selection of pathogen log reduction targets (LRTs) and HFCAs impacts the choice of treatment process trains protective of public health for the designated end use of stormwater. The following findings can be derived from this research:

- PHIPs were found in roof runoff at low detection frequencies, and their concentrations are not considerably higher than the concentrations of PHIPs found in surface water. Thus, with the proper treatment, roof runoff can be used for non-potable purposes.
- Physicochemical parameters and impervious area were not found to be important factors for determination of the presence/absence of potentially human-infectious pathogens.
- Consistent trends between microbial data and geographical location were not found despite noting some differences among study cities when *E. coli* concentrations and *Salmonella spp.* presence in roof runoff were compared.

- There might be an association between meteorological parameters and the presence/absence of potentially human-infectious pathogens based on identified correlations.
- The characterization of the presence and concentrations of potentially human-infectious pathogens obtained from this study can be used as inputs to a QMRA to develop pathogen log reduction targets and select treatment processes compatible with roof runoff end use. This will advance the status of roof runoff as an alternative water resource, thereby reducing the demand on conventional freshwater resources.
- Human MST markers are found in wet weather flows at relatively high percentages (% detection = 65.5%, median concentration = $10^{2.9}$ GC/100 mL).
- Adenoviruses, norovirus GI+GII, and enteroviruses are detected in 41.4%, 74.3%, and 38.6% of the reviewed wet weather flow measurements across the U.S at median concentrations of $10^{1.1}$, $10^{2.83}$, and $10^{0.66}$ GC/100 mL, respectively. *Giardia lamblia* and *Cryptosporidium parvum* are detected in 50% and 56.2% of the reviewed wet weather flow measurements, respectively, at much lower median concentrations compared to viral pathogens ($10^{-1.1}$ cysts/L median concentration for *Giardia lamblia* and $10^{-0.8}$ oocysts/L median concentration for *Cryptosporidium parvum*).
- Viral pathogens, specifically norovirus GI+GII, will drive the risk associated with exposure to stormwater. As a result, unit treatment processes in stormwater treatment trains should be selected and designed for the effective removal of noroviruses.
- The human MST markers dataset is better than the viral and protozoan datasets for the estimation of HFCA since viral and protozoan datasets are limited by detection levels and the range of data observed within statistical distributions.

- Human MST-based estimates of HFCA range from $<10^{-7.0}$ to $10^{-1.5}$ (median = $10^{-4.5}$) in wet weather flows and from 10^{-12} to $10^{-2.6}$ (median = 10^{-7}) in dry weather flows.
- The 95th percentile human MST-based HFCA estimated in this study ($10^{-1.5}$) falls within the 95th percentile sewage dilution range estimated by Schoen et al. (2017) and adopted by Sharvelle et al. (2017) despite using a different method of analysis. Schoen et al. (2017) sewage dilution range is 10^{-3} to 10^{-1}, where 10^{-1} is 95th percentile sewage dilution based on norovirus GII and 10^{-3} is the 95th percentile sewage dilution based on *Giardia*.
- The HFCA threshold above which the complexity of stormwater treatment trains significantly increase is 10^{-4}.
- The highest performing stormwater treatment trains with respect to treatment complexity and reliability at high pathogen LRTs and HFCA values consist of membrane filtration and at least two disinfection unit treatment processes, specifically UV and O_3 or UV and chloramine.
- A treatment train consisting of membrane filtration and only one disinfection unit treatment process, specifically O_3 or chloramine, is recommended at low HFCA values because virus LRT decreases with decreasing HFCA values.

While combining the collection of roof runoff and stormwater could augment the amount of collected water; nevertheless, the two water sources, as this study shows, have fundamentally different microbial quality with stormwater containing much more PHIPs compared to roof runoff. Thus, treating them together could prove to be both challenging and impractical.

5.2 Research Hypotheses

This section briefly summarizes whether the original research hypotheses are in agreement with the research findings.

H.1 Fecal indicator bacteria will be detected at higher frequencies and concentrations than potentially human-infectious pathogens in all study cities.

Both *E. coli* and enterococci were detected at considerably higher frequencies, 73.4% and 96.2% of the analyzed samples, respectively, than PHIPs. *Salmonella* spp. *invA*, *Campylobacter* spp. *ceuE*, and *G. duodenalis β – giardin* gene targets were detected in 8.9%, 2.5%, and 5.1% of roof runoff samples, respectively. Similarly, the concentrations of fecal indicator bacteria were considerably higher than those of PHIPs. Concentrations of both *E. coli* and enterococci ranged from <0 $\log_{10}$ to >3.38 $\log_{10}$ MPN/100 mL. Concentrations *of Salmonella* spp. *invA* and *Campylobacter* spp. *ceuE* ranged from 0-252.6 and 0-3.8 cells/L, respectively. The concentration of *G. duodenalis β-giardin* ranged from 0-15 cysts/L.

H.2 Sampling season, geographic location, overhanging trees, percent of impervious cover, roof runoff physicochemical parameters, and storm properties will have an impact on roof runoff microbial quality.

Physicochemical parameters, impervious area, the presence of hanging trees, and sampling season were not found to be important factors for determination of the presence/absence of potentially human-infectious pathogens. Consistent trends between microbial data and geographical location were not found even though some differences among study cities were noted when *E. coli* concentrations and *Salmonella spp.* presence in roof runoff were compared. This might be partially attributed to the low percent detection of PHIPs in roof runoff. There might be an association between storm properties (rainfall depth and antecedent dry period) and the presence/absence of PHIPs based on identified correlations.

H.3 HFCA in stormwater based on human MST markers will differ from those estimated based on potentially human-infectious pathogens.

Human MST-based estimates of HFCA ranged from $<10^{-7.0}$ to $10^{-1.5}$ (median = $10^{-4.5}$) in wet weather flows. PHIP-based HFCAs in wet weather flows ranged from $<10^{-7.0}$ (enteroviruses) to $10^{-0.1}$ (norovirus GI+GII). Both HFCAs were similar at the low end of the range, but they differed significantly at the high end of the range. Despite the fact that viral pathogens are found less frequently and at lower median concentrations in stormwater than wastewater, they can be detected in stormwater at concentrations almost as high as those in wastewater at the 95th percentile.

H.4 Estimates of HFCA in stormwater based on the newer, more comprehensive dataset of stormwater human MST marker and pathogen data will slightly differ from the ones estimated Schoen et al. (2017), which were based on a limited set of stormwater pathogen data.

The 95th range of PHIP-based HFCA in wet weather flows, $10^{-3.3}$ (based on *Giardia lamblia*) to $10^{-0.1}$ (based on norovirus GI+GII), is similar to the pathogen-based sewage dilutions provided by Schoen et al. (2017) 10^{-3} (based on *Giardia lamblia*) to 10^{-1} (based on norovirus GII) at the low end. The high dissimilarity at the high end could be due to the fact that the current study is based on a more recent, comprehensive dataset and both norovirus GI+GII were used in this study whereas Schoen et al. (2017) only norovirus GII. Schoen et al. (2017) remarked that using only norovirus GII could result in lower treatment requirements compared to a combined norovirus GI+GII characterization. However, when looking at the 95th percentile human MST-based HFCA estimated in this study (i.e., $10^{-1.5}$), it lies within the 95th percentile sewage dilution range estimated by Schoen et al. (2017) and adopted by Sharvelle et al. (2017) despite using a different method of analysis.

H.5 A threshold for pathogen LRTs/HFCAs exists beyond which the complexity of treatment process trains significantly changes based on metrics such as monitoring and dosing requirements.

Such threshold does exist, and it is equal to 10^{-4}. At HFCA values higher than 10^{-4}, complexity of stormwater treatment trains increases.

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APPENDIX A: SUPPLEMENTAL INFORMATION FOR CHAPTER 2

Table A.1: Forward Primers, Reverse Primers, and Probes Used in Each Droplet Digital PCR Assay

Assay	Primer or Probe	Sequence	Reference	Positive Control for ddPCR
16S rRNA gene	Forward primer	CGGTGAATACGTTCYCGG	Suzuki et al. (2000)	linearized custom gene
	Reverse primer	GGWTACCTTGTTACGACTT		
	Probe	FAM-CTTGTACACACCGCCCG-ZEN/IBFQ		
IAC	Forward primer	GCAAGCCCCAGAAACCG	Phelps et al. (2017)	linearized custom gene
	Reverse primer	CAAGATGACCGGGATTTACGA		
	Probe	VIC-TCACCCATCCACCACCT-MGB		
Giardia duodenalis	Forward primer	CATCCGCGAGGAGGTCAA	Guy et al. (2003)	*Giardia intestinalis* genomic DNA (ATCC 30888D)
	Reverse primer	GCAGCCATGGTGTCGATCT		
	Probe	HEX-AAGTCCGCCGACAACATGTACCTAACGA-ZEN/IBFQ		
mapA	Forward primer	CTGGTGGTTTTGAAGCAAAGATT	Best et al. (2003)	*Campylobacter jejuni* genomic DNA (ATCC 33560D-5)
	Reverse primer	CAATACCAGTGTCTAAAGTGCGTTTAT		
	Probe	FAM-TTGAATTCCAACATCGCTAATGTATAAAAGCCCTTT-ZEN/IBFQ		
ceuE	Forward primer	AAGCTCTTATTGTTCTAACCAATTCTAACA	Best et al. (2003)	*Campylobacter coli* genomic DNA(ATCC BAA-1061D-5)
	Reverse primer	TCATCCACAGCATTGATTCCTAA		
	Probe	HEX-TTGGACCTCAATCTCGCTTTGGAATCATT-ZEN/IBFQ		
invA	Forward primer	AGCGTACTGGAAAGGGAAAG	Kasturi and Drgon (2017)	*Salmonella enterica* genomic DNA(ATCC 700931D-5)
	Reverse primer	ATACCGCCAATAAAGTTCACAAAG		
	Probe	FAM-CGTCACCTTTGATAAACTTCATCGCA-ZEN/IBFQ		
Cryptosporidium parvum	Forward primer	ACTTTTTGTTTGTTTTACGCCG	Jothikumar et al. (2008)	*Cryptosporidium parvum* (ATCC PRA-67D)
	Reverse primer	AATGTGGTAGTTGCGGTTGAA		
	Probe	HEX-ATTTATCTCTTCGTAGCGGCG-ZEN/IBFQ		

Table A.2 The Minimum Information for Publication of Quantitative Digital PCR Experiments (dMIQE2020) checklist (The dMIQE Group and Huggett, 2020)

ITEM TO CHECK	PROVIDED	COMMENT
Column1	Y/N	Column2
1. SPECIMEN		
Detailed description of specimen type and numbers	Y	Section 2.3.1 Sample Collection
Sampling procedure (including time to storage)	Y	Section 2.3.1 Sample Collection
Sample aliquotation, storage conditions and duration	Y	Section 2.3.2 Sample Handling
2. NUCLEIC ACID EXTRACTION		
Description of extraction method including amount of sample processed	Y	Section 2.3.3.3 Sample Elution and Secondary Concentration
Volume of solvent used to elute/resuspend extract	Y	Section 2.3.3.3 Sample Elution and Secondary Concentration
Number of extraction replicates	Y	Section 2.3.3.3 Sample Elution and Secondary Concentration
Extraction blanks included?	Y	Section 2.3.3.3 Sample Elution and Secondary Concentration
3. NUCLEIC ACID ASSESSMENT AND STORAGE		
Method to evaluate quality of nucleic acids	N	relied on manufacturer Certificate of Analysis
Method to evaluate quantity of nucleic acids (including molecular weight and calculations when using mass)	Y	Section 2.3.3.3 Sample Elution and Secondary Concentration
Storage conditions: temperature, concentration, duration, buffer, aliquots	Y	Section 2.3.3.3 Sample Elution and Secondary Concentration
Clear description of dilution steps used to prepare working DNA solution	N	not applicable
4. NUCLEIC ACID MODIFICATION		
Template modification (digestion, sonication, pre-amplification, bisulphite etc.)	N	not applicable
Details of repurification following modification if performed	N	not applicable
5. REVERSE TRANSCRIPTION		
cDNA priming method and concentration	N	not applicable
One or two step protocol (include reaction details for two step)	N	not applicable
Amount of RNA added per reaction	N	not applicable
Detailed reaction components and conditions	N	not applicable
Estimated copies measured with and without addition of RT*	N	not applicable
Manufacturer of reagents used with catalogue and lot numbers	N	not applicable
Storage of cDNA: temperature, concentration, duration, buffer and aliquots	N	not applicable

6. dPCR OLIGONUCLEOTIDES DESIGN AND TARGET INFORMATION		
Sequence accession number or official gene symbol	**N**	not applicable
Method (software) used for design and *in silico* verification	**N**	references to published assays provided in Table A.1
Location of amplicon	**N**	not applicable
Amplicon length	**N**	not applicable
Primer and probe sequences (or amplicon context sequence)**	**Y**	Table A.1
Location and identity of any modifications	**N**	not applicable
Manufacturer of oligonucleotides	**N**	primers were purchased from IDT; probes were purchased from IDT or ThermoFisher
7. dPCR PROTOCOL		
Manufacturer of dPCR instrument and instrument model	**Y**	Section 2.3.3.3 Quantification of bacterial and protozoan pathogens using droplet digital PCR (ddPCR)
Buffer/kit manufacturer with catalogue and lot number	**Y**	Section 2.3.3.3 Quantification of bacterial and protozoan pathogens using droplet digital PCR (ddPCR)
Primer and probe concentration	**Y**	Section 2.3.3.3 Quantification of bacterial and protozoan pathogens using droplet digital PCR (ddPCR)
Pre-reaction volume and composition (incl. amount of template and if restriction enzyme added)	**N**	not applicable
Template treatment (initial heating or chemical denaturation)	**N**	not applicable
Polymerase identity and concentration, Mg++ and dNTP concentrations***	**N**	this information is proprietary
Complete thermocycling parameters	**Y**	Section 2.3.3.3 Quantification of bacterial and protozoan pathogens using droplet digital PCR (ddPCR)
8. ASSAY VALIDATION		
Details of optimisation performed	**N**	not applicable; published assays used
Analytical specificity (vs. related sequences) and limit of blank (LOB)	**N**	not applicable; published assays used
Analytical sensitivity/LoD and how this was evaluated	**Y**	Section 2.3.3.3 Quantification of bacterial and protozoan pathogens using droplet digital PCR (ddPCR)
Testing for inhibitors (from biological matrix/extraction)	**Y**	Section 2.3.3.3 Quantification of bacterial and protozoan pathogens using droplet digital PCR (ddPCR)
9. DATA ANALYSIS		

Description of dPCR experimental design	**Y**	Section 2.3.3.3 Quantification of bacterial and protozoan pathogens using droplet digital PCR (ddPCR)
Comprehensive details negative and positive of controls (whether applied for QC or for estimation of error)	**Y**	Section 2.3.3.3 Quantification of bacterial and protozoan pathogens using droplet digital PCR (ddPCR)
Partition classification method (thresholding)	**Y**	Section 2.3.3.3 Quantification of bacterial and protozoan pathogens using droplet digital PCR (ddPCR)
Examples of positive and negative experimental results (including fluorescence plots in supplemental material)	**N**	not included
Description of technical replication	**N**	reactions were run in duplicate
Repeatability (intra-experiment variation)	**N**	not reported
Reproducibility (inter-experiment/user/lab etc. variation)	**N**	not reported
Number of partitions measured (average and standard deviation)	**N**	not reported
Partition volume	**N**	droplet partitions are 0.85 nl
Copies per partition (λ or equivalent) (average and standard deviation)	**N**	sample concentrations are reported
dPCR analysis program (source, version)	**Y**	Section 2.3.3.3 Quantification of bacterial and protozoan pathogens using droplet digital PCR (ddPCR)
Description of normalization method	**N**	not applicable
Statistical methods used for analysis	**Y**	Section 2.3.3.3 Quantification of bacterial and protozoan pathogens using droplet digital PCR (ddPCR)
Data transparency	raw data available on request:	

* Assessing the absence of DNA using a no RT assay (or where RT has been inactivated) is essential when first extracting RNA. Once the sample has been validated as DNA-free, inclusion of a no-RT control is desirable, but no longer essential. ** Disclosure of the primer and probe sequence is highly desirable and strongly encouraged. However, since not all commercial pre-designed assay vendors provide this information when it is not available assay context sequences must be submitted [Bustin et al. (2011) Primer sequence disclosure: A clarification of the MIQE guidelines. Clin Chem 2011; 57:919-21]. *** Details of reaction components are highly desirable, however not always possible for commercial disclosure reasons. Inclusion of catalogue number is essential where component reagent details are not available.

Table A.3: Physicochemical Parameters of Roof Runoff Samples

Physicochemical Parameter	Average (Standard Deviation)	Range
TDS (mg/L)	64.7 (60.4)	0 – 376
TSS (mg/L)	25.6 (46.3)	0 – 293
VSS (mg/L)	16.9 (26.2)	0 – 147
Turbidity (NTU)	17.7 (26.4)	1 – 168.5
COD (mg/L)	73.7 (92.1)	0 – 608

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APPENDIX B: SUPPLEMENTAL INFORMATION FOR CHAPTER 3

Table B.1 95th Percentile Pathogen Log Reduction Targets for Nonpotable Uses of Stormwater Based on 10^{-1} and 10^{-3} Sewage Dilution (HFCA) for 10^{-4} ppy Infection Risk (Olivieri et al., 2021)

Sewage Dilution (HFCA)	Protozoa	Virus
10^{-1}		
Unrestricted Irrigation	4.5	6.5
Indoor Use	5.5	7.0
10^{-3}		
Unrestricted Irrigation	2.5	4.5
Indoor Use	3.5	5.0

Table B.2 Inclusion and exclusion decisions for MST marker data collected during the literature review phase.

Source Tracking Marker	Reference	Inclusion Criteria for Wet Weather Data[a]								Decision (Include / Exclude)
		Human Specific	Recovery Method & Rate	Limit of Detection	% Detect. & Conc.	number of samples	fresh samples	Composite/ EMC Samples	Multiple Locations over Time	
Esp gene of *Enterococcus faecium*	Brownell et al. (2007)	✓	NA[b]		✓		✓			Exclude
Fecal *Bacteroides spp.*	Parker et al. (2010)						✓		✓	Exclude
HS183F	Parker et al. (2010)	✓	NA[b]			✓	✓		✓	Exclude
Fecal *Bacteroides spp.*	Converse et al. (2011)						NS[c]	✓	✓	Exclude
HF183	Sauer et al. (2011)	✓	✓	✓	✓[d]	✓	✓	✓[e]	✓	Include
BacHum	Bambic et al. (2011)	✓	✓	✓	✓	✓	✓		✓	Include
HF183	Cao et al. (2017)	✓		✓	✓	✓	✓		✓	Include
HF183	Steele et al. (2018)	✓		✓	✓	✓	✓	✓[e]		Include
HF183	Clary et al. (2021)	✓		✓	✓	✓	✓		✓	Include
HF183	Gonzalez (2022, personal communication)	✓	NS[c]	✓	✓	✓	✓		✓	Include

a: green indicates high priority criteria, yellow indicates medium quality criteria, and orange indicates low priority criteria; b: not applicable; c: not specified; d: %detection is for 828 samples, but concentrations are for 214 samples; e: composite and grab samples

Table B.3. Inclusion and exclusion decisions for pathogens data collected during the literature review phase.

Pathogen	Reference	Inclusion Criteria for Wet Weather Data[a]							
		Recovery Method & Rate	Limit of Detection	% Detection & Conc.	number of samples	fresh samples	Composite/ EMC Samples	Multiple Locations over Time	Decision (Include/Exclude)
Human Polyomavirus	Brownell et al. (2007)	NA[b]				✓	NS[c]		Exclude
Enteroviruses, Rotavirus group A, Hepatitis A, Norovirus GI, Norovirus GII, Adenoviruses	Sauer et al. (2011)			✓	✓	✓	✓[d]	✓	Include
Cryptosporidium parvum, *Giardia lamblia*, *Salmonella*, *Campylobacter jejuni*, Adenovirus A,B,C,40/41, Norovirus GI, Norovirus GII, Rotavirus, Enterovirus	Bambic et al. (2011)	✓	✓	✓	✓	✓		✓	Include
Norovirus GI, Norovirus GII, *Campylobacter spp.*, *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, *Salmonella*	Steele et al. (2018)		✓	✓	✓	✓	✓[d]		Include

a: green indicates high priority criteria, yellow indicates medium quality criteria, and orange indicates low priority criteria; b: not applicable; c: not specified; d: composite and grab samples

Table B.4 Adherence of available national datasets to criteria for the selection datasets to estimate pathogen based HFCAs.

Criteria[a] / Study	Pecson et al. (2021)	Kitajima et al. (2014a); Kitajima et al. (2014b); Schmitz et al. (2016)	Simmons et al. (2011); Simmons and Xagoraraki (2011)	Francy et al. (2012)	Fong et al. (2010)
Measuring and reporting pathogen recovery for a min. of 1 influent WW sample	✓	✓		✓[b]	✓
Including the sample LOD	✓	✓	✓	✓	[c]
Recovery ≥ 5%	✓	✓		[d]	[e]
Percent of samples with pathogen conc. greater than LOD ≥ 50%	✓[f]	✓	✓[g]	✓[h]	✓
Including % detection, conc., and number of samples	✓	✓	✓	✓	✓
Analyzing fresh samples	✓	✓	✓	✓	Not mentioned
Composite samples collected from multiple locations over time	✓	Protozoa (2 plants, grab) Viruses (2 studies with 2 plants/study: plants A&B grab, plants C&D composite)	✓	✓[i]	[j]

a: criteria established by Pecson et al. (2021) and Schoen et al. (2017); green signifies that the study meets all the criteria; yellow indicates that the study meets most of the criteria and still qualifies as an acceptable dataset; red indicates that the study does not mean enough criteria to qualify as an acceptable dataset, b: % recovery for norovirus GI was not reported, c: Although the LOD was determined, it was not reported. However, all samples tested positive for adenoviruses, d: % recovery of adenovirus = 0.09% - 5.13%, e: the mean recovery ranges from 0.92% to 3.31%, f: All tested pathogens have a % detection greater than 50% except for norovirus GIA (% detection = 34) and norovirus GIB (% detection = 47), g: norovirus GI % detection = 0, h: except for norovirus GII which has 0% detection, i: grab samples were composited: 1L every 10 minutes for a period of 40 to 60 minutes, j: grab samples collected from one plant.

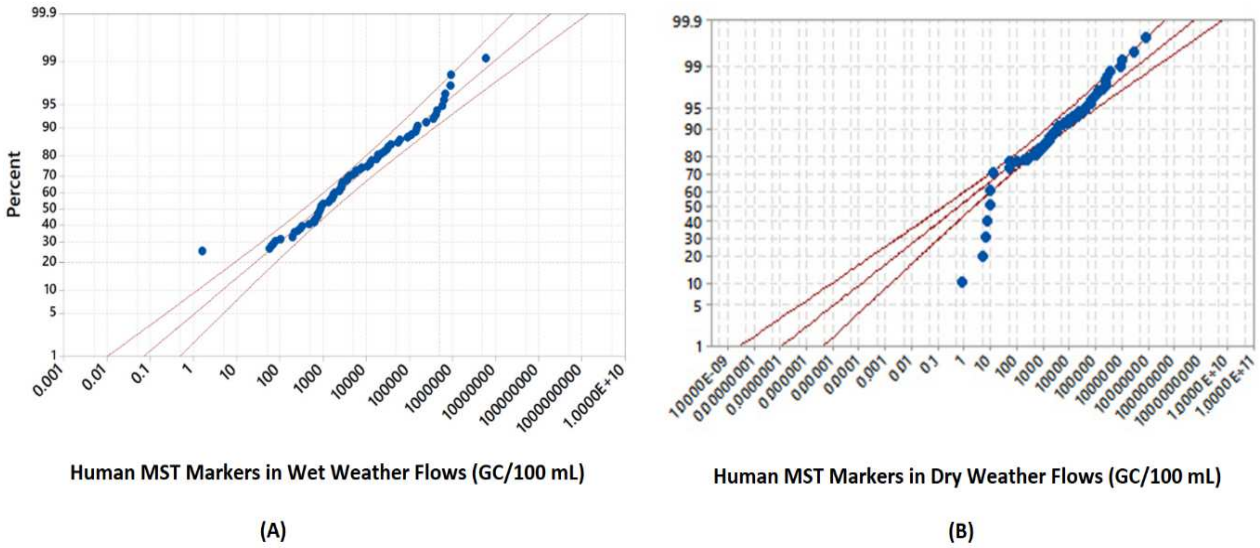

Figure B.1 Lognormal probability plots for human MST markers in wet weather (A, n = 116) and dry weather (B, n = 383) on a national scale accounting for censored data points (upper and lower red lines represent the 95% confidence interval).

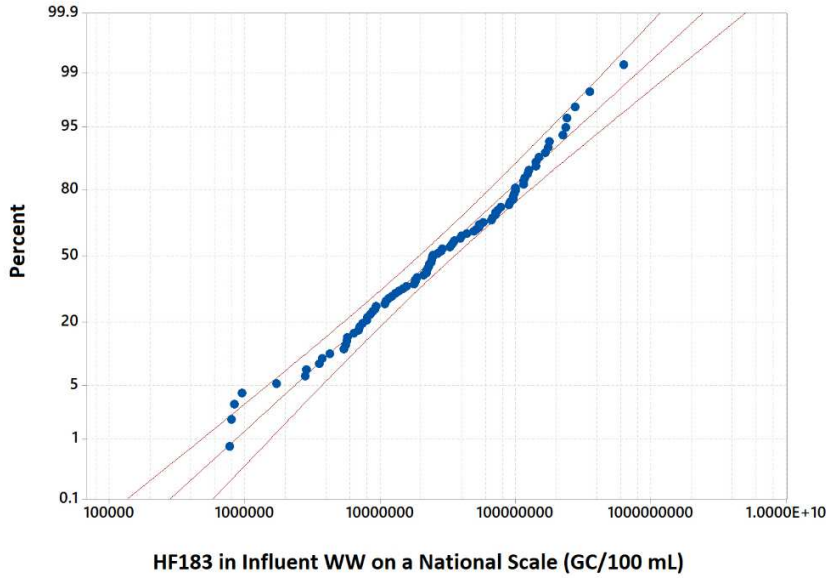

Figure B.2 Lognormal probability plot for human MST markers in influent wastewater on a national scale accounting for censored data points (upper and lower red lines represent the 95% confidence interval, p-value = 0.4, n = 91).

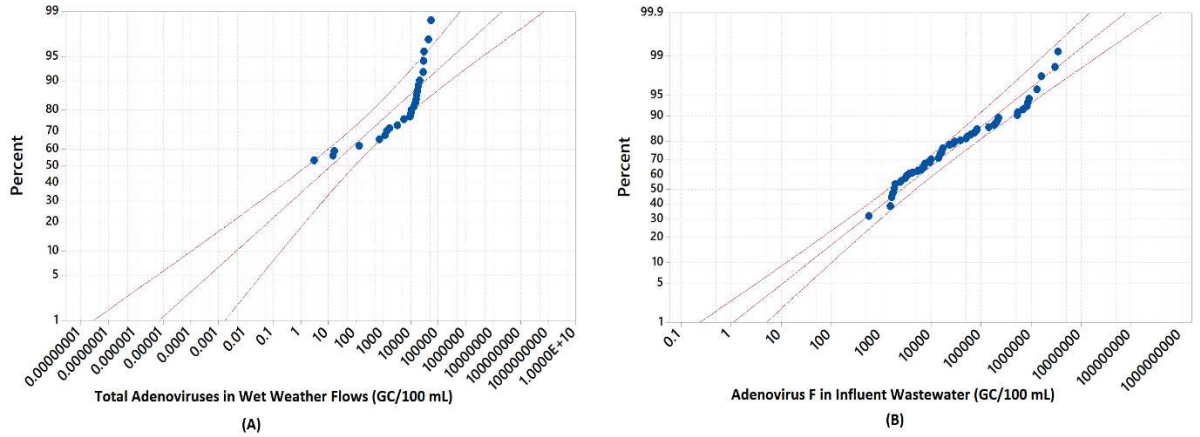

Figure B.3 Lognormal probability plots for total adenoviruses in wet weather (A, n = 70) and adenovirus f in influent wastewater (B, n = 122) accounting for censored data points (upper and lower red lines represent the 95% confidence interval).

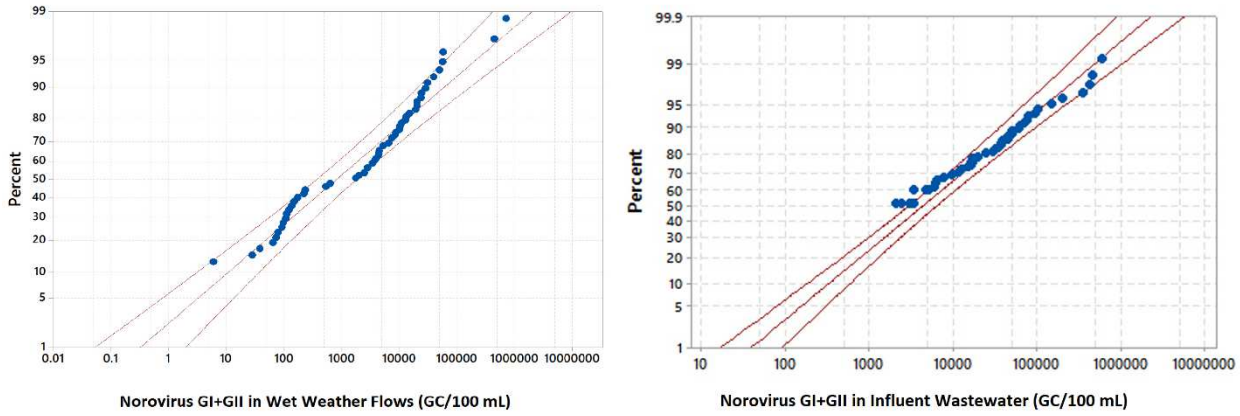

Figure B.4 Lognormal probability plots for Norovirus GII in wet weather (A, n = 69) and in influent wastewater (B, n = 122) accounting for censored data points (upper and lower red lines represent the 95% confidence interval).

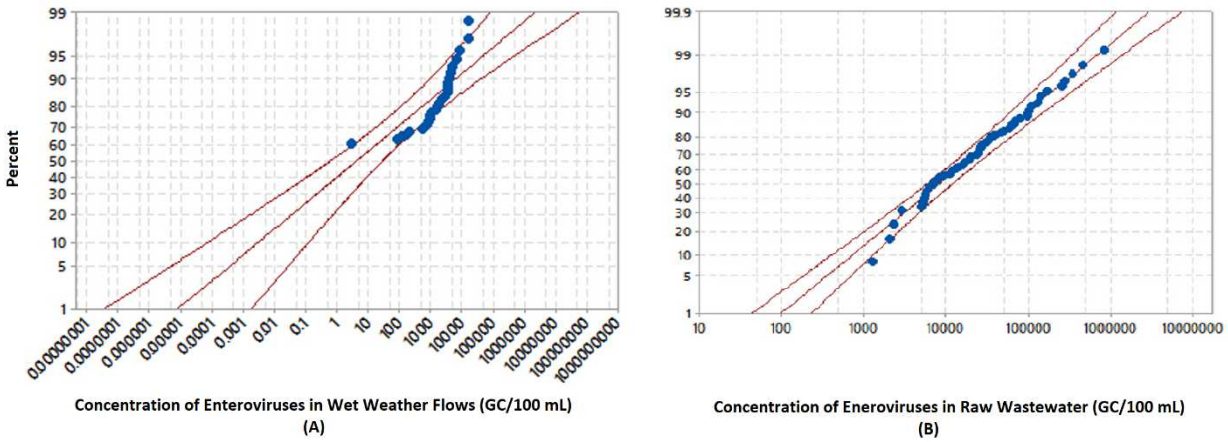

Figure B.5 Lognormal probability plot for enteroviruses in wet weather (A, n=70) and influent wastewater (B, n=122) accounting for censored data points (upper and lower red lines represent the 95% confidence interval).

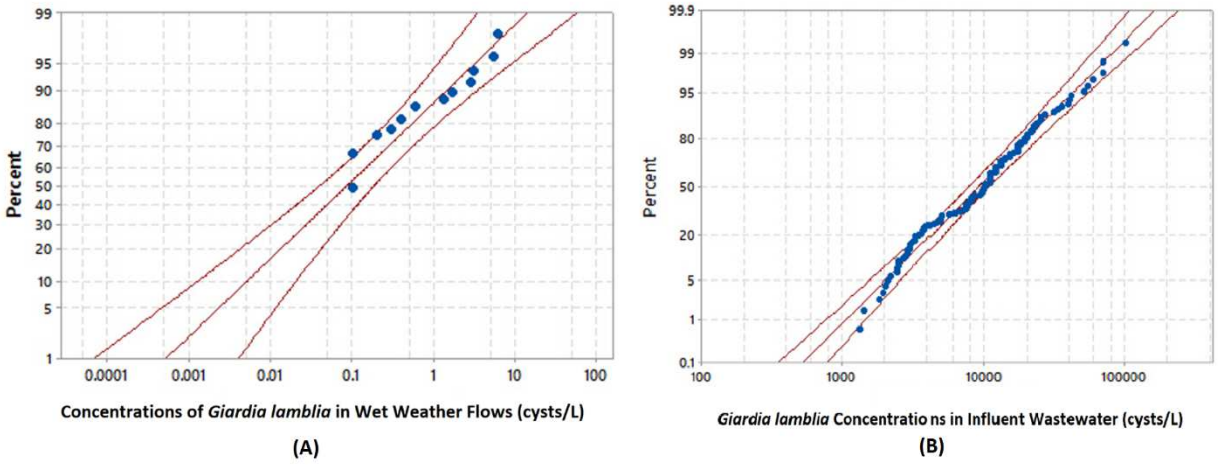

Figure B.6 Lognormal probability plots for *Giardia lamblia* in wet weather (A, n = 48) and in influent wastewater (B, n = 120) accounting for censored data points (upper and lower red lines represent the 95% confidence interval).

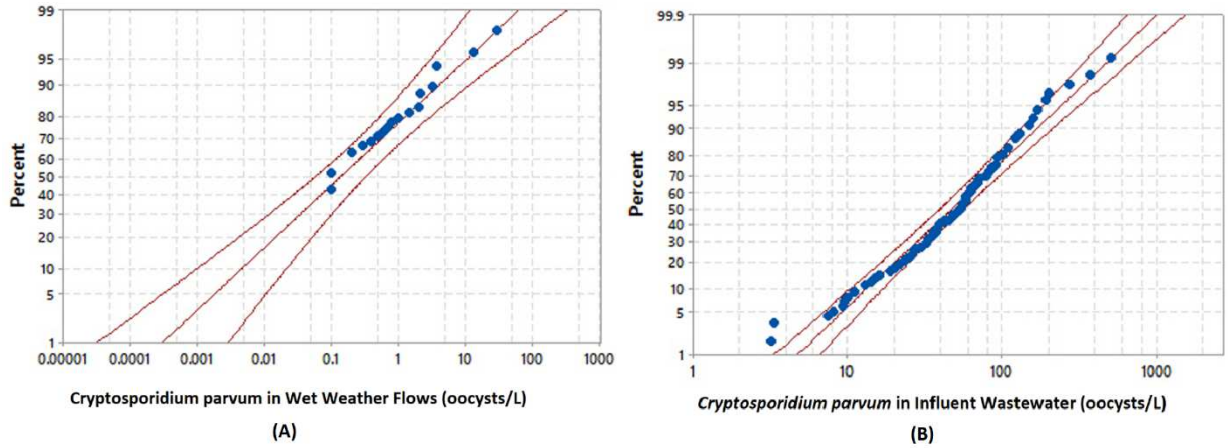

Figure B.7 Lognormal probability plots for *Cryptosporidium parvum* in wet weather (A, n = 48) and in influent wastewater (B, n = 120) accounting for censored data points (upper and lower red lines represent the 95% confidence interval).

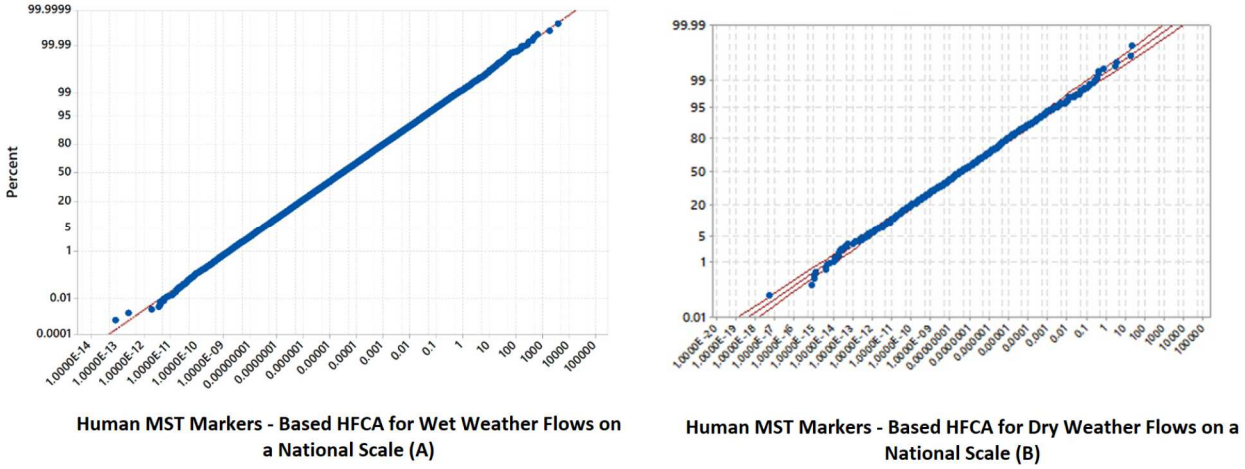

Figure B.8 Lognormal probability distributions for HFCAs for wet weather flows (A) and dry weather flows (B) based on human MST markers on a national scale accounting for censored data points (upper and lower red lines represent the 95% confidence interval, wet weather p-value = 0.14, and dry weather p-value = 0.98, n = 1000 iterations).

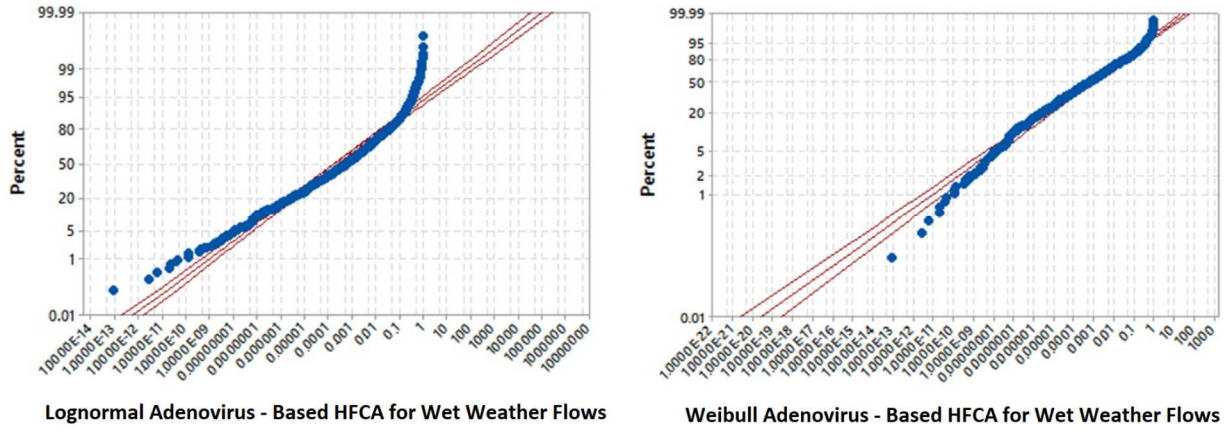

Figure B.9 Lognormal and Weibull probability plots for adenovirus-based HFCA for wet weather flows (p-value for both distributions < 0.05, the upper and lower red lines represent the 95% confidence interval, n = 747 iterations).

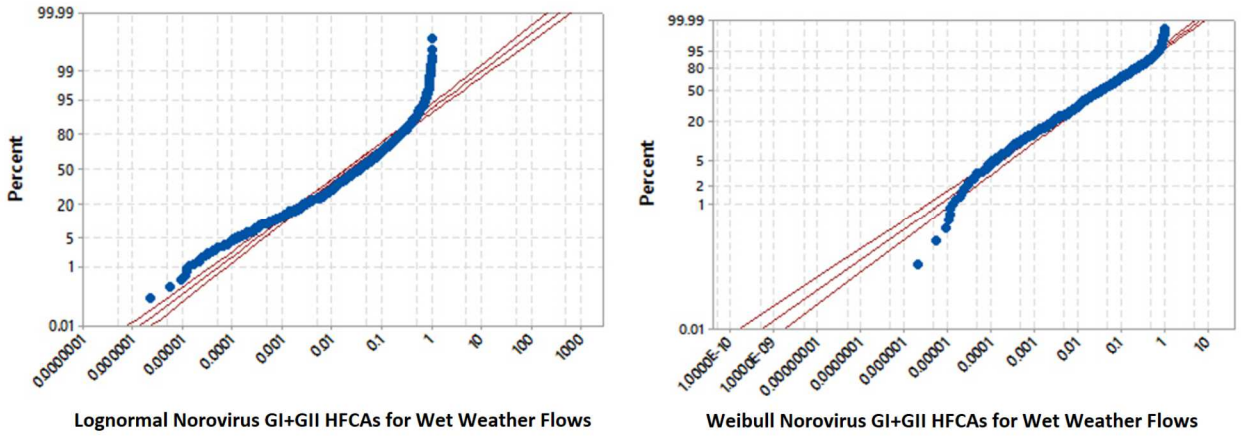

Figure B.10 Lognormal and Weibull probability plots for Norovirus GI+GII based – HFCA for wet weather flows (p-value for both distributions < 0.05, the upper and lower red lines represent the 95% confidence interval, n = 677 iterations).

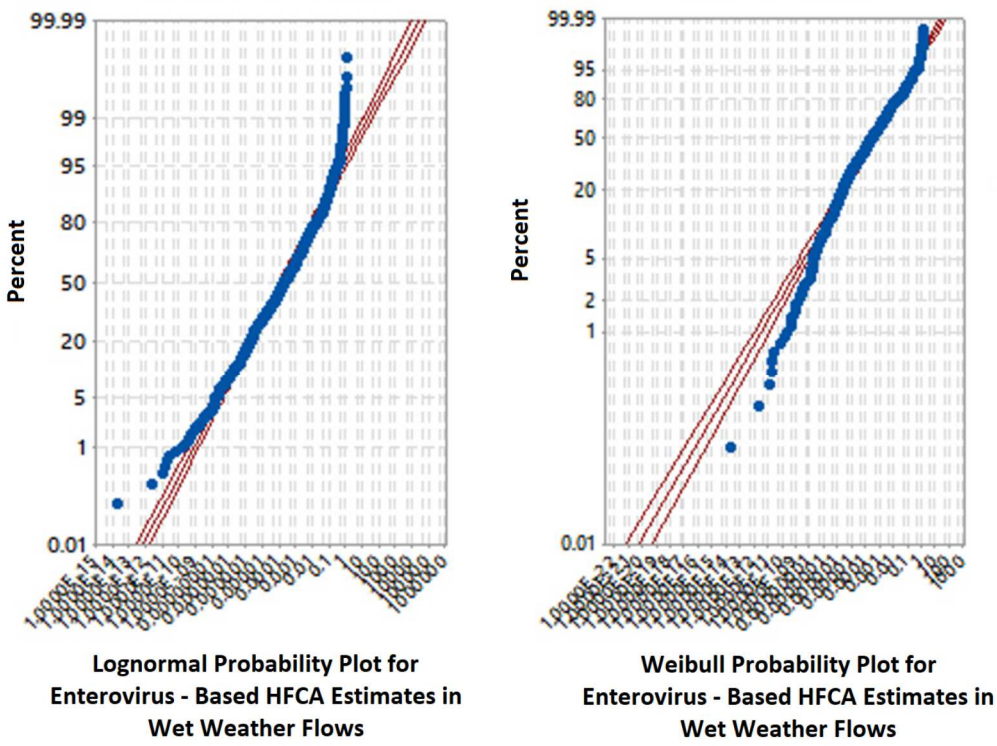

Figure B.11 Lognormal and Weibull probability plots for Enterovirus based – HFCA in wet weather flows (p-value for both distributions < 0.05, the upper and lower red lines represent the 95% confidence interval, n = 878 iterations).

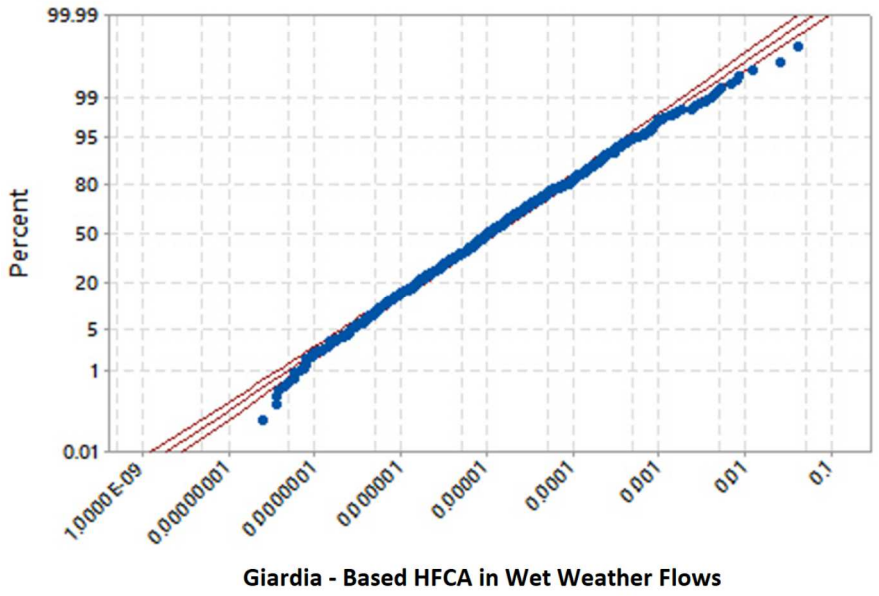

Figure B.12 Lognormal probability plot for HFCA in wet weather flows based on *Giardia lamblia*, p-value = 0.3, n = 1000 Iterations. upper and lower red lines represent the 95% confidence interval.

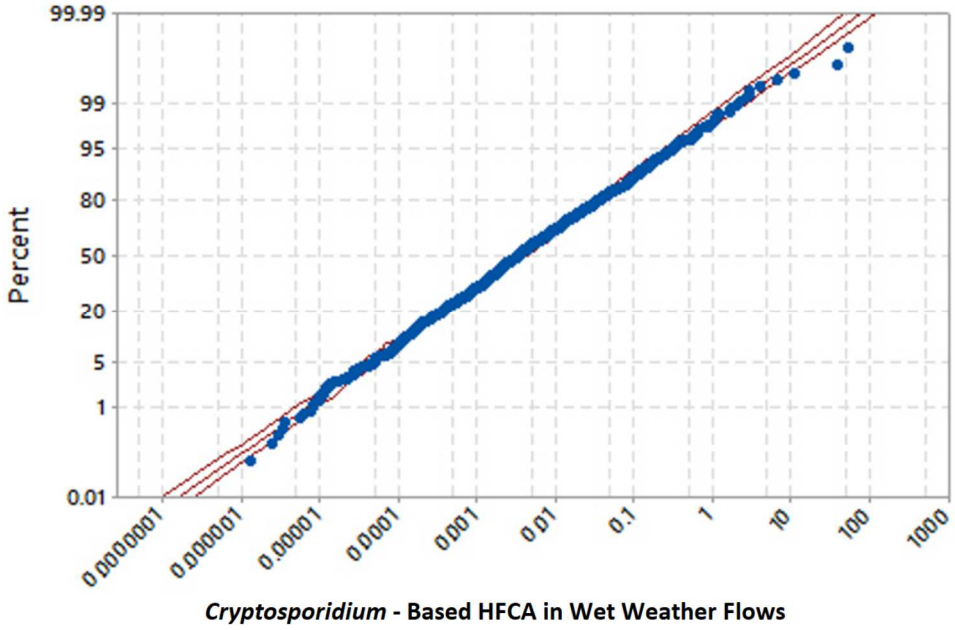

Figure B.13 Lognormal probability plot for HFCA in wet weather flows based on *Cryptosporidium parvum*, p-value = 0.5, n = 1000 Iterations. Upper and lower red lines represent the 95% confidence interval.

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APPENDIX C: SUPPLEMENTAL INFORMATION FOR CHAPTER 4

Table C.1 Pathogen LRVs Achieved by Filtration Technologies as Reported in Studies, Reports, Guidelines, and Factsheets

Filtration Technology	Bacteria	Protozoa		Viruses	Source Water	Crediting Framework or Pathogen Removal Studies	Reference
		Giardia	*Cryptosporidium*				
Slow Sand Filtration	2 (0.6 - 5)	4 (3.9 - 7.1)		2 (2 - 3)	NA[a]	Official Report based on U.S crediting framework and another official report	Sharvelle et al. (2017) based on NRMMC, EPHC, and NHMRC (2008), USEPA (2005), Harrington et al. (2001)
	NA[a]	2	> 2	2	surface water	U.S Crediting Framework	USEPA (2019)
	NA[a]	3	3	2	surface or groundwater	International Crediting Framework	Manitoba Sustainable Development (2017)
	2 - 6	0.3 - > 5		0.25 - 4	surface or groundwater	Official Report	WHO (2017)
	NA[a]	1.16 - > 2.6	0.28	1 - 5	intended for use as drinking water	Official Report based on Research Studies	WHO (2004)
	NA[a]	2.03 - 3.98	0.11 - 1.04	0.27 - 1.2	wastewater sampled from 6 plants	Official Report	Rose et al. (2004) as reported by Oakley and Mihelcic (2019)
	NA[a]	3	3	2	intended for use as drinking water	International Crediting Framework	Health Canada (2012)
	NA[a]	4		Cannot be demonstrated daily	Surface water	U.S Crediting Framework	California SWTR (2018)
Ultrafiltration	> 6	> 6		> 6 (4 - > 6)	NA[a]	Official Report based on U.S crediting framework and another official report	Sharvelle et al. (2017) based on USEPA (2005), NRMMC, EPHC, and NHMRC (2008), and Harrington et al. (2001)
	5.6 - 9	4.7 - 7.4	4.4 - 6	4.5 - 4.9	secondary effluent for norovirus and adenovirus	Peer-reviewed literature based on U.S and international crediting frameworks, peer-reviewed literature, and official reports	Soller et al. (2017) based on Kachelesky and Masterson, (1995), Jacangelo et al. (1997), USEPA (2001), EPHC, NHMRC and NRMMC (2008), and Qiu et al. (2015)
	3	4	4	0	NA[a]	Peer-reviewed literature based on official report	Soller et al. (2019) as reported by Olivieri et al. (2016)
	NA[a]	NA[a]	NA[a]	> 5.4 - 7.9	NA[a]	Official report, conference proceedings	Jacangelo et al. (1997), Dwyer et al. (1995), Trussel et al. (1998), and Kruithof et al. (1999) as

							reported by the EPA membrane filtration guidance manual (2005)
	NA[a]	1 - 4	2 - 4	0 - 4	NA[a]	U.S Crediting Framework	State-awarded removal credits as reported by the EPA Membrane Filtration Guidance Manual (2005)
Ultrafiltration	NA[a]	> 3	> 2	0	surface water	U.S Crediting Framework	USEPA (2019): Surface Water Treatment Rule Fact Sheet
	NA[a]	> 2.4	> 1.84	NA[a]	secondary WW treated effluent	Peer-reviewed literature cited in official report	Fu et al. (2010) as reported by Oakley and Mihelcic (2019): Pathogen Reduction and Survival in Complete Treatment Works/Pathogen LRVs in WW Sand Filters
	3 - 6	3 - 6		3 - 6	water to be treated by household treatment technologies	Official report based on studies reported in the scientific literature	WHO (2017) Guidelines for Drinking-Water Quality, Fourth Edition; these are LRVs achieved by household treatment technologies
	NA[a]	> 3	> 3	0	surface or groundwater	International crediting framework	Manitoba Sustainable Development (2017)
	NA[a]	> 4.7 - > 7	> 4.4 - > 7	> or = 6	NA[a]	Peer-reviewed literature cited in official report	Jacangelo et al. (1995) as reported in WHO (2004): Water Treatment and Pathogen Control
	NA[a]	> 4	NA	> 6.5	4 different source waters	Peer-reviewed literature cited in official report	Jacangelo et al. (1991) as reported in WHO (2004): Water Treatment and Pathogen Control

Table C.1 Pathogen LRVs Achieved by Filtration Technologies as Reported in Studies, Reports, Guidelines, and Factsheets (Continued)

Treatment Process	Bacteria	Protozoa		Viruses	Source Water	Crediting Framework or Pathogen Removal Studies	Reference
		Giardia	*Cryptosporidium*				
Microfiltration	NA[a]	4		cannot be demonstrated daily	Surface water	U.S Crediting Framework	California SWTR (2018)
	6 (3.5 - > 6)	> 6 (4 - >6)		1 (0 - > 2)	NA[a]	Official Report based on U.S crediting framework and another official report	Sharvelle et al. (2017) based on USEPA (2005), NRMMC, EPHC, and NHMRC (2008), and Harrington et al. (2001)
	3 - 9	4 - 7		1.5 - 4.9	NA[a]	Peer-reviewed literature based on official report and peer-reviewed literature	Soller et al. (2017) based on Reardon et al. (2005), Francy et al. (2012)
	3	4		0	NA[a]	Peer-reviewed literature based on official report	Soller et al. (2019) as reported by Olivieri et al. (2016)
	NA[a]	NA[a]	NA[a]	0 - 4	NA[a]	Official report, conference proceedings reported in U.S crediting framework	Jacangelo et al. (1997), Wilinghan et al. (1993), Schneider et al. (1999), and Trussel et al. (1998) as reported by the EPA membrane filtration guidance manual (2005)
	NA[a]	1 - 4	2 - 4	0 - 0.5	NA[a]	U.S Crediting Framework	State-awarded removal credits as reported by the EPA Membrane Filtration Guidance Manual (2005)
	NA[a]	> 3	> 2	0	surface water	U.S Crediting Framework	USEPA (2019): Surface Water Treatment Rule Fact Sheet
	2 - 4	2 - 6		0 - 4	water to be treated by household treatment technologies	Official report based on studies reported in the scientific literature	WHO (2017) Guidelines for Drinking-Water Quality, Fourth Edition; these are LRVs achieved by household treatment technologies
	NA[a]	> 3	> 3	0	surface or groundwater	International crediting framework	Manitoba Sustainable Development (2017)
	NA[a]	> 4.7 - > 7	> 4.4 - > 6.9	< 1	NA[a]	Peer-reviewed literature cited in official report	Jacangelo et al. (1995) as reported in WHO (2004): Water Treatment and Pathogen Control
	NA[a]	3.3 - 4.4	2.3 - 3.5	NA[a]	NA[a]	Peer-reviewed literature cited in official report	Karimi et al. (1999) as reported in WHO (2004): Water Treatment and Pathogen Control
	NA[a]	NA[a]	5.3	2.7 - 3.7	NA[a]	Conference proceedings cited in official report	Parker et al. (1999) as reported in WHO (2004): Water Treatment and Pathogen Control

	NA[a]	NA[a]	> 3	NA[a]	NA[a]	Peer-reviewed literature cited in official report	Yoo et al. (1995) as reported in WHO (2004): Water Treatment and Pathogen Control

a: not available

Table C.2 Doses Required to Achieve Pathogen LRVs Using UV, Free Cl_2, Chloramines, and Ozone Disinfection Based on Literature Review

Virus Log_{10} Removal (LRV)	**1**	**2**	**3**	**4**	**5**	**6**	**Crediting Framework or Pathogen Removal Studies**	**References**
UV Disinfection Dose (mJ/cm^2)	50 - 60	90 - 110	140 - 150	180 - 200	NA[a]	NA[a]	Official report based on U.S crediting framework	Sharvelle et al. (2017) based on USEPA (2006 a)
	58	100	143	186	231	276	U.S Crediting Framework	USEPA (2006 a), USEPA (2020a) using adenovirus
	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]	800	Peer-Reviewed Literature	Soller et al. (2017) based on USEPA (2006 b) and Gerba et al. (2002)
	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]	> 300	Official Report	Olivieri et al. (2016)
	10 - 56	20 - 111	29 - 167	39 - 167	NA[a]	NA	Peer-reviewed literature	Hijnen et al. (2006)[d, e]
	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]	276[f]	Official Report	Olivieri et al. (2021)
	4 – 63	7 – 120	14 – 167	22 – 222	29 - 234	211 - 235	Peer-Reviewed Literature	Malayeri et al. (2016)*
	NA	40	NA	100	NA	186	Validated UV systems	SFPUC (2020)
Protozoa Log_{10} Removal (LRV)	**1**	**2**	**3**	**4**	**5**	**6**	**Crediting Framework or Pathogen Removal Studies**	**References**
UV Disinfection Dose (mJ/cm^2)	2 - 3	5 - 6	11 - 12	20 - 25	NA[a]	NA[a]	Official Report	Sharvelle et al. (2017) based on USEPA (2006 a)
	2.5	5.8	12	22	45	85	U.S Crediting Framework	USEPA (2006 a), USEPA (2020a) based on Crypto.
	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]	800	Peer-reviewed literature	Soller et al. (2017) based on USEPA (2006 b) and Gerba et al. (2002)
	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]	> 300	Official report	Olivieri et al. (2016)[b]
	NA[a]	NA[a]	12 - 26.8	22 - 27.7	NA[a]	NA[a]	Australian Crediting Framework	WaterSecure (2017a)[g]
	2 - 3	5 - 6	11 - 12	NS[h]	NA[a]	NA[a]	Peer-reviewed literature	Hijnen et al. (2006)[i]
	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]	276[j]	Official Report	Olivieri et al. (2021)
	<0.5 - <10	<0.5 - <10	<0.5 – 28+tailing	<1 - ~60	<10	NA	Peer-reviewed literature	Malayeri et al. (2016)*
	NA	NA	40	NA	NA	100 - 186	Validated UV systems	SFPUC (2020)
Bacteria Log_{10} Removal (LRV)	**1**	**2**	**3**	**4**	**5**	**6**	**Crediting Framework or Pathogen Removal Studies**	**References**
UV Disinfection Dose (mJ/cm^2)	NA[a]	NA[a]	NA[a]	10[k]	NA[a]	800[l]	Peer-reviewed literature	Soller et al. (2017) based on USEPA (2003), USEPA (2006 b), and Gerba et al. (2002)
	3 - 6	7 - 12	10 - 17	14 - 51	NA[a]	NA[a]	Peer-reviewed literature	Hijnen et al. (2006)[m]
	0.4 – 4	0.7 – 5.7	1 – 7.8	1.1 – 8.3	1.3 – 14	1.4 – 29	Peer-reviewed literature	Malayeri et al. (2016)*

	NA	40	NA	100	NA	186	Validated UV systems	SFPUC (2020)

Table C.2 Disinfectant Doses Required to Achieve Pathogen LRV Credits Using UV, Free Cl_2, Chloramines, and Ozone Disinfection Based on Literature Review (Continued)

Virus Log_{10} Removal (LRV)	**1**	**2**	**3**	**4**	**Crediting Framework or Pathogen Removal Studies**	**References**
Free Cl_2 CT (mg.min)/L	NA[a]	1.5 - 1.8	2.2 - 2.6	3 - 3.5	Official report based on values reported in textbook	Sharvelle et al. (2017) based on Tchobanoglous et al. (2014)
	NA[a]	3	4	6	U.S Crediting Framework	USEPA (1999)[n]
	NA[a]	NA[a]	NA[a]	12[o]	Peer-reviewed literature	Soller et al. (2019)
	7	10	13	16	Australian Crediting Framework	WaterSecure (2017b)[p]
	NA[a]	NA[a]	NA[a]	6	U.S Crediting Framework	USEPA (2020b)[q]
Chloramine CT (mg.min/L)	1482	2326	3160	3949	Official Report/Pathogen Removal Study*	Keegan et al. (2012)
	NA[a]	643	1067	1491	U.S Crediting Framework	USEPA (1999)[n]
***Giardia* Log_{10} Removal (LRV)**	**1**	**2**	**3**	**4**	**Crediting Framework or Pathogen Removal Studies**	**References**
Free Cl_2 CT (mg.min)/L	2000 - 2600	NA	NA	NA	Official report based on values reported in textbook	Sharvelle et al. (2017) based on Tchobanoglous et al. (2014)
	50 – 67	99 – 134	149 – 201	NA[a]	U.S Crediting Framework	EPA (1999)[r] based on AWWA (1991)
	12[s]	NA[a]	NA[a]	NA[a]	Peer-reviewed literature based on U.S crediting framework	Soller et al. (2017) based on USEPA (1991)
	Protozoa LRVs cannot be claimed under WaterSecure (2017) protocol				Australian Crediting Framework	WaterSecure (2017b)
	NA[a]	NA[a]	149 – 201	NA[a]	U.S Crediting Framework	USEPA (2020b)[r]
Chloramine CT (mg.min/L)	615	1230	1850	NA[a]	U.S Crediting Framework	USEPA (1999)
***Cryptosporidium* Log_{10} Removal (LRV)**	**1**	**2**	**3**	**4**	**Crediting Framework or Pathogen Removal Studies**	**References**
Free Cl_2 CT (mg.min)/L	2000 - 2600	NA[a]	NA[a]	NA[a]	Official report based on values reported in textbook	Sharvelle et al. (2017) based on Tchobanoglous et al. (2014)
	800 - 900	NA[a]	NA[a]	NA[a]	Peer-reviewed literature	Soller et al. (2017) based on Nasser (2016)
	Protozoa LRVs cannot be claimed under WaterSecure (2017) protocol				Australian Crediting Framework	WaterSecure (2017b)

Table C.2 Disinfectant Doses Required to Achieve Pathogen LRV Credits Using UV, Free Cl_2, Chloramines, and Ozone Disinfection Based on Literature Review (Continued)

Bacteria Log_{10} Removal (LRV)	**1**	**2**	**3**	**4**	**Crediting Framework or Pathogen Removal Studies**	**References**
Free Cl_2 CT (mg.min)/L	0.4 - 0.6	0.8 - 1.2	1.2 - 1.8	1.6 - 2.4	Official report based on values recommended in textbook	Sharvelle et al. (2017) based on Tchobanoglous et al. (2014)
	7	10	13	16	Australian Crediting Framework	WaterSecure (2017b)[u]
Virus Log_{10} Removal (LRV)	**1**	**2**	**3**	**4**	**Crediting Framework or Pathogen Removal Studies**	**References**
Ozone CT (mg.min)/L	NA	0.25 - 0.3	0.35 - 0.45	0.5 - 0.6	Official report based on values recommended in textbook	Sharvelle et al. (2017) based on Tchobanoglous et al. (2014)
	NA	0.5	0.8	1	American Crediting Framework	USEPA (1999)[v]
	NA	NA	NA	1	American Crediting Framework	USEPA (2020)[w]
	0.85	1.23	1.63	2.01	Australian Crediting Framework	WaterSecure (2017c)[x]
	Adenovirus LRVs were reported based on USEPA (1991) guidelines				Peer-reviewed literature based on U.S crediting framework	Soller et al. (2017)
***Giardia* Log_{10} Removal (LRV)**	**1**	**2**	**3**	**4**	**Crediting Framework or Pathogen Removal Studies**	**References**
Ozone CT (mg.min)/L	4 - 4.5	8 - 8.5	12 - 13	NA	Official report based on values recommended in textbook	Sharvelle et al. (2017) based on Tchobanoglous et al. (2014)
	0.48	0.95	1.43	NA	U.S Crediting Framework	USEPA (1999)[v]
	NA	NA	1.43	NA	U.S Crediting Framework	USEPA (2020b)[w]
	9.9	20	30	NA	Australian Crediting Framework	WaterSecure (2017c)[y]
	NA	0.55[z]	1[a1]	NA	Peer-reviewed literature and U.S crediting framework	Soller et al. (2017) based on Wickramanayake et al. (1985) and USEPA (1991)

Table C.2 Disinfectant Doses Required to Achieve Pathogen LRV Credits Using UV, Free Cl_2, Chloramines, and Ozone Disinfection Based on Literature Review (Continued)

***Cryptosporidium* Log_{10} Removal (LRV)**	**1**	**2**	**3**	**4**	**Crediting Framework or Pathogen Removal Studies**	**References**
Ozone CT (mg.min)/L	4 - 4.5	8 - 8.5	12 - 13	NA	Official report based on values recommended in textbook	Sharvelle et al. (2017) based on Tchobanoglous et al. (2014)
	9.9	20	30	NA	Australian Crediting Framework	WaterSecure (2017c)[y]
	the potential max. allowable LRVs are set according to USEPA CT tables				Official report recommending values from U.S crediting framework	Olivieri et al. (2016)
	5[b1]	NA	NA	NA	Peer-reviewed literature	Soller et al. (2017)
Bacteria Log_{10} Removal (LRV)	**1**	**2**	**3**	**4**	**Crediting Framework or Pathogen Removal Studies**	**References**
Ozone CT (mg.min)/L	0.005 - 0.01	0.01 - 0.02	0.02 - 0.03	0.03 - 0.04	Official report based on values recommended in textbook	Sharvelle et al. (2017) based on Tchobanoglous et al. (2014)
	0.85	1.23	1.63	2.01	Australian Crediting Framework	WaterSecure (2017c)[x]
	the potential max. allowable LRVs are set according to USEPA CT tables				Official report recommending values from U.S crediting framework	Olivieri et al. (2016)
	NA	NA	NA	1[c1]	Peer-reviewed literature	Soller et al. (2017)

a: not available, **b**: this is the current maximum allowable LRV, **c**: Viruses were not credited with an LRV because considering validation uncertainty results in a < 39 mJ/cm^2 validated dose needed to achieve 0.5 log_{10} reduction, **d**: The LRVs are reported for adenovirus type 40, adenovirus type 2, 15, 40, and 41, adenovirus (no type 40), and rotavirus SA-11, **e**: this is based on literature review data used to calculate the inactivation rate constant k and find the UV dose required to achieve a certain LRV, **f**: Olivieri et al. (2021) assumed 10% wastewater contribution to stormwater; the model virus assumed is adenovirus. The dose can be achieved with 1 UV reactor or several reactors in series, **g**: these are validated UV doses for both *Giardia* and *Cryptosporidium* based on *Bacillus subtilis* RED = 40 mJ/cm^2 for UVT = 50% - 98%, **h**: no value due to tailing, **i**: these values are calculated for *Cryptosporidium* and *Giardia* based on data from the USEPA, **j**: Olivieri et al. (2021) assumed 10% wastewater contribution to stormwater; the dose can be achieved with 1 UV reactor or several reactors in series, **k**: UV dose of less than 10 mJ/cm^2 (8.4 mJ/cm^2) can result in 4 log_{10} removal of *E. coli* (USEPA, 2003) as reported by Soller et al. (2017); Soller et al. (2017) assumed that *Salmonella spp.* and *Campylobacter spp.* LRV = *E. coli* LRV, **l**: based on Soller et al. (2017), Soller et al. (2019), and Olivieri et al. (2016), **m**: the LRVs are reported for *Campylobacter jejuni*, *E. coli O157*, and *Salmonella typhi*; this is based on literature review data used to calculate k and find the UV dose required to achieve a certain LRV, *****: These are CT doses using 15 mg/L of monochloramine for at pH=8, temp.=10°C, turbidity of 2 NTU for the removal of adenovirus 2 from wastewater, **n**: the chlorine/chloramine dose is for pH = 6 - 9, and temp.=10°C; as temp. decreases, the required dose increases; the chloramine CT is provided for human adenovirus, **o**: the free chlorine dose for which the removal was estimated is less than or equal to 12 (mg.min)/L. This dose can achieve 4 to 5 log reduction of adenovirus and 1 to 4 log reduction of norovirus, **p**: CT values are for pH ≤ 8 (NSQD, 2018), turbidity ≤ 2 NTU, and temperature = 10°C (NSQD, 2005; WaterSecure, 2017b). WaterSecure (2017b) mentioned that the same free Cl_2 CT can be used for bacterial and viral LRVs, **q**: this is for pH=6-9 and Temp.=10°C; as pH increases the required CT increases, and as Temp. decreases, the required CT increases, **r**: The chlorine CT is for pH=8 and Temp.=10°C. As temp decreases, the required dose increases, and as pH increases, the required dose increases; the chloramine CT is for pH = 6-9, temp. = 10°C, **s**: the maximum reduction of *Giardia* at this dose is 0.5 log (Soller et al., 2017; USEPA, 1991), **t**: the free chlorine dose for which the removal was estimated is less than or equal

to 12 (mg.min)/L, **u**: CT values are for pH ≤ 8 (NSQD, 2018), turbidity ≤ 2 NTU, and temperature = 10°C (NSQD, 2005; WaterSecure, 2017b); the same free Cl_2 CT can be used for bacterial and viral LRVs, **v**: The USEPA (1999) cited AWWA (1991) as the source for CT; the values were modified by linear interpolation between increments of 5°C. This is at temp. of 10°C. As temperature decreases the required CT increases, **w**: the temp. = 10°C; as the temp. decreases, the required CT increases, **x**: these values are for wastewater at a temp. of 10°C; the WaterSecure (2017c) report recommends the same values of CT for the removal of bacteria and viruses, **y**: WaterSecure (2017c) cites USEPA (2006 b) as the source/reference; the operating temp. is 10°C; these values are for protozoa without a distinction between *Giardia* and *Cryptosporidium*, **z**: A dose of 0.55 (mg.min)/L at 5 °C can achieve at least 2 $\log_{10}$ *Giardia* removal (Wickramanayake et al., 1985) as reported in Soller et al. (2017), **a1**: using 1 (mg.min)/L results in removal of *Giardia spp*. of more than 3 $\log_{10}$ at 20 °C. This is based on USEPA (1991) guidelines, **b1**: Korich et al. (1990) as reported in Soller et al. (2017), **c1**: A CT dose of less than 1 (mg.min)/L achieves 4 $\log_{10}$ removal of *Campylobacter spp.* and *Salmonella spp*. assuming that the removal of *E. coli* is equal to that of bacterial pathogens (Sigmon et al., 2015), *Malayeri et al. (2016) reported UV doses to achieve different virus LRVs obtained from these studies: Nwachuku et al. (2005), Gerba et al. (2002), Ballester and Malley (2004), Shin et al. (2005), Linden et al. (2007), Eischeid et al. (2009), Linden et al. (2009), Baxter et al. (2007), Sirikanchana et al. (2008), Shin et al. (2009), Bounty et al. (2012), Rodriguez et al. (2013), Beck et al. (2014), Ryu et al. (2015), Boczek et al. (2016), Guo et al. (2010), Rattanakul et al. (2014), Rattanakul et al. (2015), Oguma et al. (2015), Thurston-Enriquez et al. (2003), Blatchley et al. (2008), Ko et al. (2005), Battigelli et al. (1993), Liltved et al. (2006), Lee et al. (2008), and Park et al. (2011); Malayeri et al. (2016) reported UV doses to achieve different protozoa LRVs obtained from these studies: Johnson et al. (2005), Bolton et al. (1998), Bukhari et al. (1999), Clancy et al. (2000), Craik et al. (2001), Shin et al. (2001), Belosevic et al. (2001), Zimmer et al. (2003), Bukhari et al. (2004), Clancy et al. (2004), Amoah et al. (2005), Ryu et al. (2008), Oguma et al. (2001), Beck et al. (2015), Qian et al. (2004), Campbell and Wallace (2002), Linden et al. (2002), Mofidi et al. (2002), and Craik et al. (2000); Malayeri et al. (2016) reported UV doses to achieve different bacteria LRVs obtained from these studies: Wilson et al. (1992), Butler et al. (1987), Tosa and Hirata (1999), Yaun et al. (2003), Sommer et al. (2000), Chang et al. (1985), Chen et al. (2009), and Hu et al. (2012).

Table C.3 Continuously and Routinely Monitored Parameters for Unit Treatment Processes Used in the Stormwater Treatment Trains

Unit Treatment Process	Continuously Monitored Parameters	Routinely Monitored Parameters (frequency)
Microfiltration[a, b]	turbidity	PDT (daily)[c]
Ultraviolet disinfection[d, e]	Flow rate	Lamp aging and fouling (monthly)
	Lamp status	Calibration of online sensors (weekly)
	UV intensity	Distributions of UV dose resulting from velocity profiles across the reactor
	UV transmittance	
Chlorination[f]	Free Cl_2 dose	Calibration of online sensors (weekly)
	Flow rate	
	Cl_2 residual	
	pH	
	Temperature	
	Turbidity	
	ammonia	
Chloramination[g]	Flow rate	Calibration of online sensors (weekly)
	Total or monochloramine residual	
	pH	
	Temperature	
	turbidity	
Ozonation[h]	Ozone residual (needs to be monitored at 3 locations/points)	Calibration of online sensors (weekly)
	Temperature	
	Applied ozone dose	
	Flow rate	
	Turbidity	

a: continuously monitored parameters are obtained from the USEPA Manual for Compliance with the Surface Water Treatment Rules (USEPA, 2020c), b: continuously monitored parameters are obtained from the USEPA Membrane Filtration Guidance Manual (USEPA, 2005), c: pressure decay test (Pennsylvania DEP, 2020), d: continuously monitored parameters are obtained from WaterSecure (2017a), e: routinely monitored parameters are obtained from the USEPA Ultraviolet Disinfection Guidance Manual (USEPA, 2006 a), f: continuously monitored parameters are obtained from WaterSecure (2017b) and the USEPA Disinfection Profiling and Benchmarking Technical Guidance Manual (USEPA, 2020b), g: continuously monitored parameters are obtained from USEPA (2020b) and Keegan et al. (2012), h: continuously monitored parameters are obtained from WaterSecure (2017c)

Table C.4 Inputs to the Evaluation Matrix for Suggested Treatment Process Trains at different HFCA Values

HFCA	Treatment Trains Options	System Complexity			Reliability			
		Monitoring	Dosing		Redundancy			Risk
		no. of continuously monitored controlled points	CT	dosing impacted by influent chemical quality (0/1)	no. of units that can go offline while still maintaining LRTs			no. of virus LRVs relying on 1 unit
					V[a]	P[b]	B[c]	
10^{-1}	MF, UV (186 mJ/cm^2), Cl_2 (13 mg.min/L)	12	13	1	1	2	2	0
	MF, UV (186 mJ/cm^2), chloramine (3160 mg.min/L)	10	3160	0	1	2	2	0
	MF, UV (186 mJ/cm^2), O_3 (1.63 mg.min/L)	12	1.63	0	1	2	2	0
	MF, UV (276 mJ/cm^2), chloramine (232 mg.min/L)	10	232	0	1	2	2	0
	MF, UV1 (143 mJ/cm^2), UV2 (186 mJ/cm^2)	9	0	0	0	1	1	7
10^{-2}	MF, UV (186 mJ/cm^2), Cl_2 (10 mg.min/L)	12	10	1	1	2	2	0
	MF, UV (186 mJ/cm^2), chloramine (2326 mg.min/L)	10	2326	0	1	2	2	0
	MF, UV (186 mJ/cm^2), O_3 (1.23 mg.min/L)	12	1.23	0	1	2	2	0
	MF, UV (276 mJ/cm^2)	5	0	0	1	1	1	6
10^{-3}	MF, UV (186 mJ/cm^2), Cl_2 (7 mg.min/L)	12	7	1	1	2	2	0
	MF, UV (186 mJ/cm^2), chloramine (1482 mg.min/L)	10	1482	0	1	2	2	0
	MF, UV (186 mJ/cm^2), O_3 (0.85 mg.min/L)	10	0.85	0	1	2	2	0
	MF, UV (231mJ/cm^2)	5	0	0	1	1	1	5
10^{-4}	MF, UV (186 mJ/cm^2)	5	0	0	1	1	1	4
	MF, O_3 (9.9 mg.min/L)	6	9.9	0	1	1	1	4
	MF, Cl_2 (16 mg.min/L)	8	16	1	1	1	1	4
	MF, chloramine (3949 mg.min/L)	6	3949	0	1	1	1	4

a: virus, b: protozoa, c: bacteria

Table C.5 Overall Score for Stormwater Treatment Process Trains (Options) Suggested at HFCA of 10^{-1}

Evaluation Criteria (Sub-criteria**)**	**Relative Importance**	**Normalized Weights**	**Attribute Normalized Weights**	**Alternatives (Options)**				
				1[a]	**2[b]**	**3[c]**	**4[d]**	**5[e]**
System Complexity	1	0.500						
No. of continuously monitored control points			0.333	1.00	3.67	1.00	3.67	5.00
Dosing impacted by influent chemical quality			0.333	1.00	5.00	5.00	5.00	5.00
CT			0.333	4.98	1.00	5.00	4.71	5.00
			1	2.33	3.22	3.67	4.46	5.00
Reliability	1	0.500						
No. of units that can go offline while maintaining virus LRTs			0.250	5.00	5.00	5.00	5.00	1.00
No. of units that can go offline while maintaining protozoa LRTs			0.250	5.00	5.00	5.00	5.00	1.00
No. of units that can go offline while maintaining bacteria LRTs			0.250	5.00	5.00	5.00	5.00	1.00
No. of virus LRVs relying on 1 unit treatment process			0.250	5.00	5.00	5.00	5.00	1.00
			1.000	5.00	5.00	5.00	5.00	1.00
	2	1.000	**Overall Score**	3.66	4.11	4.33	4.73	3.00
			Rank	***4***	***3***	***2***	***1***	***5***

a: MF, UV (186 mJ/cm^2), Cl_2 (13 mg.min/L), b: MF, UV (186 mJ/cm^2), chloramine (3160 mg.min/L), c: MF, UV (186 mJ/cm^2), O_3 (1.63 mg.min/L), d: MF, UV (276 mJ/cm^2), chloramine (232mg.min/L), e: MF, UV1 (143 mJ/cm^2), UV2 (186 mJ/cm^2)

Table C.6 Overall Score for Stormwater Treatment Process Trains (Options) Suggested at HFCA of 10^{-2}

Evaluation Criteria (Sub-criteria**)**	**Relative Importance**	**Normalized Weights**	**Attribute Normalized Weights**	**Alternatives (Options)**			
				1[a]	**2[b]**	**3[c]**	**4[c]**
System Complexity	1	0.500					
No. of continuously monitored control points			0.333	1.00	2.14	1.00	5.00
Dosing impacted by influent chemical quality			0.333	1.00	5.00	5.00	5.00
CT			0.333	4.98	1.00	5.00	5.00
			1	2.33	2.71	3.67	5.00
Reliability	1	0.500					
No. of units that can go offline while maintaining virus LRTs			0.250	5.00	5.00	5.00	5.00
No. of units that can go offline while maintaining protozoa LRTs			0.250	5.00	5.00	5.00	1.00
No. of units that can go offline while maintaining bacteria LRTs			0.250	5.00	5.00	5.00	1.00
No. of virus LRVs relying on 1 unit treatment process			0.250	5.00	5.00	5.00	1.00
			1.000	5.00	5.00	5.00	2.00
	2	1.000	**Overall Score**	3.66	3.86	4.33	3.50
			Rank	***3***	***2***	***1***	***4***

a: MF, UV (186 mJ/cm^2), Cl_2 (10 mg.min/L), b: MF, UV (186 mJ/cm^2), chloramine (2326mg.min/L), c: MF, UV (186 mJ/cm^2), O_3 (1.23 mg.min/L), d: MF, UV (276 mJ/cm^2)

Table C.7 Overall Score for Stormwater Treatment Process Trains (Options) Suggested at HFCA of 10^{-3}

Evaluation Criteria (Sub-criteria**)**	**Relative Importance**	**Normalized Weights**	**Attribute Normalized Weights**	**Alternatives (Options)**			
				1[a]	**2[b]**	**3[c]**	**4[d]**
System Complexity	1	0.500					
No. of continuously monitored control points			0.333	1.00	2.14	2.14	5.00
Dosing impacted by influent chemical quality			0.333	1.00	5.00	5.00	5.00
CT			0.333	4.98	1.00	5.00	5.00
			1	2.33	2.71	4.05	5.00
Reliability	1	0.500					
No. of units that can go offline while maintaining virus LRTs			0.250	5.00	5.00	5.00	5.00
No. of units that can go offline while maintaining protozoa LRTs			0.250	5.00	5.00	5.00	1.00
No. of units that can go offline while maintaining bacteria LRTs			0.250	5.00	5.00	5.00	1.00
No. of virus LRVs relying on 1 unit treatment process			0.250	5.00	5.00	5.00	1.00
			1.000	5.00	5.00	5.00	2.00
	2	1.000	**Overall Score**	3.66	3.86	4.52	3.50
			Rank	***3***	***2***	***1***	***4***

a: MF, UV (186 mJ/cm^2), Cl_2 (7 mg.min/L), b: MF, UV (186 mJ/cm^2), chloramine (1482 mg.min/L), c: MF, UV (186 mJ/cm^2), O_3 (0.85 mg.min/L), d: MF, UV (231mJ/cm^2)

Table C.8 Overall Score for Stormwater Treatment Process Trains (Options) Suggested at HFCA of 10^{-4}

Evaluation Criteria (Sub-criteria**)**	**Relative Importance**	**Normalized Weights**	**Attribute Normalized Weights**	**Alternatives (Options)**			
				1[a]	**2[b]**	**3[c]**	**4[d]**
System Complexity	1	0.50					
No. of continuously monitored control points			0.33	5.00	3.67	1.00	3.67
Dosing impacted by influent chemical quality			0.33	5.00	5.00	1.00	5.00
CT			0.33	5.00	4.99	4.98	1.00
			1	5.00	4.55	2.33	3.22
Reliability	1	0.50					
No. of units that can go offline while maintaining virus LRTs			0.25	5.0	5.0	5.0	5.0
No. of units that can go offline while maintaining protozoa LRTs			0.25	5.0	5.0	5.0	5.0
No. of units that can go offline while maintaining bacteria LRTs			0.25	5.0	5.0	5.0	5.0
No. of virus LRVs relying on 1 unit treatment process			0.25	5.0	5.0	5.0	5.0
			1.000	5.0	5.0	5.0	5.0
	2	1.000	**Overall Score**	5.00	4.78	3.66	4.11
			Rank	***1***	***2***	***4***	***3***

a MF and UV (186 mJ/cm^2), b: MF and O_3 (9.9 mg.min/L), c: MF and Cl_2 (16 mg.min/L), d: MF and chloramine (3949 mg.min/L)

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