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Evaluation of Factors Influencing the Delivery of Septic System Wastewater Effluent to Tributaries

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Abstract

There is concern that septic systems may contribute nutrients, fecal pathogens, and emerging contaminants to tributaries, and thereby impair surface water quality. The objective of this thesis was to quantify the percentage of septic effluent reaching multiple streams and to evaluate whether this percentage varies based on the stream flow conditions and the physical and socioeconomic characteristics of a subwatershed. This was addressed by broad-scale sampling in 46 subwatersheds in the Lake Erie and Lake Simcoe Basins, Ontario, with data analyzed using statistical models. It was found that the percentage of septic effluent reaching subwatershed outlets, based on acesulfame stream loads, was higher under high flow conditions and in subwatersheds with older occupied homes and lower topographic wetness index. Fecal contamination in streams, possibly associated with underperforming septic systems, was observed in smaller subwatersheds with high septic system density, small setback distances, and high topographic wetness index. The findings of this research are needed to refine estimates on the contribution of septic systems to stream contaminant loads and to inform programs for locating, constructing, and maintaining septic systems.

Keywords

Septic system, artificial sweeteners, microbial source tracking, groundwater, septic system failure, surface water

Summary for Lay Audience

Septic systems are widely used to treat household wastewater in rural areas not serviced by municipal wastewater treatment plants. Septic systems work by partially treating the household wastewater before releasing it gradually into the soil. Household wastewater contains high concentrations of contaminants that can degrade surface water quality, including nutrients (nitrogen and phosphorus), bacteria, viruses, pharmaceuticals, and other contaminants of concern. A well-functioning septic system can limit the amount of these contaminants entering groundwater and surface waters, thereby limiting their adverse impacts on the environment. However, septic systems do not always perform as designed and can release excessive amounts of contaminants to the environment, including to groundwater and surface waters. Currently, it is not clear the amount of contaminants that are reaching streams from septic systems and how this may vary between geographical areas with different physical and socioeconomic characteristics (e.g., household age, soil permeability, and household income).

This study collected water samples from streams across the Lake Erie and Lake Simcoe Basins in Ontario, Canada. The samples were analyzed for multiple tracers for human wastewater, including artificial sweeteners and human-specific DNA markers. The data were then analyzed to determine how much wastewater from septic systems was reaching the stream sampling locations. Relationships were explored between the different subwatershed characteristics and the amount of septic system wastewater reaching the streams. Using statistical models, it was found that when the flows in streams were high (from rainfall or snowmelt), in areas with older occupied homes, and in higher sloped areas, there was a greater amount of septic system wastewater reaching the streams. In addition, it was found that in smaller subwatersheds, with smaller distances between the septic system and the stream, and low sloping terrain, there was more fecal contamination in the streams, which may be associated with a higher prevalence of failing septic systems. The results of this work help understand areas where more septic system wastewater is likely to reach streams, and therefore can aid in prioritizing septic system reinspection and education programs.

Co-Authorship Statement

The candidate is responsible for the collection and analysis of field data, as well as the writing of drafts of all chapters of this thesis. Dr. Clare Robinson and Dr. James Roy provided the initial motivation for this research, provided suggestions for field work and data analysis, and provided revisions and edits for the improvement of this thesis. The co-authorship for Chapter 3 is as follows.

Chapter 3:

Authors: Evan Angus, James Roy, Christopher Jobity, Thomas Edge, Clare Robinson

Contributions

Evan Angus conducted the field data collection, data analysis, and interpretations of the results. He also prepared the draft of the chapter.

James Roy assisted in the field monitoring design, interpretation of field data and reviewed the draft of the chapter.

Christopher Jobity assisted in field data collection and laboratory analysis.

Thomas Edge analyzed microbial source tracking maker data and helped in the interpretation of this data.

Clare Robinson assisted in the field monitoring design, interpretation of field data and reviewed the draft of the chapter.

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Chapter 1

1 Introduction

1.1 Research background

Septic systems are widely used in rural areas not serviced by centralized wastewater treatment infrastructure. It is estimated that 13% of Ontario and 14% of Canadian households use septic systems for their domestic wastewater treatment (Statistics Canada, 2011). Septic systems are designed to treat and disperse wastewater into the subsurface. Septic systems function by separating the liquid fraction of wastewater from the solid fraction in a septic tank. The liquid fraction is then discharged to the subsurface through a drain field where further attenuation of wastewater constituents occurs (Amador & Loomis, 2018). In Ontario, the design and installation of septic systems is outlined by the Ontario Building Code (Ontario Building Code, O.Reg. 332/12, ss.8) and the sewage systems regulation of the Ontario Environmental Protection Act (Sewage Systems, O.Reg. 244/09). These regulations outline conditions that need to be met to achieve adequate septic system performance including system sizing requirements, horizontal setback distances between septic systems and vulnerable receptors (e.g., surface waters, water supply wells), and soil characteristics. Once installed, homeowners are required to perform regular maintenance on their septic system, such as pumping the septic tank to ensure the continued operation of the septic system. However, factors including limited mandatory reinspection programs, aging septic systems, and lack of education around septic systems can lead to increased failure rates of septic systems. Poorly performing septic systems can deliver untreated or partially treated wastewater containing high levels of nutrients (nitrogen [N] and phosphorus [P]), fecal contaminants (e.g., bacteria, viruses), and emerging contaminants (e.g., pharmaceuticals, personal care products, microplastics) into the environment.

Several studies have shown the impact of septic systems on surface water quality degradation, with many studies focusing on illustrating the elevated nutrient loading and fecal contamination caused by septic systems (e.g., Lapointe et al., 2017; Mechtensimer & Toor, 2017; Robertson et al., 1991; Withers et al., 2014.) Excess nutrient loading to fresh surface waters, particularly P loading, can cause eutrophic conditions, which can lead to harmful algal blooms, hypoxic conditions, and aquatic ecosystem degradation (Hwang, 2020). This is associated with tremendous environmental, economic, and human health costs (Smith et al., 2019). Due to the large impacts and high costs associated with elevated nutrient loads to surface waters, it is necessary to quantify the relative contributions of various nutrient sources, including septic systems, to develop effective nutrient management strategies. The research conducted in this thesis focuses on the Lake Simcoe and Ontario Lake Erie Basins, located in central and southwestern Ontario, Canada, respectively. Lake Simcoe and Lake Erie have experienced considerable water quality challenges, including eutrophication, in recent years due to high nutrient loads to the lakes (MECP, 2009). To address water quality challenges in Lake Simcoe, the Lake Simcoe Protection Plan, which was established in 2008, targets a 40% annual reduction in P loading (72 T/yr to 40 T/yr) to the lake by 2045 (MECP, 2009). Similarly, the Lake Erie Binational Phosphorus Reduction Strategy aimed to achieve a 40% reduction in total P load to Lake Erie by 2025 (Environment and Climate Change Canada. & Ministry of the Environment and Climate Change, 2018). Sources of P loads to both lakes include agriculture, landfills, leaky sewer systems, urban runoff, wastewater treatment facilities, and septic systems (MECP, 2009). It is estimated that more than 100,000 and 30,000 homes use septic systems in the Lake Erie and Lake Simcoe basins, respectively (Gao et al., 2024). Currently, there is high uncertainty regarding the contribution of these septic systems to P loads to the tributaries. It is also unclear whether the loads of P reaching tributaries from septic systems may vary between subwatersheds with different characteristics such as soil permeability, topography, average septic system age.

Recent studies conducted by Oldfield et al. (2020) and Tamang et al. (2022) presented methods to quantify the percentage of septic system wastewater effluent (herein referred to as septic effluent) reaching subwatershed outlets by normalizing the measured loads of the artificial sweetener acesulfame in streams to the number of septic systems upstream of a monitoring location. Oldfield et al. (2020) conducted low-frequency stream sampling across three subwatersheds in the Ontario Lake Erie watershed under variable flow conditions. This study found that the percentage of septic effluent reaching a subwatershed outlet is greater under high flow conditions (33%) compared to low flow conditions (2%).

Tamang et al. (2022) combined high-frequency event-based stream sampling with low-frequency sampling in nine subwatersheds in the Ontario, Lake Erie, and Lake Simcoe Basins. This study provided insight into the different pathways via which septic effluent may reach tributaries during low and high flow conditions, however, as their results were based on only acesulfame stream loads, they were only able to speculate on the relative importance of different pathways in delivering septic effluent to the streams. Septic effluent may reach streams via various pathways including slow moving groundwater transport, and more rapid pathways such as preferential pathways associated with subsurface drainage infrastructure (e.g., field tile drains) or soil/bedrock fractures, overland transport associated with poor performing septic systems, and illegal direct pipes (Maxcy-Brown et al., 2021; Seiler et al., 1999; USEPA, 2002a). It is important to understand the pathways via which septic effluent reaches a stream because more conservative wastewater constituents (e.g., nitrate, chloride, and artificial sweeteners) will be transported from septic systems to the stream via all pathways including slow moving groundwater transport. In contrast, wastewater constituents that have a high tendency to be attenuated in the subsurface (e.g., P and fecal contaminants) will more likely be delivered to surface waters only via more rapid pathways such as overland transport and illegal direct pipes.

Multiple risk assessment frameworks have been developed to identify areas where septic systems are more likely to impact the environment, including surface waters (e.g., Capps et al., 2020; Jordan et al., 2023; Oosting & Joy, 2011). Many of these risk assessment approaches assign weights to environment level characteristics (e.g. soil permeability, topographic slope, land use type) and socioeconomic characteristics (e.g. income) based on subjective expert opinions without validation with quantitative field-based measurements of human wastewater tracers. Additionally, many of the risk assessments utilize data such as septic system age or system maintenance records, which are generally not available. It is necessary to understand what characteristics of the placement of the septic system within a subwatershed influence the percentage of septic effluent delivered to the tributaries. In addition, it is necessary to understand what subwatershed characteristics are associated with poorly performing septic systems and the delivery of septic effluent to tributaries through rapid pathways. Identifying and quantifying subwatershed characteristics which influence the percentage of septic effluent reaching tributaries is needed to refine nutrient load estimates, evaluate fecal contamination risks associated with septic systems, and to prioritize septic system reinspection and education programs.

1.2 Research Objectives

There is need to understand the percentage of septic effluent reaching tributaries across multiple subwatersheds and the potential influence of different physical and socioeconomic subwatershed characteristics. To address this, the specific objectives of this thesis are:

1) Evaluate the percentage of septic effluent reaching subwatershed outlets and how it varies between flow conditions across multiple subwatersheds.
2) Evaluate the relative importance of different physical and socioeconomic subwatershed characteristics on the percentage of septic system effluent reaching subwatershed outlets.
3) Evaluate the relative importance of different physical and socioeconomic subwatershed characteristics on the delivery of septic effluent to a subwatershed outlet via rapid pathways potentially associated with poorly performing septic systems.

While the field monitoring conducted for this thesis focuses on subwatersheds in the Lake Erie and Lake Simcoe Basins, Ontario, the findings of this research are broadly applicable to other watersheds globally where septic systems are used to treat domestic wastewater.

1.3 Thesis Outline

This thesis is written in the format of an “Integrated Article”. A brief description of each chapter is presented below:

Chapter 1: Introduces the research background and states the research objectives.

Chapter 2: Reviews relevant work related to how septic systems treat and disperse wastewater, pathways that may deliver septic effluent to tributaries and human wastewater tracers that can be used to detect septic effluent in surface waters. The chapter also reviews frameworks previously developed to identify areas with potentially high septic system failure rates.

Chapter 3: Details the field work and data analysis conducted. This chapter presents field data that shows the high variability of human wastewater tracers (artificial sweeteners, human-specific microbial source tracking markers) and nutrients in streams across multiple subwatersheds. The conservative artificial sweetener acesulfame was used to calculate the percentage of septic effluent reaching subwatershed outlets. Statistical modelling was used to identify subwatershed characteristics found to be important for explaining the variability in the percentage of septic effluent reaching the outlets across multiple subwatersheds. In addition, the subwatershed characteristics influencing stream concentrations of the non-conservative microbial source tracking marker HF183 between the subwatersheds were explored.

Chapter 4: Summarizes the research findings and provides recommendations for further research.

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Chapter 2

2 Literature review

Septic systems are widely used for the treatment and dispersal of domestic wastewater in rural and suburban areas not served by centralized wastewater treatment infrastructure. In Canada and Ontario, approximately 14% and 13%, respectively, of all homes use septic systems (Statistics Canada, 2011). Furthermore, it is estimated that more than 100,000 and 30,000 homes use septic systems in the Lake Erie and Lake Simcoe Basins, Ontario, respectively (Gao et al., 2024) Wastewater effluent from septic systems (herein referred to as septic effluent) contains various contaminants of concern including nutrients (nitrogen [N] and phosphorus [P]), fecal pathogens, pharmaceuticals, and personal care products. There is concern that these contaminants may be delivered to surface waters from septic systems, particularly from poorly performing systems. However, the impact of septic systems on surface water quality is generally not well quantified and understood. This chapter presents the relevant literature on septic systems and associated risk factors related to septic system performance. In addition, this chapter presents the advancements made in using human wastewater tracers to detect septic effluent in surface waters.

2.1 Septic Systems

2.1.1 Septic system design and installation

Septic systems were first installed in rural homes in Ontario and across North America in the late 1940s following the post World War II housing boom (Amador & Loomis, 2018). In Ontario, the design and installation requirements for septic systems are set by the Ontario Building Code (Ontario Building Code. O Reg. 332/12, s.8) and the sewage systems regulation of the Environmental Protection Act (Sewage Systems, O Reg. 244/09), with post-installation inspections regulated by municipalities. Septic systems are comprised of three main elements: the septic tank, the distribution box, and a form of drain field (Amador & Loomis, 2018). The typical configuration of a septic system is shown in Figure 2-1. The septic tank is a watertight settling chamber that is placed below the ground surface. The tank is typically constructed of prefabricated concrete but can also be constructed with materials such as plastic, fibreglass, or PVC (Amador & Loomis, 2018; Boulware, 2013).

The size of the septic tank should be designed to treat at 1.3 times the maximum daily water usage by a household (Amador & Loomis, 2018; Ontario Building Code. O Reg. 332/12, s.8). The septic tank separates the heavier organics and lighter fats and oils from the liquid fraction of the wastewater.

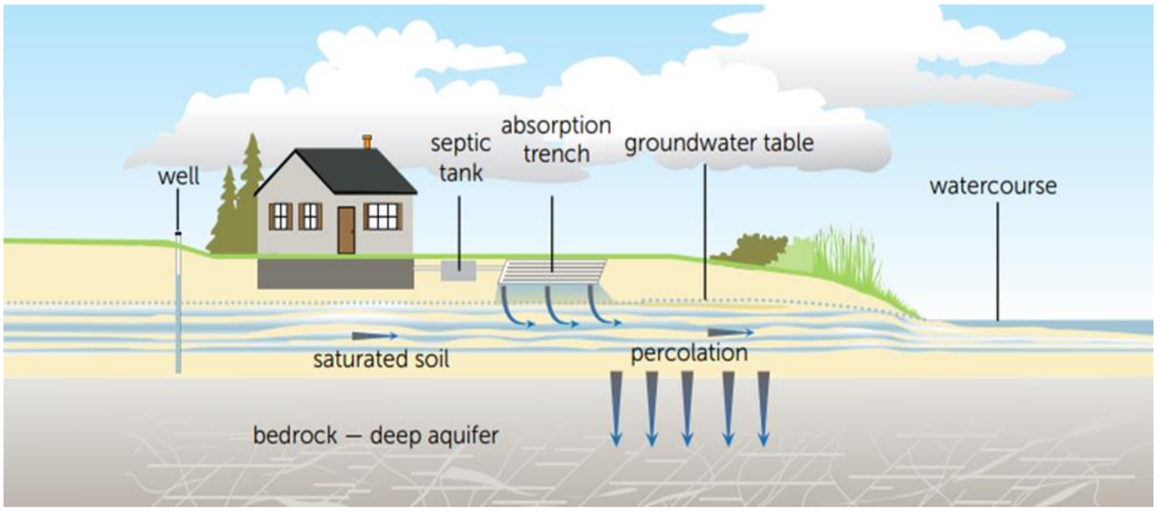

Figure 2-1: Cross-sectional view of a conventional septic system and the delivery pathway of partially treated septic system effluent (OMAFRA & RVCA, 2022)

The second element of a septic system is the distribution box, which evenly distributes the effluent from the septic tank to the drain field (also referred to as soil adsorption bed or leaching field). The third element of a septic system is the drain field which consists of perforated pipes that discharge the partially treated septic tank effluent into a bed of engineered soil media (Amador & Loomis, 2018). When correctly designed, the drain field distributes the partially treated effluent over a large area, allowing the effluent to infiltrate into the subsurface where further treatment occurs (Boulware, 2013). Although the perforated pipe style of a drain field is the most widely employed, other configurations can be used, such as a mound-style system in which the effluent is discharged into a constructed mound (USEPA, 2023). To safeguard human health and environmental health, the Ontario Building Code specifies the minimum required horizontal setback distances between the drain field and drilled wells (15 m), dug wells (30 m), surface water bodies (15 m), and homes (5 m). Additional recommended setback distances for natural structures such as trees (6 m) and gardens (5 m) are also provided. In addition, the Ontario Building Code specifies a minimum separation distance between the bottom of the drain field and the seasonally high groundwater table (600 mm), and restrictive horizons such as the bedrock layer (900 mm). The Ontario Building Code also specifies constraints on the soil infiltration rate in and below the drain fields. The drain field must not be placed in soils that have a design time of less than 1 minute or greater than 50 minutes to allow effective transmission of the effluent. In addition to providing specifications for the design and installation of conventional septic systems, the Ontario Building Code also provides specifications for other types of onsite wastewater treatment systems including pit latrines, activated treatment systems, and holding tanks.

2.1.2 Treatment of wastewater in septic tanks

The raw wastewater entering a septic tank contains many constituents such as nutrients, organics, fecal pathogens (bacteria, viruses, and protozoa), and emerging contaminants. The septic tank acts as a gravity-settling chamber in which the heavier organics and lighter oils are separated from the liquid fraction of influent wastewater. To achieve the separation of the solid and lighter fractions, the hydraulic retention time of a septic system should be at least two days (Amador & Loomis, 2018). The septic tank, when operating optimally, can remove up to 50% of organic matter and ~30% of biochemical oxygen demand, and provide log 1 removal of *E. coli* from the raw wastewater (Adegoke & Stenstrom, 2019; Amador & Loomis, 2018; Boulware, 2013). Organics in the raw wastewater are decomposed by anaerobic digestion whereby heterotrophic bacteria oxidize and solubilize the organic matter found in the setteled sludge layer (Beal et al., 2005; Bedinger et al., 1997). The removal of nutrients (N and P) in conventional septic tanks is minimal. P enters the septic tank in inorganic and organic forms, as it comes from both human waste and cleaning products used in homes (Lusk et al., 2017). The removal of P can be up to 20-30% under various hydraulic retention times with removal attributed to the settling of solids with bound or sorbed P (Aly Nasr & Mikhaeil, 2013). Ammonia typically accounts for around 90% of the total N found in raw wastewater (Brandes, 1978). As with P, anaerobic digestion processes are ineffective for N removal, and it is estimated that as little as 5% of total N is removed in septic tanks (Lusk et al., 2017). In general, limited removal of viruses and protozoan cysts occurs in septic tanks as they are unable to settle due to the longer length of time required to settle these particles due to their small size (Ferguson et al., 2009; Gerba, 2008). Bacteria removal in septic tanks is also minimal, however, some removal occurs as bacteria die off in the anaerobic liquor and via the settling of particles with attached bacteria (Feachem et al., 1983; Lusk et al., 2017). The typical concentration range of various wastewater constituents as determined from sampling in multiple septic tanks is reported in Table 2-1 (Brandes, 1978).

Table 2-1: Concentrations of various wastewater constituents in septic tanks (Brandes, 1978)**. All constituents are presented in mg/L except for total and fecal coliforms.**

	Number of samples	Range	Mean
Total Phosphorus	24	16-22	18.6
Soluble reactive phosphorus	24	3.5-21	15.2
Total Kjeldahl Nitrogen	24	140-170	153
Ammonia	25	12-160	138
Nitrite	25	0.01-0.02	0.02
Nitrate	25	0.1-0.3	0.22
Total coliforms /100ml	25	(0.003-0.9) $*10^6$	$0.25*10^6$
Fecal coliforms/100ml	23	(0.002-0.17) $*10^6$	$0.88*10^6$

2.1.3 Treatment of septic tank effluent in the subsurface

Once the liquid fraction of the effluent exits the septic tank, it is delivered to the subsurface through the drain field. The drain field is typically comprised of perforated pipes which distribute the effluent to the subsurface (Amador & Loomis, 2018). A biologically active zone called the “biomat” forms below drain fields as the delivered effluent clogs the pores of the native soil media (Beal et al., 2005; Gill et al., 2007; Lusk et al., 2017). The location of the biomat zone below a drain field can be seen in Figure 2-2. Within the biomat layer, conditions are typically favourable for the removal (retention and degradation) of wastewater constituents including nutrients and some pathogens (Beal et al., 2005).

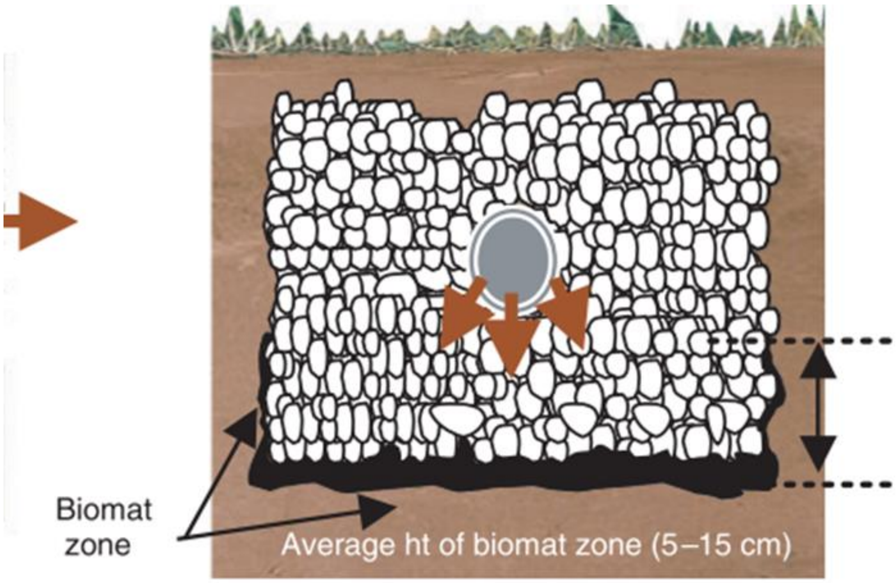

Figure 2-2: Cross-sectional view of drain field with a developed biomat zone (Beal et al., 2005).

N delivered to the drain field is primarily in the form of ammonia. Ammonia is typically nitrified and converted to nitrate after being delivered to the drain field (Beal et al., 2005; Walker et al., 1973). Whelan & Barrow (1984) showed that nitrification reactions generally do not occur in the biomat zone of the drain field, but rather occur after the effluent has passed through the biomat layer and entered more aerobic conditions in the unsaturated zone. The removal of nitrate via denitrification tends to be limited in the subsurface below drain fields, although some denitrification has been observed to occur in the unsaturated zone, especially in areas of low oxygen concentrations and a high supply of carbon (Bedessem et al., 2005). However, for many septic systems, nitrate can be transported relatively conservatively through the unsaturated zone and reach the groundwater table. Nitrate has been observed to be transported over 200 m in the groundwater downgradient of septic system drain fields (Valiela et al., 1997).

P delivered into the subsurface via the drain field is often primarily in the form of soluble reactive phosphorus (SRP) (Beal et al., 2005; Magdoff et al., 1974). The removal of P below the drain field is governed by several mechanisms such as adsorption, precipitation, and biological uptake. Robertson et al. (2019) reviewed the attenuation of P in 24 subsurface plumes below and downgradient of septic drain fields across Ontario. Noticeable differences were observed when comparing P attenuation in sites located in areas with non-calcareous sediments compared to those located in areas with calcareous sediments. Results indicated that septic systems on non-calcareous sediments had no SRP plumes extending greater than 10 m from the drain field, and within the plume, SRP concentrations were found to be 97% lower than the effluent SRP concentrations. In contrast, in sites where calcareous sediments were present, effluent SRP concentrations in groundwater plumes downgradient of drain fields were only reduced on average by 69%. This is further supported by Zanini et al. (1998) who observed high P retention in sediments located directly below the drain field (15-30 cm). These sediments had high fractions of Fe and Al, suggesting that P was mostly removed by mineral precipitation for the systems they studied. Despite the tendency of P to be sequestered to sediments below drain fields, it is important to note that a change in geochemical conditions (e.g. redox conditions) can remobilize P. Changes in redox conditions may occur below the drain field in response to conditions such as increased buildup of organic matter, limiting the diffusion of oxygen to the unsaturated zone and thereby creating reducing conditions (Zurawsky et al., 2004).

The removal of pathogens below a drain field occurs primarily in the biomat layer (Beal et al., 2005). In a pilot-scale septic system, Van Cuyk et al. (2001) found that more than 95% of bacteria and viruses were removed at depths of 60 cm in sandy soil. Alhajjar et al., (1988) studied the transport and fate of several bacterial tracers in the subsurface below a drain field. Consistent with Beal et al. (2005), they found that the removal of bacteria increased as the effluent travelled through the biomat layer.

2.2 Factors influencing septic system performance

2.2.1 Physical siting factors

Several factors can affect the performance of septic systems in treating and dispersing wastewater effluent effectively. When installing a septic system, it is important to understand the site conditions, such as topography, setback distances, soil characteristics, and groundwater table fluctuations to ensure optimal performance is achieved. As described above, attenuation and treatment of the effluent from the septic tank occur in the soil below the drain field including in a biomat layer. The soil conditions, including physical, chemical, and biological conditions, play an important role in the extent of removal of wastewater constituents (Amador & Loomis, 2018). Several important physical characteristics of the soil such as grain size, gradation, and porosity influence the removal of constituents (Amador & Loomis, 2018). For instance, soils with moderate permeability allow for the effective infiltration of the effluent, whilst still ensuring there is sufficient time for treatment to occur (Dawes et al., 2005). The topography of the site chosen for a septic system can also impact its ability to effectively treat wastewater. In sloped areas, the effluent can travel at faster rates through soils to the groundwater, which can limit the removal of constituents in the unsaturated zone (Dawes et al., 2005). In addition, it is recommended that septic systems are not placed in areas that are topographic lows as these areas may be flood-prone and have high seasonal groundwater tables (Amador & Loomis, 2018). The depth of the seasonal groundwater table at the location of the septic drain field can considerably affect the removal of effluent constituents in the subsurface. For instance, it has been observed that in areas of wet soils or low depth to the groundwater table, N removal and sorption of fecal pathogens below drain fields can be limited (Cooper et al., 2016; Humphrey et al., 2017). For this reason, guidelines including the Ontario Building Code (Ontario Building Code. O Reg. 332/12, s.8) indicate a vertical separation distance between the drain field and the seasonally high groundwater table. Similar recommendations are made regarding the optimal separation distance between the drain field and a restrictive horizon (USEPA, 2002b). Restrictive horizons may be clay, bedrock, or other boundaries that restrict the vertical flow of water. Low depths to restrictive horizons limit the percolation and travel time of the septic effluent, thereby reducing treatment. Septic effluent which encounters fractured bedrock can travel rapidly through the fractures where limited removal of constituents in the effluent occurs (Kozuskanich et al., 2014; Marshall et al., 2022). The factors described above play an important role in the performance of septic systems and need to be considered to ensure septic systems are well functioning with a high removal of constituents below the drain field.

2.2.2 System level factors

Ensuring the optimal performance of a septic system requires appropriate system design (including sizing), routine maintenance, and repairs. Septic systems have a finite lifespan and their ability to disperse effluent, and remove constituents decreases with age (Capps et al., 2020). Clayton (1974) studied septic systems of various ages through the period of 1952-1972 in Fairfax County, Virginia. Results showed that septic systems installed in the early 1950s showed the greatest failure, with 8% of systems installed in 1955 failing by 1972. A similar study conducted by Winneberger (1975) found that septic systems between the age of 26-30 years had a failure rate of 38.6% compared to a failure rate of approximately 20% of 10-year-old systems. These findings suggest that the risk of failure increases with the age of the septic system.

The sizing of a septic tank can also impact the effectiveness of the treatment. The septic tank must be sized to process at least 1.3 times the daily wastewater flow rate from the house with a minimum tank volume of 2400 L. Any additional chambers in the septic tank must be at least 50% the size of the first compartment (Ontario Building Code. O Reg. 332/12, s.8). Undersized septic systems often occur due to the addition of auxiliary appliances such as garbage disposal systems or when household occupancy increases following the home being resold and/or renovated (Clements et al., 1980; Noss & Billa, 1988). An increased volume of wastewater delivered to the septic tank can result in a decreased hydraulic retention time, overloading of the drain field, and subsequent reduction in the removal of constituents in the septic tank and below the drain field.

Septic systems should regularly be maintained with routine maintenance including pumping/desludging of the septic tank every 3-5 years or when accumulating biosolids exceed 30% of the septic tank volume (USEPA, 2005). Failing to pump out septic tanks can result in the volume of the tank being exceeded and lead to break out of septic effluent to the ground surface from the septic tank (USEPA, 2002b). Additionally, as the volume of the solids in the septic tank increases, the zone of clarified effluent is reduced, and this results in a reduced ability for solids to settle and subsequently reduces the treatment of the wastewater (Noss & Billa, 1988).

2.2.3 Socioeconomic drivers of septic system performance

Aside from physical and system operational factors, studies have shown various socioeconomic/human-related factors can influence septic system failure rates (Capps et al., 2020, 2021; Fizer et al., 2018). Firstly, the cost associated with septic system maintenance including pumping and desludging of the septic tank can often exceed $500. In a survey of homeowners that use septic systems in the Republic of Ireland, Devitt et al. (2016) found that the financial cost associated with septic system pumping/desludging was a key limitation for homeowners to maintain their systems. More broadly, previous research has shown that there has been a historical exclusion of low-income communities from municipal services including connections with centralized wastewater treatment plants (Durst & Wegmann, 2017; Marsh et al., 2015). The excluded low-income communities are required to use septic systems for which the maintenance and repair costs are the responsibility of the homeowner. This was recently highlighted in a septic system risk assessment study conducted in Georgia, U.S., in which it was found that septic systems are more widely used in communities that are in socially and economically vulnerable census areas (Capps et al, 2020). In such areas, maintenance costs associated with septic systems may be a significant barrier for homeowners and they may be perceived as a lower priority compared to other more visible home maintenance requirements such as roof repairs.

The lack of homeowner awareness and education on the best practices for septic system operation and maintenance may be another factor contributing to septic system failures. Naughton & Hynds (2014) conducted structured homeowner questionnaires to determine the level of awareness and practices related to septic systems. The results indicated knowledge gaps in the surveyed populations regarding their septic systems, including the required maintenance, and the health and environmental risks associated with septic system failure. Furthermore, less than 30% of the respondents in the study indicated that they had received guidance on septic system best management practices from a regulatory body. Similarly, Fizer et al. (2018) found that homeowners typically misunderstand the potential impact of septic systems on groundwater resources. Devitt et al. (2016) also stated that homeowners surveyed showed a lack of knowledge relating to proper maintenance practices such as what sources of wastewater are to enter the septic system and that this lack of knowledge is mostly due to limited resources and technical guidance provided to homeowners. Thus, the lack of education and resources provided to homeowners likely contributes to improper septic system operation and maintenance and the premature failure of a septic system.

2.3 Pathways for septic effluent to reach surface waters

Properly functioning septic systems deliver partially treated wastewater effluent to the subsurface in the drain field where further removal of many wastewater constituents occurs as the effluent infiltrates through the unsaturated zone including through the biomat layer (Amador & Loomis, 2018). Although well-functioning septic systems can provide a high level of removal (attenuation or retention) of less conservative constituents, more conservative constituents (e.g., chloride, nitrate, artificial sweeteners) may not be removed in the unsaturated zone. These constituents may reach the groundwater table where they will then be transported with groundwater flow and potentially reach a surface water body (pathway 1 in Figure 2-3). If a septic system is poorly located, designed, or maintained, the amount of wastewater constituents that reach surface waters via groundwater transport can be higher. Incomplete treatment of septic effluent in the subsurface can also occur when a shallow subsurface septic effluent plume is intercepted by a preferential subsurface pathway that can cause the septic effluent to travel more rapidly and directly to a surface water body (Digaletos et al., 2023). For example, this can occur when a subsurface septic effluent plume is intercepted by constructed drainage systems such as tile drains used in agricultural fields, subsurface drains located on residential properties (e.g., french drains), but also if the plume reaches urban karst features such as utility trenches and storm sewers (pathway 2 in **Error! Reference source not found.**-3). For instance, Spiese et al. (2014) measured caffeine concentrations (human-specific wastewater tracer) in tile drainage effluent in a rural watershed of Ohio, U.S., and found significantly higher caffeine concentrations in tile drainage when there were septic systems nearby. Subsurface septic effluent plumes may also be transported rapidly to surface waters if they reach bedrock fractures, or high-permeability fissures in low-permeability clay soils (Digaletos et al., 2023).

Septic systems can also underperform and potentially fail when the drain field becomes clogged, the system is hydraulically overloaded, or the groundwater table is high. When this occurs, there is a potential for septic effluent to break out from the septic tank or drain fields and pond on the ground surface. Ponded effluent may be transported to surface water via overland transport during wet weather conditions (pathway 3 in **Error! Reference source not found.**-3). Finally, raw or partially treated septic effluent may be transported to a surface water body via illegal direct drains (pathway 4 in **Error! Reference source not found.**-3). The practice of connecting a septic tank (upstream or downstream) to a pipe that discharges untreated or partially treated wastewater directly to a surface water body or drainage ditch is often termed 'hot piping'. Although illegal, the practice of 'hot piping' has been observed across North America, including in rural low socioeconomic regions of the U.S. (Maxcy-Brown et al., 2021; USEPA, 2002a). The rapid pathways described above can result in increased amounts of wastewater constituents reaching surface water bodies which pose public health and environmental health risks.

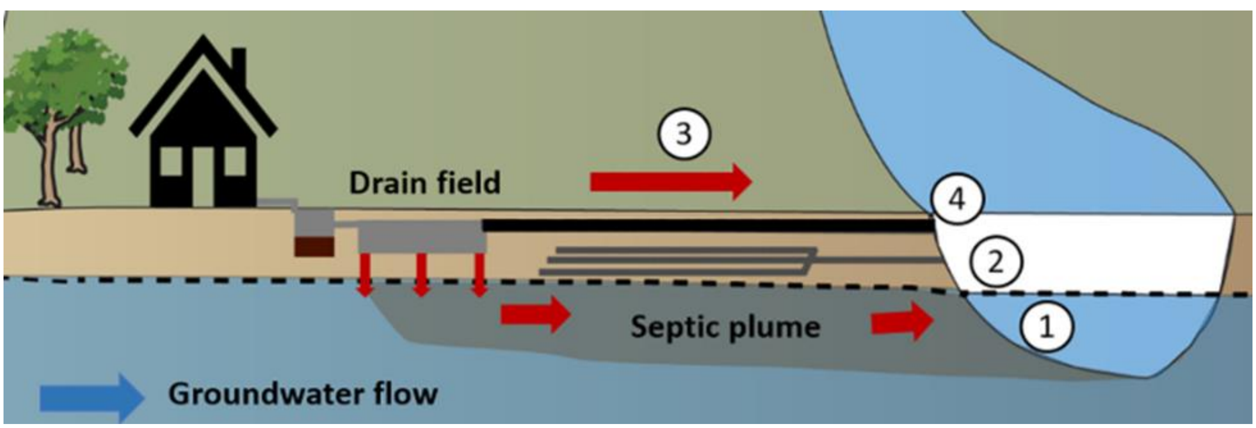

Figure 2-3: Pathways via which septic effluent may reach nearby streams: Pathway 1 represents groundwater transport, Pathways 2 represents subsurface septic plume being intercepted by subsurface drainage features, Pathways 3 represents overland

transport of septic effluent which has broken out at the ground surface, and Pathway 4 represents illegal direct pipe connecting a septic system to the stream. Figure adapted from Tamang et al. (2022).

2.4 Detecting and quantifying septic effluent constituents in surface waters

It is challenging to detect and quantify the amount of septic effluent that reaches surface water bodies because septic systems are distributed across watersheds. In addition, many of the constituents in wastewater are not specific to septic systems and are also derived from other sources such as agricultural activities. A wide range of tracers have been used to detect septic effluent in surface waters. These include microbial tracers such as *E. coli* and human-specific DNA markers (Chase et al., 2012; Digaletos et al., 2023; Georgakakos et al., 2019; Murphy et al., 2020; R. Sowah et al., 2014; Verhougstraete et al., 2015), organic constituents such as pharmaceuticals and caffeine (Glassmeyer et al., 2005; Seiler et al., 1999), and inorganic constituents such as boron (B), chloride (Cl), and bromide (Br) and their relative ratios (Bolan et al., 2023; Katz et al., 2010; Vengosh & Pankratov, 1998; Widory et al., 2005). In recent years, an increasing number of studies have used artificial sweeteners to detect human wastewater in surface waters (Gago-Ferrero et al., 2017; Snider et al., 2017; Spoelstra et al., 2020; Van Stempvoort et al., 2013; Van Stempvoort, Roy, et al., 2011; Zirlewagen et al., 2016). A suitable tracer for the detection of septic effluent in surface waters should be source-specific and widely used and found in septic effluent at concentrations above analytical detection limits. Using human-specific wastewater tracers that are conservative through the septic system treatment process and in the environment, can be valuable in providing an upper limit on the amount of septic effluent constituents in surface waters (Oldfield et al., 2020). Alternatively, using less conservative human-specific wastewater tracers can be useful in providing insight into more rapid pathways delivering septic effluent to surface waters – these pathways may be associated with higher public and environmental health risks.

2.4.1 Artificial sweeteners

Artificial sweeteners are valuable tracers for detecting human wastewater in the environment with the use of these tracers increasing over the last 15 years (Snider et al., 2017; Spoelstra et al., 2020; Van Stempvoort et al., 2011; Van Stempvoort et al., 2013). Artificial sweeteners are commonly used in food additives to provide 'sweetness' to products (Chattopadhyay et al., 2014). Non-nutritive artificial sweeteners are a class of artificial sweeteners which provide sweetness without calories (Shankar et al., 2013). Non-nutritive artificial sweeteners include acesulfame, saccharin, sucralose, and cyclamate, amongst others (Chattopadhyay et al., 2014). Non-nutritive artificial sweeteners are approved in Canada for use in a variety of household products including tabletop sugar packs, gums, beverages, salad dressings, breath freshening products, medicines as well as many other consumer products (Health Canada, 2023). Non-nutritive artificial sweeteners can be suitable tracers for human wastewater because they are widely detected in human wastewater (Mangala Praveena et al., 2019), and are certain sweeteners are conservative including through wastewater treatment (Buerge et al., 2011; Scheurer et al., 2009). For instance, studies have shown that artificial sweeteners are highly persistent in groundwater effluent plumes downgradient of septic systems (Van Stempvoort, Robertson, et al., 2011).

Acesulfame is commonly thought to be the most suitable tracer for human wastewater as it has high source specificity and is highly conservative with a long half-life and minimal sorption to sediment (Buerge et al., 2009; Storck et al., 2016). Further, due to its low analytical detection limits it is often widely detected in surface waters (Spoelstra et al., 2020). Other non-nutritive artificial sweeteners are generally not as suitable. Saccharin is not source-specific as it is widely used in the agricultural industry in swine feed (D. Li et al., 2020; Ma et al., 2017) and is known to be a metabolite of sulfonylurea-based herbicides (Buerge et al., 2011). Cyclamate is less conservative in the environment (Buerge et al., 2011). Sucralose, although highly conservative like acesulfame (Buerge et al., 2011), generally has a higher analytical detection limit and therefore is often below detection in surface waters (Spoelstra et al., 2020). It is important to note that although acesulfame is often considered to be the most suitable tracer for human wastewater, degradation of acesulfame has been observed under specific conditions. For instance, the removal of acesulfame has been observed in wastewater treatment plants under low biological oxygen demand, aerobic, and denitrifying conditions (Castronovo et al., 2017; Kahl et al., 2018). Therefore, under specific conditions found in the septic tank or the environment, some attenuation of acesulfame may occur.

Artificial sweeteners including acesulfame have been used in several studies to detect human wastewater in groundwater and surface waters. Acesulfame in groundwater and surface waters from human wastewater sources was first measured in the µg/L range by Buerge et al. (2009). Van Stempvoort et al. (2011) later detected artificial sweeteners, including acesulfame, in groundwater and surface water samples located near wastewater treatment outfalls, landfill sites, and other anthropogenic sources. Several studies have used artificial sweeteners to detect human wastewater tracers in urban tributaries where there is a wastewater treatment plant outfall upstream (e.g., Scheurer et al., n.d.; Tran et al., 2014). Comparatively fewer studies have been conducted using artificial sweeteners as a tracer for detecting human wastewater in tributaries due to septic systems. Spoelstra et al. (2020) measured acesulfame and other artificial sweeteners in 173 rural streams (one-off sampling) in Ontario, Canada, where septic systems are used upstream (no wastewater treatment plant outfall upstream). Acesulfame was detected in 91% of the stream samples analyzed. Using measured concentrations of acesulfame in the stream, Spoelstra et al. (2020) estimated that approximately 13% of the effluent from septic systems was reaching the streams. Following this work, Oldfield et al. (2020) used the artificial sweetener acesulfame to evaluate the percentage of septic system effluent reaching tributaries in three high permeability subwatersheds in the Ontario Lake Erie Basin with high densities of septic systems. They showed that the percentage of septic effluent reaching the streams (based on acesulfame stream load) was highest during high flow compared to low stream flow conditions. Tamang et al. (2022) extended this work by measuring acesulfame concentrations in nine streams in the Lake Simcoe and Lake Erie Basins that drained high and low permeability subwatersheds. They examined whether different subwatershed characteristics may explain the differences observed in the percentage of septic effluent reaching the steams. As the number of subwatersheds they sampled was relatively low, they were not able to determine whether any specific subwatershed characteristics influenced the percentage of septic effluent reaching the stream apart from home age. Their data showed that generally subwatersheds with older homes tended to have a higher percentage of septic effluent reaching the stream, but no statistical analyses were conducted.

2.4.2 Microbial tracers

Various microbial tracers have been used to detect human wastewater in groundwater and surface waters. Microbial tracers are a broad category that includes bacteria, yeasts, and spores (Keswick et al., 1982). Bacterial tracers are the most commonly used due to their relative abundance in human wastewater and the simple methods available for the analysis of some bacteria (Keswick et al., 1982). As early as 1938, fecal bacteria have been used to trace the movement of groundwater contaminated with human wastewater from pit latrines (Larson Caldwell, 1938). Numerous studies since have used microbial tracers such as fecal coliforms, *Escherichia coli* (*E. coli*), and *Clostridium perfringens* to trace septic system effluents in the environment (Henry et al., 1991; Keswick et al., 1982; Postma et al., 1992; Shadford et al., 1997).

Microbial tracers such as *E. coli* are limited in their effectiveness as human wastewater tracers as they are not source-specific (Glassmeyer et al., 2005). While modified procedures including strain labelling have been developed, these procedures are time-consuming (Bernhard & Field, 2000). In response to this challenge, Bernhard & Field (2000) developed a polymerase chain reaction (PCR) assay to identify fecal contaminant sources in water. The assay can isolate various *Bacteroides Prevotella* 16S rDNA markers from human, bovine, and other animal fecal contamination using the length heterogeneity PCR method. One of the gene markers identified in this assay is the HF183F primer. The HF183 gene marker has been extensively used to detect human wastewater contamination in surface waters as it has high specificity to human wastewater (Ahmed et al., 2008, 2016). Walters & Field (2009) evaluated the survival times of two human fecal *Bacteroides* markers (HF183 and HF134) in freshwater microcosms. The findings of their work suggested that the survival times of the human-associated markers were approximately 6 days, indicating that these markers are useful for identifying recent human wastewater contamination. The short survival times of these markers were further investigated in surface water samples by Tambalo et al. (2012) who observed a 99% reduction in all markers analyzed in less than 8 days. Ahmed et al. (2008) analyzed the specificity of the HF183 and HF134 markers on various human and animal-based wastewater samples. The HF183 marker was detected in all 52 human wastewater samples, including in septic tank wastewater, and was not detected in any of the animal wastewater samples. These results indicate HF183 has high source specificity to human wastewater and that this marker is a suitable tracer for identifying recent contamination of water by human wastewater.

The application of microbial source tracking (MST) methods such as HF183 to identify surface waters contaminated by septic effluent was investigated by Sowah et al. (2017). This study found that using typical fecal indicator bacteria such as *E. coli* and MST methods along with land use characterization can identify surface waters contaminated by human wastewater from septic systems. More recently, Billian et al. (2018) conducted column-leaching experiments to understand the potential mobility of HF183 through septic system drain fields. HF183 was detected in only around half of the collected column effluents, indicating that the mobility of HF183 below the drain field may be limited. However, it is important to note that under some circumstances, it may be possible for HF183 to travel in the subsurface. For instance, Mattioli et al., (2021) found HF183 in groundwater sampled from a water supply well where a nearby septic system was placed on highly permeable soil. The highly permeable soils may have allowed rapid subsurface transport of HF183 with limited retention. Recently, Digaletos et al. (2023) found the human-specific *Bacteroides* marker in streams downstream of rural hamlets that use septic systems. The locations where human-specific *Bacteroides* were observed indicated that septic effluent may be reaching the streams via preferential flow pathways in fine-grained overburden. Additionally, Verhougstraete et al. (2015) conducted a broad-scale sampling of tributaries across the Upper Michigan Peninsula, U.S., with samples analyzed for human-specific MST markers. Using classification and regression trees methods, they showed that higher MST marker concentrations were observed in streams at a greater concentration downstream of areas of with higher septic system usage.

2.5 Frameworks for assessing risks associated with septic systems

Multiple risk assessment frameworks have been developed to determine where septic systems are more likely to impact the environment. For instance, Carroll et al. (2006) developed a comprehensive risk assessment framework for the Gold Coast, Australia, which considered the environmental and health risks associated with septic systems. Within this risk assessment framework, subcategories of risk assessment such as environmental risk (elevated nutrient levels resulting in eutrophication), fecal contamination risks, and siting and design risks (soil type, lot size) are considered. To integrate the various data to be used in this risk assessment, a semiquantitative approach was used to define the risk of adverse environmental or health impacts. Although this risk assessment framework is comprehensive, the large amount of data required may be challenging for many jurisdictions. A less data-intensive risk assessment framework which aims to identify areas of high risk due to various environmental factors was developed by Oosting & Joy (2011). The risk factors considered in this framework include soil type, topographic slope, septic system age, lot size, proximity to surface waters, floodplains, areas of groundwater, intrinsic susceptibility, recharge areas, and proximity to water supply wells. The risk factors used in this framework are subjective as they were determined by industry experts assigning relative importance on a scale of 0-5 to each of the factors. The lack of field-based observations to validate the assigned risk weightings may lead to inaccurate weighting for some risk factors. Following the risk assessment framework developed by Oosting & Joy (2011), Capps et al. (2020) analyzed the potential for septic systems to impact the environment in the rural/suburban Athens-Clarke County, Georgia. They considered physical risk factors such as soil type and topographic slope, and socioeconomic characteristics such as septic system, age, income, and racial demographics in their analysis. They did not conduct field monitoring to validate the results of their risk assessment but had access to considerable non-publicly available data on the septic systems in the County such as the age of septic systems. Extending this work, Connelly et al. (2023) assessed the relationships between maintenance patterns of septic systems and environmental and system-level variables in the same study area. Using maintenance records provided by Athens-Clarke County, they were able to statistically test the effect of physical characteristics (e.g. topographic wetness index, distance to streams) on routine tank pumping, anomalous pumping, and repair patterns. Recently, Jordan et al. (2023) conducted a multicriteria decision analysis (MCDA) with both system-level variables (e.g., system age) and physical variables (e.g., land use type) considered. Weights were assigned to septic system characteristics such as the age of septic systems based on the previous work by USEPA (2002), Carroll et al. (2006), Capps et al. (2020), and Oosting & Joy (2011). The results of the MCDA analysis were used to classify the pollution potential of septic systems upstream of multiple tributary sampling points. These classifications were then used to predict and compare *E. coli* concentrations measured in tributary samples. These previous studies have provided valuable methodologies to predict septic system failures which may lead to adverse environmental and public health impacts including impaired surface water quality. However, the prior risk assessment frameworks developed and applied are subjective as they are mostly based on experts' perceptions and have not been validated by field measurements of the actual impacts of septic systems on surface water quality. While Tamang et al. (2022) explored the influence of subwatershed characteristics (e.g., surficial geology) on the percentage of septic effluent reaching streams in nine subwatersheds (based on using acesulfame as a human wastewater tracer), this study was not able to statistically determine the influence of different physical and socioeconomic factors due to the few subwatersheds monitored in their study.

2.6 Research gaps

From the above literature review, several studies have evaluated how environmental system level and socioeconomic factors may influence the performance of septic systems and their potential impacts on surface water quality. Many studies have investigated individual factors (e.g., groundwater table elevation) which influence the septic system wastewater treatment processes (e.g. Dawes & Goonetilleke, 2003; Noss & Billa, 1988), to make wide-scale predictions on the impact of septic systems on surface water quality or to prioritize septic system programs and policy including education and reinspection programs. However, there is a need to understand at a watershed scale what factors influence the percentage of septic effluent reaching surface water bodies including streams. Quantifying the percentage of septic system effluent reaching a stream is complex as septic systems are distributed across watersheds and many of the constituents in septic effluent (e.g., nutrients and *E. coli*) are also associated with other sources in rural watersheds. Several risk assessment frameworks have been developed to identify potential areas that are vulnerable to septic system contamination, but the factors used in these frameworks and their associated weightings have not yet been validated with field measurements (e.g. Jordan et al., 2023; Oosting & Joy, 2011). Additionally, prior studies which aim to identify areas with a high risk of septic system failure use data that are not easily accessible in all jurisdictions such as the age of septic systems and maintenance records (e.g. Capps et al., 2020; Connelly et al., 2023). As such, it can be challenging to apply these frameworks in other jurisdictions where the same level of data is not available. Finally, in assessing subwatershed characteristics that may influence the percentage of septic effluent reaching streams, it is important to understand what characteristics impact the delivery of septic effluent to streams via rapid pathways potentially from failing septic systems versus septic effluent being delivered to streams via groundwater transport potentially from well-functioning septic systems. This may be determined by using human-specific wastewater tracers that can detect septic effluent delivered to streams via all pathways (e.g., acesulfame) and from only rapid pathways (e.g., HF183).

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Chapter 3

3 Broad-scale Analysis of Factors Influencing Septic System Wastewater Effluent Inputs to Streams

3.1 Introduction

On-site wastewater treatment systems, particularly septic systems, are a widely used and cost-effective technology for the treatment and dispersal of human wastewater in rural and suburban areas not served by centralized wastewater treatment infrastructure. Private septic systems are used in 14% of Canadian and 13% of Ontario households (Statistics Canada, 2011). In the United States (U.S.), it is estimated that between 20-25% of existing households and 30% of all newly constructed households use septic systems (Capps et al., 2021). The wastewater effluent from septic systems (herein referred to as septic effluent) contains constituents that pose a threat to human and/or ecosystem health, including nutrients, pathogens, and contaminants of emerging concern (e.g., pharmaceuticals, personal care products, and microplastics) (Schaider et al., 2016). More wastewater constituents enter the environment when a septic system is poorly designed, installed, or maintained, but even well-functioning systems are not able to remove (retain or degrade) all constituents and more conservative constituents are released. As such, both well- and poorly functioning systems represent sources of wastewater constituents to the environment with the potential for constituents to pollute nearby surface waters (Withers et al., 2014).

Many jurisdictions around the world have regulations for septic system design, siting, and installation. In Ontario, Canada, septic systems regulations are included in the Ontario Building Code (Ontario Building Code, O.Reg. 332/12, ss.8) and the Environmental Protection Act (Sewage Systems, O Reg 244/09, 2009). Septic systems are designed to partially treat wastewater through two main components: the septic tank and the drain field. In a well-functioning system, raw wastewater is delivered to a septic tank where settleable solids and lighter fats and oils are separated from the liquid wastewater fraction (Lusk et al., 2017). The liquid fraction is then delivered to the subsurface through a drain field consisting of perforated pipes placed in a material of engineered soil. The soil should be well-drained and there should be a vertical separation distance between the perforated pipes and the seasonally high groundwater table (e.g., at least 0.6 m). Wastewater constituents in the liquid effluent released to the subsurface may be attenuated as they travel through the unsaturated zone including through a biomat layer. Constituents that are not attenuated may reach the groundwater table and subsequently travel downgradient with the groundwater flow. Septic system regulations typically specify minimum setback distances to reduce the risk of wastewater constituents reaching vulnerable receptors. For example, in the Ontario Building Code, the drain field must be located more that 15 m from a surface water body, 15 m from a drilled water supply well, and 30 m from a dug well. Additionally, the septic tank must be installed at least 1.2 m below the ground surface, and the slope of the drain field must not exceed 25%. Where site conditions are not ideal for septic system installation (e.g., poorly drained soil), adaptive measures such as shallow buried trenches or, if necessary, a holding tank system may be required.

Despite the regulations, not all existing septic systems are properly designed, installed, or maintained, which can lead to high release of wastewater constituents into the environment. For example, low permeability soils in a drain field may limit infiltration and cause possible breakout of the septic effluent to the ground surface (Flanagan et al., 2020; Noss et al., 1988). Similarly, the attenuation of constituents in the unsaturated zone may be limited if there is insufficient vertical separation distance between a drain field and the groundwater table (Cooper et al., 2016; Humphrey et al., 2017). The placement of a septic system on fractured bedrock can reduce treatment efficiency as the fractures can act as a rapid pathway for septic effluent to reach the groundwater table and subsequently nearby surface waters, with limited subsurface attenuation (Dano et al., 2008; Marshall et al., 2022). Septic systems must be maintained regularly to ensure optimal performance. This includes pumping out a septic tank every 3-5 years and ensuring that there is no breakout of the septic effluent to the ground surface above the drain field. Furthermore, it is recommended that a septic system is replaced every 25-30 years as increased failures are observed when this lifetime is exceeded (Clayton, 1974; Connelly et al., 2023; USEPA, 2005; Winneberger, 1975). Many homeowners, particularly in low socioeconomic areas, may face financial barriers preventing proper septic system maintenance resulting in reduced septic system performance (Capps et al., 2020; Devitt et al., 2016). Additionally, it has been noted that many homeowners are not aware of the maintenance requirements of their septic system and often neglect best practices (Devitt et al., 2016).

For well-sited and functioning septic systems, effluent constituents that are not removed in the septic tank or subsurface (unsaturated and saturated zones) can reach surface waters via groundwater transport. If a septic system is poorly sited or underperforming, effluent constituents may reach surface waters via additional rapid pathways including i) subsurface preferential transport, ii) overland transport following septic effluent breakout to the ground surface, and iii) illegal direct pipes. Subsurface preferential transport may occur when septic systems are installed in fractured bedrock areas with shallow soil depth, when a shallow subsurface septic plume is intercepted by subsurface drains, (e.g., agricultural tile drains, residential french drains) or when encountering utility trenches (Oldfield et al., 2020; Spiese et al., 2014). Raw and/or partially treated wastewater may also be delivered to surface waters via overland transport if the effluent breaks out to the ground surface. Unless a septic system is near a surface water body, this pathway is typically only active under wet conditions (Noss & Billa, 1988). Finally, raw or partially treated wastewater may be released directly into nearby surface waters (or to a nearby ditch) through an illegal direct pipe, also referred to as a 'hot pipe', where a pipe connected upstream or downstream of the septic tank releases effluent directly connected to a surface water body (Maxcy-Brown et al., 2021). Direct pipes are illegal and have been reported to occur more often in rural low socioeconomic communities (Coleman Flowers et al., 2019; USEPA, 2002a). Understanding the contribution of slow and more rapid direct pathways in delivering septic effluent to surface waters is needed for assessing the potential impacts of septic systems on surface water quality. Additionally, understanding the pathways can provide insight into the types and prevalence of septic system failures as needed to inform septic system programs and policy (e.g., septic system reinspection programs).

Recent studies have developed and applied regional scale risk assessment frameworks to evaluate the potential for failing septic systems or the potential impact of septic systems on surface water quality. A study by Capps et al. (2020) used data on physical (e.g., soil type, distance to stream, and slope of terrain), and socioeconomic factors (e.g., septic system age, poverty and race) to assess the risk of septic systems to have adverse environmental impacts in Athens-Clarke County, Georgia (314 km^2). Connelly et al. (2023) extended this work by exploring the relationships between various systems (e.g., age of septic systems) and physical/environmental level variables (e.g., distance to streams, topographic wetness index and soil type) on septic system maintenance practices (e.g., pumping, and non-routine pumping). Oosting & Joy (2011) preformed a similar risk assessment using terrain and geologic risk factors (e.g., soil type, land slope, floodplain) and design risk factors (e.g., lot size, proximity to surface waters, system age) of septic systems in Huron-Kinloss Township, Ontario, do determine areas of higher septic system failure risk. In this work, the weights were assigned based on the relative importance given by septic system professionals. While these studies present valuable approaches to quantify and map the risks associated with septic system performance, the factors and associated weightings applied were not validated with field investigations. More recently, Jordan et al. (2023) presented a similar risk assessment approach using an a GIS based multicriteria decision analysis (MCDA) for environmental/physical (e.g., slope of terrain, proximity to surface waters, soil drainage class) and system level (e.g., septic system age, septic system density) with weighting done by analytical hierarchy process methods with weights based by Oosting & Joy (2011), Carroll et al. (2006) and USEPA (2002b). The output of the MCDA was a map of the pollution potential of septic systems in the study subwatershed. The pollution potential map for the subwatershed was used to classify the areas upstream of the nine sampling locations. The pollution potential classification of upstream contributing areas was then used as a predictor of the *E. coli* concentrations measured at the nine sampling locations on six occasions, and it was found that the model with the pollution potential classification as a predictor fit best compared to alternative models. However, given that *E. coli* is not specific to human wastewater, the detection and abundance of *E. coli* in a river, particularly in more rural watersheds, may not be representative of the amount of septic effluent in the river. Additionally, this study focused on one watershed and, therefore, the study findings may not be generalizable.

Recent field studies have shown that artificial sweeteners and human microbial source tracking (MST) markers, typically at high concentrations in septic effluent (Ahmed et al., 2016; Snider et al., 2017), can be valuable tracers for tracking septic effluent in groundwater and surface waters (Digaletos et al., 2023; Snider et al., 2017; Spoelstra et al., 2020a; Van Stempvoort, Robertson, et al., 2011; Verhougstraete et al., 2015). Artificial sweeteners including acesulfame, sucralose, saccharin, and cyclamate are useful tracers as they are widely used in household consumer products such as diet drinks, medicines, and hygiene products (Buerge et al., 2009; Martyn et al., 2018). Of the artificial sweeteners, acesulfame has been the most widely used as it is relatively conservative in the environment (Buerge et al., 2011), undergoes limited attenuation in septic tanks (Snider et al., 2017) and groundwater plumes, is associated with few other sources (e.g., landfills), and can be detected at very low concentrations. Other artificial sweeteners are not as ideal as they are less conservative (cyclamate) (Buerge et al., 2011), have been linked to other sources in the environment (e.g., saccharin in pig feed) (Li et al., 2020; Ma et al., 2017), or are often below detection in surface waters (e.g., sucralose). The main challenge in using artificial sweeteners, including acesulfame as wastewater tracers, is that because they are relatively conservative, their presence does not reflect the presence of other less conservative septic effluent constituents that are more likely to be attenuated along slow or long transport pathways (e.g., groundwater). In contrast to artificial sweeteners, MST markers may be used to detect and quantify more rapid (and direct) human wastewater contamination in surface waters. These markers are less mobile in the subsurface and have high decay rates, for example, with 99% attenuation occurring in < 8 days in fresh surface waters (Tambalo et al., 2012).The genus *Bacteroides* contains species that can be used to disentangle sources of microbial contamination in waters. The specific DNA sequence, HF183, from the *Bacteroides dorei* species, has shown promise in detecting human wastewater contamination due to its source specificity to humans and short survival times outside the gut of warm-blooded hosts (Bernhard & Field, 2000; E. Li et al., 2021). The use of HF183 as a co-tracer with artificial sweeteners may allow quantification of the relative importance of slow pathways (e.g., groundwater) and more rapid pathways (e.g., subsurface drains, surface overland flow, direct pipes) in delivering septic effluent to surface waters. This can provide an indication of potential inputs of more conservative and less conservative, respectively, effluent constituents reaching surface waters.

Some studies have used human wastewater tracers, including artificial sweeteners and human MST markers, to detect and quantify septic effluent in streams using broad-scale field sampling. Verhougstraete et al. (2015) conducted a broad-scale stream sampling program across the Lower Michigan Peninsula, U.S., to explore the linkages between fecal contamination and regional-scale physical, geochemical, and hydrological characteristics. They analysed stream samples for *E. coli* and the human MST marker *Bacteroides thetaiotaomicron* and showed through classification and regression tree (CART) analysis that while fecal contamination increased with the number of septic systems, the relationship between landscape and hydrologic characteristics and septic-derived contamination in surface waters was found to be complex. More recently, Oldfield et al. (2020) and Tamang et al. (2021) used measured artificial sweetener (primarily acesulfame) concentrations in streams to estimate the percentage of septic effluent in each subwatershed that reaches the outlets of eight and four subwatersheds, respectively, in the Lake Erie Basin and Lake Simcoe Basin, Ontario, Canada. Tamang et al. (2021) indicated that the percentage of septic system effluent reaching a subwatershed outlet was higher in subwatersheds with older households compared to those with newer households, but insufficient subwatersheds (nine) were sampled to evaluate the influence of physical and socioeconomic subwatershed characteristics on the percentage of septic effluent reaching streams. Oldfield et al. (2020) and Tamang et al. (2022) also showed that conservative septic effluent tracers (e.g., artificial sweeteners) in streams tend to be higher during high flow compared to low flow conditions. While this may be partially due to the reconnection of disconnected stream reaches under high flow conditions, it may also be caused by contributions from overland transport or flushing of effluent constituents from the subsurface during wet weather conditions. Furthermore, Oldfield et al. (2020) showed that stream concentrations of conservative septic effluent tracers are higher in the early spring months compared to the reminder of the year. This seasonal variability may be due to higher groundwater discharge to streams during spring, or higher groundwater tables activating more rapid pathways including short-circuiting via subsurface drains (e.g., agricultural tile drains), or overland transport. As Oldfield et al. (2020) and Tamang et al. (2022) only used artificial sweeteners to quantify septic effluent inputs to streams, they were not able to provide direct evidence of the relative contributions of slow versus rapid pathways in delivering septic effluent to streams including how these contributions vary between flow conditions.

The primary objective of this study was to investigate the influence of physical and socioeconomic factors, as well as stream flow variability, on the percentage of septic effluent delivered to streams with consideration of the potential differences between inputs from slow and rapid transport pathways. To achieve this objective, extensive stream sampling for septic effluent tracers was conducted at the outlets of 39 subwatersheds across the Lake Erie and Lake Simcoe Basins, Ontario, Canada over a 22-month period and this was combined with previous field data from Oldfield et al. (2020) and Tamang et al. (2022). A secondary objective of this study was to add to the limited data on the percentage of septic system effluent reaching the subwatershed outlets using data for 39 subwatersheds which have not been previously sampled. Subwatersheds in the study were selected that have no other major sources of human wastewater effluent (e.g., wastewater treatment plant outfalls) and have varying physical and socioeconomic characteristics. The stream samples were analyzed for artificial sweeteners (acesulfame and saccharin), HF183 MST marker, and nutrients, with this data analyzed together with information on septic system location and physical and socioeconomic data. In providing an understanding of the relative importance of rapid versus slow pathways, the findings from this study also increase our understanding of the type and prevalence of septic system failure in subwatersheds with different characteristics. This information is needed to provide guidance on suitable areas for the installation of septic systems and to focus on management and educational awareness efforts.

3.2 Methodology

3.2.1 Study area

For this study, stream sampling was conducted at the outlets of 39 subwatersheds located in the Lake Erie Basin and the Lake Simcoe Basin in Ontario, Canada. The subwatersheds were selected because they have (i) high numbers and density of septic systems, (ii) no other major sources of artificial sweeteners and human-specific MST markers in the subwatershed (outfalls from WWTPs, landfills, leaky municipal sewage infrastructure, major recreational areas), (iii) varying physical (e.g., soil drainage class, depth to bedrock) and socioeconomic characteristics (e.g., household income, household age), and (iv) adequate site accessibility for sampling. The stream sampling data for these subwatersheds was combined with field data previously collected for three subwatersheds by Oldfield et al. (2020) and nine subwatersheds by Tamang et al. (2022).

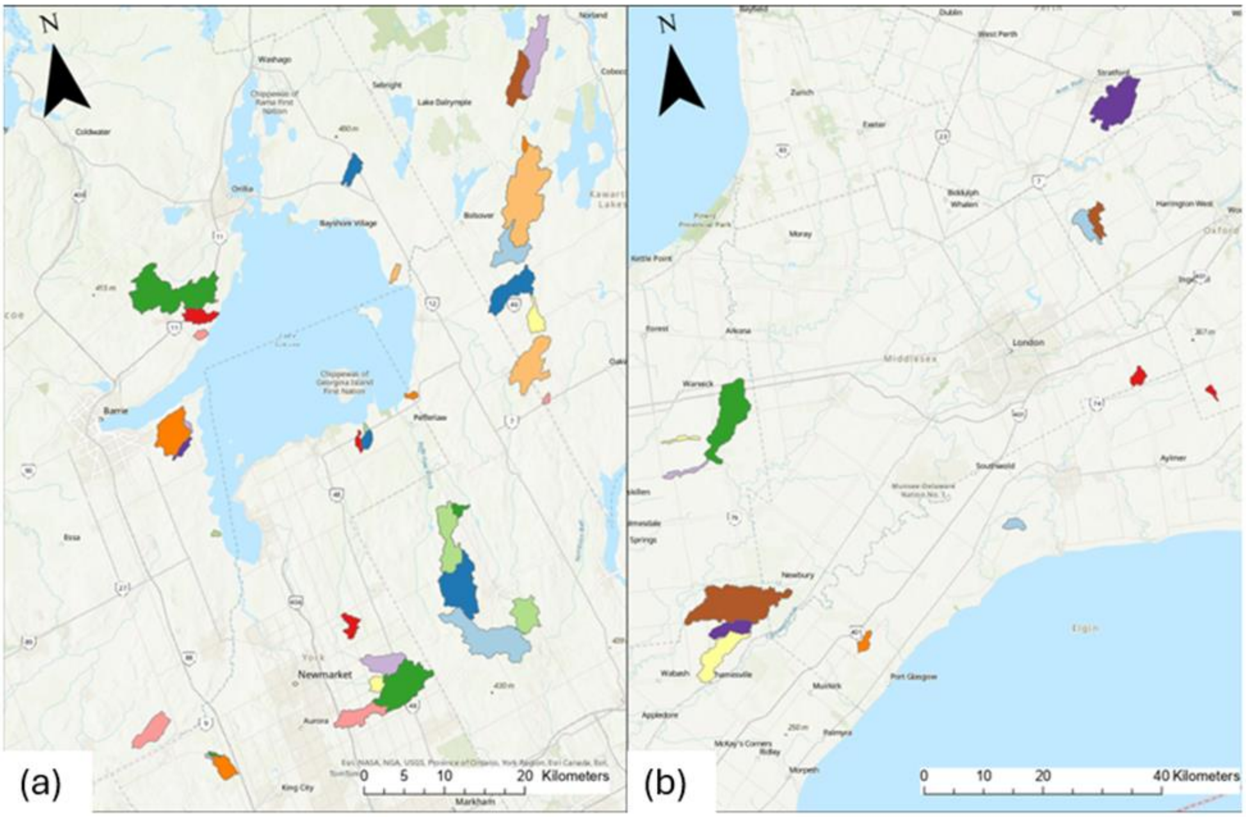

Figure 3-1: Map of the subwatersheds studied. Shown in (a) are the subwatersheds studied in the Lake Simcoe Basin. Shown in (b) are the subwatersheds studied in the Ontario Lake Erie Basin.

3.2.2 Characterization of the study subwatersheds

The boundaries of all subwatersheds were delineated using the Ontario Watershed Information Tool (OMNRF, 2018). A remote sensing approach developed and applied by Gao et al. (2024) was used to determine the locations of individual septic systems in subwatersheds. All subwatersheds were characterized by assigning physical and socioeconomic factors to each septic system within the subwatershed using publicly available geospatial data (see for data sets used, Table 3-1). The nine physical factors included (1) being on built-up land (OMNRF, 2014a), (2) on high permeability land (OGS, 2010), and the average drift thickness at the septic system location was determined (OGS, 2006). The (3) percentage on bedrock was calculated as the percentage of septic systems on less than 2 m of drift thickness in a subwatershed. A subwatershed was classified as having septic systems (4) near field tile drains if more than 20% of the septic systems were located within 100 m of a land parcel that had tile drainage (OMAFRA, 2019). The (5) septic system density was calculated by dividing the number of septic systems by the subwatershed area. The flow distance tool in ArcGIS Spatial Analyst toolbox (ESRI, 2023) was used together with the Ontario Integrated Hydrology (OIH) stream raster (STR), the digital elevation model (DEM) with filled sinks, and the flow direction raster (FDR) datasets to produce a flow distance raster (OMNRF, 2014b) for calculating the (6) setback distances (horizontal flow distance) between each septic system and the tributary. The vertical flow distance was also calculated, and the (7) slope of the flow path between each septic system and the tributary was calculated by dividing the vertical flow distance by the horizontal flow distance. The slope tool in ArcGIS was used to convert the OIH DEM with the filled sinks dataset (OMNRF, 2014b) to calculate the (8) topographic slope at the location of each septic system. From this, the topographic wetness index (9) (TWI) was calculated using:

$$\mathrm{TWI} = \ln\left(\frac{\alpha}{tan\beta}\right) \qquad (1)$$

where α is the catchment area and β is the slope in percent rise of the upstream contributing area. To calculate the specific catchment area, the flow accumulation grid was first calculated using the FDR dataset from the OIH (OMNRF, 2014b). The specific catchment area was then calculated by multiplying the flow accumulation cell count by the raster cell size.

Canadian Census of population data (Statistics Canada, 2021) was used to determine the five socioeconomic characteristics for each subwatershed including (1) mean occupied home construction age, (2) percent of dwellings occupied by renters, (3) median after-tax household income, (4) percent of low-income homes, and (5) percent of home requiring major repairs. The construction date of occupied homes was used as an indication of the age of septic systems since septic system age date data was not available. To compile the socioeconomic characteristics, census data tables for dissemination areas (DA) that intersected the study subwatersheds were accessed using the cancensus package in R (von Bergmann et al., 2021). When a subwatershed crossed multiple dissemination areas, the characteristics of a subwatershed were calculated by computing a weighted average value with weights proportional to the number of septic systems located within each DA block divided by the total number of septic systems in the subwatershed.

Table 3-1: Description of the characteristics and associated datasets used to classify the septic systems within a subwatershed.

Parameter Name	Description	Data Set	Source
Percent on built up lands	Percent of septic systems on built-up lands	SOLRIS-Built up area	(OMNRF, 2014a)
Percent on high permeability	Percent of septic systems on high permeability lands	OMAFRA soil survey	(OMAFRA, 2019a)
Drift thickness	Average drift thickness below septic systems	Overburden thickness map	(OGS, 2006)
Percent on bedrock	Percent of septic systems on less than 2 m of drift thickness	Overburden thickness map	(OGS, 2006)
Tiled	If a subwatershed has more than 20% of septic systems placed near tile drained areas	Tile drainage area	(OMAFRA, 2019b)
Septic system density	Spatial density of septic systems	Calculated	(Gao et al., 2024)
Setback distance	Average horizontal flow path distance between septic systems and stream	OIH (Calculated)	(OMNRF, 2014b)
Slope of flow paths	Average slope of flow path between septic systems and stream	OIH (Calculated)	(OMNRF, 2014b)
Topographic slope at the placement site	Average topographic slope at location of septic systems	OIH (Calculated)	(OMNRF, 2014b)
TWI	Average topographic wetness index of septic systems in a subwatershed	OIH (Calculated)	(OMNRF, 2014b)
Age of occupied homes	Weighted average of mean occupied home age from 2021	Census of Population, 2021	(Statistics Canada, 2021)
Percent renter	Weighted average of percent dwellings occupied by renters	Census of Population, 2021	(Statistics Canada, 2021)

Median after-tax income	Weighted average median after-tax household income in 2020 $	Census of Population, 2021	(Statistics Canada, 2021)
Low income	Weighted average of percent of low-income households	Census of Population, 2021	(Statistics Canada, 2021)
Percent requiring major repairs	Weighted average of percent of homes requiring major repairs	Census of Population, 2021	(Statistics Canada, 2021)

3.2.3 Field Sampling

Field sampling for this study was carried out from October 2021 to August 2023. Stream sampling was conducted during multiple seasons and flow conditions with a minimum of five sampling events conducted for each subwatershed. Stream samples were collected from the middle of the stream for analysis of artificial sweeteners (acesulfame, saccharin, sucralose, cyclamate), ammonium ([NH_4^+]), soluble reactive phosphorus (SRP), *E. coli* and human-specific MST markers (HF183, Human Mitochondrial [HumMit] markers). Samples collected for artificial sweeteners, ammonium and SRP were filtered (0.45µm cellulose acetate syringe filter) and placed in 60 mL HDPE bottles without headspace. For analysis of *E. coli* and MST markers, stream samples were collected in two 1L sterile HDPE bottles according to U.S. EPA Method 1103.1 (U.S. EPA, 2014). All collected samples were placed on ice in a cooler for transportation to the Western University laboratory. Upon return to the laboratory, the sample bottles for the SRP analysis were placed in a refrigerator and analyzed within 48 hours of sample collection. The samples collected for *E. coli* and MST marker analyses were processed in the laboratory within six hours of collection. Samples collected for artificial sweeteners, and ammonium were frozen and later shipped. The samples were kept frozen until analysis was conducted.

For all sampling locations and times, stream discharge measurements were performed. The midpoint method (Turnipseed & Sauer, 2010) was used to calculate stream discharge with the stream stage and the stream velocity measured using an OTT Hydromet MF Pro velocity meter. Stream velocity measurements were taken at 60% depth of the stream with a minimum of 20 velocity and stage measurements made across the channel width. Sampling times were classified as high flow or low flow based on hydrographs for continuously monitored tributaries located near the sampling locations shown in Appendix B.

3.2.4 Chemical analytical methods

Water samples were analyzed for artificial sweeteners at the Canada Centre for Inland Waters (CCIW), Burlington, ON, using ion chromatography (Dionex 2500 system) coupled with tandem mass spectrometry (AB Sciex QTRAP 5500 triple-quadrupole). Further details of this method are provided in Spoelstra et al. (2020). The minimum detection limit and the practical quantification limit for acesulfame, saccharin, cyclamate, and sucralose were 2, 2, 3, and 20 ng/L, and 6, 6, 8, and 60 ng/L, respectively. Ammonium was also analyzed at CCIW using a Beckman Coulter DU 720 UV / Vis spectrophotometer equipped with a 1 cm flow-through cell; the detection limit was 0.01 mg/L. SRP was analyzed at Western University using the Lachat Quikchem 8500 Series 2 Flow Injection Analysis System with a detection limit of 1µg/L. *E. coli* was enumerated at Western University using sterile membrane filtration methods (U.S. EPA, 2014). Only a select set of stream samples were run for MST analyses via HF183 and HumMit marker digital PCR (dPCR) assays on extracted DNA. Samples selected for this analysis if they had high concentrations of artificial sweeteners, *E. coli* and/or from a sampling location with a previous MST marker detection. For this analysis, up to 300 mL of unfiltered sample was filtered (0.45 µm pore size, 47 mm diameter). The filters were frozen at -30°C and transported to Edge-Water DNA Inc, Toronto, ON, for DNA extraction using the Qiagen PowerSoil Pro kit. dPCR analyses used the PCR primers and probe for *Bacteroides* HF183, HumMit and DNA standards prepared by the U.S. National Institute of Standards and Technology (SRM 2917, Plas-mid DNA for Fecal Indicator Detection and Identification). Digital PCR assays were performed using the ThermoFisher QuantStudio™ 3D Digital PCR system with details provided in Edge et al. (2021). The detection limits for HF183 and HumMit were 13 DNA copies/100 mL.

3.2.5 Data analysis

3.2.5.1 Percentage of septic system effluent reaching the subwatershed outlets

As acesulfame is the most suitable artificial sweetener for tracing septic effluent in natural waters, acesulfame stream concentrations were used to calculate the percentage of septic effluent reaching the subwatershed outlets following the method used by Oldfield et al. (2020). For this, the stream mass load of acesulfame (g/d) at each sampling time was first calculated by multiplying the acesulfame stream concentration (ng/L) by the measured stream discharge (m^3/s). The percentage of septic effluent that reached the subwatershed outlets at the time of sampling was calculated by dividing the measured acesulfame stream load by a calculated acesulfame mass released by all the septic systems upstream of the outlet. The released acesulfame mass was calculated by multiplying an estimated daily acesulfame mass released from a single septic system by the number of septic systems upstream of the sampling location. The acesulfame released from a single septic system was estimated to be 0.255 g/d/septic system. This was determined by multiplying the mean concentration of acesulfame in single household septic tanks (median = 43.8 µg/L, standard deviation = 4.2 µg/L) as measured by Snider et al. (2017) by an average per capita water usage of 221.9 L/d/Person (Gauley Associates Ltd., 2016) and an average of 2.6 persons per household (Statistics Canada, 2016). This calculation is based on several assumptions including the assumption that all septic systems release the same acesulfame mass per day. Variability in acesulfame consumption between homes and potential temporal variability in consumption is expected, however due to the large number of septic systems upstream of each stream sampling point, this variability is assumed to be smoothed out.

Although acesulfame is considered to be highly conservative, studies have shown that acesulfame can be degraded in wastewater treatment processes and in the environment (Castronovo et al., 2017; Kahl et al., 2018). As such it is possible that the approach used for calculating the percentage of septic system effluent reaching the stream may underestimate the amount of conservative wastewater constituents reaching the sampling location. In addition, using the median acesulfame concentrations previously measured in septic tanks already takes into account any acesulfame degradation that may occur in the septic tank itself. The calculation does, however, neglects potential degradation that may occur in the drain field or as acesulfame is transported through the environment to the stream sampling locations. Neglecting this possible degradation of acesulfame may also lead to an underestimation of the percentage of septic effluent reaching the stream sampling location. Despite the assumptions associated with using acesulfame stream loads to calculate the percentage of septic effluent reaching the stream, the study focuses on the relative variability in the percentage of septic system effluent reaching the multiple stream sampling locations how this varies for different flow conditions, and therefore the findings of this study are expected to still hold despite the assumptions. Statistical analyses

Relationships between the different subwatershed characteristics (Table 3-1) were tested using a Pearson's cross moment correlation. Relationships between the tracers and other constituents measured in the streams were tested using the non-parametric Spearman's rank order correlation to accommodate for samples that were below the analytical detection limit (Helsel, 2012). For tracers with limited samples below the analytical detection limit, Pearson cross-moment correlation was also calculated to assess their linear association. All correlation analyses were conducted in R (R Core Team, 2023) using the Hmisc package (Harrell Jr., 2023). The significance level used was 0.05.

The effect of subwatershed characteristics and flow conditions on the percentage of septic effluent reaching the subwatershed outlets was examined using mixed-effects models. Mixed effects models have been used in various fields including to identify nutrient concentrations by various event flow conditions (Lessels & Bishop, 2013). Prior to analysis, the percentage of septic effluent was transformed towards normality using a log transformation. The flow conditions (high and low) were reference binary coded with the high flow conditions set as the reference category (set as 0). The continuous subwatershed characteristics were Z-score standardized prior to analysis as they differ in scale. Linear Mixed Effects Regression models were constructed in R statistical software (R Core Team, 2023) using the package lme4 (Bates et al., 2015) with maximum likelihood (ML). The marginal significance of the model terms was calculated using the lmerTest package (Kuznetsova et al., 2017). Model performance and assumption checks were completed using various functions from the easystats package (Lüdecke et al., 2022). Random intercept linear mixed effect models were constructed with increasing complexity. First, a null random intercept model was fit to examine the variance accounted for by the clustering structure in the data set. The next model evaluated the influence of (high, low) flow when sampling was conducted, tested as fixed and random effects. Models were fitted using all subsets selection. Each model was evaluated using AIC and BIC; however, the AIC value was used to select the best performing model. Models within 2 AIC units of the best performing model are considered plausible and are reported. Model averaging of the models withing 2 AIC units of the top performing model was conducted using AIC weights. Each of the plausible models was evaluated for collinearity of predictors, autocorrelation, model singularity, homogeneity of error variance, normality of random effects, and normality of residuals using visual inspection. Small violations of the assumptions were deemed to be insignificant, as it has been shown that linear mixed-effects models are robust to violations, specifically the distributional assumptions of residuals (Schielzeth et al., 2020). For each of the plausible models, additional fit and performance indices such as $R^2_{marginal}$ and $R^2_{conditional}$ and bootstrapped prediction accuracy were computed.

3.2.6 HF183 data analyses

HF183 detections were classified using the risk-based water quality threshold (RBT) of 525 DNA Copies/100mL for HF183 as defined by (Boehm & Soller, 2020). Using the threshold value of 525 DNA Copies/100mL, each subwatershed was classified as “high”, “low” and “unlikely”. If any sample for a subwatershed had a HF183 concentrations above 525 DNA Copies/100mL (risk-based threshold for HF183, Boehm & Soller (2020)), the subwatershed was classified as having “high” fecal contamination, if any sample for a subwatershed had a HF183 concentration above the detection limit (13 DNA Copies/100mL) the subwatershed was classified as having “low” fecal contamination. Subwatersheds were classified as having “unlikely” microbial contamination when no samples analyzed were above the detection limit. Additionally, the subwatersheds for which no samples were analyzed due to low concentrations of artificial sweeteners and *E. coli* were also classified as “unlikely”. The relationship between the characteristics of the subwatershed and the level of HF183 detection was explored using Kruskal-Wallis non-parametric ANOVA with a Dunn post hoc test conducted in R (R Core Team, 2023).

Additionally, Spearman's rank correlations were computed between the ordinal levels of the HF183 classification and subwatershed characteristics. Finally, a principal component analysis (PCA) was conducted to explore the relationships between various subwatershed characteristics and the HF183 classification.

3.3 Results and discussion

3.3.1 Spatial characteristics of study subwatersheds

A summary of the physical and socioeconomic characteristics of the 39 subwatersheds monitored for this study and the subwatersheds monitored by Oldfield et al. (2020) and Tamang et al. (2022) are shown in Table 3-2. Distribution plots of these characteristics are provided in Appendix A. Subwatershed areas ranged from 0.045 to 77 km^2 with a mean subwatershed area of 14 km^2. The number of septic systems in the subwatersheds ranged from 10 to 2250 with a mean of 155.

Table 3-2: Summary statistics of the subwatershed characteristics considered in the statistical analyses.

	Mean	Median	SD	Minimum	Maximum
Tiled	0.46	0	0.5	0	1
Percent on high permeability (%)	41	25	38	0	100
Percent on built up (%)	44	47	33	0	100
Drift thickness (m)	60	53	51	0.66	160
Percent on bedrock (%)	19	0	42	0	200
TWI	11	12	0.53	10	13
Topographic slope of placement (%)	3.2	2.9	1.6	0.99	8
Slope of flow path (%)	0.023	0.02	0.013	0.0043	0.054
Setback distance (m)	380	350	230	58	1400
Density (#/ $km^{2)}$	33	8.8	88	1.3	580
Watershed area (km2)	14	6.4	17	0.045	77
Age of occupied homes (yrs.)	49	50	8.4	31	65
Median after-tax income ($)	87000	87000	17000	59000	130000
Low income (%)	8.5	7.7	3.4	3.4	16
Percent renter (%)	12	12	5.5	1	26
Percent requiring major repairs (%)	6.6	6.3	3.5	0	16

Pearson's cross-moment correlations indicated multiple significant (α=0.05) correlations amongst the subwatershed characteristics (Figure 3-2). For instance, a significant moderate negative correlation was observed between TWI and the topographic slope of the septic system placement ($r(44)= -0.78$, $p<.001$). This relationship is expected as the topographic slope area is an input parameter for calculating TWI. Also, as expected, there was a significant correlation between the percentage of low-income households and the median after-tax income ($r(44)= -0.75$, $p<.001$). The median after-tax household income was also found to be significantly negatively correlated with the age of occupied homes ($r(44)=-0.48$, $p<.001$) suggesting that areas where households have higher median after-tax income tend to have newer homes. The subwatershed septic system density and the percent of septic systems placed on built-up land also showed a significant correlation ($r(44)= 0.36$, $p<.05$) due to areas with more urban built-up infrastructure having higher septic system densities (e.g., rural hamlets).

It is important to note that some spurious correlations are also observed between the physical and socioeconomic characteristics. These may be a function of the Southern Ontario geographic region and the specific subwatersheds sampled. For instance, areas of higher drift thickness were found to have significantly higher median after-tax income ($r(44)= 0.74$, $p<.001$) but this relationship may be due to areas with high drift thickness being located in the southern Lake Simcoe Basin, which contains affluent suburbs of the Greater Toronto Area. Furthermore, spurious significant correlations were observed between the age of occupied homes and physical characteristics (e.g. topographic slope of the placement) as seen in Figure 3-2. In applying the statistical analyses in the following sections, these correlations between the subwatershed parameters were considered.

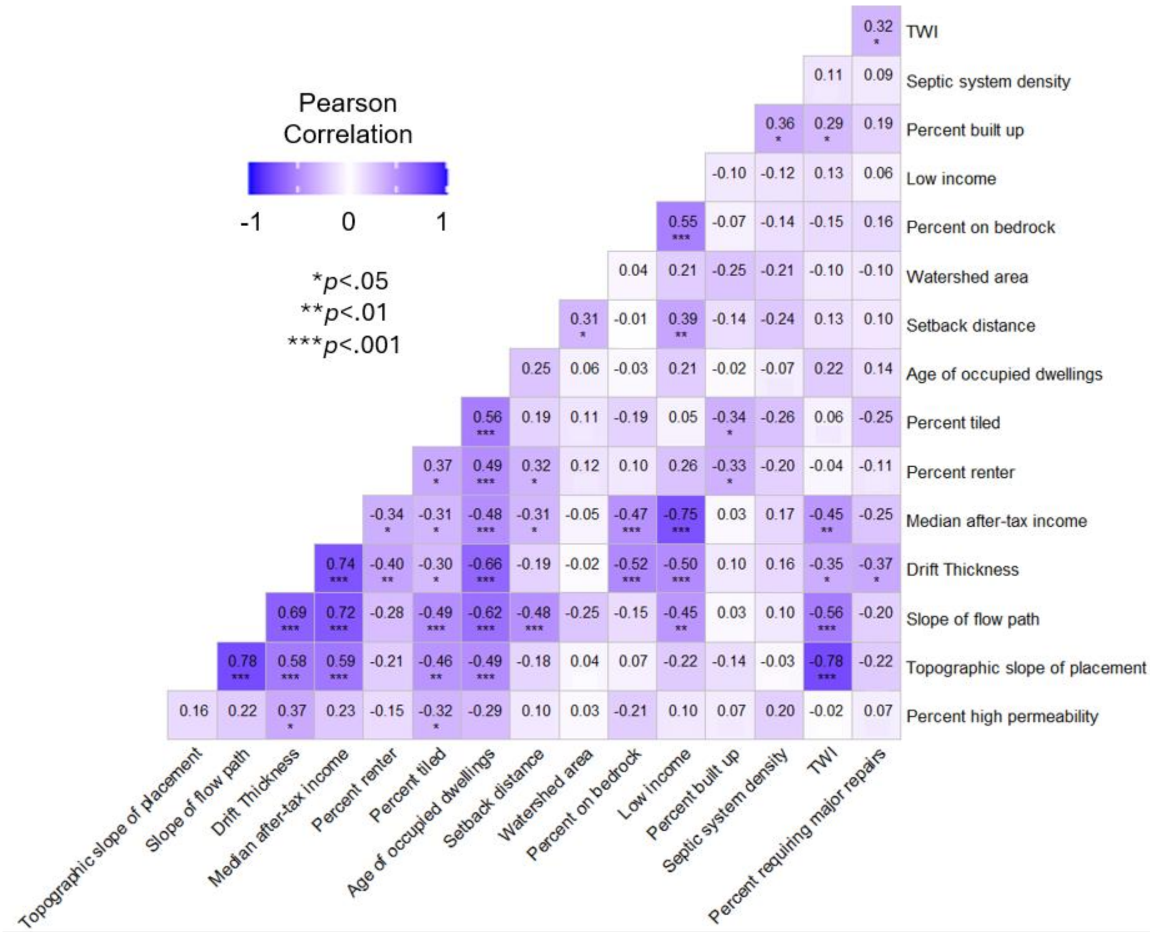

Figure 3-2: Pearson's cross-moment correlogram of the physical and socioeconomic characteristics of the study subwatersheds.

3.3.2 Septic effluent tracer results

3.3.2.1 Artificial sweeteners

The artificial sweeteners acesulfame, saccharin, cyclamate, and sucralose were analyzed for all samples collected at each subwatershed outlet. The artificial sweetener concentrations were highly variable between subwatersheds and between sampling times within a subwatershed (large interquartile range, IQR; Figure C-1, Appendix C. Acesulfame was detected in 97.5% of samples analyzed compared to sucralose being detected in only 34% of samples potentially due to the higher analytical detection limit (Figure 3-3), indicating its potential challenges as a wastewater tracer. Generally, the artificial sweeteners were detected more frequently and at higher concentrations in smaller subwatersheds compared to larger subwatersheds, which is particularly evident for acesulfame and sucralose (Figure 3-3) (both highly conservative tracers; (Buerge et al., 2011; Van Stempvoort et al., 2020)). This likely reflects less dilution of the artificial sweetener in presumably smaller streams with lower flows (Figure 3-3a) draining smaller subwatersheds, noting that the watershed area is poorly correlated with septic system density (Figure 3-2). However, it may also result from greater attenuation of artificial sweeteners in longer streams. This fact is especially applicable to cyclamate. Cyclamate has a much shorter half-life compared to other artificial sweeteners (Buerge et al., 2011; Ma et al., 2017) and therefore in larger subwatersheds cyclamate is more likely to decay. No clear trends were observed for saccharin concentrations with respect to the subwatershed area; this along with high saccharin concentrations in some subwatersheds (>1000 ng/L, subwatersheds 1, 4, and 12) may be a result of additional (non-septic) sources of saccharin, noting it is commonly included in pig feed and is a metabolite of several sulfonylurea-based herbicides (Buerge et al., 2011; D. Li et al., 2020). Acesulfame with its conservative properties and low analytical detection limit, is regarded as the best artificial sweetener to use as a wastewater tracer and is used in the data analysis conducted for this study. For all artificial sweeteners, it is possible that differences in consumption and usage rates may also contribute to some of the variation observed across subwatersheds.

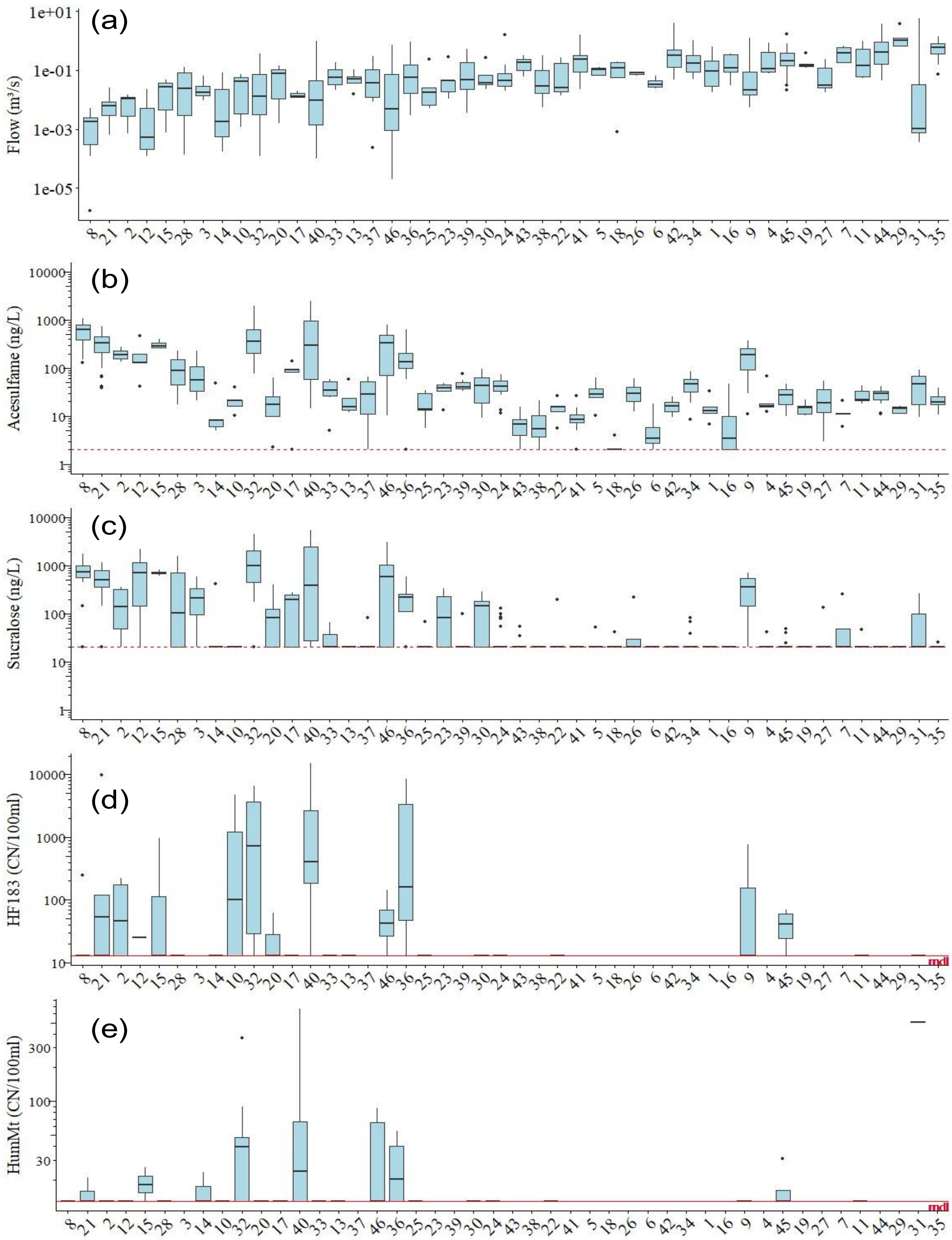

Figure 3-3: Summary of (a) flows and concentrations of (b) acesulfame, (c) sucralose and MST markers (d) HF183, and (e) HumMit measured at the outlets of the study subwatersheds. Subwatersheds are arranged by increasing subwatershed area from

left to right. Samples with concentrations below the detection limit are set at the detection limit indicated by the red dashed line. In the box plots, the horizontal line is the median, the box represents the upper and lower quartile ranges, and the whiskers extend to the maximum and minimum data with the exception of outliers.

3.3.2.2 *E. coli* and MST markers

E. coli was analyzed for all samples collected from the 39 subwatersheds sampled between 2021-2023, but not those of Oldfield et al. (2020) and Tamang et al. (2022) (i.e., samples collected in subwatersheds 34-39 and 41-44 prior 2021). *E. coli* is not a human-specific wastewater tracer and therefore the variability of the *E. coli* concentrations across subwatersheds and sampling can be influenced by several non-septic system sources including agricultural runoff. Similar to saccharin, this may indicate potential contribution from additional human non-wastewater sources. Therefore, using *E. coli* concentrations for estimating septic effluent in streams in rural subwatersheds is expected to misrepresent the level of septic effluent contamination (R. Sowah et al., 2014). *E. coli* concentrations are shown for the study subwatersheds in Figure C-2 in Appendix C.

Human-specific MST markers HF183 and HumMit were detected in 64% and 30% of the 79 samples with high artificial sweeteners and *E. coli* concentrations. Samples with low artificial sweetener and *E. coli* concentrations were not analyzed for MST markers (166 samples) and therefore the detection frequency is likely much lower. Of the samples analyzed for HF183, 21.5% were above the risk-based water quality threshold (RBT) of 525 CN/100mL as defined by Boehm & Soller (2020). The HF183 concentrations measured at the subwatershed outlets are considerably lower compared to tributary and nearshore lake sample concentrations reported by Edge et al. (2021) from a more urbanized area (City of Toronto). Cantor et al. (2017) similarly noted infrequent detection of HF183 in rural subwatersheds with confirmed upstream wastewater discharges. These lower concentrations combined with many samples below the MDL may pose potential difficulties for using human-specific MST markers in rural areas, however, it also means that the detection of HF183 or HumMit is noteworthy because it suggests that there is a nearby potentially problematic septic system from which the effluent is rapidly reaching the stream (via overland flow, direct pipe, short-circuiting pathways). This is because compared to artificial sweeteners, HF183 and HumMit markers are not overly mobile in groundwater and are non-conservative with short half-lives in surface waters (Tambalo et al., 2012; Walters & Field, 2009). As expected, due to dilution effects combined with the rapid decay of MST markers, the markers were detected more frequently and in greater magnitude in smaller subwatersheds (**Figure 3-3**; Tambalo et al., 2012; Walters & Field, 2009).

3.3.3 Relationships between septic effluent tracers and nutrients

The relationships between the artificial sweeteners, nutrients, *E. coli* and MST markers were explored with a Spearman rank order correlation to highlight the differences in their characteristics as tracers of septic effluent. The Spearman rank order correlation shows several significant correlations between artificial sweeteners, microbial tracers, and nutrients (NH_4-N and SRP; Figure 3-4). For the artificial sweeteners, a significant positive correlation was observed between acesulfame and sucralose (ρ=0.75, $p<.001$) this is expected given that these artificial sweeteners are both commonly found in household products and considered highly conservative (Buerge et al., 2009). Acesulfame and saccharin were also found to be significantly correlated, however, the magnitude of the correlation coefficient was lower than that of acesulfame and sucralose (ρ=0.38, $p<.001$). This is not unexpected because high saccharin concentrations may also be due to contributions from agricultural sources (Ma et al., 2017). Spearman correlations between cyclamate and other wastewater tracers are significant. As it is less conservative, when cyclamate concentrations are elevated, it may indicate a recent release of septic effluent that would most likely correspond to elevated concentrations of other wastewater tracers.

Comparing the artificial sweeteners with other septic effluent constituents, all artificial sweeteners were significantly correlated with SRP, with the strongest correlation observed with saccharin (ρ=0.43, $p<.001$), and a significant correlation with acesulfame (ρ=0.35, $p<.001$). This was unexpected given the multiple confounding sources of SRP in the subwatersheds compared to artificial sweeteners. The strongest correlation with SRP was *E. coli* (ρ=0.47, $p<.001$), with moderate correlations with the MST markers HF183 and HumMit (ρ=0.44, $p<.001$, ρ=0.39, $p<.001$, respectively). It is possible that the significant correlation between SRP and saccharin, *E. coli* and MST markers may indicate rapid septic effluent inputs through tile drains in wet periods, which also capture agricultural runoff containing higher levels of SRP. Several correlations between the wastewater tracers and ammonium were observed with the largest magnitude with acesulfame (ρ=0.49, $p<.001$). Additionally, moderately correlations were between NH_4-N and the MST markers (ρ=0.42, $p<.001$, ρ=0.44, $p<.001$, respectively). The correlations between acesulfame and sucralose with HF183 were not significant (ρ=0.22, $p>.05$, ρ=0.07, $p>.05$, respectively) but cyclamate and saccharin were significantly correlated with HF183 (ρ=0.39, $p<.001$, ρ=0.28, $p>.01$, respectively). The highest correlation between an artificial sweetener and HF183 was with cyclamate, which may be due to the similarities in transport properties and short half-lives of the two compounds, possibly indicating the rapid septic effluent inputs. The use of cyclamate as an indicator of rapid wastewater input from a wastewater treatment facility was explored by Zirlewagen et al. (2016), as they determined that detection of cyclamate accompanied by an increase in acesulfame concentration may indicate the presence of a recent wastewater input near a sampling location. The lack of correlation between acesulfame and sucralose and HF183 is expected given their distinctive transport behavior and reactivity in the environment and illustrates that using artificial sweeteners combined with HF183 may be a valuable technique for distinguishing the pathways (slow versus rapid) via which septic effluent is transport to subwatershed outlets. Additionally, the lack of correlation suggests that high detection of acesulfame or sucralose does not always correspond to a high HF183 concentration. The correlation between the MST markers (HF183 and HumMit) was found to be significant with moderate strength (ρ=0.55, $p>.001$). This correlation highlights potential differences between the two MST markers and may be possibly due to the lower detection frequency of the HumMit marker.

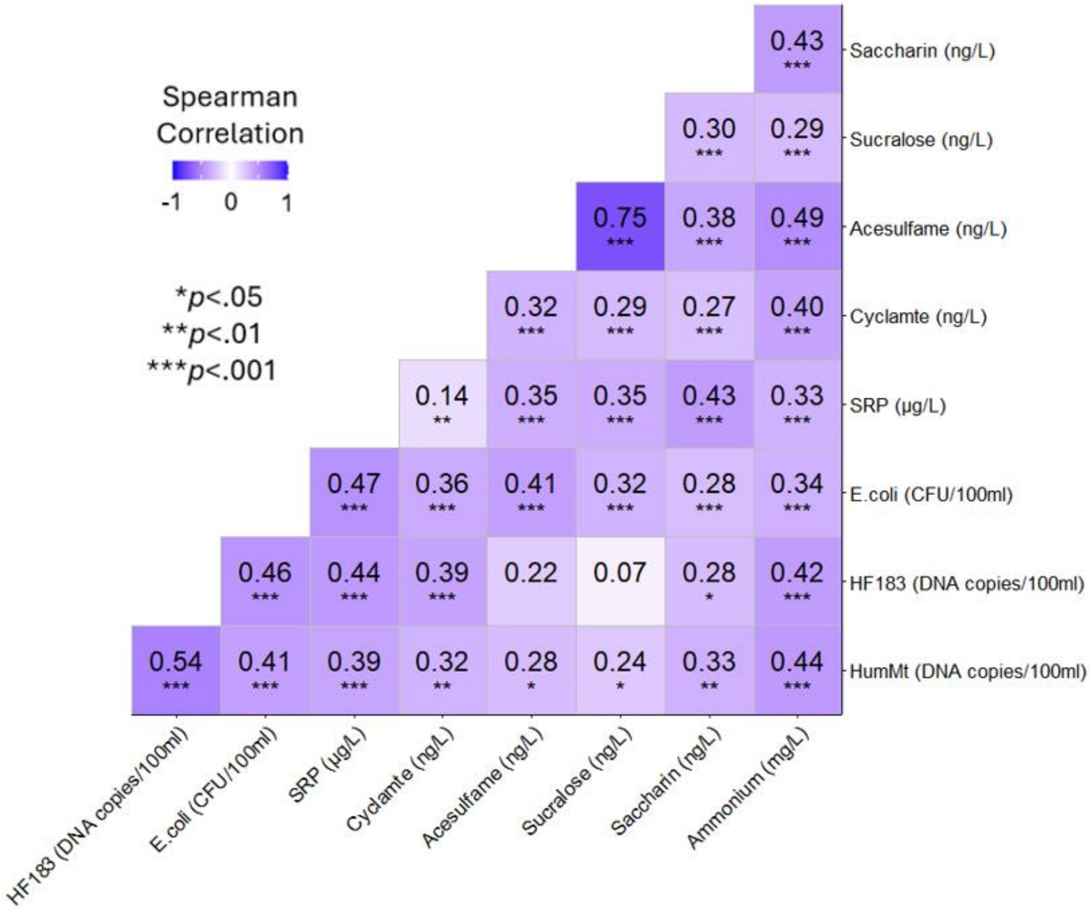

Figure 3-4: Spearman rank order correlogram for the various septic effluent tracers and additional constituents.

3.3.4 Percentage of septic system effluent reaching subwatershed outlets

The percentage of septic effluent reaching the subwatershed outlets based on the measured acesulfame concentrations and stream discharge for all sampling times are shown in Figure 3-5 with the data separated into high and low flow conditions. The percentage of septic effluent reaching the outlets considering all samples has a median of 9.2% and ranged from 0.009% to 412%. This is consistent with the findings of Spoelstra et al. (2020) who found the median of the septic system effluent across 294 samples from 173 stream sites to be 13%. The range in the percentage of septic effluent reaching the streams is consistent with the nine subwatersheds studied by Tamang et al. (2022) who found samples with percentage septic effluent reaching the stream above 250%. As shown in Figure 3-5 the variability in the percentage of septic effluent reaching the stream was large (large IQR). High variability, particularly in smaller subwatersheds, was also seen by Spoelstra et al. (2020a), who found that as the subwatershed area increased, the percentage of septic effluent reaching the stream was less variable. The data collected on the percentage of septic effluent reaching subwatershed outlets from the 39 subwatersheds analyzed in this study adds to the findings of previous works, allowing for an increased understanding of the possible range in the percentages of septic effluent reaching streams.

Consistent with Oldfield et al. (2020) and Tamang et al. (2022), the percentage of septic effluent reaching the outlets varied considerably between low flow and high flow conditions (Figure 3-5). Under low flow conditions, the median percentage of septic effluent reaching the subwatershed outlets ranged from 0.03% to 23.6% compared with 1.2 to 218% under high flow conditions. It is important to note that for some subwatersheds there are limited samples for high flow conditions (i.e. 1-3 samples), and this may create a bias when comparing the percentages between subwatersheds for high flow conditions. Several samples exceeded the 100% percentage septic effluent reaching the outlet (n=37). This was also observed by Tamang et al. (2022) and occurs because of acesulfame that is stored in the subwatershed during dry periods being released under high flow conditions as the hydrologic connectivity across the landscape and water fluxes increase (e.g. release of acesulfame stored in disconnected stream reaches or rising water tables driving flow in tile drains). It should be noted that sampling was not conducted during extreme high flow events and therefore it is possible that the percentage of septic system effluent reaching the stream is greater than reported here for these events due to increased contributions from overland flows.

Although detailed analysis of seasonal variability is not possible because not all subwatersheds were sampled in all seasons, seasonal variability in flow conditions may explain some of the variability between samples collected in a subwatershed. For instance, considering data from all subwatersheds, the median percentage of septic effluent reaching the outlets was highest in the spring (23.8%) compared to other seasons. This is consistent with the findings of Oldfield et al. (2020) who observed an increase in the percentage of septic effluent reaching the outlets in the early spring months (March, April, May) for the three subwatersheds studied. This seasonal trend may be related to increased flows observed during winter melts and spring, resulting in increased contributions, which is seen in Oldfield et al. (2020) where the highest flows occurred over this period. Note that other temporal factors may also contribute to changes in the percentage of septic effluent reaching the outlet, such as seasonal population changes resulting in periods of greater septic system usage (e.g., cottage occupancy) and artificial sweetener consumption patterns across different households.

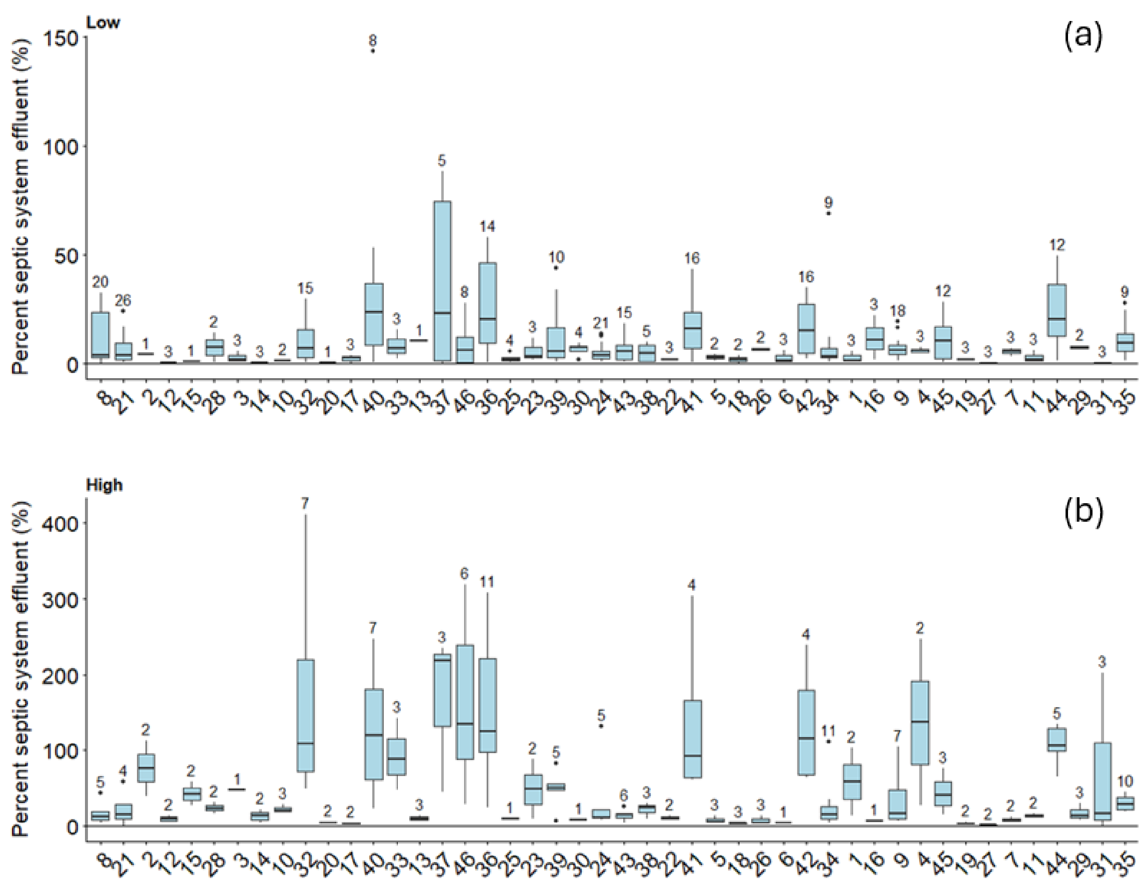

Figure 3-5: Percentage of septic effluent reaching subwatershed outlets for study subwatersheds under (a) low flow, and (b) high flow conditions, ordered by increasing subwatershed area from left to right. In the box plots, the horizontal line is the median, the box represents the upper and lower quartile ranges, and the whiskers extend to the maximum and minimum data with the exception of outliers.

3.3.5 Factors influencing the percentage of septic system effluent reaching the subwatershed outlets

Random intercept linear mixed effects models were used to explore the variability in the percentage of septic effluent reaching the subwatershed outlet between subwatersheds and between sampling events with model performance assessed using AIC, AICc, BIC and $R^2_{marginal}$ and $R^2_{conditional}$. The null random intercept model (null model) which was first fitted to compare subsequent models had an AIC of 1109 (**Table 3-3**). The level-1 model which just included the flow as fixed effect, showed an improvement over null model with a lower AIC of 939.7 (**Table 3-3**). Following this, using all subsets selection, all possible combinations of flow and various subwatershed characteristics were considered as fixed effect predictors. The top performing model (Level-2 model in Table 3-3), selected based on the lowest AIC value, included flow, TWI, and age of occupied homes as fixed effects with a random intercept for the subwatershed number. The equation of this model is given as:

$$Log\ (\%\ Septic\ system\ effluent) = 1.4 - 0.9Flow[Low]_{ij} + 0.3Age_i - 0.1TWI_i + u_i + \varepsilon_{ij} \quad (2),$$

where the percentage of septic effluent reaching the stream is indexed by i for the i^{th} subwatershed and by j for the j^{th} sample, u_i represents the random intercept for the i^{th} subwatershed and e_{ij} represents the residual error term. To address the uncertainty in all subsets model selection process, model averaging was conducted on models that were within 2 units of AIC from the top performing model, as they are considered equally plausible. The full model averaged coefficients are shown in **Figure 3-6**. The importance of subwatershed characteristics was considered by assessing how frequently they were included in the models that were within 2 AIC units of the top performing model along with the per-variable sum of weights as shown in Table 3-4.

Table 3-3: Model summaries for the null model, level-1 model and for the best predictor level-2 full averaged model. The regression coefficients (*β*), 95% confidence intervals (95% C.I) and standard error (S.E) for all model terms are shown. Model fit statistics including the AIC, BIC, $R^2_{conditional}$ and $R^2_{marginal}$ are also shown for each model.

	Null model			Level-1 model			Level-2 model		
Fixed effects	**β**	**95% C.I.**	**S. E.**	**β**	**95% C.I.**	**S. E.**	**β**	**95% C.I.**	**S. E.**
Intercept	0.8	[0.66,0.94]	0.07	1.3	[1.15,1.47]	0.08	1.4	[1.25,1.51]	0.07
Flow [Low]				-0.86	[-0.98, -0.75]	0.06	-0.9	[-0.97, -0.74]	0.05
Age							0.3	[0.20, 0.41]	0.05
TWI							-0.1	[-0.24, -0.01]	0.05
Random effects									
τ_{00}	0.15			0.19			0.09		
σ	0.53			0.35			0.35		
Fit statistics									
AIC	1109			939.7			915.1		
BIC	1121.5			956.4			940		
$R^2_{conditional}$	0.224			0.511			0.494		
$R^2_{marginal}$	-			0.237			0.366		

The effect of flow was included in the top performing model and all models that are within 2 AIC units of the top performing model, indicating that flow is an important predictor of the percentage of septic effluent reaching the outlets. The model averaged effect of low flow was found to be negative (β=-0.86, C.I. [-0.97, -0.73]) indicating that under low flow conditions the percentage of effluent reaching the outlets is lower compared to high flow conditions. As the model outcome variable is Log-transformed, the effect of flow can be interpreted as the percentage of septic effluent reaching the outlets is 86% lower under low flow conditions compared to those under high flow conditions. The significant effect of flow on the percentage effluent reaching the stream is expected given the results shown in Section 3.3.4 and previously by Oldfield et al. (2020) and Tamang et al. (2022). However, this is the first time that the magnitude of the effect has been quantified across multiple subwatersheds. The increase in the percentage of septic effluent reaching the outlets under high flow conditions is due to more pathways contributing septic effluent to the outlets during wet weather conditions, including overland transport and drains (e.g., field tile drains). In addition, disconnected compartments of the subwatershed that store acesulfame may be hydrologically reconnected with the outlets during wet weather conditions (e.g., disconnected stream reaches, and riparian zones) causing a flushing out of the acesulfame. Additionally, under wet flow conditions, there may be an increased hydraulic gradient driving groundwater discharge. It is important to note that although the models with the addition of flow included as a predictor explain the variation in the percentage of septic effluent reaching the outlets, it is possible that other temporal factors (e.g., seasonal climate, seasonal population changes, and acesulfame consumption trends) may also contribute to the temporal differences. It was not possible to analyze the effects of these additional temporal factors as it would require more data across all seasons in all subwatersheds as well as information on seasonal population changes and acesulfame consumption patterns in each subwatershed.

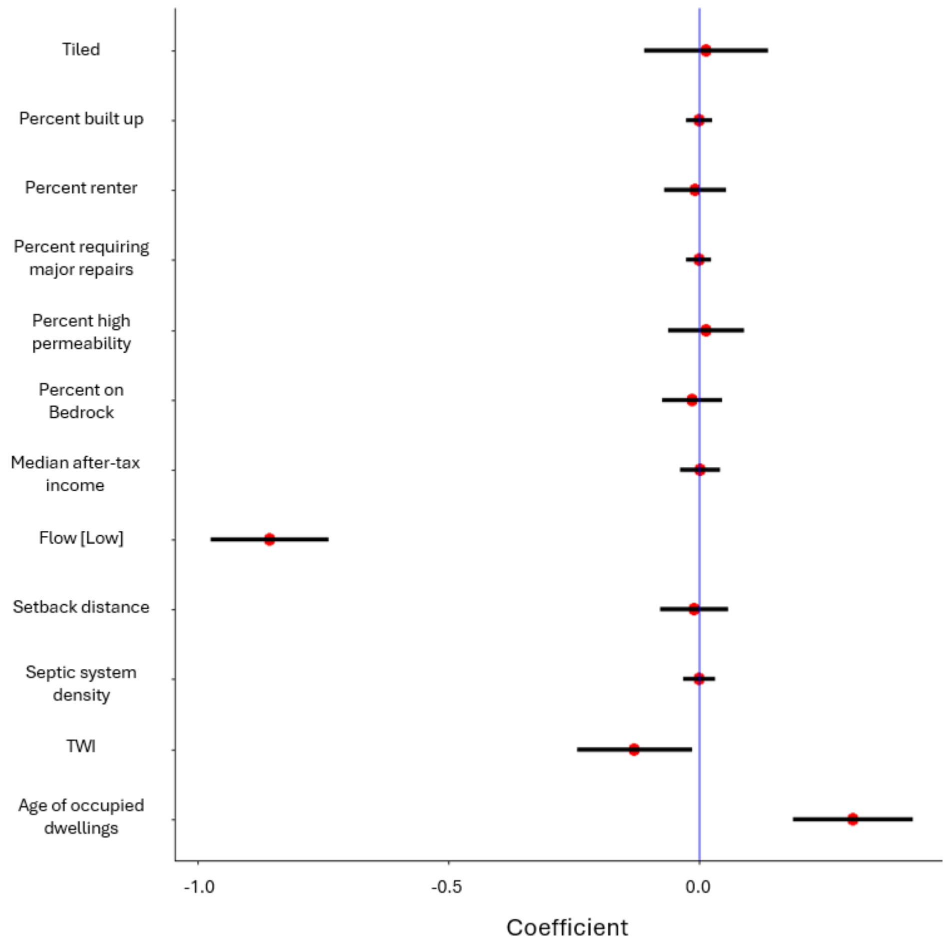

Figure 3-6: Full model averaged coefficients (β) for subwatershed characteristics included in all models that fit within 2 AIC units of the top performing model. The red dot represents the model averaged coefficient, and the black bars show the 95% confidence intervals.

The age of occupied homes was included in the top performing model and all models that are within 2 AIC units of the top performing model. The model averaged effect of the age of occupied homes was found to be positive, indicating that as the age of occupied homes increases the percentage of septic effluent reaching the outlet is expected to increase (β=0.31, C.I. [0.18, 0.42]). As the dependent variable is log-transformed and the effect of age is z-score standardized, it can be said that for an increase in one standard deviation of the age of occupied homes, the percentage of septic effluent reaching the outlet will increase by 100%. Older occupied homes are more likely to have older septic systems compared to newly constructed homes, and the typical lifespan of a septic system is 30-40 years (Pugel, 2019). This result is consistent with previous studies that have reported that the probability of septic system failure increases as the system age increases (Clayton, 1974; Winneberger, 1975). Increased failure of aging septic systems may be due to insufficient maintenance including infrequent pumping/de-sludging which can lead to less treated effluent reaching the drain field. Connelly et al. (2023) found that septic system age was an important predictor of the probability of household wastewater exceeding the volume of the septic tank. Older septic systems are more likely to be undersized due to an increase in household occupancy or appliances over time and this causes overloading of the septic tank. Additionally, the drain field associated with an aging septic system may become clogged resulting in break out of the septic effluent to the ground surface (Noss & Billa, 1988). Finally, the age of occupied homes may also be an important predictor of the percentage of septic system effluent reaching the outlets because older septic systems will have created longer groundwater septic plumes that are more likely to have reached a nearby surface water.

The TWI was included in the top performing model and all models that are within 2 AIC units of the top performing model, with the model averaged coefficient of TWI found to be negative. The model averaged coefficient indicates that increasing the TWI by one standard deviation reduces the percentage of septic effluent reaching the outlets by 25% (β=-0.13, C.I. [-0.24, -0.01]). Areas with low TWI are characterized by high topographic slopes and small upstream contributing areas. The topographic slope of the septic system placement has a strong, significant correlation with the TWI as shown in Figure 3-2, and therefore the impact of TWI may be confounded with its relationship with the topographic slope. It is possible, depending on the geological conditions, that higher topographic slopes may be associated with faster groundwater flow, meaning longer plumes and/or greater groundwater discharge to streams, and therefore higher septic effluent inputs to streams via groundwater transport. Higher slopes may also promote runoff transport over greater distances during wet periods. This result is consistent with septic system best practice manuals, which generally suggest that septic systems should be placed in areas with lower slopes due to the potential for inadequate treatment in highly sloped areas (Clements et al., 1980). Additionally, Hoghooghi et al. (2021) found that septic systems placed in areas with higher TWI were more likely to be replaced due to increased hydraulic failure. It is possible that areas with higher TWI may experience more saturated soil conditions, flooding, break out of effluent to the ground surface, and effluent being delivered to streams from nearby septic systems via overland transport during high flow conditions, however this may be limited when septic systems have a large setback distance to the stream. This effect may also not have been captured by the model due to the higher number of samples collected during low flow (n=311) compared to high flow (n=165) conditions. To test this further, additional sampling data is required for high flow conditions in the study subwatersheds.

Several additional subwatershed characteristics were included as predictors in models within 2 AIC units of the top performing model, reflecting additional possibly important characteristics. These subwatershed characteristics included tiled, percent high permeability, median after-tax income, setback distance, septic system density, and percent on bedrock. As shown in Figure 3-6, many of the model averaged coefficients for these characteristics have confidence intervals centered around zero and relatively small coefficient magnitudes. The relative importance of the subwatershed characteristics can be examined by assessing the number of models in which they are included. The number of models with each subwatershed characteristic included along with the sum of the model weights for each variable is shown in Table 3-4. Subwatershed characteristics such as percent on bedrock and percent on high permeability appear in few models (4 models) and as such they may have a smaller impact on explaining the percentage of septic effluent reaching the outlet. Despite the limited inclusion of certain variables in the models within 2 AIC units of the top performing model, certain predictor variables may be important in other study areas. For example, Capps et al. (2020) state septic system failure is more prevalent in low-income areas, however, in their study the number of septic systems used by households below the poverty line was significant (6%), compared to our study where the minimum average household income after tax in a subwatershed was $59,326 CAD, which is above the poverty line. It is also possible that other subwatershed characteristics may be important in explaining the percent septic effluent reaching the outlets, but their relationship may be non-linear (e.g., quadratic), and this was not explored.

Table 3-4: Subwatershed characteristics included in the models within 2 AIC units of the top performing model. The number of models in which they are included and the per-variable sum of model weights, which is the sum of the AIC model weights for models which the variable is included.

Subwatershed characteristic	Number of models	Per-variable sum of model weights
Age of occupied homes	15	1.00
Flow [Low]	15	1.00
TWI	15	1.00
Percent on bedrock	4	0.25
Percent high permeability	4	0.24
Setback distance	3	0.17
Percent renter	2	0.13
Tiled	2	0.12
Median after-tax income	1	0.06
Percent built up	1	0.05
Septic system density	1	0.05
Percent major repairs	1	0.05

3.3.6 Subwatershed characteristics influencing HF183 concentrations at subwatershed outlets

While the results from the linear mixed effects models presented above based on the acesulfame-derived percentage of septic effluent reaching the outlets provide insight into subwatershed characteristics that influence the amount of septic effluent reaching the stream via all pathways (because acesulfame is highly conservative), those affecting inputs via rapid pathways potentially associated with poorly performing septic systems may differ. This was explored using the HF183 concentrations at the outlets. An ordinal approach was used for this analysis due to the high number of non-detects for HF183 (46% of the samples analyzed) and because 68% samples collected were not analyzed for HF183 (as these were expected to have concentrations below detection based on concentrations of other septic effluent tracers: *E. coli* < 100 CFU/100mL and combined with artificial sweeteners < 200 ng/L). Subwatersheds were classified as 'high', 'low', and 'unlikely to have fecal contamination based on the HF183 MST concentration. Using this classification method, 8 subwatersheds were classified as “high”, 5 were classified as “low”, and 26 were classified as “unlikely”.

The relationship between subwatersheds with “high”, “low” and “unlikely” fecal (HF183) contamination levels and their subwatershed characteristics was explored using a Kruskal-Wallis test and a Spearman’s rank order correlation (Table 3-5). The Kruskal-Wallis test indicates if the subwatershed characteristics are significantly different between the subwatersheds classified as “high”, “low” and “unlikely” fecal contamination. The Spearman correlation indicates the strength and direction of a monotonic relationship between the levels of fecal contamination and the subwatershed characteristics.

Table 3-5: Kruskal-Wallis and Spearman rank-order correlation results with subwatershed characteristics and the fecal contamination level based on HF183 concentrations. The subwatershed characteristics are ordered by decreasing significance as calculated from the Kruskal-Wallis test.

	Kruskal-Wallis			**Spearman correlation**	
Parameters	χ^2	p	ε^2	ρ	p
TWI	13.52	0.001	0.36	0.551	<.001
Subwatershed area	9.43	0.009	0.25	-0.484	0.002
Septic system density	6.08	0.048	0.16	0.399	0.012
Setback distance	5.11	0.078	0.14	-0.319	0.048
Percent of built-up lands	5.11	0.078	0.14	0.215	0.188
Age of occupied homes	3.38	0.185	0.09	0.28	0.084
Percent on high permeability lands	2.58	0.275	0.07	-0.168	0.306
Percent of homes requiring major repairs	1.91	0.385	0.05	-0.062	0.706
Percent of dwellings occupied by tenants	0.65	0.721	0.02	-0.125	0.447
Percent on tile drained lands	0.29	0.867	0.01	0.062	0.707
Median after-tax income	0.24	0.888	0.01	-0.067	0.684
Drift thickness	0.16	0.925	0	0.044	0.791

From the Kruskal-Wallis test, a significant difference was found between the fecal contamination levels in a subwatershed and TWI (χ^2=13.518, p=.001, $\varepsilon^2=0.36$). Post hoc analyses revealed significant differences between the subwatersheds classified as 'high' and “unlikely” (Z=2.64, p=.012) and between the subwatersheds classified as 'low' and “unlikely” (Z=3.03, p=.007) with respect to the TWI. This is consistent with the histogram shown in Figure 3-7a and Spearman rank-order correlation test, which also showed that TWI is significantly correlated with fecal contamination levels (ρ=0.55, p<0.001). This indicates that the level of fecal contamination in a subwatershed increases with TWI. This result differs with the findings from the mixed effects model based on acesulfame data which found that the percentage of septic system effluent that reaches the outlets decreases as TWI increases. In this case, TWI appears to promote rapid pathways transporting septic effluent. Areas with high TWI are more prone to high groundwater levels and flooding, which may cause the septic effluent to breakout to the ground surface in the drain field and its subsequent overland transport to streams during wet weather conditions. Additionally, high TWI areas are more likely to require tile drainage, which could intercept the septic wastewater plume causing increased fecal contamination.

The fecal contamination level in a subwatershed was also found to be significantly different between subwatersheds with subwatershed areas (χ^2=9.43, p=.009, $\varepsilon^2=0.25$, Kruskal-Wallis test). Post hoc analyses revealed significant differences between the “high” and “unlikely” (Z=-2.51, p=.035) categories along with the “low” and “unlikely” (Z=-2.22, p=.052) categories with respect to the subwatershed area. This is consistent with the Spearman correlation which indicated that subwatershed area and fecal contamination level were negatively correlated (ρ=-0.48, p=.002, Figure 3-7b). This is likely due to higher dilution and loss during transport of HF183 in the streams with larger subwatershed areas as these streams have higher stream flows compared to smaller subwatersheds. Significant differences were also found between the levels of fecal contamination and the septic system density (χ^2=6.075, p=.048, $\varepsilon^2=0.16$). Post hoc analyses revealed that this result was due to significant differences between the 'low' and ‘unlikely’ (Z=1.46, p>.05) categories with respect to septic system density. Again, this result was also observed from the Spearman correlation test, which indicated a positive correlation between septic system density and the level of fecal contamination (ρ=0.39, p=.012). The importance of the septic system density is consistent with Verhougstraete et al. (2015) who found that the number of septic systems upstream of a stream sampling location was an important predictor of fecal indicator concentrations.

Although the setback distance was not found to be significantly different for the subwatersheds with different levels of fecal contamination at the 0.05 significance level (χ^2=5.11, p=.078, $\varepsilon^2=0.14$), these parameters were found to be negatively moderately correlated from the Spearman correlation test (ρ=-0.32, p=.048). In Figure 3-7d subwatersheds with “high” and “low” fecal contamination levels are found in subwatersheds with average setback distances less than 450 m. When septic systems are closer to the stream (smaller average setback distance) HF183 is more likely to reach the stream, especially through interception by drains and overland transport, as the HF183 is unlikely to travel through groundwater or overland flow paths when septic systems are farther from the stream due to the rapid decay and high retention of HF183 in the subsurface.

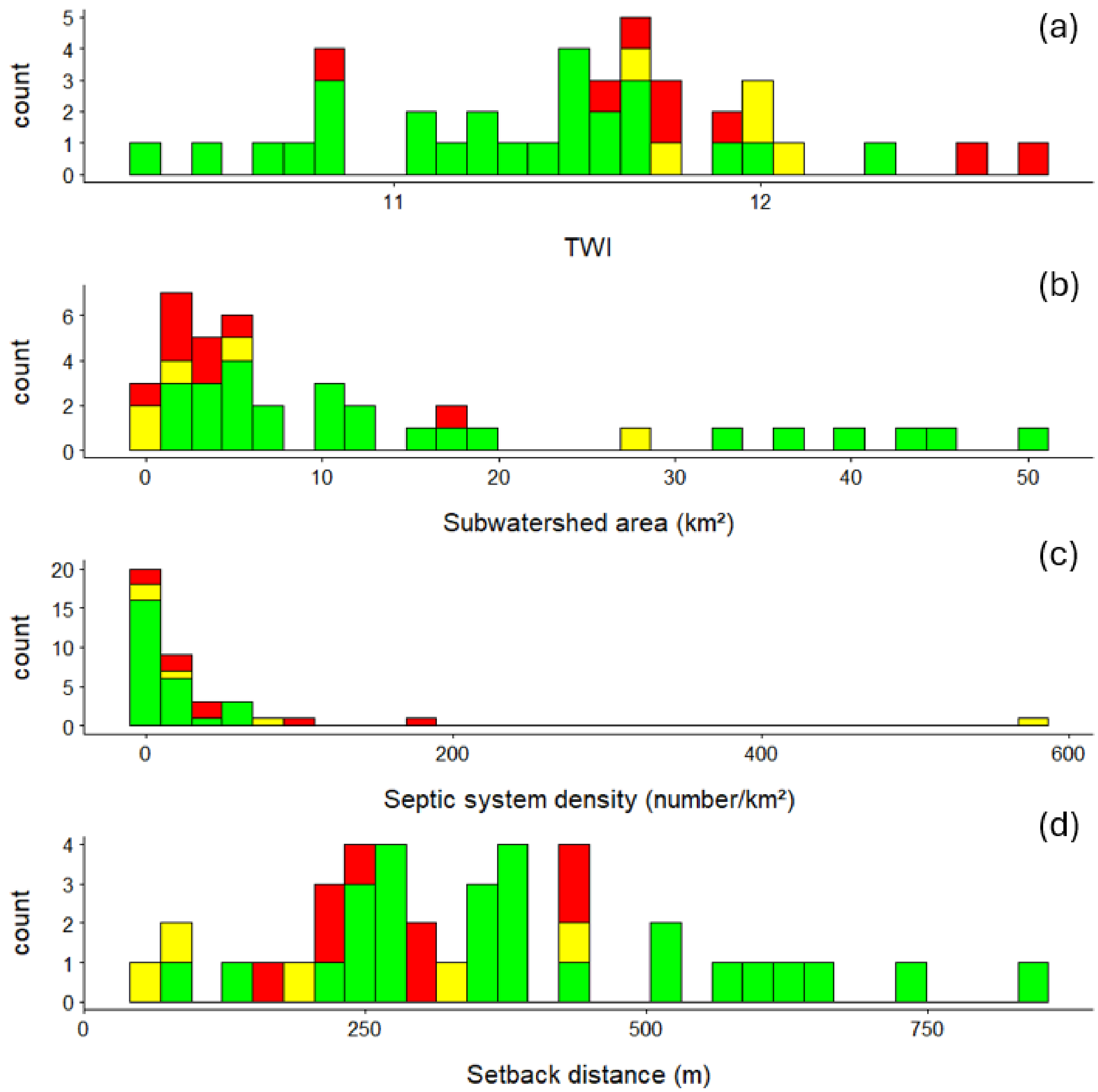

Figure 3-7: Histograms showing the distribution of subwatershed characteristics found to be significant based on the Spearman rank correlation (Table 3-5) and their associated fecal contamination level based on measured HF183 concentrations. Red indicates "high" fecal contamination level, yellow indicates "low" fecal contamination level, and green indicates "unlikely" to have detectable fecal contamination.

The above assessment ignores the potential for correlation between different subwatershed characteristics. A PCA was performed to visualize the potential associations between the subwatershed characteristics and the observed fecal contamination levels. The first two components are shown in the biplot in Figure 3-8. The principal components PC1 and PC2 explain 29.2% and 19.1% of the variance between the subwatersheds, respectively. In Figure 3-8 subwatersheds classified as 'low' and 'high' can be seen to the right side and bottom of the biplot, however they are spread across multiple quadrants. This spread of subwatersheds classified as 'high' and 'low' for fecal contamination suggests that the level of fecal contamination in a subwatershed is likely driven by several uncorrelated factors, rather than by a single factor or set of correlated factors.

Many of the subwatersheds classified as 'high' and 'low' are located at positive PC1, which in Table 3-6 is characterized by older occupied homes, with lower after-tax incomes and lower drift thickness. Additionally, many subwatersheds are characterized by negative PC2 scores, which are characterized by smaller subwatershed areas, higher septic system density, shorter setback distance, and higher TWI which have high loadings of PC2 (>|0.35|) as seen in Table 3-6. Indeed, a small cluster of several high-low subwatersheds aligns highly with TWI; likewise, there is a small cluster aligning well with the septic system density. Generally, the PCA analysis reveals the influence of similar subwatershed characteristics as in the Kruskal-Wallis test. However, here it is highlighted how the influence of several correlated factors (vectors in Figure 3-8; e.g., setback distance and septic system density) may not be distinguishable from each other. By using the PCA analysis, the drivers of 'high' and 'low' are shown to be more complex than the results of the Kruskal-Wallis suggest, and there may be a combination of factors which influence the level of fecal contamination.

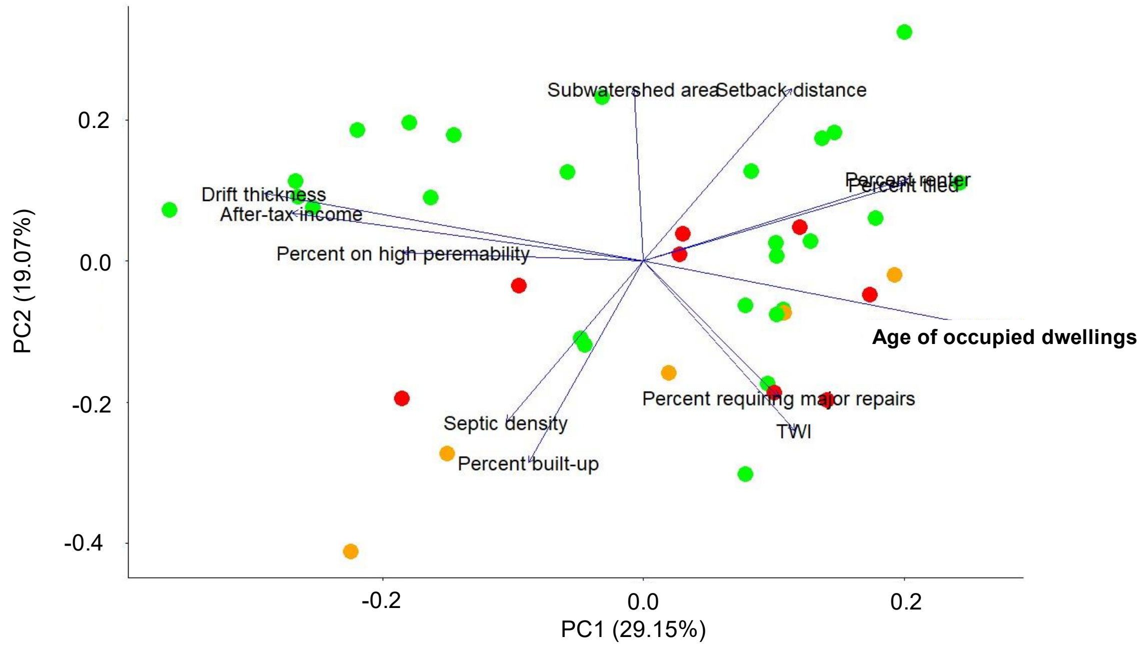

Figure 3-8: PCA biplot with PC1 and PC2. The loadings vectors for each subwatershed characteristic are shown in blue. The subwatersheds (points) are color coded by observed fecal contamination level. Red indicates "high" fecal contamination level observed at subwatershed outlet, orange indicates "low" fecal contamination level observed at subwatershed outlet, and green indicates "unlikely" to detect fecal contamination at the subwatershed outlet.

Table 3-6: Loadings for the subwatershed characteristics for PC1 and PC2.

Subwatershed characteristic	PC1	PC2
Percent tiled	0.32	0.17
Percent built-up	-0.14	-0.45
TWI	0.18	-0.38
Setback distance	0.18	0.39
Age of occupied homes	0.41	-0.15
Percent renter	0.32	0.19
Percent requiring major repairs	0.17	-0.31
After-tax income	-0.43	0.11
Subwatershed area	-0.01	0.39
Septic density	-0.17	-0.36
Percent on high permeability	-0.29	0.02
Drift thickness	-0.46	0.15

3.4 Conclusions

In this study, broad-scale sampling of multiple human wastewater tracers in streams across Ontario, Canada, were used to analyze factors that may influence the percentage of septic effluent that reaches streams. Artificial sweeteners were detected in all 46 subwatersheds and human-specific MST markers were detected in 18 subwatersheds. While the concentrations of these tracers were generally lower in larger subwatersheds compared to smaller subwatersheds, there was high variability in tracer concentrations between subwatersheds and between sampling times for an individual subwatershed. The percentage of septic effluent reaching the subwatershed outlets, as determined based on stream acesulfame concentrations, was found to be greater under high flow conditions compared to low flow conditions, consistent with the findings of Oldfield et al. (2020) and Tamang et al. (2022). The higher percentage of septic system effluent reaching the outlets under high flow conditions is thought to be due to additional hydrologic pathways delivering septic effluent to subwatershed outlets under high flow conditions. The effect of physical and socioeconomic subwatershed characteristics on the percentage of septic effluent reaching the outlets was investigated with linear mixed effects modelling using the subwatershed characteristics as fixed effects. This model showed that, in addition to flow conditions, the age of occupied homes and TWI have the greatest influence on the percentage of septic effluent reaching the outlets with the percentage increasing in subwatersheds with older homes and lower TWI. This result reflects the septic effluent reaching the outlets via all possible contributing pathways including groundwater transport as acesulfame is highly conservative. To understand the factors that may influence septic effluent being delivered to the outlets via rapid pathways potentially associated with failing septic systems, concentrations of the MST marker HF183 at the outlets were also analyzed. This showed that the levels of fecal contamination observed at the outlets (based on HF183 concentrations) were higher in small subwatersheds with high TWI, high septic system density, and small setback distances.

The findings of this study provide important insight into subwatershed characteristics that influence septic effluent inputs into streams in rural subwatersheds. This information can be used to prioritize field monitoring programs focused on assessing the impacts of septic systems on surface water quality and to inform management decisions and policies for locating, constructing, and maintaining septic systems. The study findings can also be applied to improve models developed to predict the contribution of septic systems to stream contaminants, including nutrient loads, and can be used to inform and prioritize septic system reinspection, and education and outreach programs.

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Chapter 4

4 Summary and recommendations

4.1 Summary

Septic systems are widely used to treat and disperse domestic wastewater in rural and suburban areas not serviced by centralized wastewater treatment infrastructure. Septic system wastewater effluent contains high levels of contaminants of concern including nutrients, fecal contaminants, pharmaceuticals, and personal care products (Lusk et al., 2017; Richards et al., 2017). Septic systems, particularly underperforming septic systems, may deliver these contaminants to groundwater and surface waters leading to water quality impairment and subsequent public and environmental health issues. The overall goal of this thesis was to quantify the percentage of septic effluent reaching multiple streams and to evaluate whether this percentage is influenced by physical and socioeconomic subwatershed characteristics. Understanding the conditions associated with higher septic effluent delivery to streams is important to estimate the contribution of septic systems to stream contamination, including nutrient loads, and to guide management programs and policies for locating, constructing, and maintaining septic systems.

The first objective was to evaluate the impact of septic systems on stream water quality and the percentages of septic effluent reaching the outlets of multiple streams. Stream sampling and discharge measurements were conducted at the outlets of 46 subwatersheds across the Lake Simcoe and Lake Erie Basins, Ontario, Canada, with samples analyzed for various human wastewater tracers. Using stream loads of the artificial sweetener acesulfame, the percentage of septic effluent reaching the stream was estimated for all sampling locations across the study subwatersheds. Under high flow conditions, the median percentage of septic effluent reaching the outlets was 26%, compared to only 5.6% under low flow conditions. The stream flow conditions were found to be important as a fixed effect in a mixed effects model developed to explain the variability in the percentage of septic effluent reaching the streams. Using the fixed effects model, the percentage of septic system effluent reaching the subwatershed outlet under low flow conditions was 86% lower than under high flow conditions. The lower percentage of septic effluent reaching the stream sampling sites under low flow is consistent with the findings of Oldfield et al. (2020) and Tamang et al. (2022). The higher percentage of septic effluent reaching the streams under high flow conditions is thought to be due to the activation of additional pathways that deliver septic effluent to the stream such as subsurface drains (e.g., agricultural field drains) and overland runoff.

The second objective was to identify subwatershed characteristics that may influence the percentage of septic effluent reaching the subwatershed outlets. Using a mixed effects modelling approach, key subwatershed characteristics were identified. The mean age of occupied homes within a subwatershed was found to have a positive effect on the percentage of septic effluent reaching the stream, indicating that the percentage of septic effluent reaching the outlets is higher in subwatersheds with older occupied homes. Older occupied dwellings may have older septic systems which may have exceeded their serviceable life and are expected to also be associated with longer groundwater septic plumes that are more likely to be reaching a nearby stream. The importance of system age is consistent with recent studies focused on the risk of septic system failure (Capps et al., 2020; Connelly et al., 2023). The topographic wetness index (TWI) was also found to be important for explaining the percentage of septic effluent reaching a stream. The effect of the TWI was negative, indicating that areas of higher TWI contribute a lower percentage of septic system effluent to the subwatershed outlets compared to lower TWI areas. Lower areas of TWI are generally characterized by highly sloped areas with small upstream contributing areas. The increased percentage of septic effluent reaching outlets in subwatersheds with lower TWI may be due to these areas having faster groundwater transport delivering septic effluent to streams. Additional subwatershed characteristics were identified that may be important, however, their overall impact was found to be minor for explaining the percentage of septic effluent reaching the subwatershed outlets.

The third objective was to identify subwatershed characteristics that may be associated with higher human fecal contamination in streams, likely due to rapid septic effluent inputs associated with underperforming septic systems. Samples collected in 18 subwatersheds were analyzed for the human-specific microbial source tracking (MST) marker HF183. As HF183 is not overly mobile in the subsurface and it decays rapidly, the detection of HF183 in streams suggests the septic effluent is being delivered to the stream via rapid transport pathways (e.g. illegal direct pipes, overland transport). Based on the detection and concentration of HF183 in the streams, subwatersheds were classified into ‘high’, ‘low’ and ‘unlikely’ to have fecal contamination. Using statistical analyses, it was found that smaller subwatersheds with high septic systems had greater fecal contamination levels and therefore potentially rapid pathways delivering effluent to the stream. In addition, it was found that the fecal contamination level was higher when septic systems were located in areas with high TWI. This may be due to greater septic system failures in these areas due to high groundwater tables and/or flooding. Finally, principal component analysis (PCA) was used to further explore the relationships between the subwatershed characteristics, and the levels of fecal contamination highlighted two potential groups of subwatersheds with fecal contamination. The PCA analysis revealed that there may not be a singular or singular group of subwatershed characteristics which explain the level of fecal contamination, and that there may be several mechanisms resulting in higher fecal contamination.

4.2 Recommendations

Recommendations for future work needed to further increase our understanding of contaminant inputs to streams from septic systems and how these inputs vary in areas with different physical and socioeconomic characteristics are provided below.

- Sampling was conducted at 46 subwatersheds across the Lake Erie and Lake Simcoe Basins. Expanding the sampling to more subwatersheds would aid in validating the findings of this work and confirming the subwatershed characteristics influencing inputs of septic wastewater contaminants to streams. In addition, the analysis would be improved by increased samples in each of the study subwatersheds and ensuring sufficient sampling is conducted throughout all seasons and flow conditions. This would provide additional insights into the impact of time-variant factors such as seasonality on the percentage of septic effluent reaching the stream.

- Sampling events were categorized as high or low flow based on comparing measured stream discharges and visual observations of the streams between sampling events. The categorization was also supported by long-term hydrograph data from nearby tributaries. Continuous stream discharge measurements in all the study subwatersheds would improve the quantification of the stream flow conditions.

- Canadian census data was used for assigning socioeconomic characteristics to each of the study subwatersheds. The census data for each block was assumed to be spatially invariant for the homes within the block. To better capture the spatial variability in socioeconomic characteristics higher resolution census block data could be used where available.

- Acesulfame stream loads were used to calculate the percentage of septic effluent reaching the stream. Although acesulfame is widely consumed, it is possible that acesulfame consumption patterns are not uniform. An understanding of the consumption patterns of artificial sweeteners could aid in addressing this potential uncertainty. Additionally, acesulfame degradation between the septic system and the subwatershed outlet was not accounted for. Further work should investigate the potential degradation of acesulfame to understand the extent to which the approach adopted may underestimate the percentage of septic effluent reaching the subwatershed outlet.

- In some subwatersheds for some high flow sampling times, the percentage of septic system effluent reaching the stream exceeded 100%. This is thought to be due to acesulfame that is stored or trapped in disconnected compartments in the landscape being flushed out during high flow conditions. The factors contributing to these high percentages of septic effluent reaching the streams was not explicitly investigated, and this could be further explored in future studies.

- The human-specific MST marker HF183 was only analyzed for a subset of samples due to the high likelihood that some samples would have HF183 concentrations below detection (based on *E. coli* and artificial sweetener concentrations). It would be beneficial to create an approach to handle left censored HF183 samples to allow for direct comparison with the results from the mixed model for the percentage of septic system effluent reaching the stream.

- HF183 data were able to provide insight into subwatershed characteristics associated with fecal contamination and septic system effluent reaching the stream through rapid failure pathways. It would be beneficial to apply the field sampling approach detailed here in a watershed with detailed septic system inspection data to evaluate to evaluate the relationship between observed septic system maintenance practices or issues and wastewater tracers observed in the stream.

4.3 References

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Tamang, A., Roy, J. W., Boreux, M. P., & Robinson, C. E. (2022). Variation in septic system effluent inputs to tributaries in multiple subwatersheds and approaches to distinguish contributing pathways and areas. *Science of the Total Environment*, *807*. https://doi.org/10.1016/j.scitotenv.2021.151054

Appendices

Appendix A: Characterization of study subwatersheds

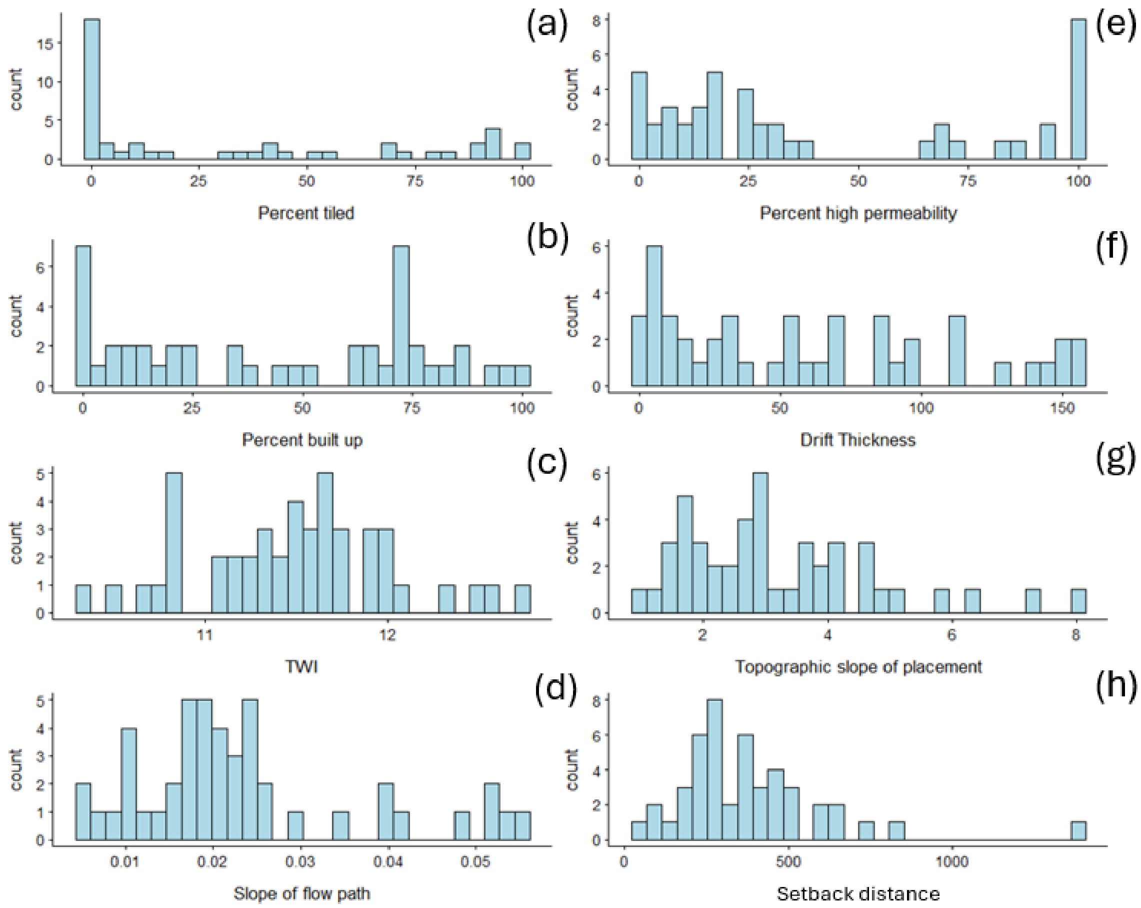

Figure A-1:Histograms of subwatersheds characteristics. (a) shows the percent on tile drained lands, (b) shows the percent on built up lands, (c) shows the TWI, (d) shows the slope of the flow path, (e) shows the percent on high permeability, (f) shows the drift thickness, (g) shows the topographic slope of the placement, and (h) shows the setback distance.

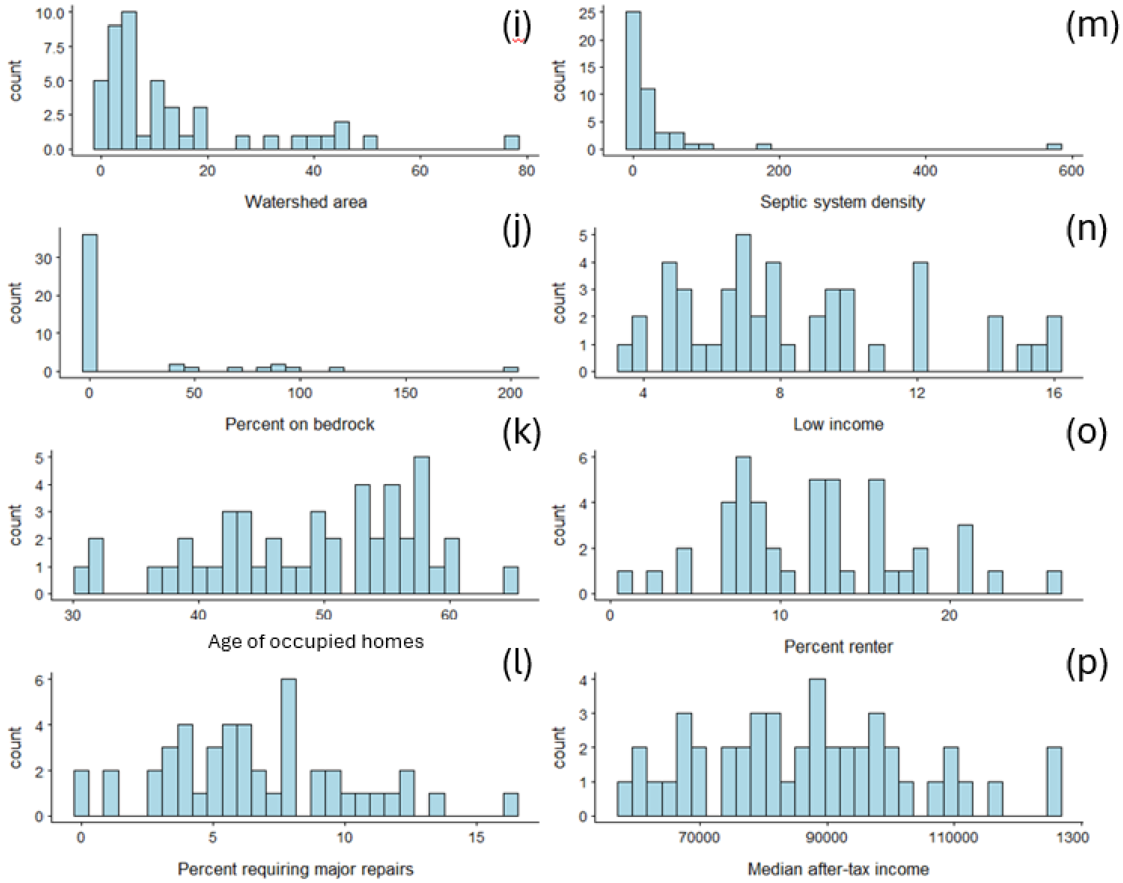

Figure A-2: Histograms of subwatersheds characteristics. (i) shows the subwatershed area, (j) shows the percent on bedrock, (k) shows the age of occupied homes, (l) shows the percent requiring major repairs, (m) shows the septic system density, (n) shows the percent low income, (o) shows percent renter, and (p)shows the median after tax income.

Appendix B: Stream discharge information

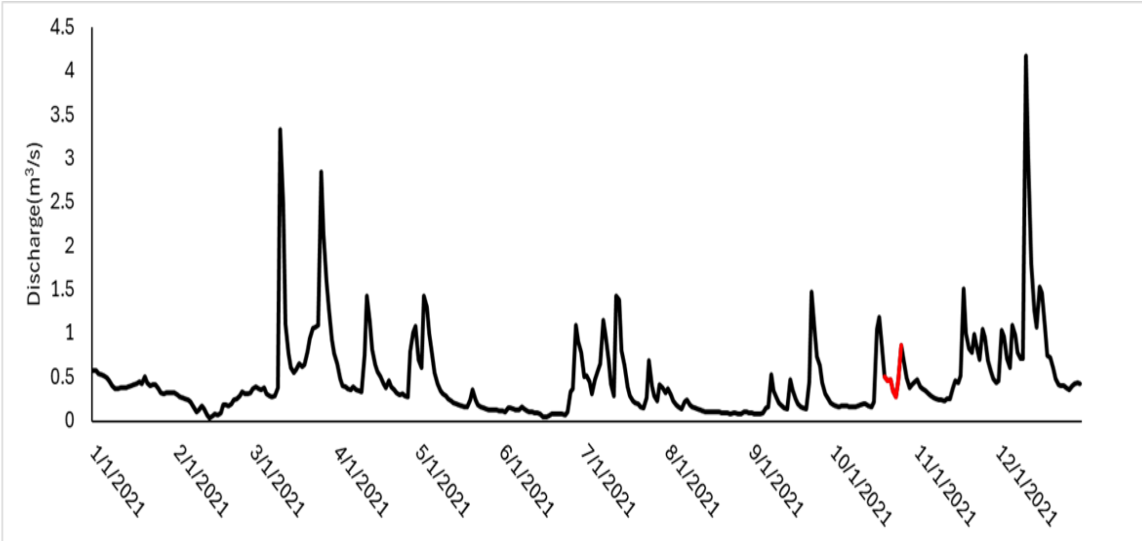

Figure B-1: Stream hydrograph data for Hawkestone creek (station id: 02EC020). Stream daily discharge data for 2021, with the red line indicating when sampling was conducted in the Lake Simcoe subwatersheds.

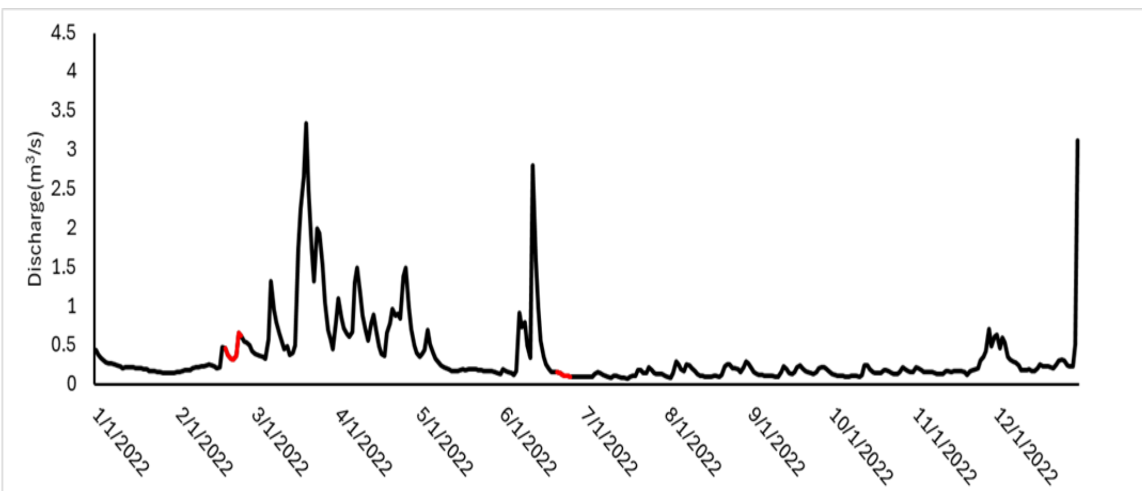

Figure B-2: Stream hydrograph data for Hawkestone creek (station id: 02EC020). Stream daily discharge data for 2022, with the red line indicating when sampling was conducted in the Lake Simcoe subwatersheds.

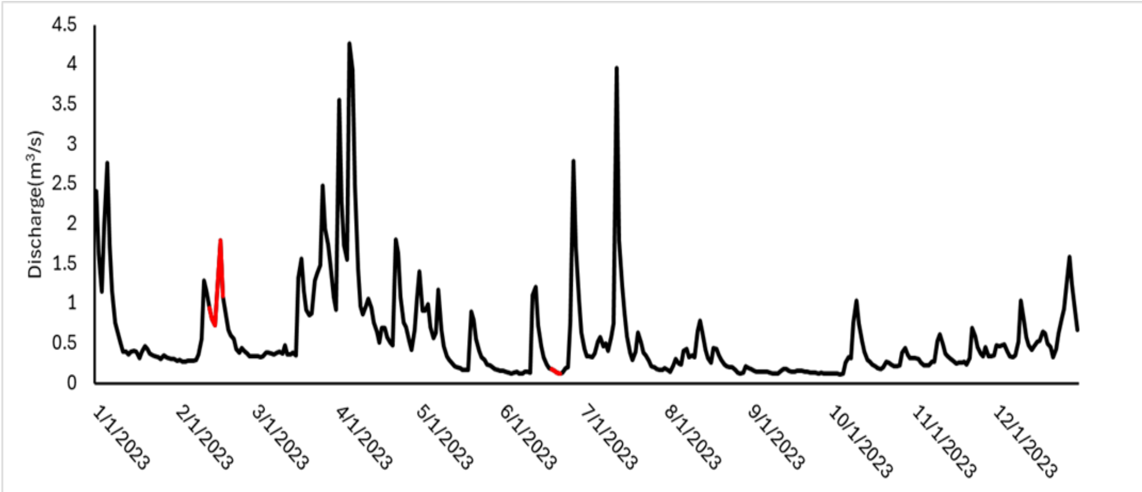

Figure B-3: Stream hydrograph data for Hawkestone creek (station id: 02EC020). Stream daily discharge data for 2023, with the red line indicating when sampling was conducted in the Lake Simcoe subwatersheds.

Appendix C: Additional wastewater tracers

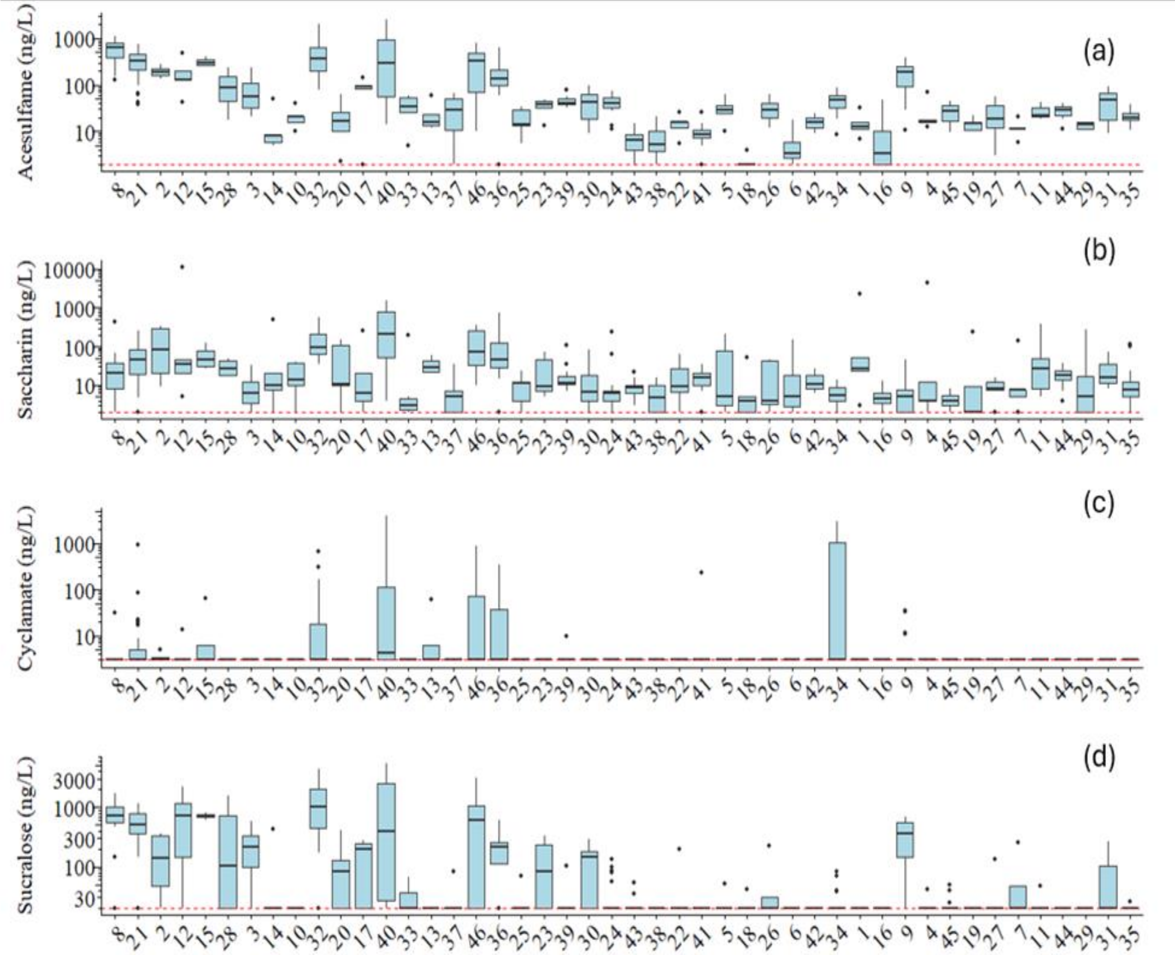

Figure C-1: Summary of concentrations of artificial sweeteners (a) acesulfame, (b) saccharin, (c) cyclamate, and (d) sucralose measured at the outlets of the study subwatersheds. Subwatersheds are arranged by increasing subwatershed area from left to right. Samples falling with concentrations below the detection limit are set at the detection limit indicated by the red dashed line. In the box plots, the horizontal line is the median, the box represents the upper and lower quartile ranges, and the whiskers extend to the maximum and minimum data with the exception of outliers.

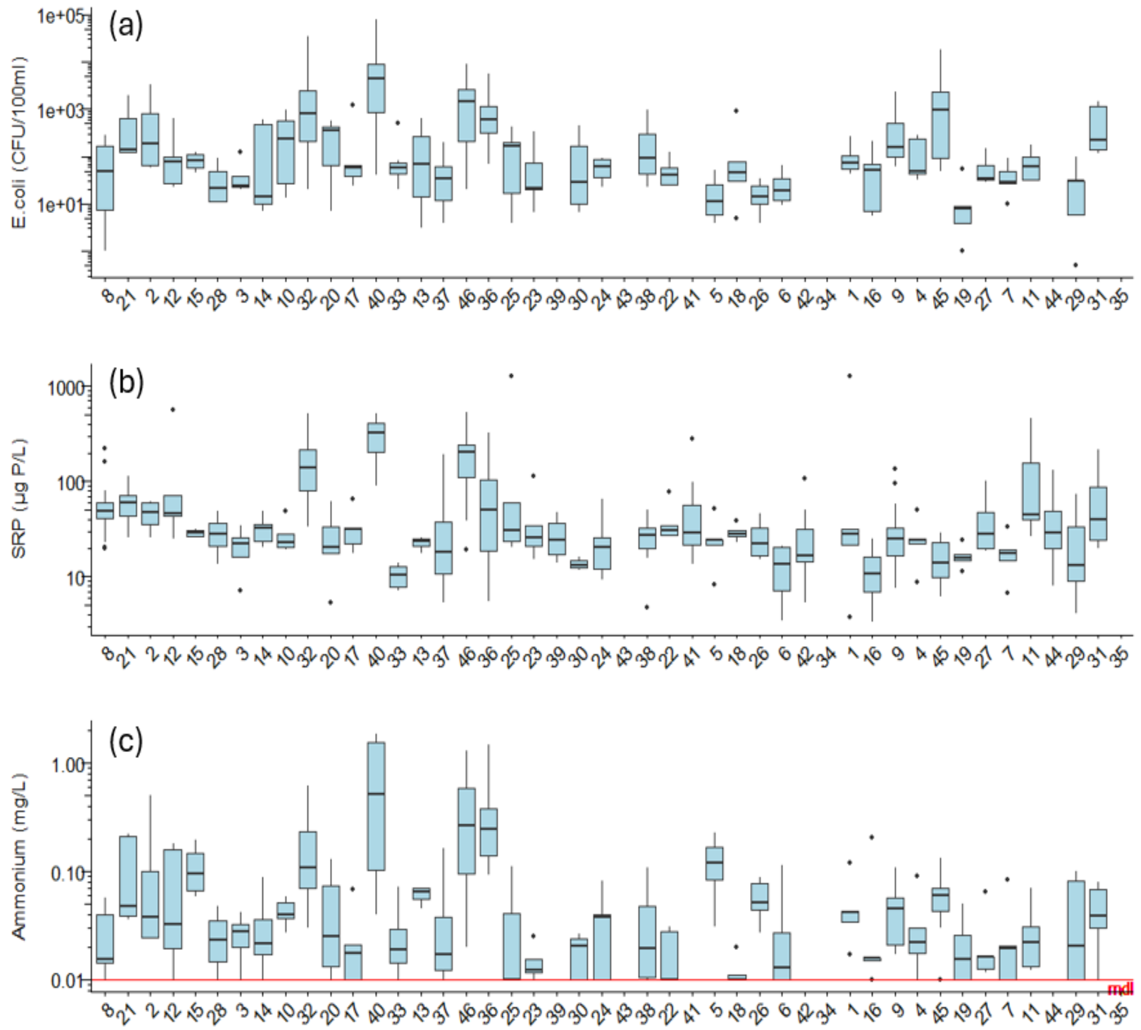

Figure C-2: Summary of concentrations of (a) *E. coli,* (b) SRP, and (c) Ammonium, at the outlets of the study subwatersheds. Subwatersheds are arranged by increasing subwatershed area from left to right. Samples with concentrations below the detection limit are set at the detection limit indicated by the red dashed line. In the box plots, the horizontal line is the median, the box represents the upper and lower quartile ranges, and the whiskers extend to the maximum and minimum data with the exception of outliers.

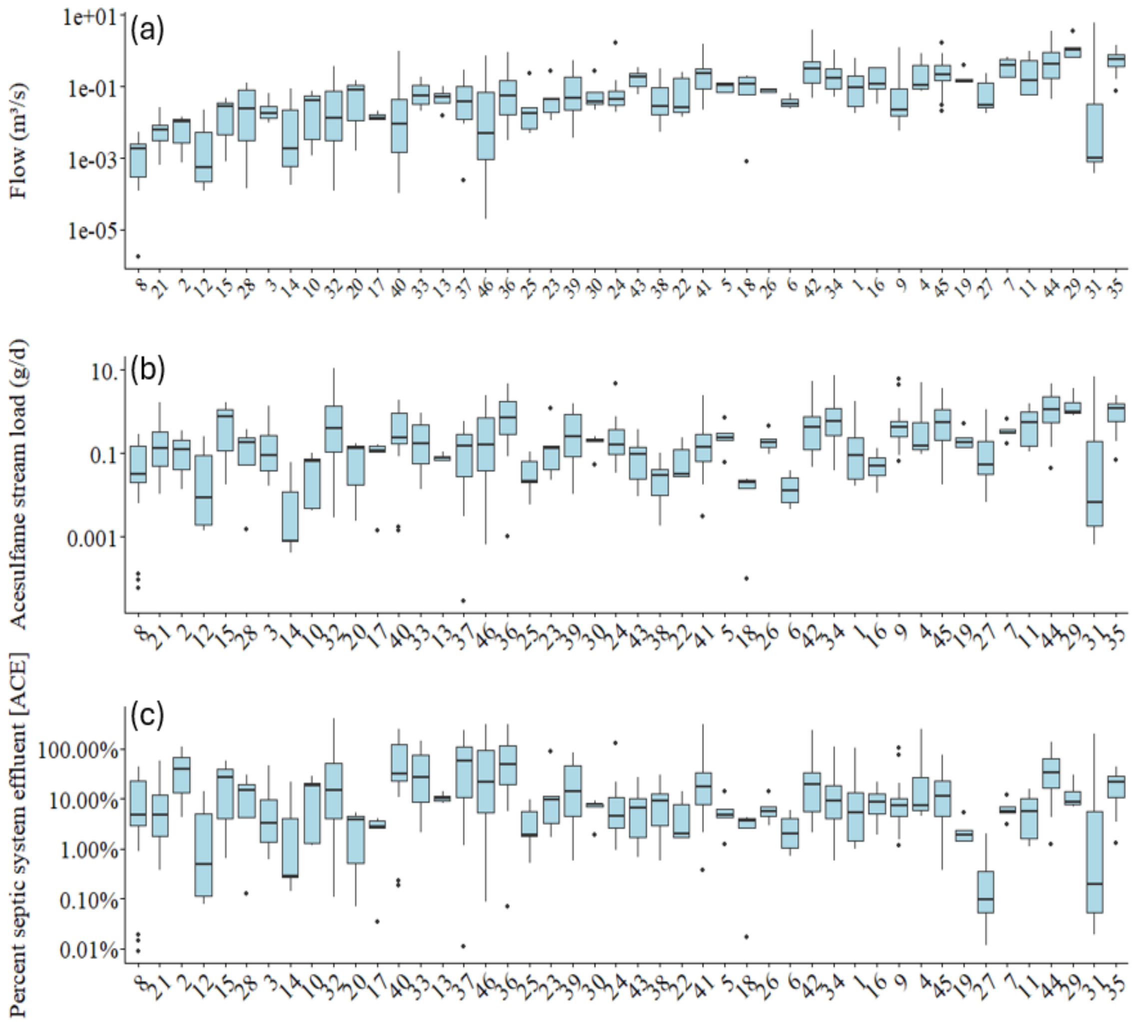

Figure C-3: Plots showing the stream flow (a), flow stream load (b) and acesulfame stream loads and (c) percetn septic system effluent reaching the stream for study subwatersheds. In the box plots, the horizontal line is the median, the box represents the upper and lower quartile ranges, and the whiskers extend to the maximum and minimum data with the exception of outliers. The y-axis is plotted on a log scale

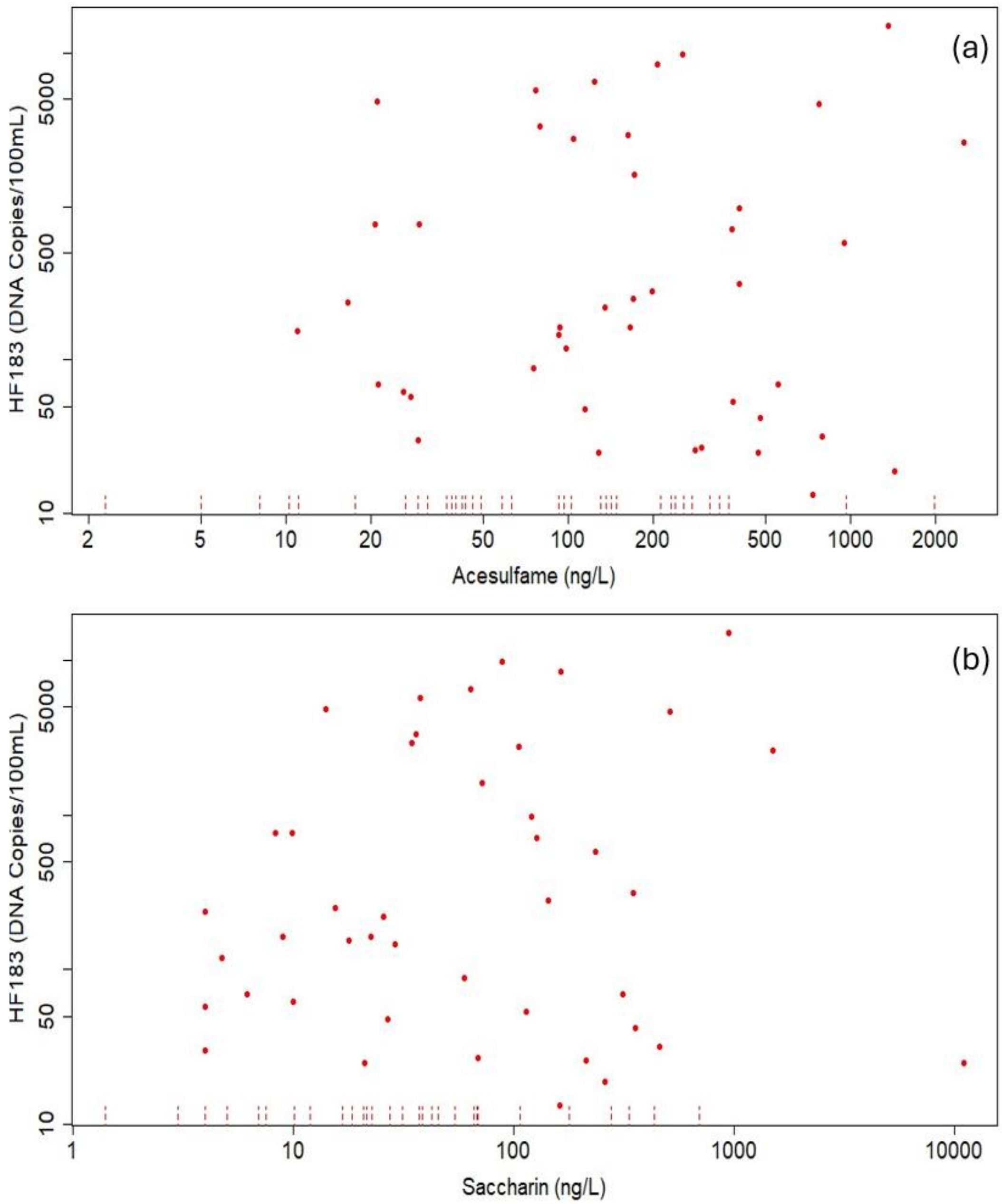

Figure C-4: Plots showing (a) acesulfame and (b) saccharin plotted against the HF183 concentration. The red dashed lines indicated points which fall below the minimum detection limit of 13 DNA Copies/100ml. Only samples with both HF183 and artificial sweeteners measured are shown.

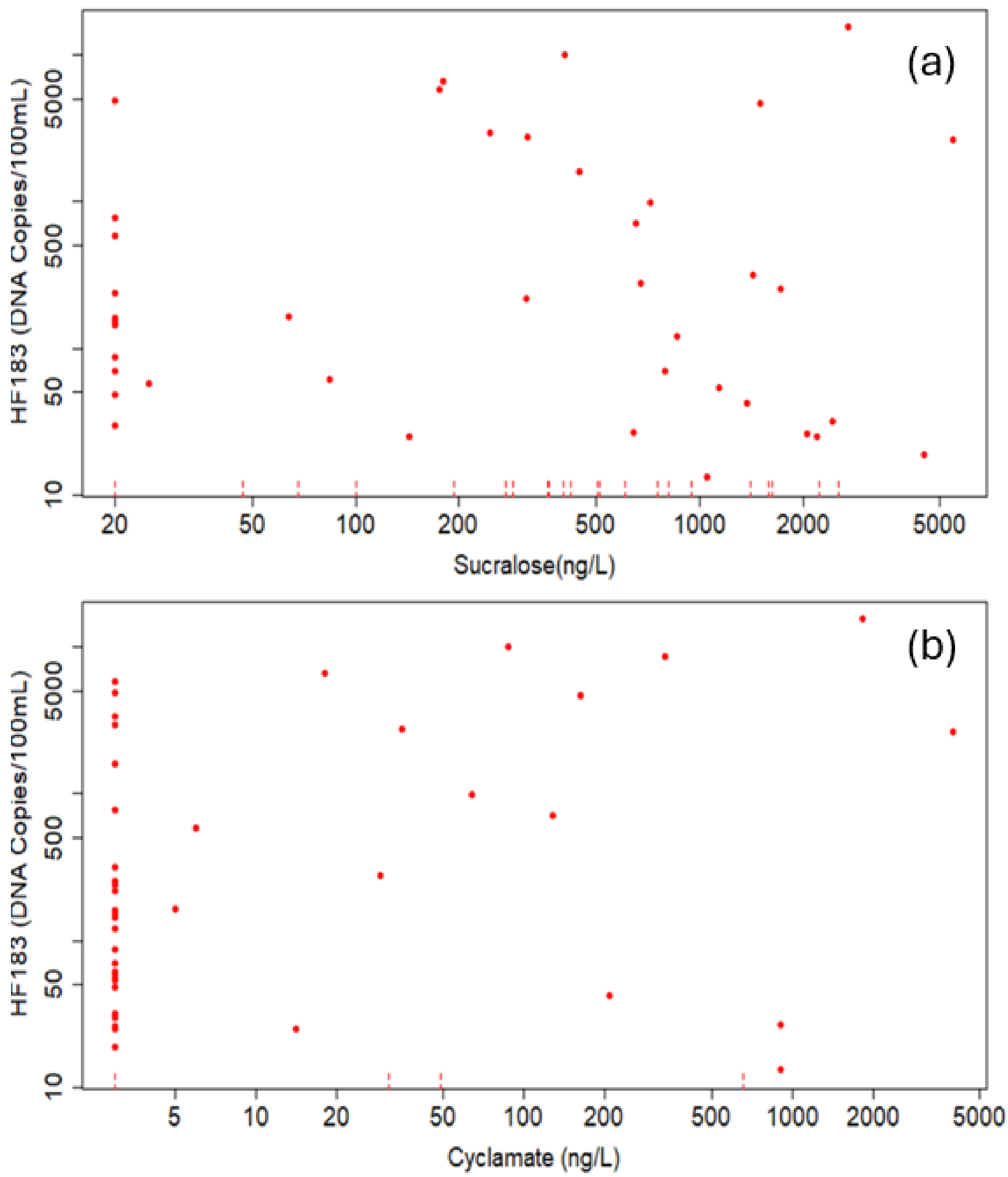

Figure C-5: Plots showing (a) sucralose and (b) cyclamate plotted against the HF183 concentration. The red dashed lines indicated points which fall below the minimum detection limit of 13 DNA Copies/100ml. Only samples with both HF183 and artificial sweeteners measured are shown

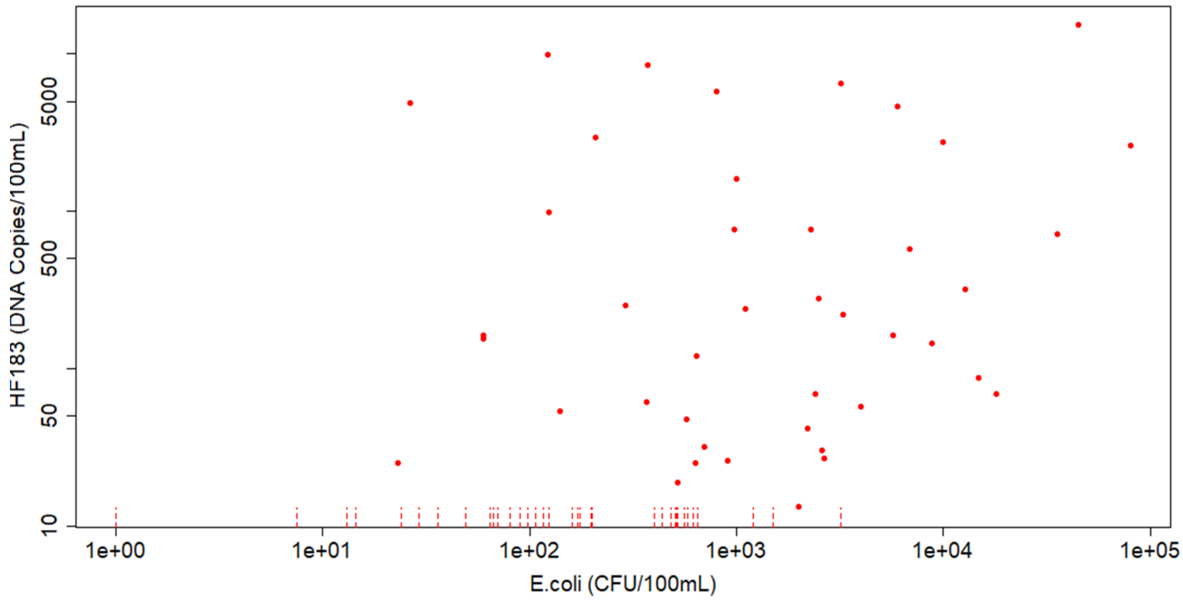

Figure C-6: Plots showing E.coli plotted against the HF183 concentration. The red dashed lines indicated points which fall below the minimum detection limit of 13 DNA Copies/100ml. Only samples with both HF183 and artificial sweeteners measured are shown

Appendix D: Additional HF183 histograms

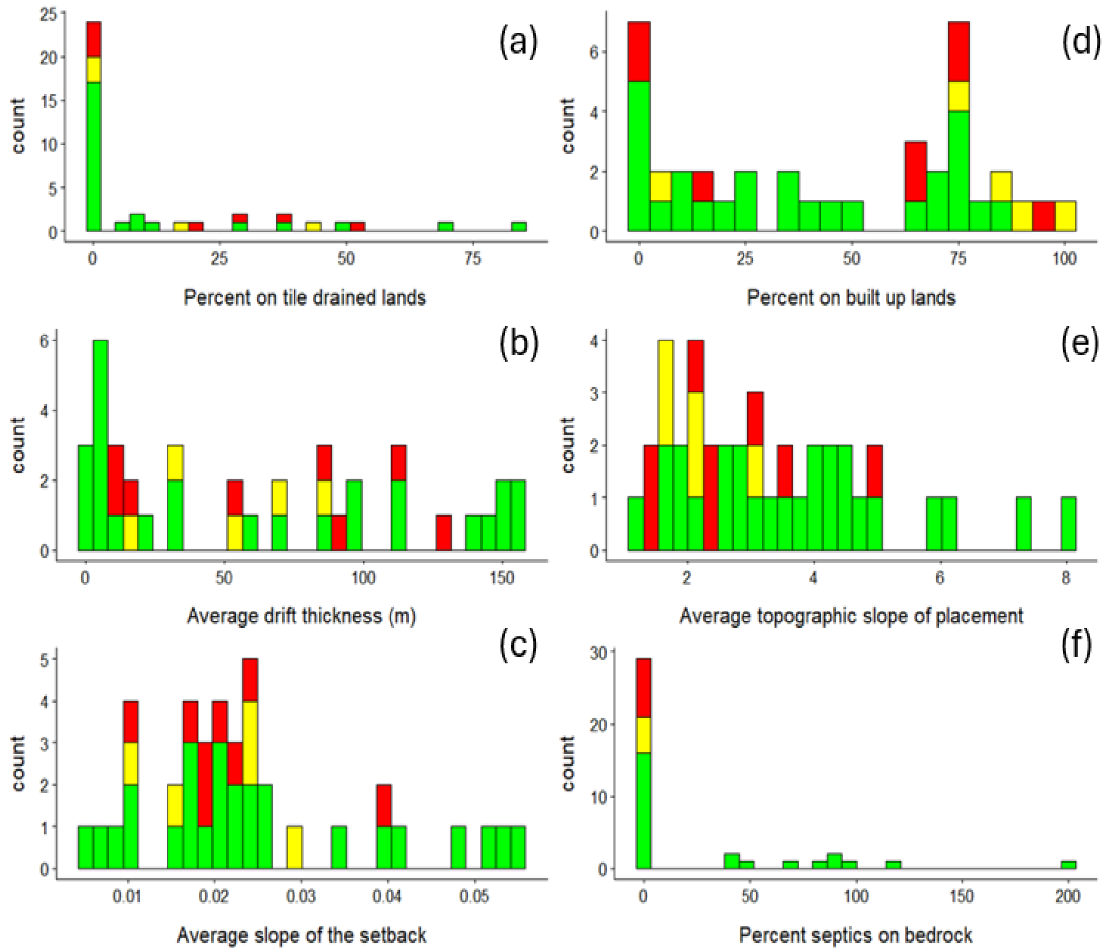

Figure D-1: Histograms showing the distribution of subwatershed characteristics, and their associated fecal contamination level based on measured HF183 concentrations for (a)percent on tiled drained soils, (b)Average drift thickness, (c) Average slope of the setback, (d) percent on built up lands, (e)average topographic slope of the placement, and (f) percent septics on bedrock. Red indicates "high" fecal contamination level, yellow indicates "low" fecal contamination level, and green indicates "unlikely" to have detectable fecal contamination.

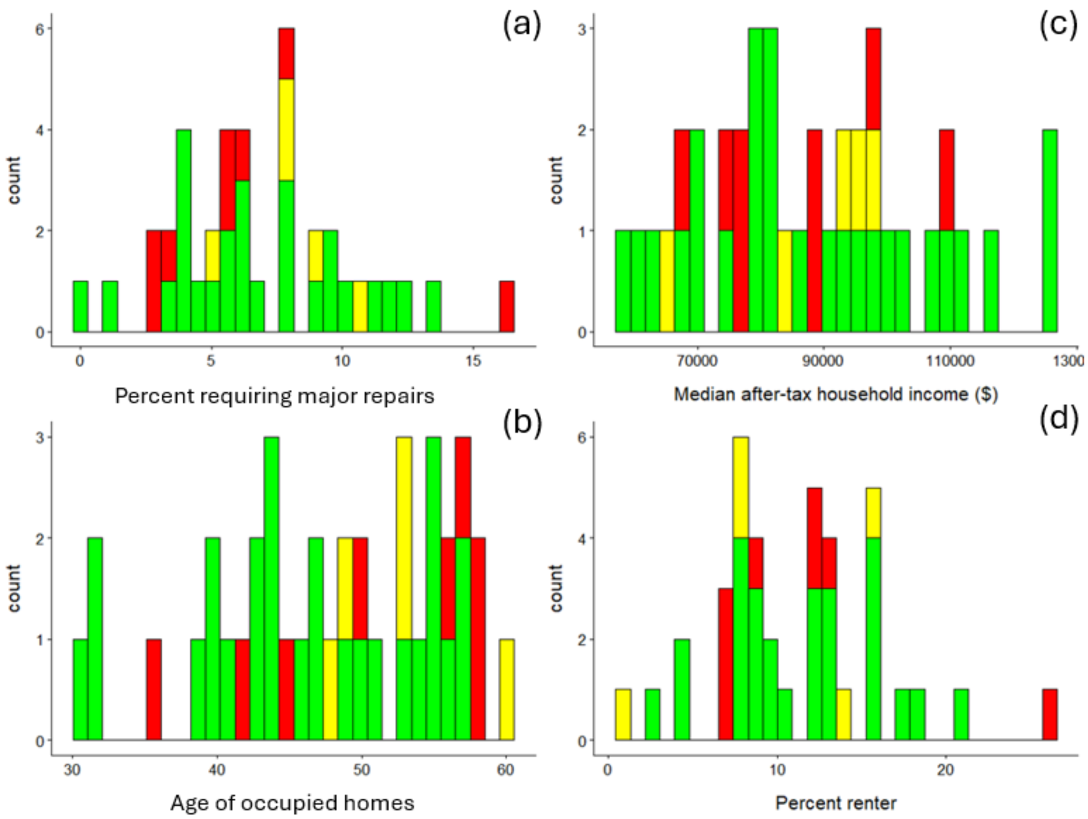

Figure D-2: Histograms showing the distribution of subwatershed characteristics, and their associated fecal contamination level based on measured HF183 concentrations for (a) percent homes requiring major repairs, (b) average age of occupied homes, (c) median after-tax income, (d) percent renter. Red indicates "high" fecal contamination level, yellow indicates "low" fecal contamination level, and green indicates "unlikely" to have detectable fecal contamination

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