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The impact of event-based *Cryptosporidium* and *Giardia* loads on drinking water risk

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Cette thèse intitulée:

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présentée par **Samira TOLOUEI**

en vue de l'obtention du diplôme de *Philosophiae Doctor*

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DEDICATION

To my beloved parents, family, my love Vahid and my beautiful Emily

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RÉSUMÉ

Des épidémies de maladies d'origine hydrique ont été documentées dans le monde entier. Deux épidémies majeures d'origine hydrique se sont produites à Milwaukee, dans le Wisconsin et à Walkerton, en Ontario, au cours des trois dernières décennies, entraînant des modifications de la réglementation en matière de protection des sources d'approvisionnement en eau potable. Les épidémies signalées se sont principalement produites à la suite d'épisodes de fortes précipitations, indiquant un lien étroit entre l'hydrologie des bassins versants et les épidémies d'origine hydrique. L'approche multi-barrières, de la source au robinet, est recommandée en tant qu'approche préventive afin de protéger la santé publique et de fournir une eau potable propre et fiable aux consommateurs.

En milieu urbain, les eaux usées non traitées ou partiellement traitées restent une source importante de contamination fécale. Cependant, les performances des stations de récupération des ressources de l'eau (StaRRE) varient également entre les conditions météorologiques sèches et humides et leurs charges n'ont généralement pas été quantifiées par temps humide. La variabilité de la qualité microbiologique des sources de contamination fécale en milieu urbain lors de précipitations doit être évaluée afin de comprendre leur impact sur les prises d'eau potable.

Cette thèse porte sur deux thèmes principaux: (1) la surveillance d'une StaRRe en tant que source de contamination fécale des parasites pathogènes, des bactéries fécales, des micropolluants des eaux usées et des matières en suspension dans des conditions météorologiques caractérisées par des régimes de précipitations variables; (2) l'application d'un modèle hydrodynamique pour évaluer les charges des contaminants sur le risque à l'eau potable par une évaluation quantitative du risque microbien (QMRA). Les objectifs généraux de ce projet étaient les suivants : (1) présenter un nouveau cadre permettant d'évaluer l'impact des sources possibles de contamination fécale sur les prises d'eau potable d'un Grand Lac par temps sec et par temps humide; et (2) étudier l'approche par QMRA en tant qu'outil de gestion permettant d'atténuer les risques pour la santé pour les décisions portant sur la protection des sources d'approvisionnement en eau. Les objectifs spécifiques étaient les suivants: (1) quantifier les sources possibles de contamination fécale à une prise d'eau potable; (2) étudier la prévalence et la variabilité temporelle des parasites pathogènes, des bactéries indicatrices, des micropolluants des eaux usées et des matières en suspension dans les échantillons d'eaux usées non traitées, dans des conditions météorologiques

allant des précipitations aux précipitations intenses; (3) identifier les marqueurs appropriés des dérivations d'eaux usées dans e milieu récepteur en décrivant les co-variations entre les parasites, les bactéries, et les micropolluants des eaux usées; (4) évaluer les relations entre les concentrations de microorganismes étudiés, de micropolluants d'eaux usées, de matières en suspension et de débits de pointe par temps de pluie; (5) étudier la variabilité des charges environnementales de microorganismes fécaux, de micropolluants d'eaux usées et des matières en suspension d'une StaRRE dans diverses conditions; (6) déterminer les facteurs les plus importants influençant les charges massives provenant de l'affluent et des effluents, en plus d'évaluer la contribution relative des processus dans l'égout; (7) classer les sources de contamination fécale à l'aide d'approches probabilistes; (8) présenter les distributions de probabilité de parasites et de bactéries fécales à l'aide de modèles probabilistes déterministes et de modélisation hydrodynamique; (8) effectuer une analyse QMRA basée sur les événements pour la planification et la gestion de la protection des sources d'approvisionnement en eau potable.

La première partie de ce projet de recherche consistait à faire un suivi d'une StaRRE desservie par un système d'égout sanitaire présentant un niveau élevé d'eaux parasites (infiltration et captage). L'influent et l'effluent ont été surveillés afin de détecter les parasites pathogènes (*Cryptosporidium* et *Giardia*), les bactéries indicatrices (*Escherichia coli*, *Clostridium perfringens*) et les micropolluants des eaux usées (caféine, carbamazépine, 2-hydroxycarbamazépine, acésulfame, sucralose et aspartame), ainsi que les matières en suspension dans diverses conditions météorologiques. L'occurrence et les concentrations de parasites cibles, d'indicateurs fécaux et de micropolluants des eaux usées dans les échantillons d'eaux usées non traitées et d'effluents traités ont été évaluées et les principaux facteurs environnementaux contrôlant leurs concentrations ont été identifiés. Les charges environnementales des paramètres étudiés provenant de l'affluent (représentant un traitement incomplet), des effluents primaires (représentant une dérivation) et de l'effluent final (représentant les conditions de fonctionnement normales) ont été estimées et la contribution des processus dans l'égout aux charges massives a été déterminée. La variabilité temporelle des paramètres étudiés dans les échantillons d'eaux usées brutes a également été évaluée au sein d'événements et entre événements. Les habitudes quotidiennes liées aux habitudes de la population étaient évidentes. Au cours de la journée, l'après-midi et le début de soirée ont été reconnus comme des moments critiques en ce qui

concerne l'impact d'une dérivation d'une StaRRE en raison de concentrations et de débits plus élevés. Les conditions météorologiques humides et la période de fonte des neiges ont également été identifiées comme des moments critiques, car les concentrations les plus élevées d'agents pathogènes ont été observées durant ces périodes. La carbamazépine a été identifiée comme un traceur approprié de la contamination fécale dans un rejet de dérivation résultant d'une infiltration/afflux et/ou d'un traitement défaillant des eaux usées par temps humide.

La deuxième partie de ce projet consistait à examiner la variabilité des concentrations de *Cryptosporidium*, *Giardia* et *E. coli* à une prise d'eau potable considérant des scénarios de référence et après des précipitations. Une approche probabiliste a été réalisée pour estimer la charge des microorganismes, quelles que soient les conditions météorologiques, afin de classer les sources possibles de contamination fécale (rivières, ruisseaux, et la StaRRE). Une approche déterministe-probabiliste a également été utilisée pour estimer les charges de microorganismes fécaux provenant de diverses sources par temps sec et humide. Un modèle hydrodynamique tridimensionnel a été utilisé en utilisant les charges estimées. Le devenir et le transport de *Cryptosporidium*, *Giardia* et *E. coli* ont été simulés de la source jusqu'à la prise d'eau potable. Les distributions des concentrations simulées pour la consommation d'eau potable ont été intégrées dans le modèle d'évaluation quantitative du risque microbien de Santé Canada. La variabilité du risque microbien dans le contexte de l'eau potable a été examinée dans diverses conditions de charge. La modélisation hydrodynamique couplée au QMRA est un outil utile pour la planification et la gestion de la protection des sources d'eau.

Cette recherche fournit des informations techniques utiles pour la communauté de l'eau potable et aux ministères de l'Environnement. Les résultats de cette étude peuvent être utilisés pour guider les municipalités dans leurs décisions relatives à leur système d'approvisionnement en eau, à leur infrastructure de traitement des eaux usées afin d'atténuer les risques et d'améliorer leur résilience.

ABSTRACT

Waterborne disease outbreaks have been documented worldwide. Two major waterborne outbreaks occurred in Milwaukee, Wisconsin and in Walkerton, Ontario over the past three decades leading to regulatory changes for source water protection and drinking water treatment. Reported outbreaks have mostly occurred following heavy rainfall events, indicating a close link between watershed hydrology and waterborne outbreaks. The multi-barrier, source to tap approach is recommended as preventive approach to protect public health and to provide clean, safe and reliable drinking water to water consumers.

In urban areas, untreated or partially treated sewage remains an important source of fecal contamination. However, the performance of Water Resource Recovery Facilities (WRRFs, also known as wastewater treatment plants (WWTPs)) also varies between dry and wet weather conditions and their loads have generally not been quantified in wet weather. The variability of the microbiological quality of urban fecal contamination sources during rainfall events should be assessed in order to understand their impact on the drinking water intakes.

This dissertation contains two main themes: (1) monitoring a WRRF as an important source of fecal contamination for pathogenic parasites, fecal indicator bacteria, wastewater micropollutants and total suspended solids under weather conditions with varying precipitation patterns; (2) combining a discharge-based hydrodynamic model with quantitative microbial risk assessment (QMRA) to evaluate the public health risk associated with drinking water following various loading conditions. The general objectives of this project were to: (1) present a novel framework in order to assess the impact of possible fecal contamination sources on drinking water intakes of a Great Lake under dry and wet weather conditions; and (2) investigate the strength of QMRA as a management tool for mitigating health risks from pathogenic contaminants for source water protection decisions. The specific objectives were to: (1) investigate the prevalence and temporal variability of pathogenic parasites, indicator bacteria, wastewater micropollutants and total suspended solids in raw sewage samples under weather conditions ranging from trace to intense precipitation; (2) identify suitable markers of sewage by-passes in receiving waters by describing co-variations between pathogenic parasites, fecal indicator bacteria and wastewater micropollutants; (3) evaluate the relationships among the concentrations of studied microorganisms, wastewater micropollutants and total suspended solids and with peak flowrates

during wet weather events; (4) investigate the varibility of environmental loadings of fecal microorganisms, wastewater micropollutants and total suspend solids from a WRRF under various conditions; (5) evaluate the excess loads from a by-pass discahrge compared to final effleunt discahrge following wet weather conditions; (6) investigate the relative contribution of sewer processes in the mass loadings from raw sewage under various flow conditions; (7) determine the most important factors influencing mass loadings from the influent and effluent in addition to evaluating the relative contribution of sewer processes in the mass loadings; (8) rank fecal contamination sources using probabilistic approaches (9) present probability distributions of parasites and fecal indicator bacteria at a drinking water intake using deterministic-probabilistic loading and hydrodynamic modeling; (10) perform event based QMRA for source water protection planning and management.

The first part of this research project was to monitor a WRRF served only by a sanitary sewer system with high level of infiltration/inflow. Influent and effluent were monitored for pathogenic parasites (*Cryptosporidium* and *Giardia*), fecal indicator bacteria (*Escherichia coli*, *Clostridium perfringens)* and wastewater micropollutants (caffeine, carbamazepine, 2-hydroxycarbamazepine, acesulfame, sucralose and aspartame) and total suspended solids under various weather conditions. The prevalence rate and concentrations of target pathogenic parasites, fecal indicators and wastewater micropollutants in raw sewage and treated effluent samples were evaluated and the most important environmental factors controlling their concentrations were identified. In addition, the environmental loadings of studied parameters from the influent (representing incomplete treatment), primary effluent (representing by-pass discharge) and final effluent (representing normal operating conditions) were estimated and the contribution of sewer processes to mass loadings arriving at a WRRF was determined. The temporal variability of studied parameters in raw sewage samples was also assessed within events as well as between events. Daily patterns related to the habits of the population were evident. Over the course of a day, afternoon and early evening were recognized as critical times with regard to the impact of the studied WRRF into Lake Ontario following a by-pass discharge because of higher concentrations and flowrates. Wet weather conditions and the snowmelt period were also identified as potential critical times as higher concentrations of pathogens were observed. Carbamazepine was identified as a suitable marker of fecal contamination in a by-pass discharge

resulting from infiltration/inflow and/or failed wastewater treatment under wet weather conditions.

The second part of this project was to examine the variability of *Cryptosporidium*, *Giardia* and *E. coli* concentrations at a drinking water intake following baseline and precipitation-driven loading scenarios. A probabilistic approach was performed for load estimation of microorganisms irrespective to weather condition to rank the possible fecal contamination sources (rivers, creeks and WRRFs). A probabilistic-deterministic approach was also used to estimate the loadings of fecal microorganisms from various sources under dry and wet weather conditions. A three-dimensional hydrodynamic model was run using the estimated loadings and the fate and transport of *Cryptosporidium*, *Giardia* and *E. coli* were simulated at a drinking water intake. Simulated probability distributions of concentrations at the studied drinking water intake were fed into Health Canada Quantitative Microbial Risk Assessment model (HC QMRA) and the variability of microbial risk in the context of drinking water was examined under various loading conditions. It was shown that coupled hydrodynamic modeling and QMRA are useful tools for source water protection planning and management.

This research provides useful technical information for the drinking water community and Environmental Ministries. Results from this study can be used to guide municipalities with decisions with regards to their water supply system, wastewater infrastructure and risk control, best management practices for risk mitigation and/or reduction and improved resilience.

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ACE	Acesulfame
ASP	Aspartame
CBZ	Carbamazepine
CBZ-2OH	2-Hydroxycarbamazepine
CAF	Caffeine
C. perfringens	*Clostridium perfringens*
CSO	Combined Sewer Overflow
DWI	Drinking Water Intake
E. coli	*Escherichia coli*
FIB	Fecal indicator bacteria
I/I	Infiltration/Inflow
QMRA	Quantitative Microbial Risk Assessment
SUC	Sucralose
TSS	Total Suspended Solids
WRRF	Water Resources Recovery Facility
WWTP	Waste Water Treatment Plant
WWMPs	Wastewater micropollutants

CHAPTER 1 INTRODUCTION

Risk-based management approaches involve employing preventive methods for conserving the quality of drinking water (WHO, 2005 and 2011). Providing detailed information about a whole water supply system and understanding each process in a system are fundamental components of a risk-based management framework (Teunis et al., 1997). In order to control and minimize the impact of waterborne pathogens on human health and local economies, examining the whole water supply system from microbial contamination sources to water consumers is required (WHO, 2011; Health Canada, 2012). In this regard, there are different types of challenges relating to source waters, including: (1) identification of fecal contamination sources, (2) quantification of microbial loads from each of those sources, (3) characterising the hydrologic and watershed factors affecting fecal loadings and (4) evaluating fate and distribution of pathogens in the water courses (Ormsbee et al., 2004).

Pathogenic protozoa have been known as the etiologic agents of nearly 381 waterborne disease outbreaks worldwide between 2010 and 2016 (Efstratiou et al., 2017). One of the largest outbreaks occurred in March-April 1993 in Milwaukee, Wisconsin, in which over 50 deaths, over 4000 hospitalizations and over 400,000 illnesses were reported (Mac Kenzie et al., 1994; Fox et al., 1996; Hoxie et al., 1997). The main reason of this outbreak was noted to be the contamination of the Milwaukee drinking water intake by *Cryptosporidium* oocysts coming from discharges of the Jones Island Water Reclamation Facility and also possibly from discharges of combined sewer overflows (Hrudey et al., 2004). It is believed that intensive rainfall, spring runoff and uncommon wind conditions caused *Cryptosporidium* to be transported to the offshore intake of Howard Avenue Water Purification Plant (Hrudey et al., 2004). *Cryptosporidium* was transported to the drinking water intake even though it was located 2.3 kilometers offshore in Lake Michigan at a depth of 12.8 meters and water quality had met the fecal indicator criteria for the state and federal standards of that time (Fox et al., 1996). In 2000, an outbreak in Walkerton, Ontario, Canada, caused the death of seven people and more than 2000 cases of gastroenteritis. The bacteria *E. coli* O157:H7 and *Campylobacter jejuni* were identified as the etiologic agents (Robert D Laing, 2002; O'Connor, 2002). This outbreak occurred in a period of intensive rainfall events like many other outbreaks.

Important lessons from other studies of waterborne pathogens in the environment were: (1) a relation exists between hydrological processes and concentrations of microorganisms with the potential to cause waterborne disease outbreaks (Dorner et al., 2007), (2) fecal contamination can reach drinking water intakes of the Laurentian Great Lakes, which have been usually located in great depth and long-distance offshore following heavy rainfall events (Edge et al., 2013), (3) fecal indicator bacteria may not be sufficiently conservative for predicting presence/absence of the pathogenic organisms in water utilities located at great distances from sources of fecal contamination (Lalancette et al., 2012).

Based on the epidemiologic studies, many of the waterborne disease outbreaks occurred following periods of intensive precipitation events (Curriero et al., 2001; Rose et al., 2001; Naumova et al., 2005; Guzman Herrador et al., 2016) due to an increase of contaminant concentrations in the surface waters. Microbial loadings from faecal contamination sources depend on the local hydro-climatology conditions and vary between dry and wet weather conditions (Åström et al., 2009; Robinson et al., 2011; Burnet et al., 2014). Heavy rainfall events are more likely drivers of risks posed to water supply systems. However, the current studies of municipal water resource recovery facilities have focused on baseline/normal weather conditions and scientific studies with regard to hydro-meteorological driven events are limited. In addition, large numbers of studies performed microbial risk assessment (associated with drinking water) using monitoring data collected in baseline/normal weather conditions (e.g. Barbeau et al., 2000a; Ryu et al., 2008; Jaidi et al., 2009; Van den Akker et al., 2011; Pintar et al., 2012; Sato et al., 2013) while contaminant loadings in to the receiving waters are many orders of magnitude higher during and following wet weather periods (Signor et al., 2005; Swaffer et al., 2014).

In Canada, the multi-barrier approach is the recommended approach to mitigate waterborne pathogens in drinking water (Health Canada, 2012). Source water protection is known as the first barrier in this approach, which protects both the quality and quantity of the municipal drinking water sources. Source water protection has several benefits such as providing safe drinking water to communities now and in the future, reducing the costs of treating and distributing municipal drinking water and decreasing costs associated with expenses of clean up when a drinking water outbreak occurs. Thus, in this study, we mostly focused on the quality of the source water particularly following heavy rainfall events to investigate the impact of possible sources of fecal

contamination on the performance of a drinking water treatment plant in various weather conditions. In other words, it was aimed to present a novel risk-based management framework in a water supply system through identifying significant sources of fecal contamination, quantifying microbial loads from those sources following normal/baseline and wet weather conditions, assessing the impact of major sources on a drinking water intake of a Great Lake and finally suggesting best management practices for risk mitigation and/or elimination.

In order to satisfy the main objectives of this research project, influent and effluent of a water resource recovery facility was monitored for pathogenic protozoa (*Cryptosporidium* and *Giardia*), fecal indicator bacteria (*Escherichia coli, Clostridium perfringens)*, wastewater micropollutants (caffeine, carbamazepine, 2-hydroxycarbamazepine, acesulfame, sucralose and aspartame) and total suspended solids under weather conditions with varying precipitation patterns. The dynamic behaviours of target microorganisms and wastewater micropollutants in raw sewage samples were assessed and their removal efficiency rates were evaluated. Furthermore, microbial and wastewater micropollutants loads from the influent (representing incomplete treatment), primary effluent (representing by-pass discharges) and final effluent (representing normal operating conditions) of the studied water resource recovery facility which is served only by a sanitary sewer system was estimated. In order to simulate the concentrations of *Cryptosporidium*, *Giardia* and *E. coli* in a drinking water intake by hydrodynamic modelling, a probabilistic-deterministic approach was used to estimate microbial loads from various fecal contamination sources including two rivers, three creeks and two water resource recovery facilities into Lake Ontario. Finally, a probabilistic quantitative microbial risk assessment was performed based on the results of the hydrodynamic modeling and local operational data in a water treatment plant to investigate the impact of various loading events on a studied drinking water intake.

CHAPTER 2 LITERATURE REVIEW

In the context of this research project on pathogens in drinking water sources and more specifically on *Cryptosporidium* and *Giardi*a including their major sources, fate and transport and on QMRA as a management tool for mitigating their impact, a literature review was conducted and is presented in the following sections. The goal of the literature review was to highlight studies and knowledge gaps that are related to the domain of this study.

2.1 Pathogens in drinking water supply systems

Potential waterborne pathogens can be classified in to three groups: bacteria, parasitic protozoa and enteric viruses. The bacterial pathogens of greatest concern are *E. coli* O157:H7, *Campylobacter*, *Salmonella*, *Vibrio cholerae*, *Yersinia enterolitica and Shigella*. Priority waterborne parasitic protozoa include *Cryptosporidium*, *Giardia duodenalis* (syn. *G. intestinalis*), *Balantidium coli and Entamoeba histolytica*. Viral pathogens of concern include: Adenovirus, Astrovirus, Calicivirus, Reovirus, Rotavirus, Hepatitis A and Enterovirus (EPA, 2001). Concentrations of these microorganisms in water supply systems are linked to fecal contamination, which originate in the intestinal tract of humans and other animals. Animal manure is the origin of several pathogenic bacteria such as *E. coli* O157:H7, *Campylobacter*, *Salmonella* and the protozoan microorganisms *Cryptosporidium parvum* and *Giardia duodenalis* (Tyrrel et al., 2003; Bowman, 2009). Human feces have also been reported to be significant sources of pathogenic microorganisms including *Salmonella*, *Cryptosporidium*, *Giardia*, *Cyclospora*, *Entamoeba histolytica*, Adenovirus, Astroviruses, Calicivirus, Enterovirus, Rotavirus, Reovirus, Hepatitis A and Hepatitis B (Arnone et al., 2007). People may be infected through infected people and/or animals, ingestion of contaminated drinking water and recreational waters and foods irrigated by contaminated waters (Xiao, 2010). Among these agents, the contamination of drinking water supplies has been identified as the most important factor related to waterborne disease outbreaks (Baldursson et al., 2011; Efstratiou et al., 2017). The World Health Organization (WHO) reported that waterborne pathogens cause illnesses such as gastrointestinal diseases and diarrhea every day and are responsible to the death of 2.2 million people yearly.

Detecting pathogenic microorganisms in natural waters is difficult and costly and thus indicator organisms are commonly monitored for evaluating source water microbiological quality (Savichtcheva et al., 2006). Conventional fecal indicator bacteria including total and fecal coliforms, *E. coli*, fecal streptococci and enterococci, have been included in many water quality regulations worldwide. For instance, the Ontario Provincial Water Quality Objectives for recreational water is 100 *E. coli* organisms per 100 mL based on a geometric mean of at least 5 samples (MOE, 1994). This criterion may be used for assessment of surface water quality as a source for drinking water. However, indicators have not always correlated well with pathogens and may also be associated with non-human sources of fecal contamination (Wu et al., 2011). Several limitations exist with regards to the use of conventional fecal indicator bacteria in place of pathogen monitoring, such as: short survival time of indicator organisms in water bodies (McFeters et al., 1974; McFeters, 1990), growth after being released to the water column (Desmarais et al., 2002), lower resistance to chemical disinfectants (Hurst et al., 2007) and weak correlation with microbial pathogens (Winfield et al., 2003; Hörman et al., 2004). A meta-analysis by Lalancette et al. (2014) showed that fecal coliforms and *E. coli* are suitable indicators of *Cryptosporidium* in source waters dominated by recent and nearby sewage systems, but not for source waters impacted by rural fecal contamination or far wastewater sources. In order to partly overcome the difficulties associated with the application of conventional fecal indicator bacteria, the use of alternative fecal indicators (e.g. *Bacteroides*, *Bifidobacterium*, spore-forming *Clostridium perfringens*, *B. fragilis* phage, FRNA coliphages) (Savichtcheva et al., 2006) and/or the combined application of alternative chemical indicators with conventional indicators have been suggested (Hagedorn et al., 2009). Human-origin chemical indicators can be used for initial screening or for cross-validation rather than replacement of microbial indicators (Hagedorn et al., 2009). Pharmaceuticals and personal care products (PPCPs), hormones and artificial sweeteners which are discharged to the aquatic environment regularly or periodically through point and non-point sources could be used for the identification of fecal sources (e.g. Glassmeyer et al., 2005; Daneshvar, 2012).

Cryptosporidium and *Giardia*, two common pathogenic parasites, were identified as the etiologic agents of 381 protozoan drinking water outbreaks between 2011 and 2016 with *Cryptosporidium* responsible for 63% and *Giardia* 37% of the outbreaks (Efstratiou et al., 2017). There is a high potential for (oo)cysts to cause infections because an infected host can shed as many as 10^{10}

(oo)cysts (Ferguson, 2005) while ingestion of only 10 *Giardia* cysts or 30 *Cryptosporidium* oocysts can result in infection (DuPont et al., 1995; Adam, 2001). In addition, (oo)cysts can be difficult to remove during conventional treatment processes and are highly resistant to chemical disinfectants used in drinking water treatment (Fayer et al., 2007b; Baldursson et al., 2011; Carmena et al., 2012). However, advanced processes such as membrane separation and ultraviolet disinfection enhance removal efficiencies (Liberti et al., 2003; Jiménez et al., 2010).

Diarrheal illnesses associated with *Cryptosporidium* and *Giardia* are called cryptosporidiosis and giardiasis, respectively. Not all ingestion of all *Cryptosporidium* and *Giardia* species/genotypes results in diarrheal illness. Among all *Cryptosporidium* species/genotypes anthropogenic *C. hominis* and zoonotic *C. parvum* were reported to be responsible for 90% of cryptosporidiosis in the humans (Coupe et al., 2006; Jothikumar et al., 2008; Geurden et al., 2009; Xiao, 2010; Ryan et al., 2014b). In addition, among species of *Giardia, G. duodenalis* Assemblages A and B (with a wide range of hosts including humans, cattle and other mammals) were mostly associated with human infections (Cacciò et al., 2005; Xiao et al., 2008).

Adeyemo et al. (2018) recently reviewed commonly used methods (microscopy, immunology based methods, flow cytometry and molecular tools) for the detection of *Cryptosporidium* and *Giardia* in environmental samples. Depending on the reaserch objective and type of samples, an appropriate method must be selected. For detection and isolation of *Cryptosporidium* and *Giardia* in water and wastewater samples immunological methods (such as Environmental Protection Agency's (EPA) Method 1623) is commonly used. In method 1623, water samples are analysed for *Cryptosporidium* and *Giardi*a detection by concentration, immunomagnetic separation and immunofluorescence assay microscopy (EPA, 2012). In order to measure concentrations with this common method effectively, careful use of the terms "organism concentration" and "limit of detection" is needed (Ongerth, 2013). Classically, the term "organism concentration" was used as the number of oo(cysts) per unit of sample volume, but now, adjusting the number of organisms found by method 1623 with the recovery efficiency is recommended (Equation 2. 1) because (oo)cysts cannot be homogeneously distributed in the water column of natural waters (Xiao et al., 2013). Ongerth (2013) analysed available data to investigate the significance of recovery efficiency measurements in describing the concentrations of the organisms. The results indicated that: (1) the recovery efficiency at a single sampling site changes widely over time, (2) it changes from one site to another, even for close sites, (3) the recovery efficiency of *Cryptosporidium* and

Giardia change independently at any single sampling site, (4) based on the water quality, the raw numbers of (oo)cysts and concentration of (oo)cysts differ usually by factor from 2 to 10. The term "limit of detection" is called the minimum number of the (oo)cysts (i.e. 1 oo(cysts)) that could be recovered in a determined sample volume (Equation 2. 2). For example, the limit of detection in a 10L sample with 50% recovery efficiency is 0.2 (oo)cysts/L.

Organism concentration = number of organisms found / (sample volume (L)×recovery efficiency) (2. 1)

Limit of detection = one organism/ (sample volume ×recovery efficiency) (2. 2)

Method 1623 can only determine the total *Cryptosporidium* and *Giardia* count in water samples and is not capable of determining the origin of host species, viability and infectivity of the detected (oo)cysts (EPA, 2012). The infectivity of (oo)cysts could be assessed with cell culture infectivity assays, which are mostly combined with other methods to assess effectively (Health Canada, 2012). Cell cultures with immunofluorescence assay (CC-IFA), cell cultures with RNA RT-PCR (Reverse transcription–polymerase chain reaction) detection (CC-RNA) and cell cultures with DNA PCR (CC-DNA) detection are commonly used for detecting infectious oocysts (e.g. Johnson et al., 2010). The dual direct detection with cell cultures and immunofluorescence assays (3D-CC-IFA), which was developed by Lalancette et al. (2010), can also be used for estimating infectious oocysts.

2.1.1 Genotyping of *Cryptosporidium* and *Giardia*

Molecular techniques are promising tools for characterizing the species/genotypes and subtypes of parasitic protozoa and consequently making a link between contamination sources and human cryptosporidiosis and giardiasis (Fayer et al., 2007b). *Cryptosporidium* and *Giardia* consist of several species/genotypes that are recognizable by molecular characterization (Xiao et al., 2008). The genus *Cryptosporidium* is made up of about 30 species and over 50 genotypes (Xiao, 2010; Ruecker et al., 2012; Ryan et al., 2016). Several types of *Cryptosporidium* species/genotypes have been detected in humans (Xiao et al., 2008; Xiao, 2010; Zahedi et al., 2016) (Table 2. 1). Among them, five *Cryptosporidium* species/genotypes including *C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, *C. canis* are known to be responsible for human cryptosporidiosis (Xiao et al., 2008; Xiao, 2010). Zoonotic *C. parvum* and anthropogenic *C. hominis* are the most common species reported in humans (Xiao, 2010; Ryan et al., 2014b) and have been identified as the main etiologic agents of waterborne disease outbreaks worldwide. However, *C. cuniculus* from rabbits

was responsible for a waterborne outbreak in the UK (Chalmers et al., 2009; Xiao, 2010; Ryan et al., 2014a). Anthroponotic transmission is a possible route for *C. hominis*, whereas the predominance of *C. parvum* in a population is due to both anthroponotic and zoonotic transmission (Plutzer et al., 2009). Several types of *Giardia* species have also been reported in the literature (Table 2. 2). Among all the *Giardia* species, *G. duodenalis* has been detected in humans, farmland animals and companion animals (Xiao et al., 2008).

Table 2. 1: Species and genotypes of *Cryptosporidium* that infect human and other hosts (Adapted from (Zahedi et al., 2016))

Species	Major host(s)	Species	Major host(s)
C. rubeyi	Squirrels	*C. suis*	Pigs
C. scophthalmi	Turbot	*C. galli*	Birds
C. huwi	Fish	*C. hominis*	Humans
C. erinacei	Hedgehogs, horses	*C. molnari*	Fish
C. scrofarum	Pigs	*C. canis*	Dogs
C. viatorum	Humans	*C. andersoni*	Cattle
C. tyzzeri	Rodents	*C. varanii*	Lizards
C. cuniculus	Rabbits	*C. baileyi*	Birds
C. ubiquitum	Ruminants, rodents, primates	*C. parvum*	Ruminants
C. xiaoi	Sheep and goats	*C. meleagridis*	Birds and humans
C. ryanae	Cattle	*C. serpentis*	Humans, snakes and lizards
C. macropodum	Marsupials	*C. felis*	Cats
C. fragile	Toads	*C. wrairi*	Guinea pigs
C. fayeri	Marsupials	*C. muris*	Rodents
C. bovis	Cattle		

Table 2. 2: Species and Assemblages of *Giardia duodenalis* (Adapted from (Feng et al., 2011))

Species	Major Host(s)
G. agilis Kunstler	Amphibians
G. ardeae Noller	Birds
G. microti Benson	Muskrats and voles
G. muris Benson	Rodents
G. psittaci Erlandsen and Bemrick	Birds
G. varani Lavier	Lizards
G. duodenalis Davaine	Mammals
Assemblage A	Humans, nonhuman primates, domestic and wild ruminants, alpacas, pigs, horses, domestic and wild canines, cats, ferrets rodents, marsupials, other mammals
Assemblage B	Humans, nonhuman primates, cattle, dogs, horses, rabbits, beavers, muskrats
Assemblage C	Domestic and wild canines
Assemblage D	Domestic and wild canines
Assemblage E	Domestic ruminants, pigs
Assemblage F	Cats
Assemblage G	Mice, rats
Assemblage H	Seals

2.2 Sources of pathogenic microorganisms

Pathogenic microorganisms in surface and groundwaters originate from both point and non-point sources such as farm animals (livestock), wildlife, water resource recovery facilities (WRRFs), urban stormwater run-off, etc. (Ferguson et al., 2008), although some opportunistic pathogens are naturally present in the environment (Visvesvara et al., 2007). In summary, common sources of fecal contamination and transport pathways of pathogenic organisms are presented in Table 2. 3. The main sources of fecal contamination in drinking water catchments with regards to

Cryptosporidium, *Giardia*, fecal and chemical indicators were reviewed and are presented in the following sections.

Table 2. 3: Common fecal contamination sources and transport pathways (EPA, 2001)

Source category	Contamination pathway	Transport process(es)
Agricultural	Livestock-feedlot	Runoff and erosion
	Livestock-manure storage	Runoff, erosion and seepage
	Crop-manure/sludge application	Runoff, erosion
	Pasture	Runoff, erosion and direct
Urban/Residential	Domestic pets	Runoff
	Wildlife	Runoff, direct
	Septic system	Leaching and interflow
	Illicit connection	Direct
	Landfills	Runoff and leaching
Forest	Wildlife	Runoff, erosion and direct
Point Sources	WRRF	Direct
	Slaughterhouse	Direct
	Combined and sanitary sewer overflows	Direct and rainfall-driven

2.2.1 Inputs from livestock

In rural areas, the primary origin of microbial contamination is fecal material from farm animals. Manure storage facilities, feedlots and grazing pasture areas are identified as the major sources of microbial pollution (Jamieson et al., 2004; Ferguson et al., 2008). Characterizing microbial loads from livestock is possible through the collection of information about farm animal populations, feedlot locations, grazing schedules, access to water courses, manure production rates, the time and rate of manure application and fecal material characteristics (EPA, 2001). In addition, considering some key factors including animal age, behaviour, shedding intensity and pathogen prevalence, catchment characteristics (including soil type, slope, green cover and riparian buffer zone) and manure management practices are suggested as well (Ferguson et al., 2008). Farm

animals density per km^2, manure production rate (kg/day), parasite prevalence and concentration in livestock feces have been documented in the literature (Dorner et al., 2004; Ferguson et al., 2008).

Cattle have been reported as the main source of waterborne disease outbreaks associated with *Cryptosporidium* (Becher et al., 2004) and hence most studies focused on pathogen loadings from cattle rather than other livestock. Other livestock including pigs, sheep, poultry, goats, horses and and companion animals (cat and dog) in farmlands have also been reported in the literature as sources of fecal contamination (Ferguson et al., 2008). A Canadian study by Lalancette et al. (2012) reported median concentrations of *Cryptosporidium* and *Giardia* in the feces of calves and cows to be 333 and 52 occysts g^{-1} and and 111 and 7 cysts g^{-1} respectively. Cattle are usually infected with four *Cryptosporidium* species including *C. parvum*, *C. bovis*, *C. ryanae* and and *C. andersoni* (Xiao, 2010). Among these species, *C. parvum* is found both in humans and animals and and is the zoonotic species identified in the transmission of infectious *Cryptosporidium* to humans (Thompson et al., 2007). In dairy cattle, pre-weaned calves are mostly infected by *C. parvum,* weaned calves *by C. bovis, C. ryanae* and and adult cattle by *C. andersoni* (Fayer et al., 2006; Fayer et al., 2007a; Santín et al., 2008). Several studies examined the occurrence of these four species and age-related distribution of these genotypes in dairy cattle (Starkey et al., 2006; Coklin et al., 2007; Feng et al., 2007; Starkey et al., 2007; Thompson et al., 2007; Halim et al., 2008; Brook et al., 2009; Huang et al., 2014) as well as in beef cattle (Feltus et al., 2008; Budu-Amoako et al., 2012). Research from China demonstrated the presence of *C. parvum* and *G. duodenalis* Assemblage E as major genotypes in dairy cattle (Liu et al., 2015; Li et al., 2016). In addition, a higher prevalence of *C. parvum* in lambs and goat kids was reported in Spain (Díaz et al., 2015). An Australian version of the study also indicated that *C. parvum* is more prevalent in pre-weaned lambs than adult sheep (Yang et al., 2009). *C. parvum* was rarely detected in pig waste (Xiao, 2010).

2.2.2 Inputs from wildlife

Wild animals play an important role in spreading fecal contamination in drinking water catchments (Xiao et al., 2000; Heitman et al., 2002; Jellison et al., 2002; Cox et al., 2005; Jiang et al., 2005; Ruecker et al., 2005; Xiao et al., 2006) and have been reported as the cause of waterborne disease outbreaks (Hrudey et al., 2004). Quantification of microbial loads and

assessment of disease in wildlife is highly challenging compared to farm animals due to the variation of wildlife density with season and and difficulty in controlling their movement in the catchment (Ferguson et al., 2008). A limited number of studies have estimated microbial loads from wild animals, although forested water supplies relying primarily on source water protection in place of filtration have been the primary source of wildlife *Cryptosporidium* data, including Melbourne, Australia (e.g. Koehler et al., 2016). It is believed that concentrations of indicators and pathogens are low in defecation of wildlife animals compared to that of livestock (Ferguson, 2005), maybe due to lower density of wildlife animals compared to farm animals. In general, a low prevalence of human infectious species was reported in wild animal fecal samples (Ruecker et al., 2012; Nolan et al., 2013; Swaffer et al., 2014). However, the importance of wildlife was highlighted when *C. cuniculus* from rabbits was found to be responsible for a recent waterborne outbreak in the UK (Elwin et al., 2012). Ferguson et al. (2008) reviewed key factors including animal type, age, behaviour, population density, volume of manure, pathogen prevalence, shedding intensity, zoonotic transfer and catchment characteristics that may influence the pathogen loadings from wildlife. In addition, they documented wildlife animal density per km^2, daily manure production rates, prevalence and concentration of pathogenic protozoa in wildlife feces. Farm animals and humans are considered to be more important sources of protozoan microorganisms in surface waters (Ono et al., 2001; Xiao et al., 2001; Ward et al., 2002; Atwill et al., 2006; Nichols et al., 2006; Fu et al., 2010; Gallas-Lindemann et al., 2013). A study by Heitman et al. (2002) in Canada showed that the prevalence rate of *Giardia* and *Cryptosporidium* was lowest in wildlife (3.28% and 0.94% respectively) as compared to other sources such as humans and livestock. However, the prevalence rate of *Giardia* was higher in aquatic mammals like beaver and muskrat. Zahedi et al. (2016) reviewed *Cryptosporidium* species and genotypes in both wild terrestrial and marine mammals, wild birds, fish, amphibians and reptiles and discussed the significance of zoonotic *Cryptosporidium* species for public health and water communities.

2.2.3 Inputs from urban areas

In urban areas, pathogens can enter water bodies from several potential sources including water resource recovery facility discharges, on-site systems (septic leachate), cross-connected storm sewers and urban stormwater. Surface sources are mostly used by drinking water intakes (DWIs) and recreational activities and therefore impaired surface water can lead to human infection.

2.2.3.1 Wastewater

Wastewater is an important source of fecal contamination in urban areas as it can lead to the deterioration of the receiving waters through treated effluent discharges from water resource recovery facilities (WRRFs, also known as wastewater treatment plants (WWTPs)) combined sewer overflows (CSOs), sanitary sewer overflows, wastewater by-pass discharges and cross-connections to storm sewers. Investigations of wastewater effluent discharges are highly needed due to the impact of treated sewage quality on the impairment of the receiving waters (Akpor et al., 2011). Nowadays, treated wastewater is reused for agricultural and industrial purposes in industrialized countries and thus the quality of the treated sewage is also a concern.

Combined sewer systems convey untreated domestic wastewater, industrial sewage and rainfall induced runoff to WRRFs to be treated and released into the water body; however, during extreme rainfall and snowmelt, when the WRRFs are at their full capacity, untreated sewage may be discharged into the nearby rivers via CSOs or by-passes. Statistical process control analysis indicated that 80% of the peak concentrations of *E. coli* at DWIs were a result of upstream CSO discharges occurring by precipitation >10 mm or spring snowmelt (Madoux-Humery et al., 2016). Sanitary sewer systems usually transfer only sanitary flows to the WRRFs. Other flows such as infiltration flow from groundwater through network defects and rapid inflow following heavy rainfall events and snow thaws may be added to the sanitary flow (EPA, 2014). Sanitary sewer overflows may occur in separate sanitary sewer systems. Raw sewage not only can pose a health risk, but also can cause eutrophication of water courses because it contains high quantities of nutrients (Rechenburg et al., 2006). Infiltration/inflow, equipment and pipe failure, cross connections, blockage and breaks in the sewer lines are known to be responsible for the occurrence of sanitary sewer overflow and by-pass discharges (EPA, 2002). Sanitary sewer overflows and by-pass discharges have been determined as a major source of fecal pollution (EPA, 2004) and hence their impact on drinking water should be assessed.

Current studies have mostly investigated parasites, fecal and chemical indicators during different treatment stages of WRRFs and evaluated their removal efficiencies mostly during dry weather conditions (Table 2. 4 and Table 2. 5). However, the performance of WRRFs depend on weather conditions and are different between dry and wet weather conditions. The dynamics of fecal indicators were also evaluated at the WRRF influent with regard to WRRF efficiency and

management (Lucas et al., 2013) and CSO discharges (Madoux-Humery et al., 2013). Yet, the variability of raw sewage microbiological quality and loadings should be characterized during heavy rainfall events to understand the imapct of by-pass discharges and incomplete treatment on the DWIs. Concentrations and loadings of fecal and chemical indicators were evaluated in CSO discharges (Phillips et al., 2012; Madoux-Humery et al., 2013; Madoux-Humery et al., 2015), river waters (Daneshvar, 2012) and stormwater collection systems (Sauvé et al., 2012) and suitable indicators of fecal contamination were identified. However, co-variations among pathogenic parasites, fecal indicators and WWMPs have not been addressed in relation to precipitation and flowrates. Hence, data are needed to fill knowledge gaps in this area of study.

2.2.3.2 Septic systems and cross-connected storm sewers

On-site wastewater systems (septic systems), which are the oldest and most widely used treatment system, have usually been evaluated as poor as compared to centralized wastewater treatment plants. They sometimes are associated with the deterioration of waterbodies as a result of failure and surface and subsurface malfunctions. Septic systems are an important source of fecal contamination in surface and especially ground waters. The mean concentrations of *Cryptosporidium*, *Giardia* and *E. coli* in effluent discharges of septic systems in Australia were reported to be 8.7×10^4 oocysts/L, 486 cysts/L and 7.0×10^7 CFU/L respectively (Ferguson et al., 2008).

It is believed that interconnections between sanitary sewer systems and storm sewer networks could be potential sources of surface water contamination (Boyd et al., 2004; Sercu et al., 2009; Kuroda et al., 2012; Hajj-Mohamad et al., 2019) and groundwaters (Ellis et al., 2002; Wolf et al., 2006). Sauer et al. (2011) monitored 45 stormwater outfalls in four watersheds within metropolitan Milwaukee and analysed samples for a human-specific Bacteroides marker (HF183) to investigate if storm water samples were contaminated by human waste. In about two-thirds of the outfalls, the concentration of HF183 was high (>5000 copy number, i.e. CN, per 100 ml) or moderate (1000–5000 CN per 100 ml), indicating extensive sewage pollution of the storm sewer systems.

2.2.3.3 Stormwater/Urban runoff

Stormwater has also been identified as one of the potential sources of fecal contamination in urban areas (Selvakumar et al., 2006). Several factors including rainfall, illicit connections of the sanitary sewer system to the storm sewer system and backflow from combined sewer systems have been known as the main reasons of the stormwater contamination. The origin of microbial pollution in stormwater is attributed to human and animal wastes. Urban animals such as dogs, cats and rodents are responsible for the microbial contamination of the floodwater resulting from rainfall and therefore collecting information about the companion animals density in urban areas is needed (Ferguson et al., 2008). Microbial contamination in stormwater originates from illicit connections and backflow from combined sewer systems. Pathogens such as bacteria, enteric viruses and pathogenic protozoa that are commonly observed in WRRF samples can be found also in stormwater (Noble, Griffith et al. 2006, Rajal, McSwain et al. 2007, Cizek, Characklis et al. 2008, Sercu, Werfhorst et al. 2009, Sauer, VandeWalle et al. 2011). A summary of studies examining fecal indicator bacteria and reference pathogens in urban stormwater are presented in Table 2. 6.

Table 2. 4: Pathogenic protozoa and indicator bacteria in WRRFs (also known as WWTPs)

Author (year)	Monitoring description and investigation area	Findings
Rechenburg et al. (2006)	Monitoring influent and effluent of six WWTPs (two simple and small plants, two plants with moderate technology and two modern plants with tertiary treatment technology) and CSO discharges under normal and wet weather conditions for a period of 15 months and analysing samples for fecal indicators (*E. coli*, total coliform, fecal streptococci) and parasites *(Cryptosporidium* and *Giardia*). Investigation area: North-Rhine Westphalia-Germany	Under regular condition, removal efficiency of microorganisms was found to be dependent on the treatment technology of the plants. The reduction of bacteria for the small and modern plants was $1.8\log_{10}$ and $3\log_{10}$ respectively and reduction of *Giardia* for modern plants was about $3.2\log_{10}$. Reduction of *Cryptosporidium* was not measured due to detecting few numbers of oocysts at the raw sewage. The level of total coliform and *Giardia* at the downstream of sewer overflows was nearly $2\log_{10}$ higher compared to treated effluent following dry weather conditions. The annual loads of fecal organisms from CSOs were considerably higher than from treated effluent.
Arnone et al. (2006)	Examining indicator organisms (total coliform, fecal coliform, *E. coli*, enterococcus and fecal streptococcus) and parasites (*Cryptosporidium* and *Giardia*) in three CSO outfalls for two overflow events. Investigation area: Atlanta, GA & Louisville, KY	*Giardia* was detected in 96% of the CSO outfalls, but *Cryptosporidium* was found in only 12% of the samples. Limited correlations was found between *Giardia* and both enterococcus ($R^2 = 0.51$) and fecal streptococcus ($R^2 = 0.45$). CSOs were notably contributed to the *Giardia* load, while the contribution of CSO to *Cryptosporidium* load was insignificant.

Table 2. 4: Pathogenic protozoa and indicator bacteria in WRRFs (also known as WWTPs) (cont'd)

Author (year)	Monitoring description and investigation area	Findings
Kistemann et al. (2008)	Monitoring influent and effluent of six WWTPs (two bigger plants equipped with tertiary treatment, two moderate plants with secondary treatment and two compact plants) during 1 year for several fecal indicators including *E. coli*, total coliform, fecal streptococci, *C. perfringens* and *Giardia*. Investigation area: South west of Germany	The median concentration of *Giardia* at the influent and effluent of six WWTPs was in the range of 1618-46696 cyst/100L and 4-226 cyst/100L respectively. Microbial reduction was dependent on the size and capacity of the sewage systems and varied between 1.9 $\log_{10}$ to 3.5 $\log_{10}$. *Giardia* removal in small plants was inadequate (<1.5 $\log_{10}$) compared to bigger plants (>3$\log_{10}$). The total microbiological removal efficiencies were improved with final sand filtration and extensive intermediate settling.
Kay et al. (2008)	Analysing 1933 samples to fecal indicators (total coliforms, enterococci and fecal coliform), which were collected from 162 different sewage discharge sites. Samples were collected at the different process of wastewater treatment facilities including raw sewage, primary, secondary and tertiary treated sewage in the base flow and high flow conditions. Investigation area: UK and Jersey	Under base flow condition, geometric mean of fecal indicators were 1.3×10^3 CFU fecal coliform /100 mL, 5.5×10^3 CFU total coliform /100 mL and 3×10^2 CFU enterococci /100 mL; and under high flow condition 9.1×10^2 CFU fecal coliform /100 mL, 3.8×10^3 CFU total coliform /100 ml and 2.1×10^2 CFU enterococci /100 mL respectively. Under base flow condition, reduction of fecal indicator in primary effluents compared with raw sewage were 0-67.66%; in secondary effluents compared with primary-treated effluent were 95.22-99.29% and in tertiary effluents compared with secondary-treated effluent were 93.24- 99.92%.

Table 2. 4: Pathogenic protozoa and indicator bacteria in WRRFs (also known as WWTPs) (cont'd)

Author (year)	Monitoring description and investigation area	Findings
Fu et al. (2010)	Monitoring different stages of three WWTPs (primary, secondary, sedimentation, filtration, membrane ultra-filtration and ozone/chlorine disinfection) for *Cryptosporidium, Giardia and* fecal indicators including somatic coliphages and fecal coliforms during three years (2005 – 2007). Investigation area: Beijing, China	Concentrations of *Cryptosporidium* and *Giardia* in raw sewage were 33–600 oocysts/L and 130–3600 cysts/L, in primary effluent were 67–333 oocysts/L and 533–2033 cysts/L, in secondary effluent were 0–9 oocysts/L and 0-33 cysts/L and in tertiary effluent were 0–0.4 oocysts/L and 0-2.1 cysts/L respectively. Log removals of *Cryptosporidium* and *Giardia* from primary treatment process were 0.12 $\log_{10}$ and 0.18 $\log_{10}$ respectively. Efficiency of oxidation ditch process for removal of *Cryptosporidium* and *Giardia* (2.17 $\log_{10}$ and 2.60 $\log_{10}$) was higher than anaerobic-anoxic-oxic process (1.79 $\log_{10}$ and 2.04 $\log_{10}$) and conventional activated sludge process (1.52 $\log_{10}$ and 1.68 $\log_{10}$). Efficiency of membrane ultrafiltration for removal of *Cryptosporidium* and *Giardia* (>1.84 $\log_{10}$ and 2.4 $\log_{10}$) was higher than conventional flocculation sedimentation (o.77 $\log_{10}$ and 0.73 $\log_{10}$) and sand filtration process (0.92log10 and 0.82log10) as the tertiary treatment. Fecal coliforms did not correlate well with pathogenic protozoa. However, soamtic coliphages showed good correlation with *Cryptosporidium* ($R=0.73$, $p<0.001$) and *Giardia* ($R=0.63$, $p<0.001$) and thus was known as good indicator of wastewater.
Ajonina et al. (2012)	Examining *Cryptosporidium* at the influent and effluent of a WWTP seasonally for dry weather periods and assessing removal efficiency of the plant. Investigation area: North Germany	*Cryptosporidium* occurred in 100% of the influent and 80% of the effluent samples and *Cryptosporidium* concentration reported to be higher during autumn and winter compared to summer. *Cryptosporidium* in raw sewage and treated effluent ranged from 50 to 1280 oocysts/L and 30 to 1170 oocysts/L respectively. *Cryptosporidium parvum* was dominant parasite.

Table 2. 4: Pathogenic protozoa and indicator bacteria in WRRFs (also known as WWTPs) (cont'd)

Author (year)	Monitoring description and investigation area	Findings
Madoux-Humery et al. (2013)	Monitoring two sewage CSO outfalls for eleven events resulting from precipitations in summer and fall seasons and mixture of precipitation and snowmelt in late winter and early spring in order to examine the variability of *E. coli* and wastewater micropollutants in CSOs during events. Investigation area: Great Montreal, Canada	Among different types of events, snowmelt time was known as a critical period with respect to impose of higher health risk by CSOs to DWIs due to long duration of this period and lack of any management strategies to restrict overflows. *E. coli* (with median concentration of 1.5×10^{6} *E. coli*/100mL in outfall OA and 5.1×10^{4} *E. coli*/100mL in outfall OB) was determined as the best indicator of fecal contamination in CSOs compared to that of wastewater micropollutants.
Gallas-Lindemann et al. (2013)	Collecting samples from influent and effluent of WWTPs, recreational swimming areas, raw water, stream, running water, drinking water and groundwater during 2009-2011 and analysing samples for *Cryptosporidium* and *Giardia*. Investigation area: France	The average *Cryptosporidium* at the influent and effluent of studied WWTPs (48 oocysts/L and 1.7 oocysts/L respectively) was less than *Giardia* (218 cysts/L and 4.4 cysts/L respectively). Study confirmed the prevalence of *Cryptosporidium* and *Giardia* in all sampling locations and showed that parasites can be considered as major threat for public health as they enter and distribute in the aquatic environment.

Table 2. 4: Pathogenic protozoa and indicator bacteria in WRRFs (also known as WWTPs) (cont'd)

Author (year)	Monitoring description and investigation area	Findings
Lapen et al. (2016)	In order to have an accurate quantitative *Cryptosporidium* infection risk associated with surface waters, samples were collected from South Nation river and Grand river basins (main river stems, tributaries, agricultural drainage streams, water treatment plant intakes and effluent of a WWTP) and were analysed for the concentration and species/genotype of *Cryptosporidium* oocysts. Investigation area: Canada	Mean concentration of *Cryptosporidium* oocyst in WWTP outflow (in Grand river basin) during four seasons was 45.02 (oocyst/ 100 L). Out of 12 occurrences of *C. hominis* and 12 occurrences of *C. parvum* 3 and 4 were observed at the effluent of studied WWTP (in Grand river basin). In addition to both *C. hominis* and *C. parvum*, *C.andersoni*, *C.ubiquitum*, Fox Muskrat I, Skunk, *C. felis*, *C. muris*, Chipmunk were also detected in WWTP outflow.

Table 2. 5: Summary of studies of chemical indicators in WRRFs

Author (year)	Monitoring description and investigation area	Findings
Miao et al. (2005)	Examining the distribution of CAF, CBZ and five of its metabolites (CBZ-EP, CBZ-2OH, CBZ-3OH, CBZ-10OH, CBZ-DiOH) in both aqueous and solid phases of various treatment processes of a WWTP. Investigation area: Ontario/Canada	In untreated biosolids the concentrations of CBZ, CBZ-2OH, CBZ-3OH and CBZ-DiOH were 69.6, 1.9, 1.6 and 7.5 µg/kg (dry weight) and in treated biosolids the concentrations were 258.1, 3.4, 4.3,and 15.4 µg/kg (dry weight), respectively. CBZ-EP and CBZ-10OH were not present in any of the biosolid samples. In untreated wastewater, the mean concentrations were 356.1ng CBZ/L, 39.2 ng CBZ-EP/L, 59.0 ng CBZ-2OH/L, 55.4 ng CBZ-3OH/L, 22.2 ng CBZ-10OH/L, 1001.2 ng CBZ-DiOH/L and in treated wastewater were 251.0 ng CBZ/L, 19.1 ng CBZ-EP/L, 70.4 ng CBZ-2OH/L, 69.2 ng CBZ-3OH/L, 32.5 ng CBZ-10OH/L, 1081.2 ng CBZ-DiOH/L. The removals of CBZ and CAF from the aqueous phase were 29% and 99.9% respectively. However, CBZ metabolites were not removed effectively.
Buerge et al. (2009)	Collecting samples from the untreated and treated wastewater, rivers, lakes, groundwater and tap waters and examining them for the four artificial sweeteners including acesulfame, cyclamate, saccharin and sucralose. Investigation area: Switzerland/Zurich	Acesufame was consistently observed in influent and effluent of studied WWTPs, in several surface waters, groundwater and tap water. The acesulfame was not removed through treatment processes and was quite persistent in surface waters. The highest concentration of acesulfame in groundwater was up to 4.7 µg/L and hence was introduced as an ideal chemical indicator of domestic wastewater in groundwater. The mean removal of cyclamate and saccharin were 99% and 90% in wastewater treatment plants with activated sludge whereas no elimination was observed for acesulfame and sucralose.

Table 2. 5: Summary of studies of chemical indicators in WRRFs (cont'd)

Author (year)	Monitoring description and investigation area	Findings
Scheurer et al. (2009)	Examining the prediction power of six tracer markers (acesulfame, sucralose, carbamazepine, diatrizoic acid, 1H-benzotriazole and 4-methyl analogue) as potential wastewater markers. In addition, studying the impact of advanced treatment processes for the removal of studied markers. Investigation area: Germany Baden- Württemberg	The ratios of acesulfame, sucralose and carbamazepine in the influent and effluent were stable (ACE/CBZ=45 and 40, SUC/CBZ=1.8 and 1.7, ACE/SUC=24 and 24 in the influent and effluent respectively), indicating the stability of these tracers in the studied wastewater treatment plants. Applying further treatment steps like ozonation and activated carbon, consumption pattern in various countries and seasonal variations may shift these ratios. Additional treatment with activated carbon enhanced the elimination of carbamazepine, 1H-benzotriazole and 4-methyl analogue in studied WWTPs. By limiting influence of point sources and regional differences acesulfame and carbamazepine seem to be the strongest markers of wastewater.
Sim et al. (2011)	Collecting samples from the influents and treated effluents of WWTPs including 12 municipal (M-WWTPs), 4 livestock (L-WWTPs), 4 hospital (H-WWTPs) and 4 pharmaceutical manufactures (P-WWTPs) and analysing samples for 24 different pharmaceuticals (Non-steroidal anti-inflammatory drugs, stimulant, Anti-seizure, Antibiotic) Investigation area: Korea	At the influents and effluents, the concentration of caffeine were in the range of 2500- 60400 ng CAF/L and 859-76600 ng CAF/L and the concentration of CBZ were 167-10100 ng CBZ/L and 1750-51700 ng CBZ/L respectively. At the influent, the concentrations were in the rank order of L-WWTPs> P-WWTPs> H-WWTPs> M-WWTPs. Due to the different fate and transport of studied pharmaceuticals in WWTPs, the concentration distributions in the effluents were different from that in the influents. The highest daily loads from the influents were noticed at M-WWTPs due to their high flowrates.

Table 2. 5: Summary of studies of chemical indicators in WRRFs (cont'd)

Author (year)	Monitoring description and investigation area	Findings
Gao et al. (2012)	Examining fate and transport of fifteen pharmaceuticals (tetracycline, demeclocycline, chlortetracycline, oxytetracycline, doxycycline, meclocycline, sulfadiazine, sulfamerazine, sulfamethazine, sulfamethoxazole, tylosin, acetaminophen, erythromycin-H2O, lincomycin, caffeine and carbamazepine) in different stages of a conventional municipal WWTP and sludge phases by a high-performance liquid chromatograph coupled to a tandem mass spectrometer. Investigation area: Michigan/ USA	Among the fifteen pharmaceuticals, sulfamerazine, tylosin, erythromycin and demeclocycline were not detected in the influent of studied WWTP. The other target pharmaceuticals were detected in the studied WWTP and sludge. Relatively higher removal efficiency rates (>99%) were observed for chlortetracycline, tetracycline, sulfamerazine, acetaminophen and caffeine and lower removal efficiency rates for doxycycline, oxytetracycline, sulfadiazine and lincomycin (<50%). The removal efficiency rate of sulfamethoxazole was about 90%. A net increase of mass of 41% from the influent was noticed for carbamazepine. Mass balances indicate that biotransformation was the major process for the removal of pharmaceuticals (22% to 99%). While for the investigated pharmaceuticals, the contribution of sorption to sludge was approximately insignificant (7%).

Table 2. 5: Summary of studies of chemical indicators in WRRFs (cont'd)

Author (year)	Monitoring description and investigation area	Findings
Gan et al. (2013)	Testing wastewater, surface water, coastal waters, groundwaters, tap waters and precipitation samples to seven artificial sweeteners including acesulfame, sucralose, cyclamate, saccharin, aspartame, neotame and neohesperidin dihydrochalcone Investigation area: China	Higher concentrations (1 and 15 µg/L) were noticed for sucralose and acesulfame at the treated effluent. Sucralose and acesulfame were introduced as the most persistent artificial sweeteners in the studied WWTPs. Among studied artificial sweeteners acesulfame, sucralose, cyclamate and saccharin were usually detected in surface waters; acesulfame in surface waters and tap waters. Neotame and neohesperidin dihydrochalcone were not usually detected at the collected samples and the observed concentrations were usually low.
Subedi et al. (2014b)	Examining fate and transport of four artificial sweeteners (sucralose, aspartame, saccharin, acesulfame) in influent, primary effluent, effluent, suspended particulate matter and sludge of a WWTP. Investigation area: New York / USA	All studied artificial sweeteners were detected (100%) in the range of 0.13-29.4 µg/L, 0.4 27.7 µg/L, 0.11-29.6 µg/L, 0.08-0.65 µg/L in the influent, primary effluent, effluent ar sludge respectively. The fraction of the total mass of aspartame, acesulfame, saccharin and sucralose sorbed t suspended particulate matter were 50.4%, 10.9% and 0.8% respectively. The daily loadings of sucralose, acesulfame and saccharin into water bodies per 1000 peop were 17.6 g/d, 1.22 g/d and 1.07 g/d respectively. The removal efficiency rate of saccharin and aspartame were 90.3% and 68.2% respectivel However, sucralose and acesulfame barely removed from the studied WWTPs (<2%).

Table 2. 5: Summary of studies of chemical indicators in WRRFs (cont'd)

Author (year)	Monitoring description and investigation area	Findings
Tran et al. (2018)	Reviewing 60 emerging contaminants (ECs) including antibiotics, pharmaceuticals, personal care products, hormones and artificial sweeteners in the influent, treated effluent, sludge and biosolids of WWTPs from different geographical regions including Asia, Europe and North America	Concentrations of most of reviewed ECs were lower in raw sewage in European and North American countries than in Asian region. Occurrence and fate of ECs was mostly studied in dissolved phase than in particulate phase (i.e. sewage sludge and biosolids). Intra-day, inter-day and inter-season sampling in addition to composite sampling was suggested.

Table 2. 6: Summary of studies on pathogens, fecal and chemical indicators in urban stormwater

Author (year)	Description and investigation area	Findings
Arnone et al. (2005)	Samples were collected from three urban stormwater and six CSO outfalls and were analysed for *Giardia*, *Cryptosporidium and* six fecal indicators. Investigation area: USA	Geometric means of *Cryptosporidium* and *Giardia* in urban stormwater samples were in the range of 5-31 oocysts/100L and 5-377 cysts/100L respectively. *Cryptosporidium* and *Giardia* were prevalent in 40% and 60% of the stormwater samples respectively. Smaller volume of samples for stormwater analysis was suggested when the level of turbidity is higher.
Chong et al. (2012)	The composite samples were collected from urban stormwater runoff over different wet weather events by auto-sampler and were analysed for heavy metal, toxicity, fecal indicators and pathogens. Investigation area: Australia	Several pathogens including bacteria and viruses were present in wet weather samples. The numbers of *E. coli* and enterococcus in three wet weather events ranged from 1.33×10^{2} to 1.07×10^{4} CFU *E. coli*/100mL and 1.27×10^{2} to 3.11×10^{4} CFU enterococcus /100mL respectively.
Sidhu et al. (2012)	Six study sites were monitored for enteric bacteria, viral pathogens and fecal indicators. Samples were collected in the base-flow and in stormwater runoff. Investigation area: Australia	Adenovirus, *Campylobacter,* polyomavirus, *Campylobacter coli, C. jejuni*, *Salmonella enterica and human*–specific HF183 *bacteroides marker* were detected mostly in the dry and wet weather samples. The level of *E. coli* was in the range of 7.67×10^{1}-1.73×10^{3} MPN/100mL in dry weather samples and 4.67×10^{2}-8.93×10^{3} MPN/100 mL in wet weather samples.

Table 2. 6: Summary of studies on pathogens, fecal and chemical indicators in urban stormwater (cont'd)

Author (year)	Description and investigation area	Findings
de Man et al. (2013)	In order to estimate microbial risk associated with urban floodwater ingestion, 23 events based urban stormwater samples (originated from storm sewers, combined sewers and from stormwater runoff generated by rainfall) were collected from 18 locations in the Netherlands. The collected samples were analysed for pathogens (*Cryptosporidium*, *Giardia*, Campylobacter, noroviruses and enteroviruses) and indicator organisms (*E. coli* and *enterococci*). Investigation area: Netherland	The level of *Cryptosporidium* and *Giardia* was in the range of 0.1-9.8 oocysts/L and 0.1-142 cysts/L respectively. *Cryptosporidium*, *Giardia*, noroviruses, enteroviruses and Campylobacter were prevalent in 30%, 35%, 61%, 29% and 35% of the collected samples respectively. Infection risk from exposure to floodwater originating from CSOs, from storm sewers and from stormwater runoff reported to be 33%, 23% and 3.5% respectively.
Staley et al. (2016b)	Culturable *Escherichia coli* and ampicillin-resistant *E. coli* levels, microbial source tracking markers (Bacteroidales spp., human, ruminant/cow, gull and dog) and chemical source tracking markers associated with human wastewater (caffeine, carbamazepine, codeine, cotinine, acetaminophen and acesulfame) were quantified in storm water outfalls and sites along the Humber river. Investigation area: Ontario/Canada	The mean concentrations of the human fecal marker in storm water outfalls were 4.22 $\log_{10}$ CN/100 mL for human and 0.46 $\log_{10}$ CN/100 mL for gulls. At the studied sites the observed maximum concentrations of caffeine, acetaminophen, acesulfame, *E. coli* and the human fecal markers were 34800 ng/L, 5120 ng/L, 9720 ng/L, 5.26 $\log_{10}$ CFU/100ml and 7.65 $\log_{10}$ CN/100ml respectively. It suggests the sewage contamination of the storm water outfalls and the Humber river due to cross connection problems.

Table 2. 6: Summary of studies on pathogens, fecal and chemical indicators in urban stormwater (cont'd)

Author (year)	Description and investigation area	Findings
Hajj-Mohamad et al. (2019)	Water samples were collected from 30 sampling points of two watersheds (with unintended sewage connection to stormwater collection system) during dry weather condition. Samples were analysed for fecal coliforms, *E. coli*, general Bacteroidales (human-specific Bacteroides 16S rRNA gene sequence (HF183) and human-specific mitochondrial DNA (Hmt)) and WWMPs (CBZ (carbamazepine), CAF (caffeine), THEO (theophylline), ACE (acetaminophen)). Investigation area: Greater Montreal Region/Canada	WWMPs were detected at all samples, suggesting the contamination of urban stormwater system with human fecal contamination. HF183, Hmt, CAF, THEO and ACE were proposed as appropriate markers for determining sewer cross-connections. For identifying cross-connected sewers with possibility of high *E. coli* concentrations in stormwater collection system, threshold of concentrations more than 3 Log HF183 (copies 100 mL−1), 2Log Hmt (copies 100 mL−1), 2Log CAF (ng/L), 2 Log THEO (ng/L), 1 Log ACE (ng/L) was proposed.

2.3 Fate and transport of surface water pathogens

Understanding the fate and transport of pathogenic organisms on the land surface, within stream networks and water reservoirs is needed to estimate microbial risk for the development of management priorities for risk mitigation. Investigating fate and transport of microorganisms in terrestrial and aquatic systems is more challenging as a result of the complexity of natural environmental systems and the large number of physical, chemical and biological factors that influence pathogen survival and migration (Bradford et al., 2013). Fundamental factors, which are controlling pathogens survival and transport, were reviewed for watersheds (Ferguson et al., 2003a; Jamieson et al., 2004; Dorner, 2005), in agricultural settings (Bradford et al., 2013) and in lakes and/or reservoirs (Brookes et al., 2004).

Pathogen survival depends on the nature of the pathogens and type of environment (water, soil, manure) (Bradford et al., 2013). Generally, visible and ultraviolet solar radiation, salinity, pressure, inorganic (ammonia) and organic nutrients are significant agents that influence on die-off rates and the transport of pathogenic microorganisms in aquatic environments (Ferguson et al., 2003b; Brookes et al., 2004). Abiotic factors including attachment, temperature, moisture content and biotic factors such as competition, predation and biofilms have been mainly reported as important factors that influence the inactivation of pathogens on the land surface (Bradford et al., 2013). Among several factors, light and temperature are the major controlling factors that influence the inactivation of the pathogens in soils, animal wastes and water; however, for some of the organisms, predation might be an important mechanism (Brookes et al., 2004; Burnet et al., 2017). Water temperature has a large impact on the die-off rate of waterborne pathogens; as such oocysts can persist in cold waters for several months (Robertson et al., 1992; Medema et al., 1997; Fayer et al., 1998), but inactivate more rapidly in warm waters (Schijven et al., 2005). *Cryptosporidium* reaches the highest viability at 4ºC and its viability decreases as temperature exceeds 4ºC (Walker et al., 1999). First order kinetics are commonly used to simulate the inactivation of pathogens in natural waters (Equation 2. 3) (Chauret et al., 1998; Jenkins et al., 1999; Davies et al., 2005; Ives et al., 2007), in soils (Jenkins et al., 2002; Darnault et al., 2004; Nasser et al., 2007) and in animal feces (Robertson et al., 1992; Olson et al., 1999).

$$N = N_0 \exp(-\mu t) \qquad (2.\ 3)$$

In this equation, N is the number of microorganisms at time t (CFU), N_0 is the number of microorganisms at time t_0 (CFU), μ is the constant of die-off rate h^{-1} and t is time.

2.3.1. The impact of hydrologic and hydrodynamic processes on the fate and transport of pathogens

Waterborne disease outbreaks associated with pathogenic parasites have been reported worldwide (Baldursson et al., 2011; Efstratiou et al., 2017). Most of the outbreaks have occurred following storm events, suggesting a connection between watershed hydrology and waterborne disease outbreaks (Hrudey et al., 2002; Hunter, 2003). Studies have evaluated the relationship between hydrologic events and the microbial density in surface waters and confirmed increases of pathogenic parasites and fecal indicators in the source waters (Atherholt et al., 1998; Kistemann

et al., 2002; Signor et al., 2005; Burnet et al., 2014; Swaffer et al., 2014). An increase of approximately 1.5 log of *E. coli* concentrations at DWIs was noticed between dry and wet weather conditions (Madoux-Humery et al., 2016).

Besides hydrologic events, hydrodynamic processes also have a great impact on the fate and transport of microorganisms in receiving waters. Pathogens are dispersed in lakes and water reservoirs through hydrodynamic processes including advection (horizontal and vertical) and dispersion (turbulent and shear). Inflows, wind currents and internal waves are responsible for horizontal advection of pathogens in lake and water reservoirs. In stratified reservoirs, wind currents only have an impact on the surface layer while inflows can influence at any depth. Internal waves can produce internal currents, which can be active at different depths and in different directions (Brookes et al., 2004). Internal waves are also responsible for producing vertical movement in water reservoirs and facilitate the movement of microorganisms to offshore intakes through vertical advection (Deen et al., 2000). The behaviour of riverine inflow is important from the point of view of hydrodynamic modelling. Hydrodynamic distribution of microorganisms in lakes and water reservoirs depends on the speed of inflow and insertion depth (Brookes et al., 2004). The density of the fluid for inflows drives certain processes. For example, warm inflows usually flow over the lake surface and cold dense inflows move down to lower level and flow toward the deepest points. Therefore, dense underflows may be a major threat to drinking water reservoirs (Brookes et al., 2004). Riverine inflows increase following heavy rainfall events and therefore the level of microorganisms discharging in tothe receiving waters can be high. Rainfall events can also cause reservoir short-circuiting and consequently increase the possibility of viable pathogens reaching drinking water intakes (Hipsey, 2005).

2.4 Fate and transport modelling

Both field monitoring and computer modelling are commonly employed for tracking microorganisms in the environment. Field monitoring is an effective method for characterizing the risk from waterborne pathogens to water bodies, but high costs and high temporal and spetial variability of pathogens in ecosystems have made its function limited (Shirmohammadi et al., 2006). Computer models are usually employed as an alternative to intensive field monitoring methods as they can be used to test various management practices, thereby saving time and reducing costs (Shirmohammadi et al., 2006; Cho et al., 2016). Hydrologic and hydrodynamic

models (coupled to a water quality model) are commonly used to simulate fate and transport of reference pathogens on the land surface, stream networks, estuaries, coastal waters, water reservoirs and lakes. In other words, hydrologic models simulate fate and transport of pathogens at the watershed level while hydrodynamic models are responsible for simulating the fate and distribution of microorganisms within aquatic environments considering variation of density, bathymetry, meteorological data, tides, velocity and surface elevation.

At the watershed level, a hydrologic model is commonly linked to a water quality model to estimate pathogen loads from the catchment to the drinking water reservoir. Cho et al. (2016) critically reviewed the models associated with microbial water quality of surface waters and discussed the components of current models at the watershed scale. Several research studies have examined the fate and transport of indicator organisms at the watershed level. Some of these research studies including MWASTE (Moore et al., 1989), COLI (Walker et al., 1990) and SEDMOD (Fraser et al., 1998) have not considered stream routing in modelling processes and therefore are known as fecal indicator loading models. Others such as the Soil and Water Assessment Tool (SWAT) (Sadeghi et al., 2002; Coffey et al., 2010; Bougeard et al., 2011; Coffey et al., 2013; Niazi et al., 2015), the Hydrological Simulation Program Fortran (HSPF) (Moyer et al., 2003; Im et al., 2004; LaWare et al., 2006; Desai et al., 2011; Seong et al., 2013), SIMHYD (Haydon et al., 2006; Holz, 2010), the Watershed Assessment Model (WAM) (Tian et al., 2002; Collins et al., 2004) simulate fate and transport of microbial indicators on the land surface and stream network. At the watershed level, studies on modelling pathogenic protozoa loads from a catchment coupled to stream routing are relatively scarce (Walker et al., 1999 ; Medema et al., 2001; Dorner et al., 2004; Dorner et al., 2006; Ferguson et al., 2007). Pathogen models at the watershed scale have been reviewed and the summaries are listed in Table 2. 7.

The application of 1D, 2D or 3D hydrodynamic models has received more attention for aquatic environments (rivers, lakes, coastal waters, bays, estuaries and reservoirs), for recreational waters and DWIs. Similar to hydrologic models, hydrodynamic models are usually coupled with a water quality model for a wide range of objectives. Deterministic and/or probabilistic approaches have been used for load estimation when enough field data are available from the point of discharge of fecal pollution into the aquatic system. In deterministic approaches, the processes are defined in physical terms without considering a random component (Pitt et al., 2002) and a set of model

inputs (that are assumed to be known with certainty) are used to provide a set of output values (Portielje et al., 2000; Boano et al., 2006). However, it has been reported that many deterministic models are not capable of producing reasonable results for many fundamental biological constituents (McIntyre et al., 2003); as they are simplified from complicated environmental processes and will always provide results with some degree of uncertainty (McIntyre et al., 2004). In probabilistic approaches, deterministic relationships and random variables are selected. Probabilistic approaches are effective tools to be used for estimating microbial loads because: (1) the uncertainty of the system can be presented by probability distribution functions, which are more meaningful than single-point values; (2) it is easy to incorporate randomly driven parameters into a system.

The hydrodynamic-based studies examined various fate mechanisms (McCorquodale et al., 2004) and critical controlling factors (Hipsey et al., 2004; Hellweger et al., 2008a) in aquatic environment to simulate the fate and transport of pathogens to DWIs (Sokolova et al., 2012), evaluate the influence of climate and population changes on the water quality of DWIs (Jalliffier-Verne et al., 2015) and to provide recommendations for the management of fecal contamination sources (Sokolova et al., 2013; Jalliffier-Verne et al., 2016a). In addition, hydrodynamic modelling was recently combined with quantitative microbial risk assessment to evaluate public health risk and to provide recommendations for managing beaches and drinking water sources (Sokolova et al., 2015; Eregno et al., 2016). A summary of these studies is illustrated in Table 2. 8. This table indicates that literature studies have mostly simulated fate and transport of microbial contamination at DWIs following normal operation conditions; wet weather impacts and its negative consequences on DWIs have not been as widely addressed.

Table 2. 7: Summary of pathogen models at the watershed scale

Author (year)	Model description, findings and limitation
Walker and Stedinger (1999)	A numerical generalized watershed loading function (GWLF) model that can predict *Cryptosporidium* loadings from treated sewage and dairy calves. Hydrologic model, stream and reservoir routing and first order oocysts inactivation considered in this model. Results show that *Cryptosporidium* oocyst loading from treated effluent was greater than from dairy cattle. Some process such as entrapment and filtration, which can decrease oocysts concentration in run-off were neglected in modelling process.
Medema et al. (2001)	Modelling emission of *Cryptosporidium* and *Giardia* in surface water (discharged from untreated and treated sewage) and their distribution through surface water by combining PROMISE and WATNAT models in Netherland. Results suggest that predicted *Cryptosporidium* and *Giardia* level at the surface water was lower than observed concentration. (oo)cysts attachment to lake and river sediments was not considered in modelling process.
Dorner et al. (2004) & Dorner et al. (2006)	A probabilistic pathogen loading model (developed using β distributions and Γ distributions for pathogen prevalence and shedding intensity respectively) coupled to hydrologic model WATFLOOD in order to estimate *Cryptosporidium, Giardia, Campylobacter, E. coli* and *E. coli* O157:H7 loadings at the watershed scale. Results indicate that when pathogens enter stream from land sources subsurface flow play an important role in transition of microorganisms toward stream compared to overland flow. Moreover, stream sediments have also been identified as great source of pathogens in storm events.
Ferguson et al. (2007)	A Process-based model (pathogen catchment budgets-PCB) that can be used to estimate *Cryptosporidium*, *Giardia and E. coli* loads generated within an exported from the catchment in dry, intermediate and wet weather conditions. Model contains 5 modules including hydrological module, a land budget module, on-site sewage system module, sewage treatment plant module and an in-stream transport module. Results suggest that organism excretion rates from human and non-human sources and mobilization rates of manure are major factors that control model output.

Table 2. 7: Summary of pathogen models at the watershed scale (cont'd)

Author (year)	Model description, findings and limitation
Tang et al. (2011)	A SWAT 2005 model was applied to simulate *Cryptosporidium* oocyst transport in ungauged agricultural catchments on daily basis. The results show that temperature and the timing of manure application in relation to the run-off events are the most important factors that control *Cryptosporidium* transport in the study catchment.
Whelan et al. (2014)	The framework for risk analysis in multimedia environmental systems (FRAMES) linked to a QMRA model was applied to evaluate the impact of different manure-based contaminant sources on microbial risk (*Salmonella eterica*, *Cryptosporidium* spp. and *Escherichia coli* O157:H7) associated with recreational waters. The results show that this approach with combination of various factors enable regulators to understand their systems and implement best management practices effectively.
Sterk et al. (2016)	Fecal microorganisms fate and transport module was combined with a conceptual rainfall-runoff model (Wageningen Lowland Runoff Simulator ,WALRUS) to evaluate the impact of climate change on the concentration of *Cryptosporidium* and *Campylobacter* in run-off reaching surface waters. The results indicate that some processes such as run-off fluxes, pathogen release and dilution are influenced by the climate change but overall the impact of climate change on the concentrations of *Campylobacter* and *Cryptosporidium* in run-off from land to surface waters and consequently on infection risks associated with recreational water is limited.

Table 2. 8: Examples of hydrodynamic modelling of fate and transport of pathogenic microorganisms in the context of drinking water

Author (year)	Model description	Findings
McCorquodale et al. (2004)	In order to assess the impact of storm water flows on the recreational waters, a 3D hydrodynamic model of Princeton Ocean Model (POM) with bacteria fate–transport submodel were applied. The adopted methodology was to evaluate the concentration of fecal coliform, *Enterococci* and *E. coli* in near real time.in beaches. Investigation area: Lake Pontchartrain, Louisiana, USA	It was found that storm water runoff discharges last for 2 to 3 days after a heavy rainfall event (>12mm). The settling process was significant, as 30% of the bacteria were attached to fine sediments. However, based on the laboratory study the die-off rate was slow. A framework as a management tool was presented to update swimming advisories on a timely basis.
Hipsey et al. (2004)	The water quality model CAEDYM (in which *Cryptosporidium* module is implemented) was coupled to 3D ELCOM hydrodynamic model in order to examine *Cryptosporidium* dynamics in lakes and reservoirs. Processes such as oocyst inactivation (via natural mortality and exposure to various bands of UV), settling, resuspension and aggregation onto particles were included in the *Cryptosporidium* fate module. Oocyst advection, mixing, waterbody inflow and thermal dynamics were also modeled by ELCOM. Investigation area: Myponga drinking water reservoir, South Australia	*Cryptosporidium* didn't attach to inorganic particles and settled according to Stoke's sedimentation dynamics as free-floating oocysts. Hence, it was found that the reduction of oocyst due to settling process was insignificant. Relative to the timescales for transport, inactivation rate was minor. Natural mortality and UV-B band (280–320 nm) in the surface layer (10-50 cm) were identified as significant processes in decreasing the infectivity potential of oocysts.

Table 2. 8: Examples of hydrodynamic modelling of fate and transport of pathogenic microorganisms in the context of drinking water (cont'd)

Author (year)	Model description	Findings
Hellweger et al. (2008b)	Time variable 3D hydrodynamic model (ECOMSED) coupled to water quality model (RCA) was applied to assess the fate and transport of *E. coli* in surface waters. Investigation area: Boston's Charles River	The predominant source of *E. coli* to the basin was identified. The hydrodynamics caused by dam, wind conditions and die-off rate were identified as the primarily driver of the spatial and temporal patterns.
Sokolova et al. (2012)	The concentrations of *Cryptosporidium*, norovirus and *E. coli* O157/H7 in the fecal contamination sources around a Lake were estimated using the measured concentrations of *E. coli* and *Bacteroidales* genetic markers for the endemic and epidemic conditions. A 3D hydrodynamic model MIKE 3 FM coupled to microbiological model ECO Lab was used to simulate fate and transport of microorganisms in the lake and to estimate the contributions from various sources of contamination to the pathogen concentrations at the water intake. Investigation area: Sweden	The source with the highest concentration of pathogen was not necessarily the main contributor of pathogen concentration at the water intake. The concentrations of pathogen at the DWI were strongly variable over time, indicating the variability of imposed risks to drinking water supply. Under epidemic conditions, the estimated concentrations in the fecal contamination sources were up to 5 log units higher than under endemic condition. Under epidemic condition, the on-site sewers were identified as the most important contributor of the both norovirus and *Cryptosporidium* concentrations at the water intake. Yet, under endemic condition, the most important contributor of norovirus and *Cryptosporidium* concentration at the water intake were on-site sewers and the cattle grazing area respectively.

Table 2. 8: Examples of hydrodynamic modelling of fate and transport of pathogenic microorganisms in the context of drinking water (cont'd)

Author (year)	Model description	Findings
Sokolova et al. (2013)	The load of *E. coli* from various fecal contamination sources into Lake Rådasjön were calculated using the available data and assumptions. A 3D time-dependent hydrodynamic model MIKE 3 FM coupled to microbial water quality model ECO Lab to simulate fate and transport of fecal contamination in the lake and to investigate the contribution of various fecal contamination sources to the concentration of *E. coli* at the water intake, taking the decay of the *E. coli* into account. Investigation area: Sweden	The river Mölndalsån and the discharges from the on-site sewers were identified as the main contributor of *E. coli* concentrations at the water intake. The measured and simulated *E. coli* concentration at the water intake demonstrated that the highest and the lowest concentrations of *E. coli* in the Lake occurred during the period of October-March and summer months respectively. Vertical temperature, wind speed and direction were introduced as two major factors that govern transport of faecal contamination to the water intake.
Sokolova et al. (2015)	The norovirus concentrations at the water intake were estimated using the observed concentrations at the contamination source and hydrodynamic modelling. The results of 3D hydrodynamic model were used as input in QMRA model taking into account the health target. Finally the required levels of treatment at the drinking water treatment plant were calculated for the various environmental loading scenarios. Investigation area: Sweden	The average estimated concentration of norovirus in the studied DWI was between 4.8×10^{2} and 7.5×10^{3} genome equivalents L^{-1}; and the average required reduction by water treatment facility was between 7.6 and 8.8 Log_{10}. The treatment performance of studied drinking water treatment plant was adequate to control all tested loading scenarios, but was strongly related to chlorine disinfection.

Table 2. 8: Examples of hydrodynamic modelling of fate and transport of pathogenic microorganisms in the context of drinking water (cont'd)

Author (year)	Model description	Findings
Jalliffier-Verne et al. (2016a)	In order to provide appropriate advices to the CSO management, the effects of cumulative CSO discharges were investigated on the degradation of downstream DWIs using a 2D hydrodynamic model. Investigation area: Quebec, Canada	High concentration of fecal contamination at DWIs depended on the seasons, location of overflows, discharged concentrations, accumulation of overflows and dispersion processes rather than dilution process. High concentrations at DWIs were induced with upstream high concentration discharges even from a small CSO. Hence, specific CSOs which have great impact on the peak concentrations of DWIs should be targeted for source water protection planning. For the risk analysis the cumulative impacts of CSOs should be considered rather than individual outfalls.
Jalliffier-Verne et al. (2016b)	A 2D hydrodynamic model (the Dispersim module of the Hydrosim softeware) was performed to evaluate the impacts of climate and population changes on the water quality of DWIs. Developed hydrodynamic model simulated the dispersion and transport of *E. coli* from CSO discharges in the urban river used as a drinking water source. Investigation area: Quebec, Canada	Drinking water treatment classes was mostly sensitive to population growth than river flow and hence municipalities should have strategies for controlling the anticipated increases in the loads. Although greater dilution was expected during high flow condition, higher mean concentrations of *E. coli* at the DWIs during high flow conditions may be due to the increase of stream velocity and consequently greater dispersion of *E. coli* toward the water intakes. The location of the DWIs relative to the fecal contamination sources and mixing processes were identified as the most important controller of *E. coli* concentrations at DWIs.

Table 2. 8: Examples of hydrodynamic modelling of fate and transport of pathogenic microorganisms in the context of drinking water (cont'd)

Author (year)	Model description	Findings
Eregno et al. (2016)	Three-dimensional hydrodynamic and transport models (GEMSS-HDM) coupled with QMRA model to estimate public health risk associated with recreational beaches during and after rainfall events. Hydrodynamic model was calibrated using *E. coli* as indicator of fecal contamination with different decay rates. The concentrations of norovirus, *Campylobacter*, *Salmonella*, *Giardia* and *Cryptosporidium* were estimated at the local beaches using their relation with *E. coli* in sewage discharges from previous studies. Investigation area: West of Oslo, Norway	On the first day after the rainfall event, the risk level for viral reference pathogen was higher at all recreational beaches and severe at two small and big beaches, but acceptable for the parasitic and bacterial reference pathogens. Mitigation measures and in-depth assessment of risk was suggested in two small and big beaches.

2.5 Quantitative microbial risk assessment

Quantitative Microbial Risk Assessment (QMRA) is a technical process that assesses the probability of adverse health effects that can take place from exposure to microorganisms (Brown et al., 1996). Risk analysis includes three subsets of risk assessment, risk management and risk communication (Soller, 2006). Risk assessment is identification/quantification and estimation of adverse health risk from exposure to risky substances (National Research Council, 1983; Hoppin et al., 1993). The risk management component is about organizing appropriate management practices based on the results of the risk assessment (Soller, 2006). Risk communication is the practice of sharing information and experience among risk assessors, risk managers, consumers and other interested groups (WHO, 1999).

A critical review conducted by Soller (2004) of 1100 QMRA studies revealed that two approaches including directly using epidemiologic data and indirectly using models, are most commonly used to perform QMRA. Based on this review article, the direct approach is often used for risk estimation while the indirect approach helps investigators obtain valuable information for decision making and conducting best management practices regarding environmental variation in whole water supply systems (Soller, 2006). In the modelling approach, deterministic and probabilistic methods have commonly been applied to predict human health risk from exposure to recreational and drinking waters. In a deterministic model, it is assumed that each parameter value is constant and quantifiable with high level of certainty (Signor, 2007) and therefore results are presented as a single point-estimate. However, variability (parameters' conditional and inherent variation) and uncertainty (incomplete knowledge about parameters, data and models) often exist in a system (Signor, 2007). Uncertainty can be mitigated through more data collection and research while elimination of variability is impossible. Variability and uncertainty of a system can be reflected by probabilistic approaches, in which probabilities are incorporated into an individual level and are assessed by iterative procedures such as Markov Chain Monte Carlo analysis (Koopman et al., 2002).

In order to estimate microbial risk from exposure to drinking water, four main steps including hazard identification, exposure assessment, dose-response assessment and risk characterisation should be followed (Haas et al., 1999). These steps are summarized in Figure 2. 1.

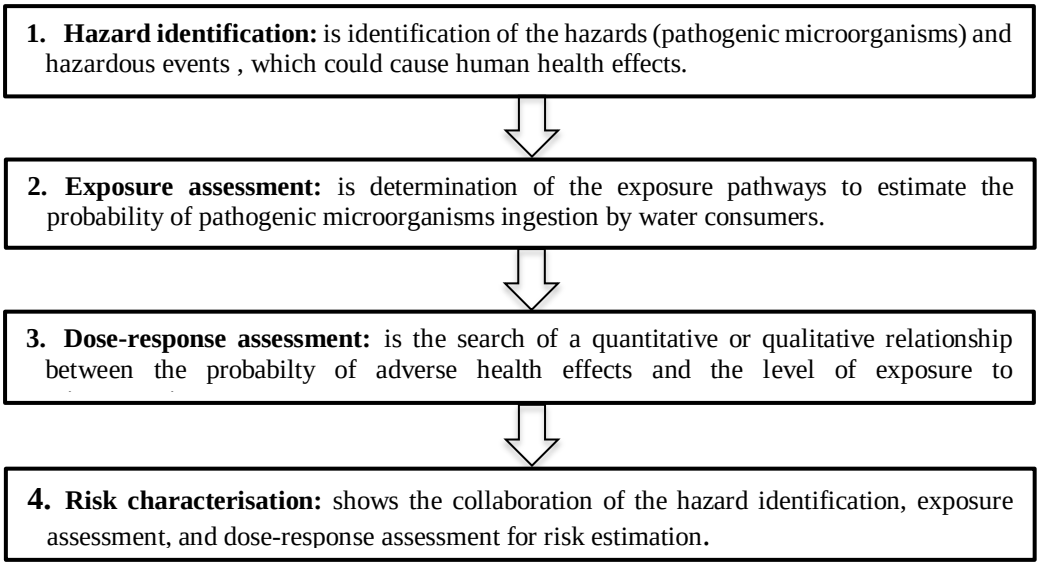

Figure 2. 1: Quantitative Microbial Risk Assessment (Haas et al., 1999)

theoretical pathogen dose for a single exposure is simply described by Equation 2. 4 (Signor, 2007):

$$D = \mu V \qquad (2.\ 4)$$

In this equation, D refers to the pathogen dose ingested by a drinking water consumer, μ is the concentration of pathogens in drinking water and V is the consumed water volume in one exposure period. The probability of infection is related to ingested dose and is usually calculated by two common models; the single-hit the Exponential and the Beta-Poisson dose-response models (Haas et al., 1999). The Exponential model is based on the assumption that organisms are distributed in water randomly and any organism has the same probability of survival in a host (r) (Equation 2. 5). In the Beta-Poisson model, the probability of any organisms survival in host is beta distributed and hence two parameters (α, β) are included in the model (Equation 2. 6) (Haas et al., 1999).

$$P_{\inf} = 1 - e^{rD} \qquad (2.\ 5)$$

$$P_{\inf} = 1 - (1 + \frac{D}{\beta})^{-\alpha} \quad \text{when } \alpha \le \beta \text{ and } \beta \ge 1 \qquad (2.\ 6)$$

P_{inf} is the probability of infection for a person per exposure, r is the probability of survival of an organism in a host (in the range of [0,1]) and α, β are the parameters of the β distribution. Selection of an appropraite dose-response model is a key factor in implementing an effective QMRA. The application of an exponential model for the reference pathogens *Cryptosporidium* and *Giardia* and Beta-Poisson model for Rotavirus, *Campylobacter* and *E. coli* O157 was suggested in the HC QMRA tool (Health Canada, 2012).

2.5.1 QMRA and risk management

QMRA has a high potential to be used as a management tool for controlling a water supply system and setting critical goals in a system (Hrudey et al., 2006; Medema et al., 2006). The implementation of water safety plans (WSP), which require a full risk-based approach for hazards management in a whole water supply system (source to tap) (WHO, 2011) is recommended in the WHO guidelines for safe drinking water (WHO, 2009). Petterson et al. (2016) recently reviewed the studies that applied QMRA in drinking water systems and highlighted their relevance to WSP. The review shows that for understanding a system, QMRA models have been applied from source to tap and the sensitive components of the system were identified. Source water variation and treatment failure were usually identified as the significant components of a whole system (Teunis et al., 1997; Jaidi et al., 2009). Neverthless, the Canadian version of QMRA models has not been usually conducted in full water supply systems and have mostly focused on treatment dimensions rather than integrating the quality of the source water (Dunn et al., 2014). In addition, a review of the literature reveals that current QMRA studies have been mainly conducted for baseline/normal weather conditions and the application of QMRA for wet weather events has received less attention (Table 2. 9), while concentrations at source waters are elevated during wet weather periods (Kistemann et al., 2002; Burnet et al., 2014).

An event-based QMRA approach has been introduced as an effective method for evaluating short term risk. McBride et al. (2013) implemented discharge-based QMRA to estimate public health risk from exposure to stormwater-borne pathogens in recreational water. They measured seven reference pathogens (*Cryptosporidium*, *Giardia*, *Salmonella*, Norovirus, Rotavirus, Enterovirus and Adenovirus) in 12 outfalls (residential stormwater, agricultural runoff, commercial/industrial stormwater, CSOs, forested land runoff, mixed use stormwater (residential, commercial and agricultural)) in wet weather periods to investigate the impact of short term events (during and

just after a rainfall event) on the recreational water users by assuming a simple dilution factor from the discharge point to the exposure point. Eregno et al. (2016) combined discharge-based hydrodynamic modelling with QMRA to estimate public health risk associated with bathing water during and after heavy rainfall events. They calibrated a hydrodynamic model using *E. coli* as an indicator of fecal contamination and simulated the average concentration of reference pathogens (norovirus, *Campylobacter*, *Salmonella*, *Cryptosporidium* and *Giardia*) at local beaches using the relative relationship between observed *E. coli* and reference pathogens in Norwegian sewage. The simulated concentrations were used as an input to a QMRA model to estimate the probability of infection of bathers during the three consecutive days after the rainfall events. In context of drinking water, Sokolova et al. (2015) also combined discharge-based hydrodynamic modellling with QMRA to evaluate the ability of a drinking water treatment plant for providing safe drinking water to water consumers following various loading conditions. The norovirus concentrations were monitored in contamination sources and their transport within the water source under different loading scenarios was simulated using a hydrodynamic model. QMRA was performed to calculate the required reduction of norovirus at a drinking water treatment plant based on the estimated concentrations in source water. Finally, the estimated treatment performance was compared with the calculated required reduction to test if the treatment performance is sufficient to deal with various loading conditions.

Table 2. 9: Summary of research studies, which performed QMRA in the context of drinking water

Author (year)	Monitoring discription	Model description and remarks
Barbeau et al. (2000a)	Collecting raw water samples from 45 utilities in Quebec, Canada and analysing samples for *Cryptosporidium parvum* and *Giardia lamblia.*	Implementing a Monte Carlo simulation technique in order to quantify the microbial risk posed by parasites to the drinking water consumers of St. Lawrence River watershed. The mean annual risk of infection with *Cryptosporidium* was higher than with *Giardia.*
Signor (2007)	Collecting samples from the river inlet in to the drinking water reservoir (in southern Austerilia) through seven rainfall-induced runoff events and evaluating the presence of the *E. coli, Campylobacter jejuni and Cryptosporidium parvum.*	Performing probabalistic QMRA to explore the impact of parameter variability, event conditions and model uncertainites on the quality of treated water and consequently human health risk. Based on the results of the sensitivity test, the outcome of the QMRA model was highly dependent on the source water pathogen concentrtaion. Moreover, short duration events had a gtreat impact on the risk elevation.
Ryu et al. (2008)	Measuring occurrence of the *Cryptosporidium, Giardia and* fecal indicator in 5 sampling sites including surface waters (three sites) and water treatment plants (two sites) in dry weather periods in Arizona, the United States.	Employing deterministic QMRA to estimate relative microbial health risk. The microbial risk met the acceptable level of risk 10^{-4}.

Table 2. 9: Summary of research studies, which performed QMRA in the context of drinking water (cont'd)

Author (year)	Monitoring discription	Model description and remarks
Jaidi et al. (2009)	Monitoring raw water of the two filtration plants in dry weather period from January 2000 to June 2006 and analyzing samples for *Cryptosporidium* and *Giardia.*	Building a Monte Carlo probabilistic QMRA model to estimate relative microbiological risk posed by pathogenic parasites to drinking water consumers, using the results of the raw water analysis and water treatment plant operational data. Input parameters particularly probability distribution function of protozoan occurrence in source water, ozone inactivation and granular filtration hade a great impact on the outcome of the QMRA model.
Van den Akker et al. (2011)	Monitoring *Cryptosporidium* and *Giardia* and several microbial indicators in the influent and effluent of three WWTPs discharged to Lake Burragorang, Sydney, following dry weather periods.	Using probabilistic QMRA to estimate the relative health risks that could be posed by three WWTPs to drinking water consumers in baseline and various hazardous event scenarios including failure in water filtration plant, failure in the Lake (short circuiting of flood waters) and failure in sewage treatment plants. The microbial risk was lower than accepted level of risk 10^{-4}.
Pintar et al. (2012)	Collecting water samples from the surface water of Grand river and tributaries within Waterloo Region, Ontario, over a three year period and analyzing samples for *Cryptosporidium*, following dry weather periods.	Building probabilistic QMRA based on the results of the monitoring, water treatment plant operational data and literature data in order to estimate infectious risk posed by *Cryptosporidium* to water consumers. Public health risk from exposure to drinking water was very low.

Table 2. 9: Summary of research studies, which performed QMRA in the context of drinking water (cont'd)

Author (year)	Monitoring discription	Model description and remarks
Xiao et al. (2013)	Monitoring *Cryptosporidium* in 10 sites at the mainstream of river, 9 sites in backwaters area of tributaries, 4 sites in backwater areas of cities and 5 WRRFs discharged to the Three Gorges Reservoir (TGR), China, following dry and wet weather periods.	Performing probabilistic QMRA to estimate annual health risk, considering four exposure pathways including directly drinking, food dish washing, swimming and diving and residues from tooth-brushing. Results showed that, health risk could be posed by the TGR reservoir to drinking water consumers and recreational users.
Sato et al. (2013)	Collecting samples from nine different watersheds of Sao Paulo State, Brazil, in dry weather periods, analyzing samples for parasitic protozoa *Cryptosporidium* and *Giardia.*	Running Monte Carlo simulation to estimate public health risk, considering children and adult exposure. Results indicated that, population served by these water sources could be infected by *Cryptosporidium* and *Giardia.*

CHAPTER 3 RESEARCH OBJECTIVES, HYPOTHESES AND METHODOLOGY

3.1 Critical review of previous research and problem identification

As discussed in previous sections, in a multi-barrier approach, microbial risk estimation and management cannot be completed without examining the whole water supply system from sources to drinking water consumers. Source water protection, which addresses the need to maintain the quality of the water supply, is the first barrier in this approach. Source water quality changes with time and weather conditions and wet weather is recognized as one of the major factors that causes the impairment of receiving water quality and can lead to waterborne disease outbreaks. However, studies on the link between source water quality, weather conditions and QMRA in the whole water supply system are scarce.

Since pathogen concentrations cannot be continuously measured at DWIs, hydrodynamic models linked with deterministic loading models have been used to estimate the transfer of contaminants to the point of exposure. The majority of studies simulating pathogen and indicator concentrations at DWIs have been conducted during dry weather conditions. However, there is a need to estimate pathogen and indicator loads from potential sources of fecal pollution under various loading conditions (baseline loadings *versus* precipitation-driven loading events) in order to evaluate the variability of the risk associated with those sources. For instance, the impact of a WRRF on the receiving water is varible under normal operating conditions, incomplete treatment and by-pass discharges and these variabilities should be assessed in quantifying microbial risk. The peak concentrations of pathogens at DWIs are rarely detected by routine monitoring processes. Hence a coupled event based hydrodynamic model with QMRA gives us an opportunity to test the impact of various loading events on DWIs.

Water resource recovery facilities are identified as one of the important sources of fecal pollution in urban areas (note section 2.2.3.1). However, the prevalence of pathogens, fecal indicator bacteria and wastewater micro-pollutants, their concentrations and removal through different stages of wastewater treatment processes have been mostly discussed during dry weather conditions (or weather conditions were not specified). The performance of a WRRF depends on hydrologic conditions and varies between dry and wet weather conditions. Hence, there is a need

to characterize the variability of concentrations in untreated discharges in order to evaluate the impact of potential peak concentrations at DWIs following by-pass discharges.

Standard enumeration of fecal indicator bacteria (*E. coli* and *Enterococci*) is usually performed for microbiological analyses as pathogenic parasite analysis is time consuming, expensive and complex. However, fecal indicators are not always well correlated with pathogens and hence the combined application of alternative chemical indicators with fecal indicators has been proposed for initial screening and/or as a supplementary cross-validation tool. Co-variations between fecal indicator bacteria and wastewater micropollutants (WWMPs) have been described in CSO discharges, river waters and stormwater collection systems. However, co-variations between pathogenic parasites and WWMPs have not been widely addressed.

3.2 Research objectives

In this study we were mostly interested in presenting a novel risk-based framework for source water protection planning and management. The main goals of this research project were to:

1. Evaluate the impact of potential fecal contamination sources particularly WRRF discharges, on a DWI of a Great Lake under dry and wet weather conditions.
2. Assess the utility of QMRA as a management tool for mitigating health risks from pathogenic contaminants.

On a more detailed level, the specific objectives were to:

1. Evaluate the prevalence rate and dynamic behaviour of pathogenic parasites (*Cryptosporidium* and *Giardia*), fecal indicator bacteria (*E. coli* and *C. perfringens*), WWMPs (caffeine (CAF), carbamazepine (CBZ), 2-hydroxycarbamazepine (CBZ-2OH), acesulfame (ACE), sucralose (SUC) and aspartame (ASP)) and TSS in a WRRF under various weather conditions;
2. Estimate the varibility of target parasites, FIB, WWMPs and TSS mass loadings from a WRRF in the case of normal operation conditions, incomplete treatment and by-pass discharges;
3. Evaluate the relationship between the varibility pattern of parasites, fecal indicator bacteria (FIB), WWMPs and TSS concentrations with the peak flowrate;
4. Investigate the excess loads from a by-pass discharge compared to a final effluent discharge following wet weather conditions;

5. Identify appropriate markers of sewage by-passes in receiving waters through describing co-variations between parasites, FIB and WWMPs;
6. Determine the most important governing factors of mass loadings from the WRRF influent and effluent discharges;
7. Evaluate the relative contribution of sewer processes (net deposition or net resuspension of particle associated contaminants, biological activity such as inactivation, predation, biodegradation, etc) in the mass loadings from raw sewage under various flow conditions;
8. Rank various sources of fecal contamination including rivers, creeks and effluent discharges;
9. Investigate the variability of *Cryptosporidium*, *Giardia* and *E. coli* probability distribution functions at a DWI under dry and wet weather conditions;
10. Investigate microbial risk associated with drinking water under baseline and precipitation-driven loading events.

The objectives of this study were based on the four main hypotheses:

1. The dynamic behaviour of parasites, FIB, WWMPs and TSS in raw sewage vary during a course of a day, from dry weather condition to wet weather condition and from one event to another event in relation to flowrate;

Originality*: This is the first study to investigate the temporal variability of pathogenic parasites combined with wastewater micropollutants in raw sewage*

2. Parasites and indicator loadings from a WRRF (fed by separate sewer system) into a receiving water are variable and affected by the extent of infiltration/inflow into a sewage system;

Originality*: This is the first study to estimate the variability of mass loadings from a WRRF served only by sanitary sewer system and impacted highly with infiltration/inflow*

3. Microbial risk associated with drinking water is higher for precipitation-driven loading condition compared to baseline loading condition;

Originality: A probabilistic-deterministic loading *approach coupled with a hydrodynamic and a QMRA model was never applied to evaluate the impact of various loading events on a DWI.*

4. The short term microbial risk associated with WRRF by-passes can be quantified by event-based QMRA;

***Originality**: The impact of by-pass discharges on a DWI was never investigated by event based QMRA*

The results of this study were presented in three sections as three articles. The first article, which has been recently published in the journal *Water Research*, is on investigating temporal variability of parasites, FIB and WWMPs in a WRRF under various weather conditions. The second article, which has been submitted to the *Journal of Water and Health*, is a study of weather effects on parasite, indicator bacteria and wastewater micropollutant loads from a WRRF influent and effluent. In the final paper, microbial risk was assessed through event-based pathogen loading and hydrodynamic modelling. The third article was submitted to the journal *Science of the Total Environment*.

3.3 Methodology

In order to fulfill the main and specific objectives of this research project, the most important fecal contamination sources that could have an impact on a downstream DWI were identified, the influent and effluent of a WRRF (served by sanitary sewer system with high level of infiltration/inflow following heavy rainfall events) was monitored under trace precipitation (dry weather) and wet weather conditions (i.e. 2 days cumulative rainfall prior to sample collection in the range of <3mm to 32mm) and a discharge based hydrodynamic model coupled with a QMRA model was performed. The link among these project components is illustrated in Figure 3. 1.

In summary, time proportional composite samples from the influent and grab samples from the effluent were collected following four wet weather evets (one in April, two in June and 1 in September) and two trace precipitation events (May and September) in 2014. The 2-day cumulative rainfall prior to sample collection was in the range of trace to 32mm. Collected samples were analysed for pathogenic parasites (*Cryptosporidium* and *Giardia*), FIB (*E. coli* and *C. perfringens*), WWMPs (CAF, CBZ, CBZ-2OH, ACE, SUC, ASP) and TSS. Detailed information about sampling is presented in section 4.3.2 and Figures 3. 2 to 3. 5.

A probabilistic approach was used to identify the relative importance of fecal contamination sources regardless of weather conditions. In order to consider the impact of fecal contamination sources on an intake, *Cryptosporidium*, *Giardia* and *E. coli* loadings from rivers, creeks and

effluent discharge of WRRFs were estimated following dry and wet weather conditions and used as input in a three-dimensional MIKE-3 hydrodynamic model. In addition, the impact of sewage by-pass discharges on the studied DWI was assessed. Finally, event-based QMRA model was performed to evaluate the impact of upstream loadings on downstream drinking water intake. Detailed information about load estimation, event-based hydrodynamic model and QMRA is provided in sections 6.3.2, 6.3.3 and 6.3.4.

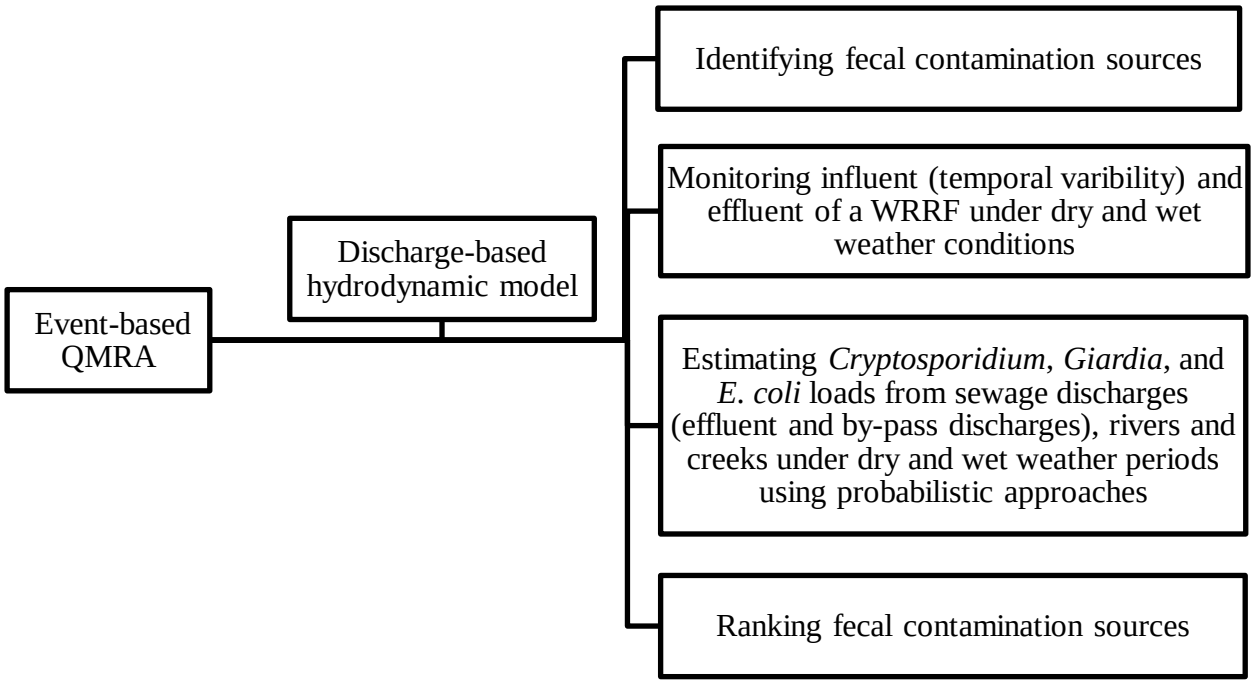

Figure 3. 1: The link among fecal sources, hydrodynamic model and QMRA

Figure 3. 2: Sampling from the influent of a WWRF (June 2014)

Figure 3. 3: Sampling from the effluent of a WWRF (June 2014)

Figure 3. 4: Collecting samples from the influent and effluent (June 2014)

Figure 3. 5: Preliminary analysis and pre-treatment of samples in WRRF (September 2014)

CHAPTER 4 ARTICLE 1: TEMPORAL VARIABILITY OF PARASITES, BACTERIAL INDICATORS AND WASTEWATER MICROPOLLUTANTS IN A WATER RESOURCE RECOVERY FACILITY UNDER VARIOUS WEATHER CONDITIONS

This chapter presents the manuscript published in the journal *Water Research*. The manuscript contains investigation on temporal variability of target parasites and indicators in a water resource recovery facility under dry and wet weather conditions.

Temporal variability of parasites, bacterial indicators and wastewater micropollutants in a water resource recovery facility under various weather conditions

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4.1 Abstract

Wastewater discharges lead to the deterioration of receiving waters through treated effluents and by-passes, combined and sanitary sewer overflows and cross-connections to storm sewers. The influence of weather conditions on fecal indicator bacteria, pathogens and wastewater micropollutants on raw and treated sewage concentrations has not been extensively characterized. However, such data are needed to understand the effects of by-pass discharges and incomplete treatment on receiving waters. A water resource recovery facility was monitored for pathogenic parasites (*Cryptosporidium* oocysts, *Giardia* cysts), fecal indicator bacteria (*Escherichia coli, Clostridium perfringens)* and wastewater micropollutants (caffeine, carbamazepine, 2-hydroxycarbamazepine, acesulfame, sucralose and aspartame) during 6 events under different

weather conditions (snowmelt and trace to 32mm 2-day cumulative precipitation). Greater intra- and inter-event variability was observed for *Giardia, E. coli and C. perfringens* than for studied WWMPs. Even with the addition of inflow and infiltration, daily variations dominated concentration trends. Thus, afternoon and early evening were identified as critical times with regards to high concentrations and flows for potential by-pass discharges. Peak concentrations of *Giardia* were observed during the June wet weather event (1010 cysts/L), with the highest flowrates relative to the mean monthly flowrate. Overall, *Giardia, E. coli and C. perfringens* concentrations were positively correlated with flowrate ($R>0.32$, $p<0.05$). In raw sewage samples collected under high precipitation conditions, caffeine, carbamazepine and its metabolite 2-OH-carbamazepine were significantly correlated ($p<0.05$) with *Giardia*, *E. coli* and *C. perfringens* demonstrating that they are useful markers for untreated sewage discharges. Data from the study are needed for estimating peak concentrations discharged from wastewater sources in relation to precipitation or snowmelt events.

4.2 Introduction

No less than 381 waterborne disease outbreaks associated with parasitic protozoa were documented worldwide between 2011 and 2016 (Efstratiou et al., 2017). Besides the cryptosporidiosis outbreak in Milwaukee, Wisconsin, major waterborne outbreaks have also occurred in Canada over the past two decades. Water supplies in Walkerton, Ontario and North Battleford, Saskatchewan were contaminated by pathogens identified as *E. coli* O157:H7, *Campylobacter jejuni* and *Cryptosporidium parvum* (R.D Laing, 2002; O'Connor, 2002). Many reported outbreaks have occurred following heavy rainfall events that led to the contamination of drinking water sources (Hrudey et al., 2002; Hunter, 2003).

Drinking water intakes of the Great Lakes were assumed to have low vulnerability due to their great depth, distance from shore and large dilution potential and are considered as good sources of drinking water (Canadian Public Health Association, 1986). Yet, the Milwaukee outbreak resulted from contamination of the offshore intake by sewage-derived *Cryptosporidium* oocysts introduced into Lake Michigan (MacKenzie et al., 1994). The Great Lakes region is expected to experience more extreme precipitation events as a result of climate and land use change (Patz et al., 2008). Some of the greatest uncertainties associated with global change are in relation to future sewage discharges and local contaminant mass loads (Jalliffier-Verne et al., 2015).

Waterborne pathogens enter the Great Lakes from a variety of point and non-point sources, that represent potential threats for the production of drinking water delivered to more than 40 million people (Patz et al., 2008). Water Resource Recovery Facilities (WRRFs, also known as wastewater treatment plants) have been identified as major point sources of pollution in the Great Lakes Basin with billions of liters of sewage from combined sewer overflows (CSOs) and sewage by-passes being discharged into the Great Lakes yearly (Podolsky et al., 2013). It is important to not only investigate the quality of treated effluent discharges, but raw sewage as well. Discharge concentrations of faecal microorganisms vary over orders of magnitude (Madoux-Humery et al. 2013) and storm events can lead to discharges with limited or no treatment.

In a WRRF served by a separate sewer system, flows originate from three major components: base sanitary flow, infiltration flow from groundwater through network defects and rapid inflow during intense storm events and snowmelt (EPA, 2014). Inflow and infiltration flow present important challenges including reduced treatment efficiency or sewage by-passes (Brière, 2014). WRRF by-passes are common during intense storm events in the Great Lakes Basin and they are a major source of fecal pollution (EPA, 2004; Podolsky et al., 2013).

Available literature has focused on evaluating the prevalence of pathogenic parasites and fecal indicator bacteria (FIB) in WRRFs, their concentrations and removal rates during different stages of treatment processes, their seasonal variation and the impact of effluent discharges on the microbiological quality of drinking water reservoirs (Ottoson et al., 2006; Cheng et al., 2009; Ajonina et al., 2012; Cheng et al., 2012; Ajonina et al., 2013; Gallas-Lindemann et al., 2013; Burnet et al., 2014). The prevalence of infectious *Cryptosporidium* has also been observed in raw sewage and secondary treated effluents (Rose et al., 2004; Lalancette et al., 2012). The prevalence and removal rates of *Giardia* have usually been higher than those of *Cryptosporidium* (Kitajima et al., 2014b; Taran-Benshoshan et al., 2015). Parasites and FIB are eliminated through treatment processes with efficiencies depending on the size, type and capacity of the treatment facilities (Garcia-Armisen et al., 2008; Kistemann et al., 2008; Fu et al., 2010). The efficiency of conventional wastewater treatment has been shown to be limited for parasite removal (Fu et al., 2010; Kitajima et al., 2014b) and advanced processes such as membrane separation and ultraviolet disinfection are required for improved removal efficiency (Liberti et al., 2003; Jiménez et al., 2010).

Most studies that have examined pathogen and indicator occurrence and removal during wastewater treatment have been conducted during dry weather conditions (or weather conditions were not specified); however, WRRFs are subject to hydrologic events and their performances vary between dry and wet weather conditions. (Lucas et al., 2012). Secondary and tertiary treatments were reported to be less effective for FIB removal under high flow conditions (Kay et al., 2008). While evaluating the variability of a WRRF removal efficiency rate during various weather conditions is important, characterizing the variability of concentrations is even more critically needed when it comes to untreated or partially treated discharges. There is a need for data on the varibility of raw sewage microbiological quality during intense storm events in order to understand potential peak concentrations of by-pass discharges.

Sewer sediments have been identified as a sink for bacteria, wastewater micropollutants and total suspended solids and their contribution to CSO loads have been determined (Gasperi et al., 2010; Passerat et al., 2011; Madoux-Humery et al., 2015). However, little is known about the role of sewer sediments with regards to loads of bacteria, micropollutants and total suspended solids (TSS) from sanitary sewer networks with high levels of infiltration and inflow. Data on the relative contribution of sewer sediments to concentrations arriving at WRRFs may be especially critical when it comes to implementing management practices for sewer lines and estimating the impact of sewage by-passes on receiving waters.

Since pathogenic parasite analyses are time consuming, expensive and complex, standard enumeration of FIB (*E. coli*, *Enterococci*) is usually performed as a surrogate for pathogens. However, fecal indicators are often not correlated with pathogens (Wu et al., 2011). In order to partly overcome the difficulties associated with the use of conventional FIB, the combined application of alternative chemical indicators during initial screening and/or as a supplementary cross-validation tool has been suggested (Hagedorn et al., 2009). Fecal microorganisms originate from both human and non-human sources; hence, the application of human-origin wastewater micropollutants (WWMPs) has garnered more attention in recent years (Daneshvar et al., 2012; Sauvé et al., 2012). However the relationships between pathogenic parasites and WWMPs have not been widely addressed. The presence of suitable WWMPs in source waters (e.g. drinking water intakes downstream of a WRRF) indicates fecal pollution from human sources and the potential for human pathogen presence.

Table 4. 7. contains information on WWMPs that are useful markers of wastewater contamination in aquatic environments. The objectives of the present study were to: (1) investigate the prevalence and temporal varibility of pathogenic parasites (*Cryptosporidium* and *Giardia*), FIB (*E. coli* and *C. perfringens*) and WWMPs (Caffeine (CAF), carbamazepine (CBZ), 2-hydroxycarbamazepine (CBZ-2OH), Acesulfame (ACE), Sucralose (SUC) and Aspartame (ASP)) under weather conditions ranging from snowmelt and trace to intense precipitation; (2) investigate/describe co-variations between pathogenic parasites, FIB and WWMPs to identify appropriate markers of sewage by-passes in receiving waters; and (3) determine the relationship between the varibility pattern of parasites, FIB, WWMPs and TSS concentrations during wet weather events with flowrates. These data are needed to simulate events such as by-pass discharges that occur during peak flows and are not usually captured by routine monitoring programmes. To the best of our knowledge, this is the first study to investigate the temporal variability of pathogenic parasites with wastewater micropollutants in raw sewage. Moreover, few data are available on the variability of influent concentrations during wet weather periods. The variability of untreated wastewater concentrations are needed to understand the impact of fecal pollution sources on downstream drinking water intakes, especially given the higher risk of gastrointestinal illnesses for consumers whose drinking water sources have been impacted by untreated wastewater discharges following precipitation events (Jagai et al., 2015).

4.3 Material and methods

4.3.1 Study site

The studied WRRF is located on the north shore of Lake Ontario, Canada. It receives sewage from a drainage area of 32 964 ha (sewer length of 2110 km) and has a daily capacity to treat 518 000 m^3 of wastewater collected from approximately 1 million people (Kambeitz, 2015personal comunication). Wastewater from residential, industrial, commercial and institutional facilities is treated using preliminary, primary and secondary treatment (conventional activated sludge with phosphorus removal through precipitation with ferrous chloride), followed by chlorine disinfection. After screening and grit removal, raw sewage is conveyed to primary clarifiers through three Channels. Raw sewage from Channels 1 and 2 are treated in plant 3 and Channel 3 is treated in Plants 1 and 2. The by-pass gate is located after the primary clarifier of Plant 3.

Treated effluents are discharged into Lake Ontario in which several drinking water intakes are located. Although the sewer system is separated, studies conducted in this area have shown that additional flows may enter the sanitary sewer system through inflow and infiltration following heavy storm events (ColeEngineering, 2011). Under heavy rainfall conditions, high flowrates lead to the discharge of primary treated effluents (with or without disinfection) into Lake Ontario, thereby representing a direct input of potential pathogens into a drinking water supply.

4.3.2 Sample collection

Influent and effluent of the WRRF were monitored under various weather conditions. In order to determine monthly thresholds that were indicative of an increase of flowrate from increased inflow and infiltration during wet weather, the relationship between historical flowrates and precipitation was examined. Wet weather conditions were defined based on (i) 2-day cumulative rainfall >10 mm prior to sample collection and (ii) flowrates above a threshold determined by historical data analysis for each month of the year. For the purpose of this study, "dry" weather conditions were defined as events with only trace amounts of rainfall (<3 mm) and flow rates below the determined threshold. A total of four wet weather events (one in April, two in June and 1 in September 2014) and two trace precipitation events (May and September 2014) were monitored. The April precipitation event occurred during a snowmelt period. The 2-day cumulative rainfall prior to sample collection is shown in Table 4. 8 and ranged from trace to 32mm. The return periods for the monitored wet weather events were below 2 years based on historical meteorological data. Detailed information regarding sampling is presented in Section 4.7. 1 and Table 4. 8.

4.3.3 Analytical methods and physico-chemical analyses

Raw sewage and treated effluent samples were analysed for *Cryptosporidium* and *Giardia* following U.S. EPA Method 1623.1 adapted for wastewater matrixes (McCuin et al., 2005; EPA, 2012). *Escherichia coli* and *C. perfringens* were enumerated by membrane filtration following MOE LSB E3371 and EPA/600/R-95/178 (1996) standards, respectively. Pharmaceuticals from raw sewage and treated effluents including CAF, CBZ, CBZ-2OH and ASP were analysed by on-line solid phase extraction coupled to liquid chromatography atmospheric pressure chemical ionization and tandem mass spectrometry (SPE-LC-APCI-MS/MS) (Morissette et al., 2015). SUC

and ACE were analysed by online solid-phase extraction coupled with liquid chromatography-heated electrospray ionization tandem mass spectrometry (SPE-LC-HESI/MS/MS). Detailed information about analytical methods is presented in Section 4.7.2.

Temperature and pH were measured with a HI 991001 portable device (Hanna Instruments Canada, Laval, QC, Canada). TSS were determined by Standard Method 2540D (American Public Health Association et al., 1997). Further details regarding the limits of detection (LOD) of the analytical methods are listed in Table 4. 9.

4.3.4 Statistical analyses and calculations

Statistical analyses were carried out with STATISTICA software (version 12). Normality of the variables was tested with a Shapiro-Wilk W test. Given that the majority of raw and $\log_{10}$ transformed data were not normally distributed, non-parametric tests were performed. Kruskal-Wallis and Mann-Whitney U statistical tests were used to assess differences and characterize variability. Nonparametric Spearman correlation test was also applied to describe covariations between parasite, FIB and WWMP concentrations as well as between flowrate and measured concentrations. The significance level was set to alpha = 5% for all statistical analyses.

EPA's ProUCL software (Singh et al., 2013) was used to impute values below the LOD. For the majority of the studied parameters values were above the LOD, except for *Cryptosporidium*, *Giardia* and aspartame. Unless stated otherwise, *Giardia* concentrations were not adjusted by the recovery efficiency. Recovery efficiency data are used to discuss uncertainties in *Giardia* measurements as a result of variable recoveries within and between events.

Two-day cumulative rainfall data were calculated using Thiessen Polygons through hourly data obtained from seventeen rain gauges within a 30 km distance. A normalizing technique was applied to understand the relationship between peak concentrations and peak flowrates by quantifying concentration and flowrate patterns regardless of temporal variability. Total influent flowrates and concentrations of each parameter measured during wet weather events were divided by their respective peak values (C/C_{peak} and Q/Q_{peak}) and the boxplots of the normalized concentrations were presented for the last deciles of normalized flowrate ($0.9<Q/Q_{peak}<1$). The normalized boxplots of C/C_{peak} are a visual representation of the likelihood that contaminant peaks will occur with peak flows during wet weather.

4.4 Results and Discussion

4.4.1 Inflow and infiltration influence on flowrate

The time of day and the amount of inflow and infiltration (as a result of groundwater table depths, precipitation and snowmelt) influence the WRRF's flowrate. To evaluate the impact of precipitation and snowmelt on the flowrate, ΔQ (Channel 2's event flowrates minus the average flowrate for its corresponding month) and historical hourly flowrates for the corresponding months in Channel 2 are shown in Figure 4. 1a and 4. 1b, respectively. The difference relative to the mean flowrate for each event (as mean monthly flowrates also vary) helps to illustrate how each event was influenced by precipitation or snowmelt. ΔQ is generally higher for the events with 2-day cumulative rainfall >15mm prior to sample collection regardless of the time of day events occurred, suggesting a direct impact of rainfall during those events (Ev1, Ev3 and Ev5). In this study, event sampling was generally triggered in the afternoon or evening (Table 4. 8). The median value of ΔQ was highest for Ev3 (June 12th, daytime, 2-day cumulative rainfall of 24mm) and lowest for Ev6 (September 10th, night time, trace precipitation) ($p<0.05$), further demonstrating the effect of precipitation on flowrates. The flowrate in Channel 2 is also higher during spring months (Figure 4. 1b), especially in April following snowmelt and frequent precipitation events that likely resulted in higher groundwater and infiltration levels. It should be noted that the flowrate measured in Channel 2 is part of total plant influent flowrate and is variable; its fraction relative to total influent flowrate was in the range of 0.01-0.7 (in the period of March to September 2014), but generally represents 42% of the total plant influent.

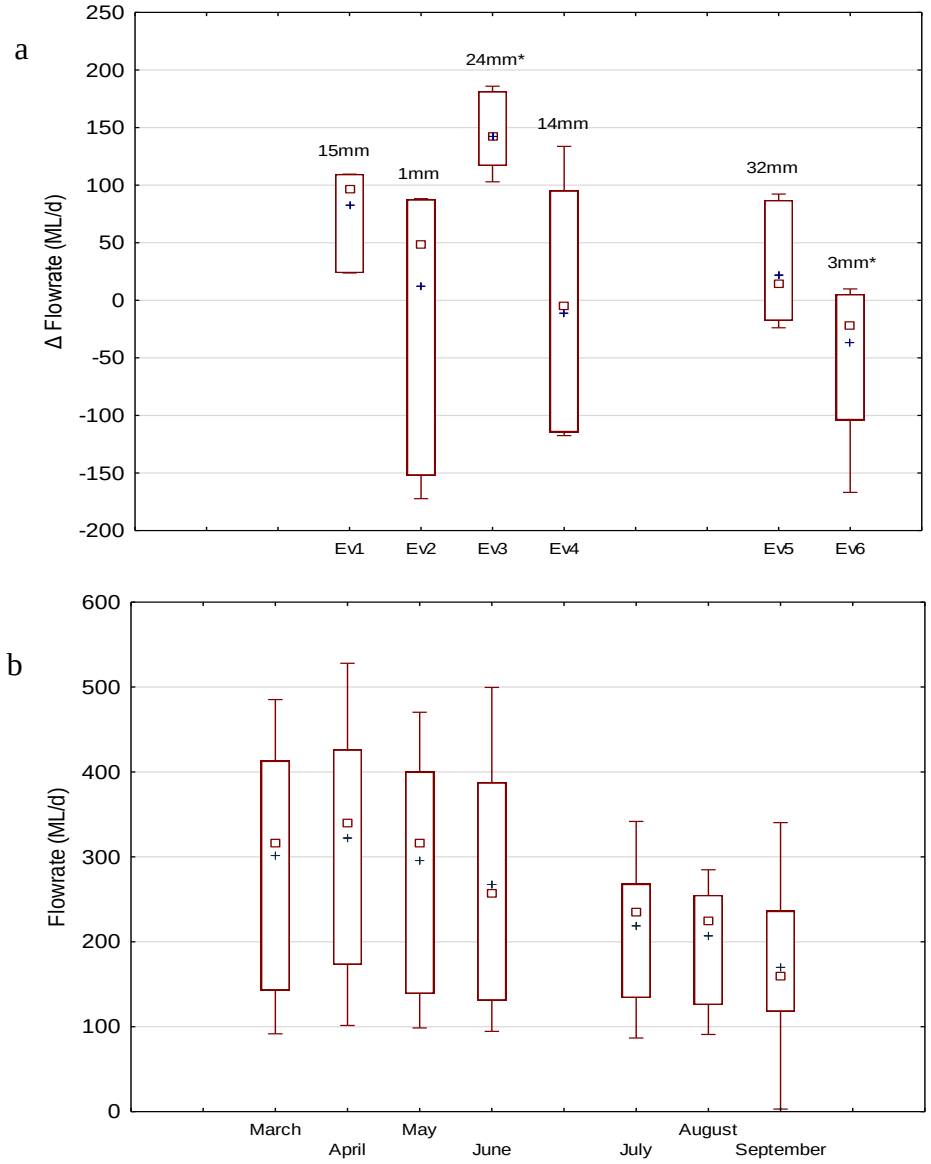

Figure 4. 1: Flowrate conditions during each event in comparison to historical flowrates in chronological order. Boxplots of (a) ΔFlowrate (event flowrate - mean flowrate in Channel 2 for the corresponding month) and (b) Hourly historical flowrate for each month in Channel 2 in 2014 . Boxplots illustrate 10th and 90th percentiles, median values (☐), mean (+) and whiskers (minimum and maximum values). Ev1 (April), Ev3 (June), Ev4 (June), Ev5 (September) are wet weather events and Ev2 (May) and Ev6 (September) are trace precipitation weather events respectively. Two-day cumulative rainfall prior to sample collection is indicated on top of each boxplot. * indicates a significant difference (i.e. $p<0.05$) with the other boxplots.

4.4.2 Prevalence and contaminant concentrations in influent and effluent samples

The concentrations of parasites (*Cryptosporidium* and *Giardia*), FIB (*E. coli* and *C. perfringens*), WWMPs (CAF, CBZ, CBZ-2OH, ACE, SUC and ASP) and TSS in raw sewage and treated effluent discharges are shown in Table 4. 1. We found a higher prevalence of *Giardia* as compared to *Cryptosporidium* in raw sewage (88.6% compared to 8.6%) and in effluent samples (100% compared to 30%). The prevalence rates for parasites in raw sewage were indeed lower than in effluent samples and it relates to the complexity of the raw sewage matrix, which causes recovery efficiencies to drop compared to treated effluents. This has been previously reported by others (Rose et al., 2004; Lalancette et al., 2012). Concentrations of *Cryptosporidium* and *Giardia* in raw sewage varied from below LOD to 10 oocysts/L and from below the LOD to 1010 cysts/L, respectively. In treated effluents, they ranged from below LOD to 0.2 oocysts/L and from 0.1 to 11.1 cysts/L, respectively. The *Giardia* and *Cryptosporidium* concentrations in raw sewage and treated effluents fall within the ranges documented in the literature (Table 4. 10). Additional discussion is presented in Section 4.7.3 Supplementary Information.

E. coli and *C. perfringens* were detected in 100% of the raw sewage and treated effluent samples. Median concentrations in raw sewage samples were 2.6×10^{6} CFU/100mL and 4×10^{4} CFU/100mL for *E. coli* and *C. perfringens*, respectively. They were generally 5 and 2 logs lower in treated effluents, respectively. The observed concentrations are in the range of reported values in the literature (Table 4. 11).

With the exception of ASP, which was detected in 92.7% of raw sewage samples and was absent in the treated effluents, micropollutants including CAF, CBZ, CBZ-2OH, ACE and SUC were found in all raw sewage and treated effluent samples. The absence of ASP in effluent samples is likely due to its higher degradability (Kokotou et al., 2012; Lange et al., 2012). ASP concentrations in raw sewage samples of two wastewater treatment plants in China were in the range of 44-53 ng/L but they were absent in treated effluents (Gan et al., 2013). In the studied WRRF, the ASP median concentrations were significantly lower than other artificial sweeteners of ACE and SUC ($p < 0.05$ in Mann-Whitney U test), potentially indicating different consumption patterns of ASP compared to other artificial sweeteners in addition to higher degradation rates.

Following ingestion, ASP is eliminated to a larger degree from the human body (Nabors, 2001), whereas a large amount of ACE and SUC pass in an unchanged form (Buerge et al., 2009).

In contrast to ASP, we observed an average increase of 26% for CBZ during wastewater treatment (Table 4. 1). CBZ is a highly persistent micro-pollutant not easily degraded or adsorbed during wastewater treatment processes and its concentrations have been shown to increase at rates as high as 100% in treated effluents (Miao et al., 2003; Clara et al., 2004; Gao et al., 2012; Bahlmann et al., 2014). Carbamazepine forms N-glucuronides and its concentration increase in treated effluent could be related to the partial cleavage of N-glucuronide conjugates during treatment (Bahlmann et al., 2014). In the present study, the CBZ-2OH concentration was usually higher than that of its parent CBZ compound and may be the result of the different nature of their glucuronides. Interestingly, a higher concentration was reported in other studies for CBZ-DiOH (another metabolite of carbamazepine) in raw sewage and treated effluents compared to that of its parent CBZ compound (Miao et al., 2003; Miao et al., 2005; Hummel et al., 2006; Leclercq et al., 2009; Bahlmann et al., 2014). In contrast to CBZ, which forms N-glucuronides, all hydroxylated metabolites of CBZ especially CBZ-2OH and CBZ-3OH are excreted as O-glucuronides (Bahlmann et al., 2014). The cleavage of the O-glucuronides of the hydroxylated metabolites of CBZ occurs relatively quickly in the sewer network (before they enter the WRRF) (Leclercq et al., 2009; Bahlmann et al., 2014). In our study, the length of sewer network is about 2110 km, thus it is more likely to observe higher concentrations of CBZ-2OH in its free form in raw sewage. WWMPs are regularly discharged into Lake Ontario from the studied WRRF and are expected to be found at drinking water treatment plant intakes that are influenced by sewage discharges. A comparison of measured concentrations with other studies is discussed in Section 4.7.4.

Table 4. 1: Concentrations (mean± standard deviation (number of samples), median, minimum and maximum) of parasites, fecal indicator bacteria and wastewater micropollutants in raw sewage and treated effluent samples collected during trace precipitation and wet weather conditions

Parameters	Raw sewage			Treated effluent		
	Mean± SD (N)	Median	Min-Max	Mean± SD (N)	Median	Min-Max
Cryptosporidium (oocyst/L)[1]	-	-	LOD-10	-	-	LOD-0.2
Giardia (cyst/L)[2]	232.5±277.7(35)	110	LOD-1010	3.4±4(10)[4]	2.2	0.1-11.1
E. coli (CFU/100 mL)	$3.5\times10^{6}\pm3.4\times10^{6}$(92)	2.6×10^{6}	2.6×10^{5}- 2.7×10^{7}	122.4±319(18)	13	2-1000
C. perfringens (CFU/100 mL)	$6.2\times10^{4}\pm6.1\times10^{4}$(97)	4×10^{4}	2.4×10^{3}- 3.2×10^{5}	501.3±153(19)	542.5	225-750
CAF (ng/L)	$5.5\times10^{4}\pm1.8\times10^{4}$(55)	5.2×10^{4}	$(1.9 -9.2)\times10^{4}$	199.5±204.7 (18)	78	37-537
CBZ (ng/L)	182.5±40 (55)	180	79-270	229.6±36.5(18)	228	176-300
CBZ-2OH (ng/L)	691.9±195.8 (55)	713	270-1059	550.5±103.4(18)	554	378-740
ACE (ng/L)	$1.2\times10^{4}\pm6.1\times10^{3}$(55)	1.3×10^{4}	$(0.8-24.3)\times10^{3}$	$7.9\times10^{3}\pm6.1\times10^{3}$(18)	7.4×10^{3}	$(0.8-20.5)\times10^{3}$
SUC (ng/L)	$1.6\times10^{4}\pm1.3\times10^{4}$(55)	1.3×10^{4}	$(4.4-80.5)\times10^{3}$	$1.3\times10^{4}\pm4.1\times10^{3}$ (18)	1.3×10^{4}	$(3.6-18.8)\times10^{3}$
ASP (ng/L)[3]	$1.2\times10^{3}\pm1.4\times10^{3}$(55)	676	61-8007	-	-	-
TSS (mg/L)	674.6±373.7(92)	705	84-1600	4.6±2.5(18)	3.5	3-11
pH	7.3±0.2(99)	7.3	6.6-7.8	7.1±0.4 (18)	7.1	5.9-7.6
Conductivity (µS/cm)[4]	1312.5±320.6 (42)	1449	458-1800	1431.1±247.8 (9)	1555	1099-1655

1: The concentration of *Cryptosporidium* was below the limit of detection in 32 (out of 35) raw sewage samples and 7 (out of 10) treated effluents; 2: The concentration of *Giardia* was below the limit of detection in 4 (out of 35) raw sewage samples; 3: The concentration of ASP was below the limit of detection in 4 (out of 55) raw sewage samples and in 18 (out of 18) treated effluents 4: are available in Ev1, Ev2 and Ev5 only; LOD: Limit of detection

4.4.3 Temporal variability of contaminant concentrations in raw sewage

Parasite, FIB and WWMP concentrations were assessed between and within events that occurred under various rainfall and flowrate conditions.

4.4.3.1 Intra-event variability

Within single events, higher variations were observed for microbial contaminants than for WWMPs (Figure 4. 2 and Figure 4. 3). For *E. coli*, *C. perfringens* and *Giardia* differences were as high as 2, 1.9 and 1.5 orders of magnitude, respectively. In contrast, WWMPs varied by 1 order of magnitude or less, except for ASP (max. 1.2 orders of magnitude).

For FIB, a similar trend was observed for both *E. coli* and *C. perfringens* over the course of a day (Figure 4.4). Under wet weather conditions (Ev1, Ev3, Ev4 and Ev5), their concentrations decreased overnight (0:00 to 7:00 AM), then started to increase at 7:00 AM to reach their peak values in the early afternoon. Under trace precipitation weather conditions (Ev2 and Ev6), the onset of increases occurred later. The daily variation of fecal pollution and the occurrence time of peak FIB concentrations is associated with diurnal defecation patterns as well as with residence time of the sewer system (Mara et al., 2003). During wet weather, inflow to the sewershed increases the flowrates and velocities and thereby shortens the travel time of fecal contaminants within the sewer network (Madoux-Humery et al. 2013). No clear temporal trends were observed for TSS over the course of a day (Figure 4. 6).

As many samples were negative for *Cryptosporidium* in the influent (3 of 35 samples were positive), no temporal trends could be observed. For *Giardia*, peak concentrations occurred during the day (Figure 4. 6) rather than at night, demonstrating a weak potential for a temporal trend that is heavily influenced by the uncertainties of the methods and also potentially the sporadic spatial occurrences throughout the sewershed.

For WWMPs, little variation was observed for CBZ and its metabolite CBZ-2OH within single events (Figures 4. 3, 4. 4 and 4.7. 1). Little or no daily variation was also reported for CBZ in a WRRF effluent in the USA (Nelson et al., 2010) and in raw sewage in the Greater Montreal area (Madoux-Humery et al., 2013). A trend was observed for CAF, SUC and CBZ when considering all monitored events (Figure 4. 4). Their concentrations decreased overall from 0:00 AM to 7:00 AM and then increased from 7:00 AM to 6:00 PM for both CAF and SUC and from 7:00 AM to

3:30 PM for CBZ. Within-day fluctuations of WWMPs (ACE, CBZ-2OH and ASP are also shown in Figure 4. 6) may be related to their consumption patterns, half-lives in the body, excretion pathways and retention time in the sewer system (Madoux-Humery et al., 2013). Our findings further indicate that the impact of untreated or partially treated sewage discharges into Lake Ontario will be greater with regards to WWMPs if they occur in the late afternoon or early evening.

4.4.3.2 Inter-event variability

Comparing events, Figures 4. 2 and 4. 3 show the variability of concentrations for the samples collected under various weather conditions. Higher inter-event fluctuations (up to 2 orders of magnitude) were observed for *Giardia*, *E. coli* and *C. perfringens* as compared to those of WWMPs. The lowest variation was observed for CBZ and its metabolite CBZ-2OH ($\leq$0.6 orders of magnitude).

Among events, the lowest median value of *Giardia* was observed in the September wet weather event (Figure 4. 2) ($p<0.05$ in Kruskal-Wallis test followed by Mann-Whitney U test) when mean monthly flows into Channel 2 were at their lowest (Figure 4. 1b). The maximum value observed for *Giardia* (1010 cysts/L) was during the first June wet weather event with a 2-day cumulative rainfall of 24mm prior to sample collection. For *E. coli*, median concentrations were significantly higher under wet weather conditions than under trace precipitation weather conditions (Mann-Whitney U test,$p<0.05$). Differences between median *C. perfringens* concentrations under trace precipitation and wet weather conditions were insignificant ($p>0.05$). The median concentrations of *C. perfringens* were significantly higher in the spring (April wet weather and May trace precipitation) events as compared to the other events ($p<0.05$ in Kruskal-Wallis test followed by Mann-Whitney U test) (Figure 4. 2).

Wastewater inflow and sewer deposit resuspension may contribute to the conveyance of fecal pollution. Wastewater concentrations of fecal microorganisms are orders of magnitude higher than inflow (stormwater) concentrations (Madoux-Humery et al. 2013). Sewer deposit resuspension is related to the type and configuration of the sewershed, rainfall intensity and antecedent dry period (Madoux-Humery et al., 2015). Spore-forming *C. perfringens* can be associated with settleable particles and they have a long survival in surface waters and sediments (Edwards et al., 1998; Lisle et al., 2004; Krometis et al., 2007; Mueller-Spitz et al., 2010). The

flowrate is generally higher in April and May due to the higher level of infiltration/inflow following the snowmelt period and higher water table (Figure 4.1). Sewer sediments could become a source of *C. perfringens* spores that accumulated during lower flow periods.

Snowmelt has been recognized as a critical time with regards to source water protection because of few restrictions on raw sewage discharges from sewer overflows and lower temperatures affecting drinking water treatment efficiencies (Madoux-Humery et al., 2013). The probability of a by-pass event is higher during and following the snowmelt period with rain on snow. The spring period was identified as a vulnerable time for the water treatment plants located in Lake Ontario as a result of higher parasite concentrations at offshore intakes (Edge et al., 2013). At the studied WRRF, by-passes are discharged following primary treatment sometimes with and sometimes without disinfection (depending on whether sufficient time is available for disinfection). It is assumed that the high concentrations of fecal microorganisms following a by-pass event are mitigated because raw sewage is treated to some extent after primary treatment. However, not all microorganisms are reduced following primary treatment (Katayama et al., 2004; Kay et al., 2008). *Giardia* log removal through primary treatment has been reported to be in the range of 0.12 to 0.65 (Robertson et al., 2000; Cacciò et al., 2003; Fu et al., 2010).

Among the selected WWMPs, the median concentration of ACE was significantly lower during wet weather conditions ($p<0.05$ in Mann-Whitney U test) perhaps as a result of its higher solubility (587500 mg/L at 25°C) as compared to other WWMPs (Table 4. 7). Among events, the variability of CAF, CBZ and CBZ-2OH concentrations was significantly higher ($p<0.05$) in the September-wet weather event with 2-day cumulative rainfall of approximately 32mm prior to sample collection (Figure 4. 3). Differences among concentrations may be related to their different excretion patterns (influenced by the time of sampling as illustrated in Table 4. 8) and can be influenced by hydraulic conditions in the sewer system. For example, sewer system sediments were identified as both source and sink of WWMPs in urban water systems (Hajj-Mohamad et al., 2014; Madoux-Humery et al., 2015). Discharge patterns, dilution processes, degradation rates, sorption and desorption processes were recognized as key controlling factors regarding the fate and transport of WWMPs in aquatic environments, including within combined sewer systems (Scheytt et al., 2005; Hajj-Mohamad et al., 2017). WWMPs that are highly soluble and degradable would tend to not accumulate in sewer sediments and are more likely to be diluted by the addition of stormwater into the sewer system. In contrast, WWMPs that

accumulate in sewer sediments could see concentrations increase with the addition of stormwater as increased flowrates lead to sediment resuspension and desorption.

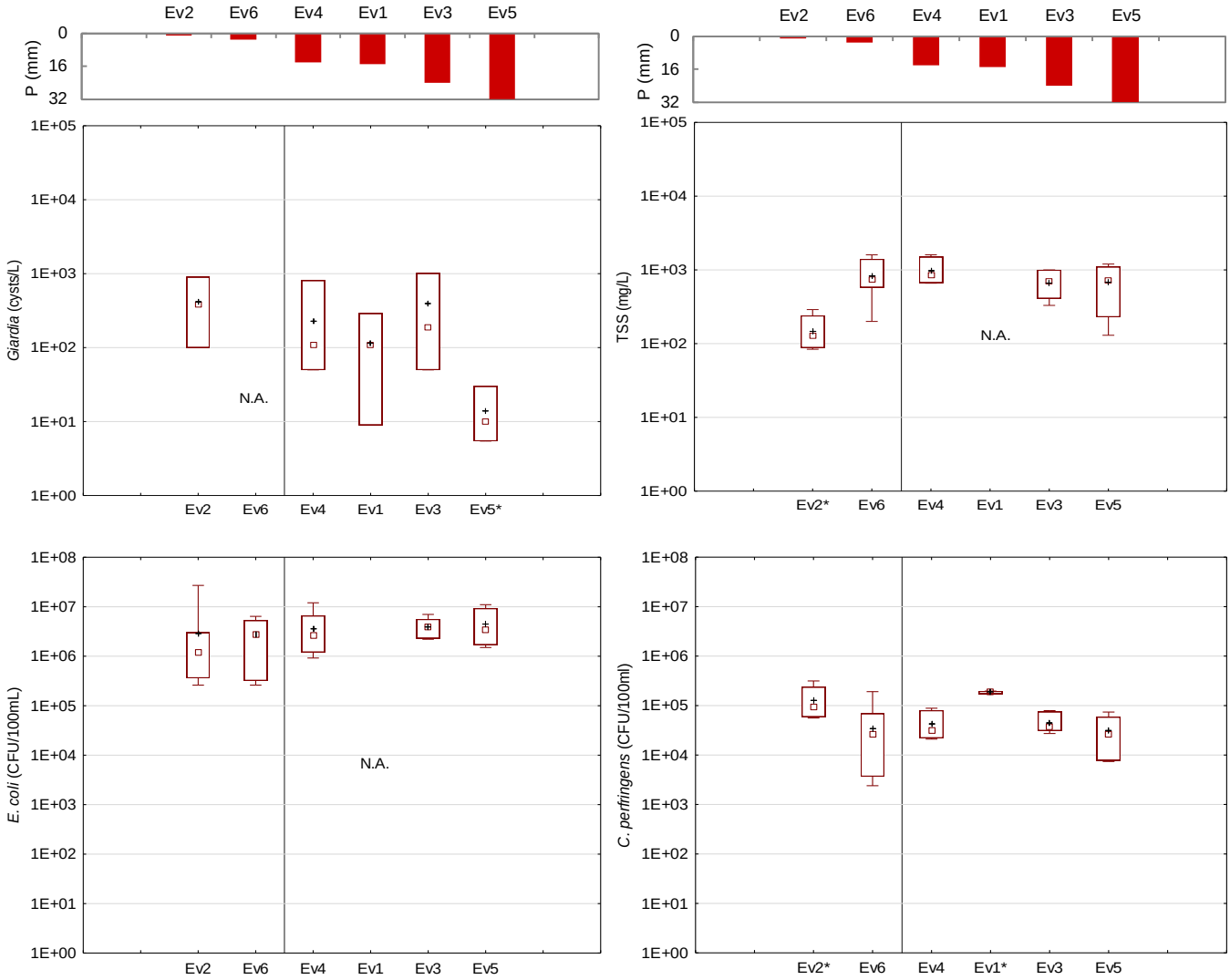

Figure 4. 2: Temporal variations of *Giardia*, *E. coli*, *C. perfringens* and TSS concentrations in raw sewage under trace precipitation (Ev2 (May) and Ev6 (September)) and wet weather conditions (Ev1 (April), Ev3 (June), Ev4 (June), Ev5 (September)). Boxplots illustrate 10th and 90th percentiles, median values (☐), mean (+) and whiskers (minimum and maximum values). Two-day cumulative rainfall prior to sample collection is indicated on top (P(mm), events in order of increasing precipitation). * indicates a significant difference (i.e. $p<0.05$) with the other events. N.A.: Not available. Vertical lines separate trace precipitation from wet weather events.

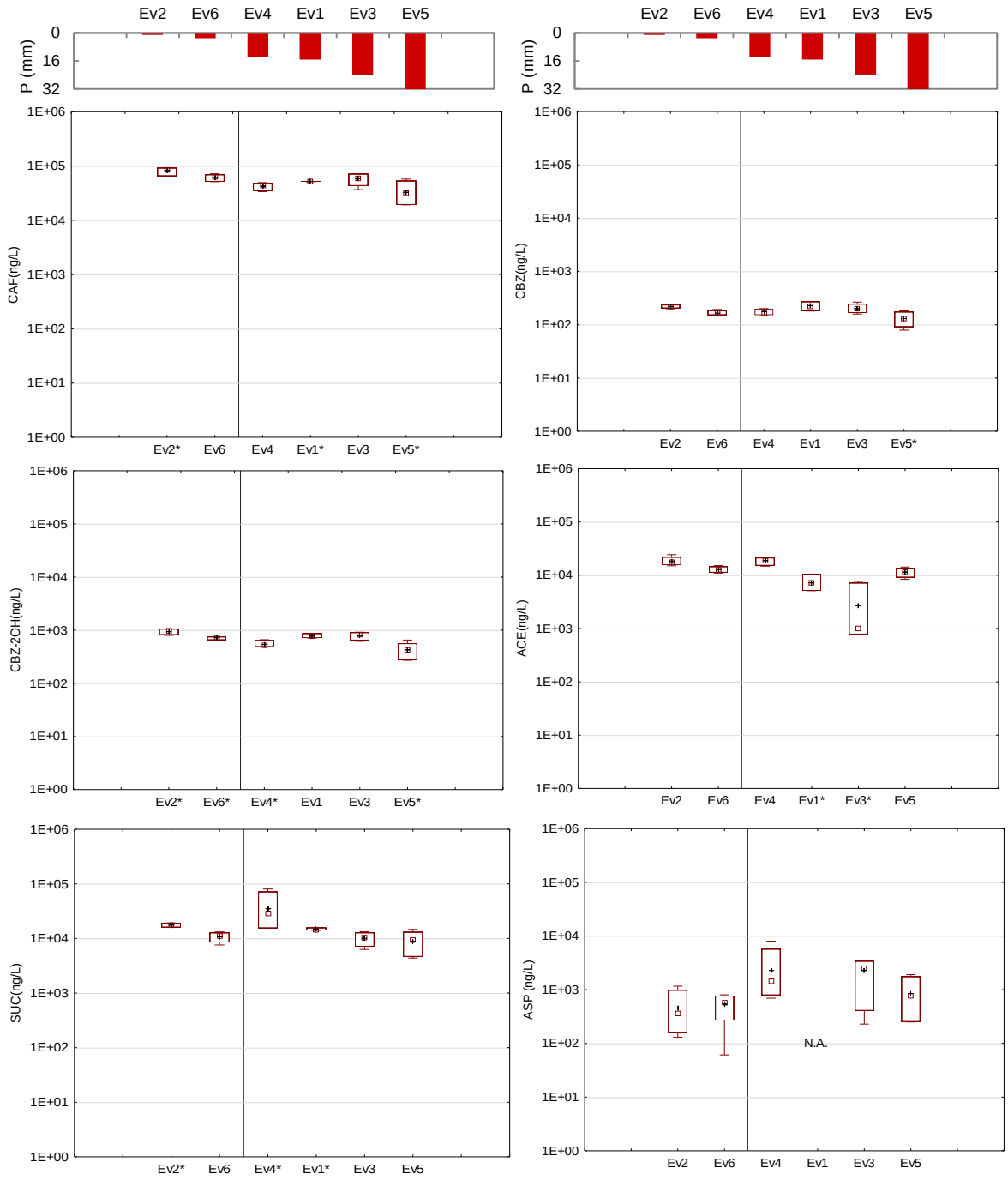

Figure 4. 3: Temporal variations of wastewater micropollutant (WWMP) concentrations in raw sewage under trace precipitation (Ev2 (May) and Ev6 (September)) and wet weather events (Ev1 (April), Ev3 (June), Ev4 (June), Ev5 (September)). Boxplots illustrate 10th and 90th percentiles, median values (☐), mean (+) and whiskers (minimum and maximum values). Two-day cumulative rainfall prior to sample collection is indicated on top (P (mm), events, in order of increasing precipitation). * indicates a significant difference (i.e. $p<0.05$) with the other events. N.A.: Not available. Vertical lines separate trace precipitation from wet weather events.

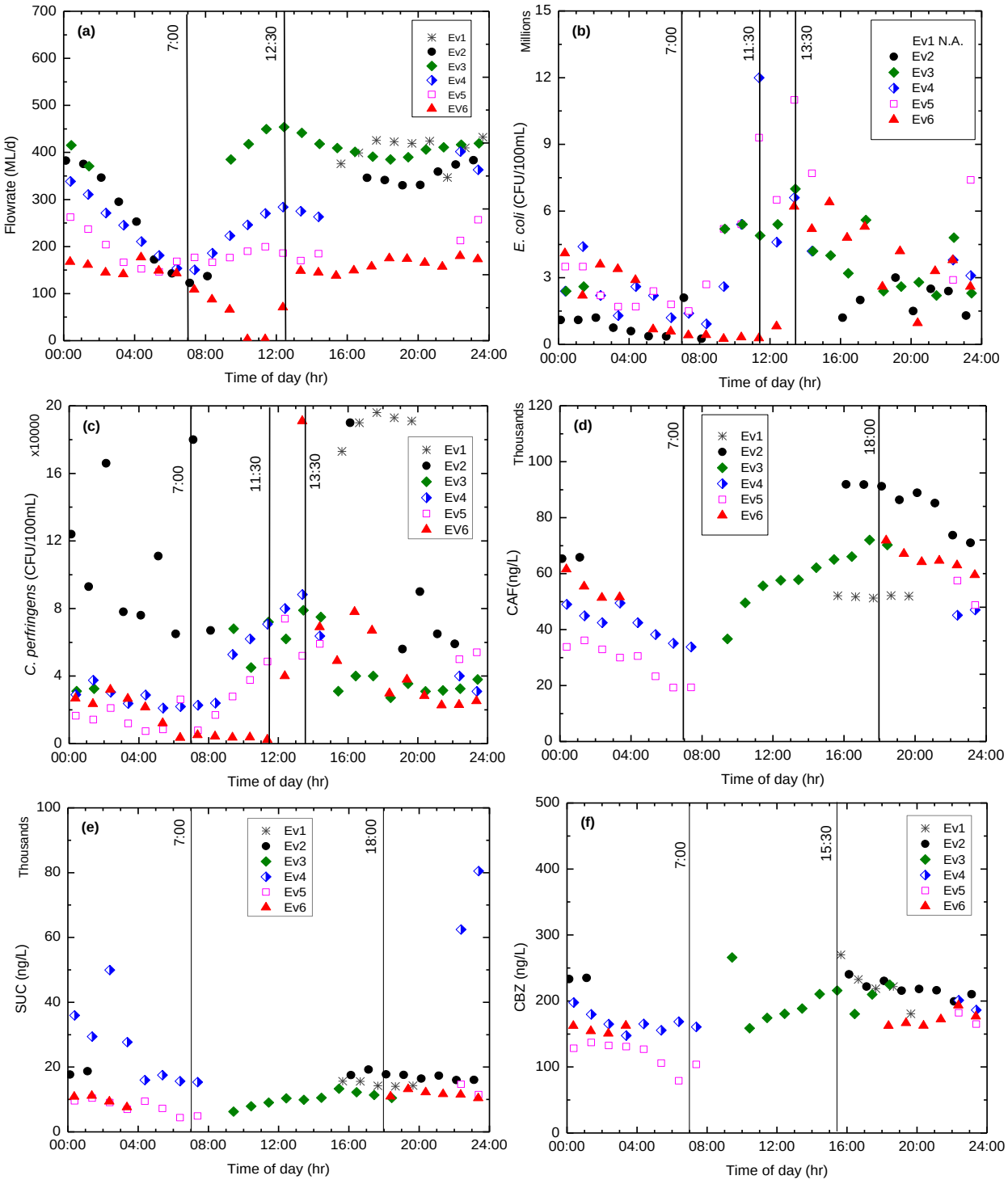

Figure 4. 4: Within-event temporal variations in (a) flowrate, (b) *E. coli*, (c) *C. perfringens*, (d) CAF, (e) SUC and (f) CBZ in raw sewage. Ev1 (April), Ev3 (June), Ev4 (June), Ev5 (September) are wet weather events and Ev2 (May) and Ev6 (September) are trace precipitation events. N.A.: Not available. Vertical lines show the times of day that transitions of increasing or decreasing concentrations occur.

4.4.3.3 Relationship between concentrations and flowrates

When studying contaminants in urban sewersheds, it is useful to consider whether contaminant concentrations are flow or mass limited. The concepts of mass-limited and flow-limited events have been described by Cristina et al. (2003) for characterizing the dynamics of pollutant transport in urban areas. A mass limited concentration means that when the source of the contaminant is exhausted (or diluted in the case of sewage) there is no longer enough mass available in the system to be carried by hydraulic transport; thus, the concentration will decrease as flow increases. With flow limited contaminants, the source of the contaminant remains and the flowrate is the critical factor for pollution concentrations and loads (Piro et al., 2014). Although these concepts have generally been applied to stormwater driven systems, they are also useful in the context of sewage flow driven systems because they can help explain trends and relationships between contaminant concentrations and peak flows.

Flowrates were weakly yet significantly correlated to *E. coli* ($R=0.32$, $p<0.05$), *C. perfringens* ($R=0.48$, $p<0.05$) and *Giardia* concentrations ($R=0.4$, $p<0.05$), suggesting similar sources as well as fate and transport dynamics for microbial contaminants in the sewer network. When considering only the period from 7:00 am to early afternoon, the correlations between flowrate and fecal indicator bacteria were stronger (*E. coli*: $R=0.67$, $p<0.05$, *C. perfringens*: $R=0.70$, $p<0.05$). The association of FIB with settleable particles and sediment resuspension (during increasing flowrate conditions) presumably influences microbial fate and transport in the sewer network (Gonçalves et al., 2009; Passerat et al., 2011). In general, microbial contaminants were not strongly mass limited and increasing flowrates led to higher concentrations even when greater dilution of sewage would be expected. Possible explanations are: 1) less die-off as a result of shorter travel times, 2) peak flows corresponding to peak fecal excretion periods and 3) higher flows leading to greater net resuspension or less net deposition.

No significant correlations were observed between flowrate and ASP or SUC. CAF, CBZ and CBZ-2OH were positively correlated with flowrates ($R=0.34$, 0.66, 0.53, respectively, $p<0.05$). In contrast, TSS and ACE were negatively associated with flowrates ($R=-0.28$, -0.33, respectively, $p<0.05$). ACE is highly soluble and is diluted by higher flows. If some sediment resuspension occurred during flowrate increase in the sewer network, TSS concentration was more mass limited as compared to the other contaminants for the events monitored. The absence

of correlation between TSS and *Giardia* or *E. coli* ($p>0.05$) suggests that they were not associated with fecal contamination, likely because they were released from different sources (Madoux-Humery et al. 2013).

By-pass discharges typically occur when flowrates are at their highest. From a drinking water source protection perspective, it is important to understand how concentrations vary in relation to peak flows because it is logistically difficult to deploy for sampling when by-passes occur as they are less frequent and more difficult to predict. In order to determine the degree to which peak contaminant concentrations were associated with peak flows, box plots of normalized concentrations (C/C_{peak}) of fecal microorganisms, WWMPs and TSS in raw sewage were illustrated for the last decile of normalized flowrate ($0.9<Q/Q_{peak}<1$) (Figure 4. 5). The concentration variations (C/C_{peak}) were lower for CAF, CBZ, CBZ-2OH and SUC (with the median values in the range of 0.79-0.9) and higher for ASP and ACE (with the median values of 0.61 and 0.49 respectively). The fluctuation of C/C_{peak} was also higher for *Giardia*, *E. coli, C. perfringens* and TSS with median values of 0.3, 0.59, 0.73 and 0.46 respectively. Evaluation of peak concentrations of fecal contamination sources are needed to estimate peak concentrations at drinking water intakes in order to ensure that drinking water treatment plants are appropriately designed (Dorner et al., 2006). Peak concentrations of *Giardia, E. coli and C. perfringens* may not be observed during peak flowrates and hence they are more mass limited than flow limited with regards to their origins in sewer networks as compared to CAF, CBZ, CBZ-2OH and SUC.

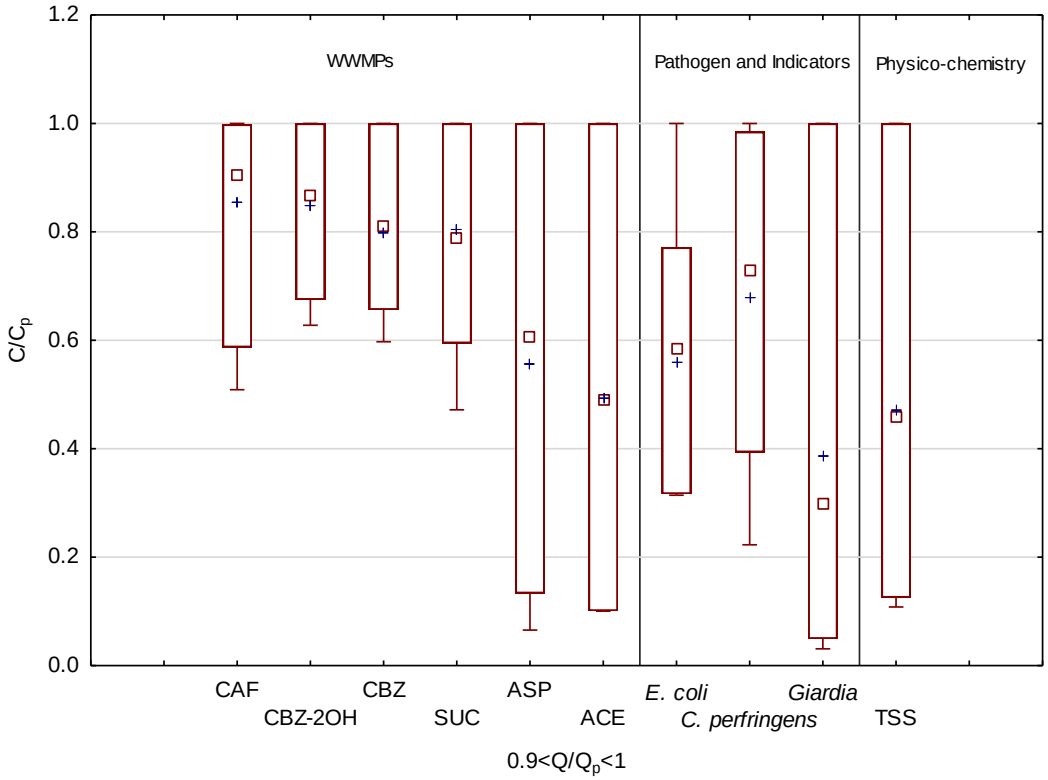

Figure 4. 5: Variation of C/C_{peak} for parasites, FIB and WWMPs for the wet weather data set when total influent flowrate approached its peak value ($0.9 \leq Q/Q_{peak} \leq 1$). Boxplots illustrate 10th and 90th percentiles, median values (•), mean (+) and whiskers (minimum and maximum values).

4.4.4 Indicators of fecal pollution in surface waters impacted by by-passes

Selection of appropriate indicators of fecal pollution should be carried out by considering their sources, fate and transport characteristics (Sauvé et al., 2012). These were shown to be variable in sewer systems under wet weather conditions (Gasperi et al., 2010; Passerat et al., 2011), indicating the important role of local hydro-meteorology. Suitable indicators of fecal pollution in a by-pass discharge and/or WRRF effluent in the case of a treatment failure were assessed by examining the correlations between concentrations in raw sewage under trace precipitation and wet weather conditions (Table 4. 2). This information provides a reliable indication of the potential for the presence of pathogens in source waters.

Under wet weather conditions, *Giardia* was not correlated with bacterial indicators but was significantly and positively correlated with CBZ, CBZ-2OH ($R \geq 0.45$, $p<0.05$) and CAF ($R=0.4$, $p<0.05$). CBZ is more persistent than CAF and its removal is marginal through conventional wastewater treatment as a result of its refractory behaviour (Miao et al., 2003; Sauvé et al., 2012;

Tran et al., 2018). The half-life for CAF was shown to range between 0.8 and 5 h in wastewater with biological processes (Buerge et al., 2003). *Giardia* cysts are also environmentally resistant; therefore a more stable marker is more conservative and useful. Given the correlations and poor removal through wastewater treatment, CBZ and its metabolite CBZ-2OH appear to be the most suitable markers of fecal contamination in a by-pass discharge and/or failed treatment following wet weather conditions. Under wet weather conditions, many WWMPs were strongly intercorrelated (Table 4. 2). Significant correlations were previously observed between ACE and CBZ concentrations in surface waters (R^2=0.94) and in riverbank filtration wells (R^2=0.85) (Scheurer et al., 2011).

A significant correlation (R=0.79, $p<0.05$) was observed between *Giardia* and *E. coli* (n=7) for events with only trace precipitation, but not for wet weather events. Survival rates differ among fecal microorganisms; for instance die-off rates of fecal coliforms, *Salmonella typhimurium* and *Giardia* in sediments of two constructed wetlands were reported as 0.15 $\log_{10}$ day^{-1}, 0.31 $\log_{10}$ day^{-1} and 0.37 $\log_{10}$ day^{-1} respectively (Karim et al., 2004). Thus, over time, *Giardia* and *E. coli* would accumulate in the sewer network at different rates, which could lead to differences in their relative contributions from the sewer network during wet weather.

Overall, TSS were significantly negatively correlated with *C. perfringens*, CAF, CBZ, CBZ-2OH and ACE (R=-0.47 to -0.61, $p<0.05$) and were positively correlated with ASP (R=0.51, $p<0.05$). Positive correlations would be expected for parameters with similar origins and pathways.

Daneshvar et al. (2012) proposed CBZ as a marker of cumulative wastewater discharges into surface waters and CAF as recent marker of fecal contamination. Correlations among fecal microorganisms and WWMPs change according to weather conditions and potentially the time of day. These variations may relate to changes in terms of source including varying excretion patterns, sources within the network (fecal inputs and sewer sediments) and different travel times as affected by flowrates and velocities (from sewage and additional inflow during precipitation events) and fate and transport behaviours of studied parameters within the sewer network.

Table 4. 2: Spearman's rank correlation of parasites, FIB and WWMPs in raw sewage (numbers); *: denotes a significant correlation i.e. p<0.05

	Parameter	Type of dataset	Fecal indicators		WWMPs					
			E. coli	*C. perfringens*	CAF	CBZ	CBZ-2OH	ACE	SUC	ASP
Parasite	*Giardia*	All	0.15 (28)	**0.42(33)***	**0.57(33)***	**0.46(33)***	**0.57(33)***	-0.04(33)	**0.4(33)***	0.15(33)
		DW**	**0.79 (7)***	0.18(7)	-0.43(7)	-0.21 (7)	0.11(7)	-0.46(7)	0.14(7)	0.07(7)
		WW***	0.24 (21)	0.39(26)	**0.4 (26)***	**0.45(26)***	**0.47(26)***	**-0.62(26)***	0.32(26)	0.14(26)
Fecal indicators	*E. coli*	All		**0.31 (92)***	0.08 (50)	0.12(50)	0.1(50)	**-0.37(50)***	-0.23(50)	0.27(50)
		DW		0.24(41)	-0.23(20)	-0.41(20)	-0.32(20)	-0.36(20)	-0.42(20)	0.38(20)
		WW		**0.76 (51)***	**0.6 (30)***	**0.54(30)***	**0.54(30)***	-0.33(30)	-0.07(30)	0.13(30)
	C. perfringens	All			**0.6 (55)***	**0.8(55)***	**0.75(55)***	0.08(55)	**0.4(55)***	-0.22(55)
		DW			**0.73(20)***	**0.73(20)***	**0.78(20)***	**0.76(20)***	**0.79(20)***	**-0.57(20)***
		WW			**0.7 (35)***	**0.77(35)***	**0.75(35)***	**-0.4(35)***	-0.4 (35)	-0.09(35)
WWMPs	CAF	All				**0.64(55)***	**0.88(55)***	**0.47(55)***	**0.34(55)***	0.01(55)
		DW				**0.72(20)***	**0.82(20)***	**0.75(20)***	**0.79(20)***	-0.21(20)
		WW				**0.74(35)***	**0.89(35)***	**-0.35(35)***	0.23(35)	**0.37(35)***
	CBZ	All					**0.74(55)***	0.05(55)	**0. 5(55)***	-0.15(55)
		DW					**0.85(20)***	**0.68(20)***	**0.87(20)***	-0.34(20)
		WW					**0.74(35)***	**-0.43(35)***	**0.36(35)***	0.01(35)
	CBZ-2OH	All						**0.29(55)***	**0.31(55)***	-0.06(55)
		DW						**0.72(20)***	**0.85(20)***	-0.31(20)
		WW						**-0.5 (35)***	0.15(35)	0.26(35)
	ACE	All							0.08(55)	**-0.46(55)***
		DW							**0.78(20)***	-0.43(20)
		WW							-0.31(35)	-0.3(35)
	SUC	All								0. 06(55)
		DW								-0.36(20)
		WW								0.15(35)

4.5 Conclusions

A total of 864 separate microbial and chemical analyses were conducted for the quantification of pathogenic parasites, fecal indicator bacteria and WWMPs in raw sewage and treated effluents under various hydro-meteorological conditions. Our conclusions with regards to the studied contaminants in sewage influents and effluents are the following:

- In raw sewage and treated effluents, *Giardia* cysts were more prevalent than *Cryptosporidium* oocysts. Parasites were more prevalent in treated effluents than in raw sewage as a result of the higher recovery efficiencies in the treated effluent from reduced water matrix effects.
- The concentration of carbamazepine (CBZ) increased in treated effluents. The observed CBZ increase was possibly related to the partial cleavage of N-glucuronide conjugates during wastewater treatment. ASP was the only WWMP to be consistently removed to below its limit of detection in treated effluents.
- Given the significant correlation between CBZ and *Giardia*, *E. coli* and *C. perfringens* in raw sewage samples ($R \geq 0.45$, $p < 0.05$), CBZ appears to be an appropriate chemical marker of human related fecal pollution resulting from untreated or partially treated wastewater discharges such as WRRF by-passes. Hence, the presence of CBZ in source waters indicates the potential for human pathogen presence.
- Intra and inter event variations were more pronounced for microbial contaminants (*Giardia, E. coli* and *C. perfringens*) than for WWMPs under both dry and wet weather conditions.
- Even with the addition of stormwater from inflow to the sewer network, observed concentrations of microbiological contaminants and WWMPs in raw sewage were driven largely by daily patterns related to human behaviour. Concentrations were only moderately influenced by precipitation events.
- When considering the dilution potential of infiltration and inflow during wet weather, the concentrations of microorganisms in raw sewage were not strongly mass limited. Peak contaminant concentrations were also observed during wet weather events. Thus, dilution of raw sewage could be partly offset by reduced travel times and fewer losses from deposition or degradation within the sewer network during periods with higher flow.
- For the WRRF studied, higher concentrations were observed in the afternoon and early evening and can be considered as critical periods with regards to untreated or partially

treated sewage discharges. However, critical periods for other sewersheds might vary depending on the size and configuration of the sewershed, the population served and the time of travel within the sewer network.

- WRRF by-pass discharges are more likely to occur during periods of high flow. For systems with frequent by-pass flows, it is important to consider when by-passes occur and the relationship between flowrates and concentrations. An increase in flow will not necessarily represent an equivalent increase in sewage dilution even when the increase is partly from stormwater inflow and infiltration.

4.6 Supplementary information

4.6.1 Sample collection

Influent sampling was performed using an Automated Isco portable auto-sampler 6712 FR (Fiberglass and Refrigerated, Teledyne ISCO, Lincoln NE, USA) logged to a flowmeter of the plant and to a digital cellular modem (transmitting text message alarms at the beginning and at the end of sampling). Raw sewage (following preliminary screening and grit removal) was collected in time-proportional composite samples in 1L polypropylene bottles based on rainfall and flowrate thresholds in Channel 2. Unless stated otherwise, the flowrate means the flowrate of the Channel 2. The sampling strategy was as follows: during events, two 1 L composite samples were collected every hour for the first 7 hours and then one 1L composite samples was collected every hour for the next 10 hours. To fill the 1 L bottle, 100 mL of sewage was collected every 6 minutes. Samples from the first 7 hours of the event were analysed for pathogenic protozoa (*Cryptosporidium* and *Giardia*), FIB (*E. coli*, *C. perfringens*), WWMPs (CAF, CBZ, CBZ-2OH, ACE, SUC and ASP) and total suspended solids (TSS). The final 10 hours of the events were analysed for FIB and TSS and 3 hours for WWMPs only. During the September event under trace precipitation conditions, 1L composite samples were collected hourly during a 24 h period and analysed for FIB, TSS and WWMPs only. Filtration of WWMP samples and measurement of temperature, conductivity and pH were performed on-site.

Effluent grab samples were collected in 10L collapsible container low density polyethylene bottles (Cole Parmer) following chlorination and before de-chlorination processes. Whenever possible,

samples were collected considering the calculated plant residence time. For safety and security reasons, samples could not be collected at night.

4.6.2 Analytical methods

U.S. EPA Method 1623.1 adapted for wastewater matrixes was used to analyse raw sewage and treated effluent samples for *Cryptosporidium* and *Giardia* (McCuin et al., 2005). In total, 2 out of 7 raw sewage samples for each monitored event as well as 1 out of 2 effluent samples were examined for recovery rate assessment using matrix spikes with modified (oo)cysts (ColorSeed, BTF). Raw sewage samples (100 mL) were mixed with Tween 80 (final concentration 1%) and centrifuged (1400 x g; 20 min). Treated effluent samples were filtered through Envirocheck HV filter cartridges after a pre-elution step with 5% solution of sodium hexametaphosphate. Concentrated raw sewage and treated effluent samples were then processed by immunomagnetic separation (Dynabeads, Invitrogen) and stained with fluorescently labeled monoclonal antibodies (Easystain, BTF) before enumeration of stained (oo)cysts by epifluorescence microscopy.

MOE LSB E3371 and EPA/600/R-95/178 (1996) standards were also performed to analyse the collected samples for *Escherichia coli* and *C. perfringens* respectively. Collected samples were filtered through 0.45 µm cellulose membranes. *Escherichia coli* and *C. perfringens* were enumerated following incubation in aerobic and anaerobic chambers at $44.5\,^{0}C$ for 24 h on mFC-BCIG agar and mCP agar (supplemented with D-cycloserine, Polymyxin B sulfate, 4.5% $FeCl_3.6H_2O$ solution, 0.5% Phenolphthalein diphosphate solution and 0.075% Indoxyl–β–D–Glucoside solution) respectively.

The SPE-LC-APCI-MS/MS method used in this study has been previously described (Morissette et al., 2015). For the SPE-LC-HESI-MS/MS method, the instruments used were the same with a Heated Electrospray Ionization (HESI) source instead of an Atmospheric Pressure Chemical Ionization (APCI) source. Details regarding this method are described in Table 4. 3 Compound-dependent MS parameters for all targeted compounds are also detailed in Table 4. 4.

Table 4. 3: Gradient elution conditions used for the SPE-LC-HESI/MS/MS method

Loading pump				Analytical pump			
Time (min)	A (%)	B (%)	Flow rate (µL min^{-1})	Time (min)	A (%)	B (%)	Flow rate (µL min^{-1})
On-line SPE loading				Column equilibration			
0.00	100	0	1000	0.00	60	40	500
1.20	100	0	1000	1.20	60	40	500
Loop wash then SPE column wash				Elution and chromatographic separation			
1.30	0	100	1500	4.50	5	95	500
5.50	0	100	1500	5.10	5	95	500
SPE column conditioning				Column equilibration			
5.51	100	0	1500	5.11	60	40	500
7.00	100	0	1500	7.00	60	40	500

Mobile phases➔ A: H2O+0.1%NH_4OH; B: MeOH+0.1% NH_4OH

Columns➔ Analytical column: Hypersil GOLD C8 (100x2.1mm; 3µm), Thermo Scientific; SPE column: Hypersil GOLD C8 (20x2.1mm; 5µm), Thermo Scientific; Analytical column temperature: 30ºC

Table 4. 4: MS/MS optimized parameters for all selected compounds

Compound	Precursor ion (m/z)	Product ion (m/z)	Source PI/NI	TL (V)	CE (eV)
CAF	195	138	APCI	80	19
	$[M+H]^+$	110	PI	80	23
CBZ	237	194	APCI	77	18
	$[M+H]^+$	192	PI	77	23
CBZ-2OH	271	180	APCI	80	20
	$[M+H]^+$	253	PI	80	36
ASP	295	120	APCI	82	33
	$[M+H]^+$	235	PI	82	15
ACE	162	133	HESI	65	39
	$[M-H]^-$	78	NI	65	16
SUC	395 (397)	359	HESI	59	14
	$[M-H]^-$	361	NI	59	14
CAF*	198	140	APCI/PI	81	18
CBZ*	247	204	APCI/PI	82	20
SUC*	401	365	HESI/NI	60	15

*: Labelled internal standard; PI: Positive ionization; NI: Negative ionization; TL: Tube lens; CV: Collision energy

4.6.3 Prevalence and concentrations of parasites in influent and effluent samples

In treated effluents of the same WWTP, Edge et al. (2013) reported comparable prevalence rates of 40% and 90% as well as maximum concentrations of 0.43 oocysts/L and 28.3 cysts/L for *Cryptosporidium* and *Giardia*. For both *Cryptosporidium* and *Giardia*, higher recovery rates were observed in treated effluent than in raw sewage. In treated effluents, they ranged from 9.1% to 76.8% and from 32.3% to 66.3%, respectively, while in raw sewage, they varied between 0% to 44.7% and between 0% to 91.9%, respectively. Although these recovery performances appear to be highly variable, they are nonetheless common in wastewater matrices. For ten WWTPs across the US reported *Cryptosporidium* recoveries varied from 0% to 83.8% in raw sewage and from 0% to 62.6% in secondary treated effluent samples (McCuin et al., 2006). In a Canadian study, mean recovery efficiencies for *Cryptosporidium* and *Giardia* in raw sewage were 18% and 13% and they increased in treated effluents with 26% and 36%, respectively (Lalancette et al., 2012). We show that the recovery efficiency for *Cryptosporidium* and *Giardia* is highly variable even at short (hourly) time scales both in influent and effluent samples. Given this high variability, recovery efficiency should ideally be measured for every water sample (Ongerth, 2013). However, there are also uncertainties associated with measured recovery efficiencies (i.e. errors in counting and losses during sample processing) (Emelko et al., 2010). As such, *Giardia* results are interpreted considering that uncertainties can be as high as one and a half orders of magnitude (based on recovery efficiency data applied to measurements), but with a median uncertainty of 0.5 orders of magnitude.

4.6.4 Concentrations of wastewater micropollutants in influent and effluent samples

The observed concentrations of CAF, CBZ, ACE and SUC in the influent and effluent were consistent with previous studies, while the CBZ-2OH concentration was higher (up to one order of magnitude higher) than reported values from other studies (Tables 4. 5, 4. 6). In two Canadian studies the concentration of CBZ-2OH was in the range of 59-121 ng/L in raw sewage and 70.4-132.3 ng/L in treated effluent (Miao et al., 2003; Miao et al., 2005). Differences in WWMP concentrations measured in WWTPs could be related to different usage patterns, per

capita water consumption, combined versus separated sewer networks and treatment processes (Daneshvar et al., 2012). CBZ, CBZ-2OH, ACE and SUC are persistent pollutants in water resource recovery facilities and are found in high concentrations both in raw sewage and treated effluents.

Table 4. 5: The average value of CAF, CBZ and CBZ-2OH in WRRFs

Country/Region	Year	CAF (ng/l)		CBZ (ng/l)		CBZ -2OH(ng/l)		References
		Inf	Eff	Inf	Eff	Inf	Eff	
Canada/Great Montreal	2010	7482[a]		229[a]				Madoux-Humery et al. (2013)
Canada/Ontario	2010-2011			4.15-22.6	4.54-12.3			Hoque et al. (2014)
Canada/Ontario	2003	63200	68	356.1	251	59	70.4	Miao et al. (2005)
Canada/Ontario	2002			368.9	426.2	121	132.3	Miao et al. (2003)
Canada/Ontario	Not specified	70066.7	46.7	539.3	453.3			Zhao et al. (2008)
Southwest Germany	2010			320-1200	53-1000			Scheurer et al. (2011)
USA/Michigan	2010	41204	76	110	155			Gao et al. (2012)
Korea	2008	20100	859	1920	1750			Sim et al. (2011)
Korea	2010	45457-72471.2	<4.6-1857.8	1668.8-2085.4	108.3-362.8			Lee et al. (2013)
Korea	2009	7559	38	173	154			Lee et al. (2011)
German and Portuguese	2014			470-1900[a]	520-2000[a]			Bahlmann et al. (2014)

Inf: Influent; Eff: Effluent; a: Median value

Table 4. 6: The range of artificial sweeteners in WRRFs (Adapted from Lange, Scheurer et al., 2012)

Country/Region	Year	ACE (µg/l)		SUC(µg/l)		ASP(µg/l)		Reference
		Inf	Eff	Inf	Eff	Inf	Eff	
Canada/Ontario	2010-2011			0.011-0.018	0.35-0.48			Hoque et al. (2014)
Sweden	2006/2007			3.5-7.9	1.8-10.8			Brorström-Lundén et al. (2008)
Sweden	2009			1.7-3.2	2.3-2.5			Neset et al. (2010)
Switzerland /Zurich	2008	12-43	14-46	2.0-9.1	2-8.8			Buerge et al. (2009)
Germany Baden-Württemberg	2009	35-47	26-28	0.82	0.6-0.7			Scheurer et al. (2009)
Southwest Germany	2010	8.2-37	11-39	0.44-1.5	0.4-1.53			Scheurer et al. (2011)
USA AZ	2009				1.5-4.3			Torres et al. (2011)
USA (FL, TX, CA, IL, MI)	2009/2010				27[a]			Oppenheimer et al. (2011)
USA (NC)	not specified				11.9			Mead et al. (2009)
USA (NC)	2013	0.05-2.27	0.59-4.33	15.7-46.1	15.5-55.3	0.01-0.44	0.02-0.22	Subedi et al. (2014b)
China	2011	16-17	15-17	1.9-2.1	1.5-1.8	0.04-0.05	n.d	Gan et al. (2013)
USA	not specified				0.8-1.8			Ferrer et al. (2013)

Table 4. 7: Physical properties of the studied wastewater micropollutants

	CAF	CBZ	CBZ-2OH	ACE	SUC	ASP
Use	Stimulant	Antiepileptic	CBZ metabolite	Artificial Sweeteners		
Molecular formula	$C_8H_{10}N_4O_2$	$C_{15}H_{12}N_2O$	$C_{15}H_{12}N_2O_2$	$C_4H_5NO_4S$	$C_{12}H_{19}Cl_3O_8$	$C_{14}H_{18}N_2O_5$
Solubility at 25 ^{0}C (mg/L)	21600[a]	16.8[a]	-	587500[a]	109800[a]	10000[a]
pK_a	10.41[a]	14[b]	9.3[c]	2[d]	11.8[e]	4.11[a]
Log K_{ow}	-0.07[a]	2.46[a]	2.25[f]	-1.33[a]	-1[a]	0.07[a]

Table 4. 8: Sampling scheme

Weather (site)	Dry Weather Events		Wet Weather Events			
Date (Event, Month)	2014/05/28 (Ev2, May)	2014/09/14 (Ev6, September)	2014/06/17 (Ev4, June)	2014/04/14 (Ev1,April)	2014/06/12 (Ev3, June)	2014/09/10 (Ev5, September)
2days-cumulative rainfall prior to sample collection (mm)	1	3	14	15	24	32
Flowrate threshold (ML/d)	<300	<180	>250	>350	>250	>180
Sampling onset	4:07 PM	6:22 PM	10:23 PM	3:39 PM	9:26 AM	10:20 PM

Table 4. 9: Summary of analytical methods and their limits of detection (when applicable)

Parameters	Analysis	Method	Detection limit
Parasites	*Cryptosporidium* and *Giardia*	Method 1623.1	1 (oo) cyst/100mL
FIB	*E. coli*	MOE LSB E3371	10 CFU/100 mL
	Clostridium. perfringens	Membrane filtration	1 CFU/100 mL
Micro-pollutants	CAF, CBZ, CBZ-2OH, ACE,SUC, ASP	Online SPE LC MS/MS	31, 12, 30, 546, 12, 75 (ng/L), respectively
Physico-chemical	TSS	Standard-2540D	-
	pH and conductivity	Standard Method	-

Table 4. 10: The average value of *Giardia* and *Cryptosporidium* in WRRFs

Country/Region	Year	*Giardia* (cyst/l)		*Cryptosporidium* (oocyst/l)		Reference
		Influent	Effluent	Influent	Effluent	
Canada/Qc	2001-2002	3 140	56			Payment 2003
Canada/Qc	Not specified	1552	349	26	10	Payment et al. (2001)
Canada, Great Montreal	2009	LOD-9010 [a]	LOD-472[a]	LOD-533 [a]	LOD-89[a]	Lalancette et al. (2012)
Germany	2009-2010	107-383	0-9	2.5-183	0.2-6.3	Gallas-Lindemann et al. (2013)
North Germany	2011			694	962	Ajonina et al. (2012)
Germany	Not specified	211-508	0.22-4.5			Kistemann et al. (2008)
Beijing, China	2005-2007	(8-16) $\times 10^2$	<0.033-2	(1.4-2.4)$\times 10^2$	<0.033-1.5	Fu et al. (2010)
Sweden	Not specified	1300	0.4	5	0.13	Ottoson et al. (2006)
Arizona, USA	2011-2012	(4.8–6.4)$\times 10^3$	33-190	74-100	12-13	Kitajima et al. (2014a)
Urope, Italy	2000	(2.1-41)$\times 10^{3a}$	_			Cacciò et al. (2003)
Spain	2007	89-8305[b]	79-2469[b]	6-350[b]	2-390[b]	Castro-Hermida et al. (2008)

LOD: Limit of detection; a: range of observed data; b: geometric mean

Table 4. 11: The average value of *E. coli* and *C. perfringens* in WRRFs

Country/ Region	Year	*E. coli* (CFU/100ml)		*C. perfringens* (CFU/100ml)		Reference
		Influent	Effluent	Influent	Effluent	
Canada/Qc	2001-2002	(6.7-13.7)×10^{5a}	(1.4× 10^{2}-2.6×10^{5})[a]	(7.1-19.7) × 10^{3a}	(4.3-24.8) ×10^{2a}	Payment (2003)
Canada/Great Montreal	2010	1.6 × 10^{6a}				Madoux-Humery et al. (2013)
Canada	Not specified	1.6×10^{6}	1.4×10^{6}	12782	5897	Payment et al. (2001)
Paris/France	2008-2012	(9.1-14) × 10^{6b}				Lucas et al. (2013)
		(7.5-10.7)× 10^{6b}				
South west of Germany	Not specified	(9-13) × 10^{6}	(4-85)× 10^{3}	(5-33)× 10^{4}	(2-40)× 10^{2}	Kistemann et al. (2008)
Sweden	Not specified					Ottoson et al. (2006)
Arizona, USA	2011-2012		<1-225[a]			Kitajima et al. (2014a)

a: Median values; b: MPN/100ml

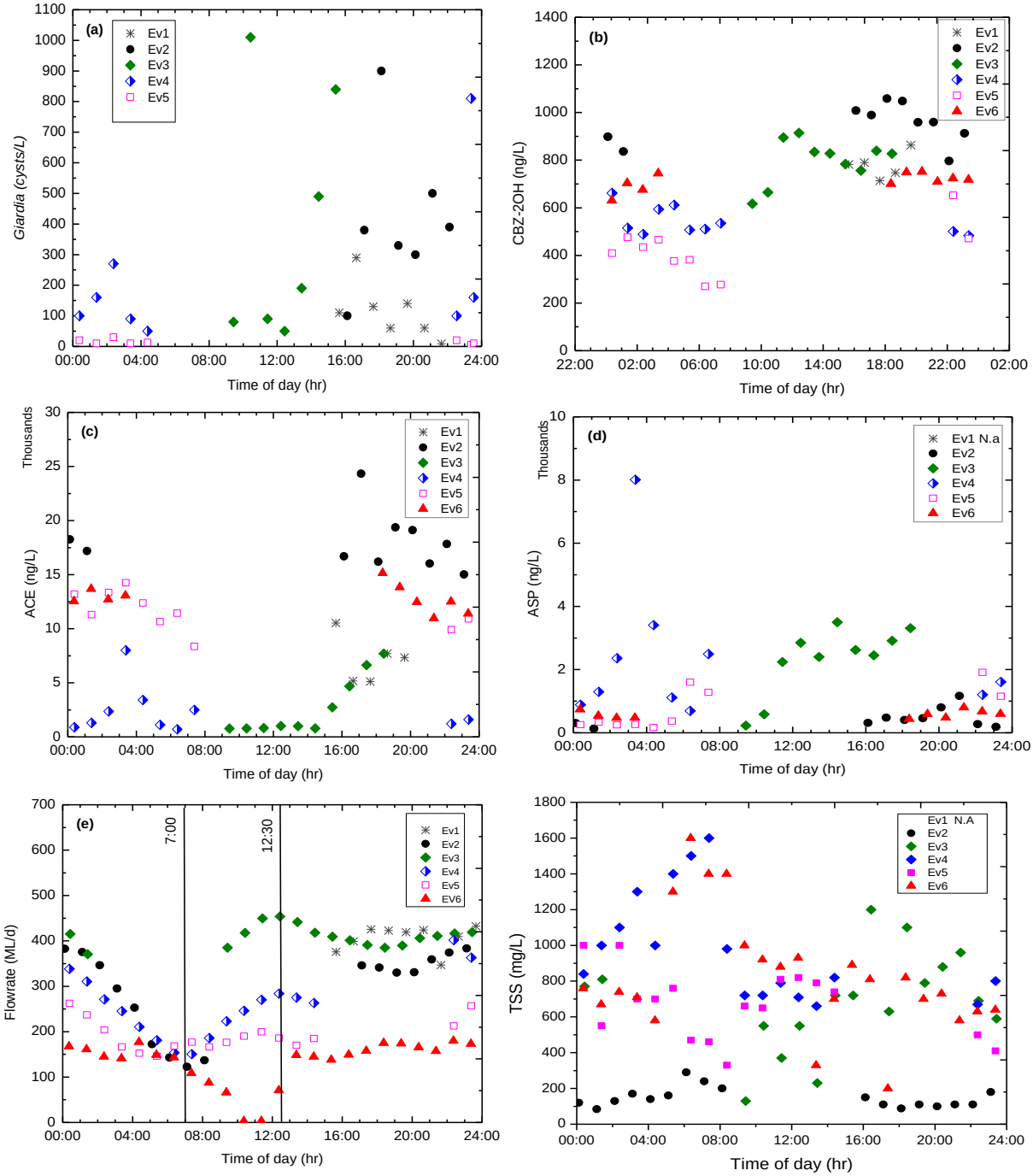

Figure 4. 6: Temporal variability of (a) *Giardia*, (b) CBZ-2OH, (c) ACE, (d) ASP, (e) flowrate and (f) TSS in raw sewage during all events. Ev1 (April), Ev3 (June), Ev4 (June), Ev5 (September) are wet weather events and Ev2 (May) and Ev6 (September) are trace precipitation weather events, respectively. N.A: Not available

CHAPTER 5 ARTICLE 2: WEATHER EFFECTS ON PARASITES, INDICATOR BACTERIA AND WASTEWATER MICROPOLLUTANT LOADS FROM A WATER RESOURCE RECOVERY FACILITY INFLUENT AND EFFLUENT

This chapter presents the manuscript submitted to the *Journal of Water and Health* in January 2019. The manuscript discusses the loadings of parasites, indicator bacteria and wastewater micropollutants from a water resource recovery facility under various weather conditions.

Weather effects on parasite, indicator bacteria and wastewater micropollutant loads from a water resource recovery facility influent and effluent

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5.1 Abstract

The variability of fecal microorganisms and wastewater micropollutants loads in relation to influent flowrates was evaluated for a water resource recovery facility in support of a vulnerability assessment of a drinking water source. Incomplete treatment and by-pass discharges often occur following intense precipitation events that represent conditions that deviate from normal operation. Parasites, fecal indicator bacteria and wastewater micropollutants concentrations and flowrate were measured at the water resource recovery facility influent and effluent during dry and wet weather periods. Influent concentrations were measured to

characterize potential by-pass concentrations that occur during wet weather. Maximum influent *Giardia* and *C. perfringens* loads and maximum effluent *E. coli* and *C. perfringens* loads were observed during wet weather. Influent median loads of *Cryptosporidium* and *Giardia* were 6.8 log oocysts/day and 7.9 log cysts/day per 1000 people. Effluent median loads were 3.9 log oocysts/day and 6.3 log cysts/day per 1000 people. High loads of microbial contaminants can occur during water resource recovery facility by-passes following wet weather and increase with increasing flowrates; thus, short-term infrequent events such as by-passes should be considered in vulnerability assessments of drinking water sources in addition to the increased effluent loads during normal operation following wet weather.

Keywords: Water resource recovery facility, by-pass discharges, *Cryptosporidium*, fecal indicator bacteria, *Escherichia coli*, wastewater micropollutants

5.2 Introduction

Waterborne disease outbreaks are of concern for the health, environment and the economy of a society (Corso et al., 2003; Baldursson et al., 2011). In order to prevent waterborne disease outbreaks, the application of a source-to-tap multi-barrier approach for drinking water supply systems is recommended (WHO, 2011; Health Canada, 2012). Characterizing the variability of source water quality and implementing adequate source water protection strategies are essential for the prevention of waterborne disease outbreaks (Signor et al., 2005).

The variation of source water quality and contaminant loads depends on many factors including land use and meteorological conditions (Charron et al., 2004; Huang et al., 2013; Jalliffier-Verne et al., 2015). Meteorological conditions (i.e. rainfall events or snowmelt) influence the quality of stormwater runoff (Parker et al., 2010), sediment transport (Wu et al., 2009), resuspension of sewer sediments (Passerat et al., 2011) and efficiency rates of water resource recovery facilities (WRRFs, also known as wastewater treatment plants) (Lucas et al., 2013). Heavy rainfall events are regularly associated with peak concentrations of pathogens in surface waters (Atherholt et al., 1998; Kistemann et al., 2002; Signor et al., 2007; Burnet et al., 2014) and they increase the frequency of by-pass discharges and combined or sanitary sewer overflows (CSOs and SSOs). WRRF influent is conveyed from combined sewer systems collecting both stormwater and/or influent from separate sewer systems that collect sewage and additional flows through inflow and

infiltration. Inflow/Infiltration (I/I) into sewer lines during wet weather periods are a common cause of by-pass discharges from WRRFs served only by separate sewer systems. By-pass discharges are a concern for urban areas as they contribute to beach closures and the contamination of drinking water supplies (EPA, 2004).

Several studies have investigated the concentrations, the loadings and removal efficiencies of pathogens, microbial indicators and wastewater micropollutants (WWMPs) in different stages of WRRFs (Kistemann et al., 2008; Fu et al., 2010; Ajonina et al., 2012; Gallas-Lindemann et al., 2013; Burnet et al., 2014; Subedi et al., 2014a) and CSOs (Benotti et al., 2007; Madoux-Humery et al., 2013; Al Aukidy et al., 2017). Concentrations of WWMPs have been shown to be approximately 1 log higher in CSO discharges than in treated effluent discharges (Phillips et al., 2012), although continuously discharged effluents remain the main source of environmental contamination of WWMPs (Madoux-Humery et al., 2015). Studies examining the variability of pathogens, fecal indicators and WWMPs loadings from a WRRF (fed by a separate sewer system) under various operating conditions, particularly, by-pass discharges are rare even though they are needed for source water protection planning and setting management priorities (Signor et al., 2005). Limited attention has been paid to the characterization of pathogen loads from by-pass discharges, possibly because of the difficulties in collecting representative samples during these transient events. Åström et al. (2009) estimated pathogen and fecal indicator loads from the effluent and emergency discharges of local WRRFs as well as combined and sanitary sewer overflow discharges using literature data for microbial concentrations and removals. To our knowledge, similar studies have not been performed on estimating parasites, fecal indicators, WWMPs and total suspended solid (TSS) loads from by-pass discharges. These data are needed to assess microbial loads from potential sources for microbial risk analyses, particularly, wastewater treatment performance data are not relevant when treatment does not occur or is incomplete.

In a WRRF served by a combined sewer system, fecal indicators and WWMP concentrations are influenced by sewer processes such as deposition or resuspension of sewer sediments. Sewer sediments were identified as a reservoir for fecal indicator bacteria (FIB), WWMPs and TSS and the importance of their contribution to the loads from CSO discharges have been evaluated (Hajj-Mohamad et al., 2014; Madoux-Humery et al., 2015). When considering the dilution potential of

inflow/infiltration during wet weather in a WRRF fed by a separate sewer system, the concentrations of microorganisms in untreated wastewater were not strongly mass limited and peak contaminant concentrations were observed during wet weather periods (Tolouei et al., 2019). However, the contribution of sewer processes as a result of inflow/infiltration following heavy rainfall events to contaminant loadings is still unknown. Data on the variability of contaminant loads during wet weather periods are needed to estimate the vulnerability of drinking water treatment plants influenced by the loads from wastewater effluents as de facto or unplanned wastewater reuse is common (Rice et al., 2015) and seldom acknowledged. To the authors' best knowledge, this is the first study to examine the relative influence of inflow/infiltration on parasites, FIB, WWMPs and TSS loads into a WRRF.

In the present study, parasites (*Cryptosporidium* and *Giardia*), FIB (*E. coli* and *C. perfringens*) and WWMPs (CAF, CBZ, CBZ-2OH, ACE, SUC and ASP) were investigated as they are usually present in WRRF discharges (Buerge et al., 2003; Kistemann et al., 2008; Fu et al., 2010; Weyrauch et al., 2010; Ajonina et al., 2012; Gallas-Lindemann et al., 2013; Burnet et al., 2014; Subedi et al., 2014a). The main objective of this study was to investigate the impact of variable weather conditions on contaminant loads from a WRRF serving a separate sewer system. The specific objectives were to: (1) evaluate the most important factors influencing mass loadings from the WRRF influent and effluent; (2) investigate the importance of sewer processes in the mass loadings arriving at a WRRF under various flow conditions; (3) estimate the variability of parasites (*Cryptosporidium* and *Giardia*), FIB (*E. coli* and *C. perfringens*), WWMPs (CAF, CBZ , CBZ-2OH, ACE, SUC and ASP) and TSS mass loadings from a WRRF in the case of failure, by-pass discharges and normal operation conditions under weather conditions ranging from trace to intense precipitation; and (4) assess the excess loads from a by-pass discharge compared to that of final effluent discharges during wet weather conditions. Although the loads estimated are specific to the system under investigation, other similar sewer systems are expected to have similar behaviour with regards to wet versus dry weather conditions. Furthermore, data on loads per capita from wastewater discharges are needed for comparing among WRRF loads and their impacts worldwide.

5.3 Material and methods

5.3.1 The study site

The studied WRRF has a capacity of 518 000 m^3 per day and is fed by a separate sewer system. It receives the sewage from residential (approximately 1 million residents), industrial and commercial facilities in the Greater Toronto Area, Canada (Kambeitz, 2015, personal communication). The WRRF treats raw sewage through primary, secondary treatment (activated sludge processes with phosphorus removal) and chlorine disinfection. Studies from this region (ColeEngineering, 2011) and historical data indicate that inflow/infiltration during wet weather periods are a challenge for local WRRFs. Raw sewage is conveyed to primary clarifiers after preliminary treatment (screening and grit removal) by three channels. Plant 3 treats the raw sewage from Channels 1 and 2 and Plants 1 and 2 from Channel 3 (Figure 5. 6). Primary treated effluent (with or without disinfection) from Plant 3 is discharged into Lake Ontario when the flowrate exceeds the treatment capacity of the plant. From 2007 to 2014, the studied WRRF experienced 11 by-pass events mostly following heavy rainfall (>80% of by-pass events).

5.3.2 Sample collection and analytical methods

WRRF influent (following preliminary screening and grit removal) and effluent were monitored between April 2014 and September 2014. Time-proportional composite samples from the influent (using ISCO 6712FR fiberglass and refrigerated portable auto-samplers in 1 L polypropylene bottles) and grab samples from the effluent (in 10 L collapsible container low density polyethylene bottles) were collected under various weather conditions. Sampling was initiated based on rainfall and flowrate thresholds. The relationship between historical flowrates and rainfall data were determined to establish monthly thresholds that are representative of inflow/infiltration events. Wet weather conditions were defined as 2-day cumulative rainfall prior to sample collection > 10 mm and flowrates above the determined threshold from the historical data analysis for each month of the year. Conditions with only trace amounts of rainfall (< 3 mm) and flowrate below the threshold (also determined through historical data analysis) were defined as “dry” weather conditions. Four wet weather events (one in April, two in June and 1 in September) and two trace precipitation weather events (May and September) were monitored during the sampling campaign. The 2-day cumulative rainfall prior to sample collection ranged

from trace amounts to 32mm. For the 4 wet weather events, return periods were below 2 years. Collected samples were analysed for parasites (*Cryptosporidium* and *Giardia*), fecal indicator bacteria (*E. coli* and *C. perfringens*), WWMPs (CAF, CBZ, CBZ-2OH, ACE, SUC and ASP) and TSS. Detailed information regarding sampling and analytical methods is provided by Tolouei et al. (2019).

5.3.3 Calculations

5.3.3.1 The contribution of sewer processes to mass loadings arriving at a WRRF

In a WRRF served by a separate sewer system with high level of inflow/infiltration, the total mass loadings into the WRRF during wet weather periods ($L_{inf\text{-}WW}$) is equal to the loadings during dry weather period ($L_{inf\text{-}DW}$), plus loadings as a result of sewer processes (L_{SP}) and inflow/infiltration ($L_{I/I}$) (Equation 5.1). Sewer processes include net deposition or net resuspension of particle associated contaminants depending on flow conditions, but also include biological activity such as inactivation, predation, biodegradation, etc. One can assume that parasites, FIB, WWMPs and TSS concentrations are negligible in inflow/infiltration ($L_{I/I} \approx 0$) as compared to sewage, thus loads as a result of sewer process can be calculated using Equation (5.2). In this study, the mass loadings into the WRRF during dry weather were calculated using the median values of the observed concentrations and total flowrate at the WRRF influent in the September dry weather event for two reasons: (1) more data were available in this month (n=24), (2) flowrate data for the September dry weather event (as compared to the May dry weather event) were not affected by other sources of stored water such as infiltration as a result of a higher water table (Figure 5. 7). For each wet weather event, loadings were also calculated from the median concentrations and total flowrates (i.e. from all channels) observed at the WRRF influent. Finally, the contribution of sewer processes was estimated for each wet weather event. This calculation was not conducted for *Cryptosporidium* and *Giardia* due to insufficient data.

$$L_{inf-WW} = L_{inf-DW} + L_{sp} + L_{I/I} \qquad (5.1)$$

$$L_{SP} = L_{inf-WW} - L_{inf-DW} \quad \text{If } L_{I/I} \approx 0 \quad . \qquad (5.2)$$

$$L_{inf-DW} = \sum_{i=1}^{n} C_{inf-DW}(i) \times Q_{inf-DW}(i) \quad (5.3)$$

$$L_{inf-WW} = \sum_{i=1}^{n} C_{inf-WW}(i) \times Q_{inf-WW}(i) \quad (5.4)$$

Where L_{inf-DW} [log-units/day], L_{inf-WW} [log-units/day], L_{SP} [log-units/day] and $L_{I/I}$ [log-units/ day] are the contaminants mass loadings from WRRF influent under dry and wet weather conditions, from sewer processes and from inflow/infiltration, respectively; C_{inf-DW} [log-units/ liter] and C_{inf-WW} [log-units/liter] are the observed concentrations at the WRRF influent under dry and wet weather conditions, respectively; Q_{inf-DW} [liter/day] and Q_{inf-WW} [liter/day] are the total influent flowrates under dry and wet weather conditions, respectively; n is number of data.

5.3.3.2 Removal efficiency rates and concentrations at the primary effluent (with disinfection)

For the sampling events under dry and wet weather conditions, removal efficiencies were calculated by averaging monitored concentrations in the influent and effluent. Concentrations in the primary effluent (with disinfection) following by-pass discharge were calculated (Eq 5.5) based on the log removal values provided in Tables 5. 1 and 5. 4. For the contaminants with poor total removal efficiency rates (≤70%) (i.e. CBZ, CBZ-2OH, ACE and SUC), the primary treatment removal efficiencies (with disinfection) were assumed to be negligible.

$$C_{by-pass} = C_{inf} - R_{pt} + R_{dis} \quad (5.5)$$

C_{inf} [log-units/ liter] and $C_{by-pass}$ [log-units/liter] are the concentrations in the influent and by-pass discharges, R_{pt} and R_{dis} are contaminant log removals by the primary treatment and disinfection processes, respectively. Since the prevalence rate of *Cryptosporidium* in the influent (8.6%) and effluent (30%), as well as ASP in the effluent (0%) were low, calculations were not performed for these contaminants.

Table 5. 1: Estimated removal efficiency rates through primary treatment and disinfection processes*

Parameters	Primary treatment	Disinfection
Giardia	0.3 log	0.4 log
E. coli	0.5 log	1.5 log
C. perfringens	0.6 log	0.5 log
CAF	0.12 log	~ 0
TSS	0.7 log	~ 0

*Based on reported range of removals (refer to Table 5. 4) and observed total removal efficiency rates

5.3.3.3 Mass loadings from influent, primary effluent (with disinfection) and treated effluent

The daily loads of parasites, FIB, WWMPs and TSS from primary effluent (with disinfection) following a by-pass discharge were estimated using the median, 10^{th} and 90^{th} percentiles of historical data (2008-2014) for the by-pass flowrate (Q_{by}) and duration (D_{by}) as well as estimated concentrations in the primary effluent. Although these by-pass flowrates are unique to the WRRF and depend on the plant capacity available, the state of the sewer network and local hydrometeorological factors, the approach can be generalized to other systems. The percentiles of the historical data (d10, d50 and d90) for the by-pass flowrates were 58ML/day, 136ML/day and 240 ML/day for corresponding durations of 3h, 5h and 16.5 h, respectively. Contaminant loadings from primary effluents were estimated considering three scenarios using: a) 10^{th} percentiles of Q_{by} and D_{by}; b) the median of Q_{by} and D_{by}; and c) 90^{th} percentiles of Q_{by} and D_{by}.

Loadings from influent, by-pass and effluent discharges per 1000 people were calculated using equations 5.6 to 5.8 and the per 1000 people basis is to compare with other studies such as (Burnet et al., 2014). Two ratios were calculated to: a) compare by-pass discharge to effluent discharge during wet weather (F_1) and b) compare effluent discharge during dry weather with the sum of wet weather effluents (effluent and by-pass discharges, F_2). The F_1 and F_2 ratios were computed using the median, 10^{th} and 90^{th} percentiles.

$$L_{inf}/1000\ \text{people} = (C_{inf} \times Q_{inf}).\frac{1000}{pop} \quad (5.6)$$

$$L_{by-pass}/1000\ \text{people} = (C_{by-pass} \times Q_{by-pass}).\frac{1000}{pop} \quad (5.7)$$

$$L_{eff}/1000\ \text{people} = (C_{eff} \times Q_{eff}).\frac{1000}{pop} \quad (5.8)$$

$$F_1 = \frac{L_{by-pass-WW}}{L_{eff-WW}} \quad (5.9)$$

$$F_2 = \frac{L_{eff-DW}}{L_{by-pass-WW}+L_{eff-WW}} \quad (5.10)$$

where L_{inf} [log-units/day], $L_{by\text{-}pass}$ [log-units/day] and L_{eff} [log-units/day] are contaminant loads from influent, by-pass discharge and effluent per 1000 people; C_{inf}, $C_{by\text{-}pass}$ and C_{eff} [log-units/liter)] are the concentrations in the influent, by-pass and effluent; Q_{inf}, $Q_{by\text{-}pass}$ and Q_{eff} [liter/day] are the total flowrate in the influent, by-pass discharge and effluent; pop is the population; DW and WW refer to dry and wet weather respectively. For influent and primary effluent analyses, *Cryptosporidium* and *Giardia* concentrations were not adjusted by the recovery rates in the influent as recovery data were not available for each sample, whereas their concentrations were corrected by the recovery rates for the effluent loading analyses.

5.3.3.4 Statistical analysis

Statistical analyses were performed using the STATISTICA software (Version 12). Given that the majority of loads were neither normally, nor log-normally distributed, non-parametric Mann-Whitney U tests were performed to assess differences between loadings under dry and wet weather conditions. The differences and regressions were considered to be significant at alpha = 5%. EPA's ProUCL software (Singh et al., 2013) was used to impute left-censored data (i.e. values below the limit of detection, n=4 below limit of detection for *Giardia* and ASP). The variation of loadings under dry and wet weather periods were demonstrated in boxplots in which boxes present 10^{th} and 90^{th} percentiles and whiskers illustrate minimum and maximum values, median (square in box) and mean (+ in box).

5.4 Results and discussion

5.4.1 Treatment removal efficiency rates

Variable removal efficiencies were observed (Table 5. 2). For all monitored conditions, removal efficiencies for *Giardia* ranged from 72.6% to 99.9% and in most instances (i.e. 80% of the time), it was ≥97%. Removal efficiencies for *E. coli* and *C. perfringens* varied from 99.9% to 99.99% and from 98.2% to 99.7%, respectively. Removal efficiency of pathogens and microbial indicators vary with plant sizes and treatment conditions (Fu et al., 2010). The observed removal efficiencies for *Giardia*, *E. coli* and *C. perfringens* are consistent with published removal rates in the literature, which is unsurprising given the wide ranges of reported removal rates (Ottoson et al., 2006; Kistemann et al., 2008; Fu et al., 2010; Kitajima et al., 2014a).

Treatment processes effectively removed both TSS and CAF (Table 5. 2). This observation is consistent with previous observations showing higher removal of CAF in WRRFs (Miao et al., 2005; Lee et al., 2011; Sim et al., 2011; Gao et al., 2012; Lee et al., 2013). In contrast, CBZ, CBZ-2OH, ACE and SUC were not notably removed in this WRRF and even negative removal efficiencies were observed for these WWMPs (Table 5. 2). In other Canadian and non-Canadian studies, low and even negative removal efficiencies were similarly reported for CBZ, CBZ-2OH, ACE and SUC (Miao et al., 2003; Miao et al., 2005; Scheurer et al., 2009; Hoque et al., 2014; Subedi et al., 2014a). The lower removal efficiency of CBZ from WRRFs was explained by its poor biodegradability (Kasprzyk-Hordern et al., 2009) and the increase of the CBZ concentration in the WRRF effluent was attributed to the hydrolysis of carbamazepine glucuronide conjugate and cleavage of the free parent compound (Radjenović et al., 2007). The test of biodegradability of ACE and SUC in activated sludge of a typical WRRF under laboratory conditions confirmed their persistence as no degradation was observed within 7h of incubation at 15 °C (Buerge et al., 2009). In a fate study, SUC was identified as a compound resistant to microbial degradation, soil sorption, hydrolysis, chlorination, ozonation and UV-photolysis (Soh et al., 2011). ACE and SUC have been suggested as ideal indicators of wastewater contamination in groundwater and surface waters because of their chemical properties (Buerge et al., 2009; Oppenheimer et al., 2011).

Table 5. 2: Total removal efficiencies of parasites, indicator bacteria, total suspended solids and wastewater micropollutants

Parameters	Total removal efficiency rate	Treatment Rank
E. coli	99.9-99.99	High (>99%)
CAF	99.1-99.9	
Giardia	72.6-99.9*	Moderate (>90%)
C. perfringens	98.2-99.7	
TSS	94.3-99.5	
CBZ	-48.5-2.52	Poor (≤70%)
CBZ-2OH	-34.5-39.5	
ACE	-5.5-70	
SUC	-59.1-51.7	

* *Giardia* removal efficiencies were ≥ 97% 80% of the time

5.4.2 Flowrate influence on concentrations

The influence of flowrate on FIB, WWMP and TSS concentrations in the influent and effluent of the studied WRRF (served by separate sewer system) was characterized by log concentration-log flowrate (log C – log Q) plots for all data (wet and dry weather) (Figure 5. 1). This type of analysis was also used for WWMPs, hormones and indicator bacteria in raw sewage and treated effluent of WRRFs served by combined sewer systems as well as in CSOs (Phillips et al. 2012, Madoux-Humery et al. 2015). The slope in log C – log Q plots indicates the importance of dilution on concentrations, with slopes greater than -0.7 showing that concentrations decrease at a slower rate than the increase in flowrates. Influent flowrate data are from Channel 2.

In the influent, *Giardia*, *E. coli* and *C. perfringens* concentrations increased significantly ($p<0.05$) with flowrate (Figure 5. 1). The observed patterns suggest that dilution processes did not affect the loadings of the fecal microorganisms. Higher concentrations with higher flowrates can be explained by several confounded processes: 1) shorter travel times in the sewer network lead to decreased microbial inactivation, 2) higher flowrates occur at times of day that correspond to human defecation patterns and 3) less sedimentation occurs in the sewer network and higher flowrates may also lead to sewer sediment resuspension.

For WWMPs, the slopes of CAF, CBZ, SUC and CBZ-2OH in log C – Log Q plots were in the range of 0.4-0.44 in the influent (Figures 5. 1 and 5. 8). ACE, however, displayed a slope of -1.1, indicating that it was strongly influenced by dilution (including from inflow and/or infiltration). Among the studied artificial sweeteners (ACE, SUC, ASP), dilution processes only affected the loadings of ACE. The observed behaviour may, in part, be explained by the higher solubility of ACE as compared to the other artificial sweeteners studied (SRC, 2015). A clear and significant trend was not observed for ASP (Figure 5. 8).

For TSS, the slope (-0.6) in log C - Log Q plot remained above -0.7 in the influent, reflecting the contribution of the non-wastewater sources to its loads and reducing the effect of dilution (Phillips et al., 2012; Madoux-Humery et al., 2015). TSS in sewer lines may originate from wastewater, sewer deposit resuspension and to a lesser extent, inflow. Solids are deposited in the sewer system during low flows and are mobilized by high flows, which also enhance the transport of particulate compounds (Phillips et al., 2012).

In contrast to increasing influent concentrations with flowrate in this study of a separate sewer system, a decrease of hormones, WWMPs and FIB concentrations with increasing flowrates was reported in combined sewer systems (Phillips et al., 2012; Madoux-Humery et al., 2015). Although inflow/infiltration cause the flowrate to increase in the influent of a WRRF served by separate sewer systems, combined sewer systems have more potential for dilution. Thus, the dilution of raw sewage could be a more important factor in controlling contaminant concentrations and loads in WRRF influents fed by combined sewer systems and concentrations and loads from separate sewer systems are more strongly influenced by human defecation patterns.

In the effluent, the concentration of *Giardia* cysts was inversely related to flowrate (Figure 5. 1). *Giardia* cysts are environmentally resistant to degradation; *Giardia* die-off rates in water and sediment are reported to be 0.029 $\log_{10}\text{day}^{-1}$ and 0.37 $\log_{10}\text{day}^{-1}$, respectively (Karim et al., 2004). Thus, it seems that the loading of *Giardia* was principally influenced by dilution in the WRRF effluent or that treatment efficiency of *Giardia* was not greatly influenced by higher flowrates. In contrast, the concentrations of *E. coli* and *C. perfringens* in effluents increased with flowrate, suggesting that reduced treatment efficiency was more important than dilution. This trend was previously reported for both *E. coli* and *Enterococci* in Parisian WRRFs that showed

decreased treatment efficiency as a result of the decrease of hydraulic retention times during wet weather periods (Lucas et al., 2013).

For WWMPs, the slope of the log C – log Q plots for CAF, CBZ, CBZ-2OH, ACE and SUC ranged from -2.3 to -0.03 in the effluent (Figures 5. 1, 5. 8), but the regression was only significant for ACE. The half-life for CAF was shown to range between 0.8 and 5 h in wastewater (with biological processes), biodegradation being the most important process for the elimination of CAF through treatment facilities (Buerge et al., 2003; Pérez et al., 2005). Hence, biodegradation appears to be a dominant factor influencing CAF loads in the effluent. CBZ is less biodegradable with little removal observed in treatment facilities due to their refractory behaviour (Miao et al., 2003; Tran et al., 2018). ACE and SUC with lower biodegradability ($k_{biol} < 0.06$) and lower log K_{ow} are also more persistent in WRRFs (Lange et al., 2012; Subedi et al., 2014a; Tran et al., 2018). Dilution appears to influence the loadings of ACE in the effluent due to its higher solubility (587500 mg/L at 25 $^{\circ}$C). A study by Madoux-Humery et al. (2015) also demonstrated that, in contrast to *E. coli*, concentrations of caffeine, carbamazepine, theophylline and acetaminophen were inversely correlated to flowrate in the effluent of a WRRF served by combined sewer system. It should be noted that in our study, effluent log C – Log Q plots are from less data points ($n \leq 6$) and hence further data are needed to analysis the relationship between flowrate and concentrations thoroughly.

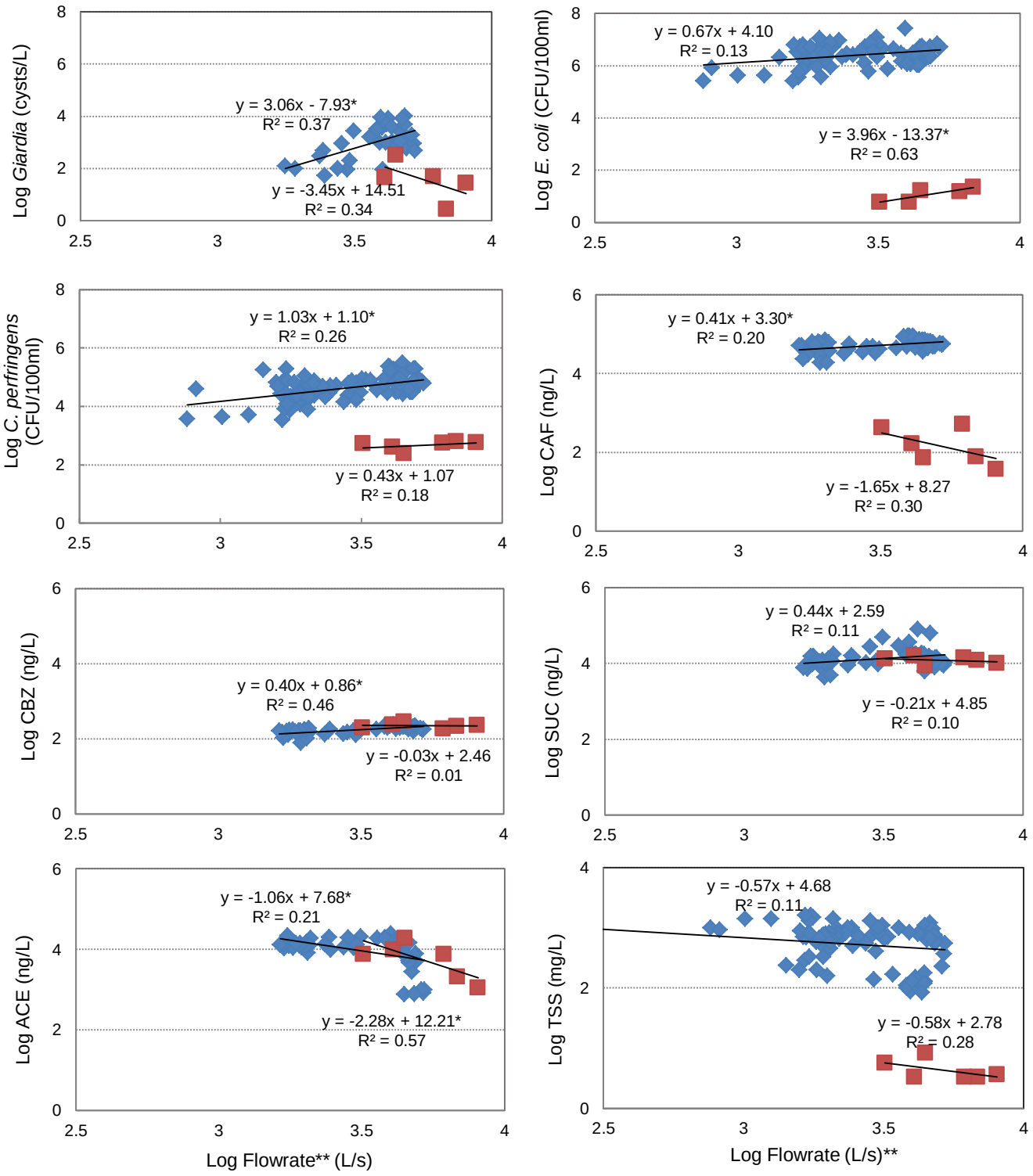

Figure 5. 1: Concentrations in the influent (diamonds) and in the effluent (squares); *: The regressions were significant at $p<0.05$;**: indicates flowrate in Channel 2

5.4.3 Source contribution for the contaminant loads into a WRRF

The relative contribution of sewer processes varied among contaminants and events (Figure 5. 2). Depending on flowrates, sewer process contribution to *E. coli*, *C. perfringens* and TSS loads from the influent varied from 10% to 49%, from 21% to 83% and from 15% to 24%, respectively. A recent study by Madoux-Humery et al. (2015) demonstrated that sediment resuspension contributed to FIB and TSS loads measured in CSOs. The contribution of sewer deposit resuspension ranged from 10% to 70% for *E. coli* loads, 40% to 80% for *Enterococci* loads and from 26% to 82% total suspended solids loads from CSO discharges (Chebbo et al., 2001; Gasperi et al., 2010; Passerat et al., 2011). The contribution of sewer deposit resuspension depends on pollutant type, sewershed type and configuration, rain intensity and antecedent dry weather period. It has been demonstrated that the contribution of sewer deposit resuspension to the TSS load from CSOs varied significantly among rain events (10%-70% for low-intensity events) and were higher for the high intensity events (>60%) (Gasperi et al., 2010). In this study of a sewershed with separate storm and sanitary sewers, the effects of sewer sediment resuspension were less pronounced than in combined sewers, likely as a result of the lower variability of wet weather flows and a closer association between higher flowrates and human excretion patterns. Increased loads with higher flow in this study are also the result of less deposition during higher flows and shorter travel rates leading to less biodegradation within the sewer network.

In the case of WWMPs, the contribution of sewer processes was observed for CBZ, SUC and ASP (Figure 5. 2). The contribution of sewer processes to the loads of CBZ under wet weather condition was higher than that of CAF. This can be explained, at least in part, by lower biodegradability of CBZ in comparison to readily biodegradable CAF (Tran et al., 2018). Sewer sediments are known to act as a reservoir for CBZ in combined sewer systems (Madoux-Humery et al., 2015). Hajj-Mohamad et al. (2017) further showed that the sorption coefficient (log k_{dapp}) of native suspended and settled sediments from a combined sewer system were higher for CAF (0.3±0.2 $L.Kg^{-1}$ and 0.0±0.1 $L.Kg^{-1}$, respectively) than for CBZ (0.1±0.1 LKg^{-1} and -0.1±0.1 LKg^{-1}, respectively), while desorption constants of CAF were lower than those of CBZ. Among the studied artificial sweeteners, the contribution of sewer processes to the loadings of ACE was limited (negative bars in Figure 5. 2). This can be relatively explained by higher dilution of ACE as a result of its higher solubility following higher flow conditions. Compared to ACE and SUC,

the contribution of sewer processes was higher for ASP, as the latter has relatively lower water solubility (10,000 mg/L) and higher log K_{ow} (0.07). Subedi et al. (2014a) detected ASP in 92% of influent suspended particles. In their study, the fraction of total ASP sorbed to suspended particulate matter was 50.4% and was higher than that of ACE and SUC. Their sorption coefficient was based on the concentrations measured in influent (ng/L) and suspended particulate matter (ng/kg.dw) and reported as 289, 5.1 and 4540 L/kg for ACE, SUC and ASP respectively.

The flowrate of the WRRF depends on human activities and inflow/infiltration (as a result of precipitation, snowmelt and groundwater table depths). It usually increases during the day before decreasing at night (Brière, 2012) and during the spring (following the snow melt period, Figure 5. 7). Interestingly, for the majority of the microbial indicators and WWMPs studied, higher contributions of sewer processes were estimated for the Ev1 and Ev3 (events that occurred in spring and daytime respectively). This could be partly related to higher fecal loads associated with the higher flows (from human defecation patterns) in addition to more resuspension of sewer sediments, less degradation and less deposition with higher flows.

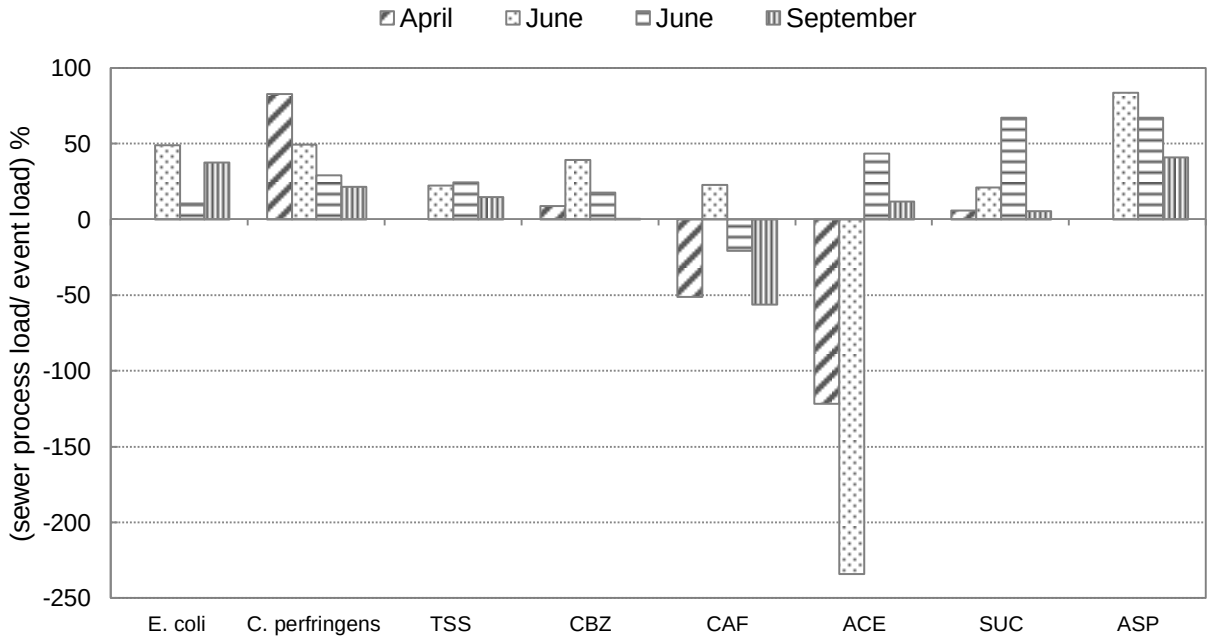

Figure 5. 2: Relative contribution of sewer process load to loads of indicator bacteria, wastewater micropollutants and total suspended solids during four wet weather events. E. coli, TSS and ASP data were not available for the April wet weather event

5.4.4 Mass loadings variability

Mass loadings from a WRRF depend on the population size, water usage and flowrate patterns, type of treatment and weather conditions. Here, we determined mass loadings during normal operation conditions of the WRRF as well as in the case of failure and by-pass discharge during various weather conditions. Daily loads of pathogenic parasites, FIB, WWMPs and TSS from the influent and effluent as well as estimated daily loads from the primary effluent following scenarios of by-pass discharge duration and flowrate (by-a (10^{th} percentile by-pass), by-b (median by-pass), by-c (90^{th} percentile by-pass)) are illustrated in Figures 5. 3 and 5. 4. The impact of WRRFs on the receiving waters could possibly be from the treated effluent and by-pass discharges during wet weather conditions; thus contaminant loads from the by-passes were quantified to estimate the extra imposed load from the WRRFs into receiving waters during wet weather periods compared to normal operation condition.

5.4.4.1 Influent mass loadings under dry and wet weather conditions (representing incomplete treatment)

All meteorological conditions considered, influent median loads per 1000 people were 6.8 log oocysts/day, 7.9 log cysts/day, 13.2 log CFU *E. coli* /day and 11.4 log CFU *C. perfringens* /day. The median load of *Giardia* was significantly higher during the dry weather events monitored and *E. coli* during wet weather events ($p<0.05$ in Mann-Whitney U test test). However, the maximum loads of *Giardia* and *C. perfringens* (8.9 log cysts/day/1000 people and 12.5 log CFU/day/1000 people) were observed during wet weather period (Figure 5. 3).

Overall, the median loads of WWMPs per 1000 people were 4.6 log mg CAF/day, 2.1 log mg CBZ/day, 2.6 log mg CBZ-2OH/day, 3.9 log mg ACE/day, 3.9 log mg SUC/day and 2.6 log mg ASP/day respectively. For the studied WWMPs, the maximum loads were generally observed during wet weather periods, CAF and ACE being the exceptions (Figure 5. 4). The median load of TSS from the WRRF influent was 5.5 log g /day and 5.7 log g /day during dry and wet weather conditions respectively (Figure 5. 3). TSS median loadings from the influent was significantly lower for dry weather events ($p<0.05$ in Mann-Whitney U test test). It should be noted that return periods of monitored events were 2 years or lower, meaning that larger precipitation events with higher return periods could lead to higher loads during by-pass events.

5.4.4.2 Effluent mass loadings under dry and wet weather conditions (representing normal operating conditions)

Overall, the median loads of *Cryptosporidium* and *Giardia* into Lake Ontario per 1000 people were 3.9 log oocysts/day and 6.3 log cysts/day and indicator bacteria were 7.8 log CFU *E. coli*/day and 9.3 log CFU *C. perfringen*s/day. These are similar to the mean loads of *Cryptosporidium, Giardia* and *E. coli* reported from effluent discharges of a WRRF in Luxembourg (4.3 log oocysts/day/1000 people, 6.2 log cysts/day/1000 people and 8.7 log MPN/day/1000 people, respectively) (Burnet et al., 2014). The median *C. perfringens* loads from effluent were significantly lower during dry weather events as compared to wet weather loads ($p<0.05$ in Mann-Whitney U test) (Figure 5. 3). The difference between *Giardia* and *E. coli* median mass loadings from the effluent under dry and wet weather conditions were insignificant ($p>0.05$ in Mann-Whitney U test). While the low number of data (n=2) for *Giardia* in effluent samples precludes further conclusions, maximum mass loadings were observed during wet weather conditions for *C. perfringen*s and *E. coli*.

For WWMPs, the median loads from effluent discharged into Lake Ontario per 1000 people were 1.6 log mg CAF/day, 2.0 log mg CBZ/day, 2.4 log mg CBZ-2OH/day, 3.4 log mg ACE/day and 3.8 log mg SUC/day. As was observed for FIB, the maximum loads of all studied WWMPs into Lake Ontario occurred during wet weather events, ACE being the exception (Figure 5. 4). The median mass loading of TSS from effluent discharge was 3.3 log g TSS/day/1000 people. Mass loadings of CBZ and CBZ-2OH from effluent discharge of the studied WRRF were comparable to values (2.3 log mg CBZ/day and 1.7 log mg CBZ-2OH/day per 1000 people respectively) reported in another Canadian study (Miao et al., 2005). Total mass loadings of ACE and SUC from effluent discharge and sewage sludge of a WRRF in USA was reported as 3.04-3.13 log mg ACE/day/1000 people and 4.23-4.26 log mg SUC/day/1000 people respectively (Subedi et al., 2014a).

Results demonstrated that loads vary according to the meteorological conditions and hence routine monitoring which is based on regular sampling dates does not adequately describe event-based contaminant discharges important for quantifying the risks at drinking water intakes.

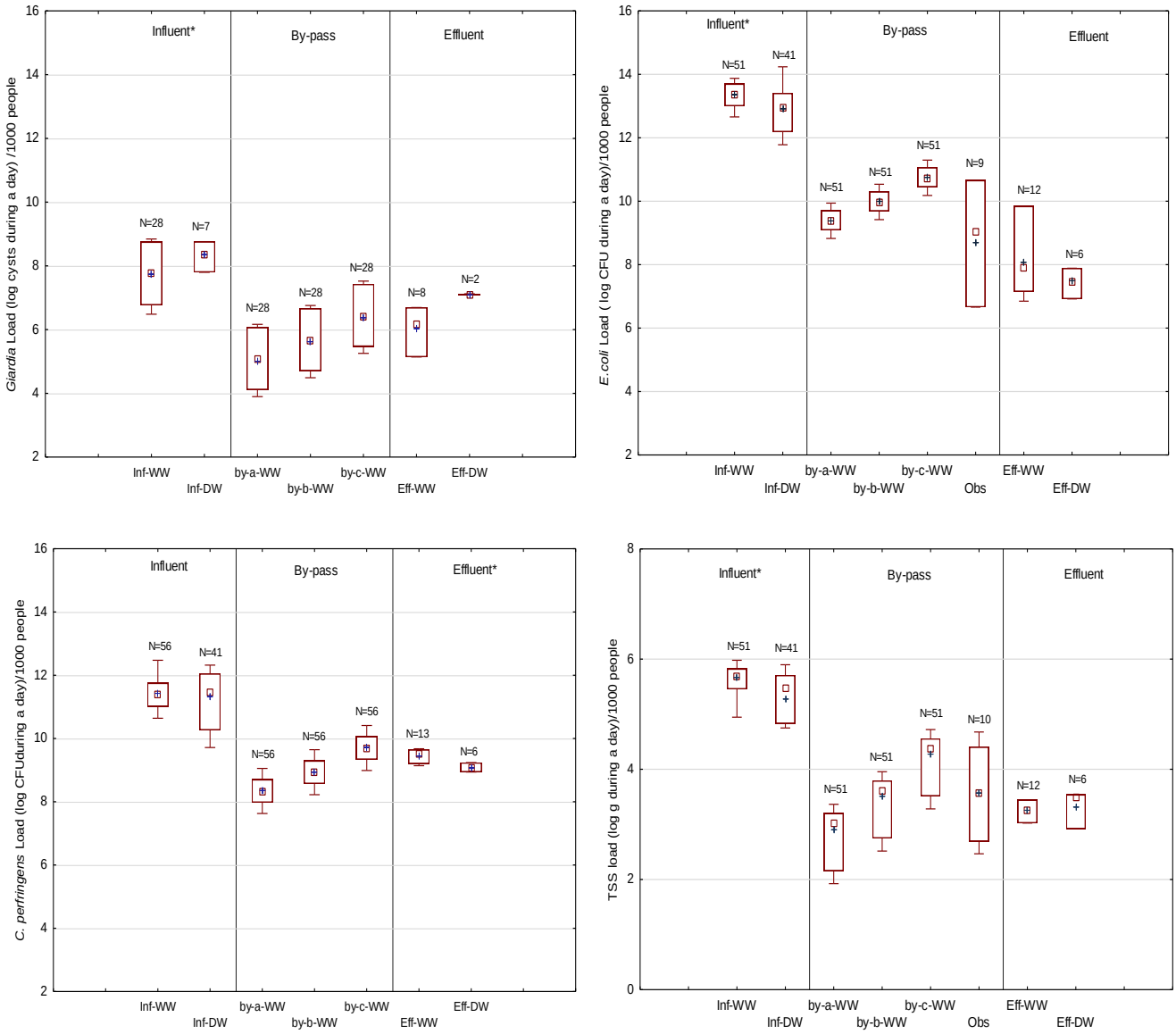

Figure 5. 3: Observed *Giardia*, *E. coli*, *C. perfringens* and TSS mass loadings from the influent and effluent and estimated mass loadings from by-pass discharges. Boxplots represent the 10th and 90th percentiles, median values (•), mean (+) and whiskers (minimum and maximum values). by-a-ww: 10^{th} percentile by-passes (Q_{by}=58 ML/day and D_{by}=3h); by-b-ww: 50^{th} percentile by-passes (Q_{by}=136ML/day and D_{by}=5h); by-c-ww: 90^{th} percentile by-passes (Q_{by}=240 ML/day and D_{by}=16.5 h) respectively. Obs: are the observed by-passes through historical data *: indicates a significant difference ($p<0.05$) between wet and dry weather conditions.

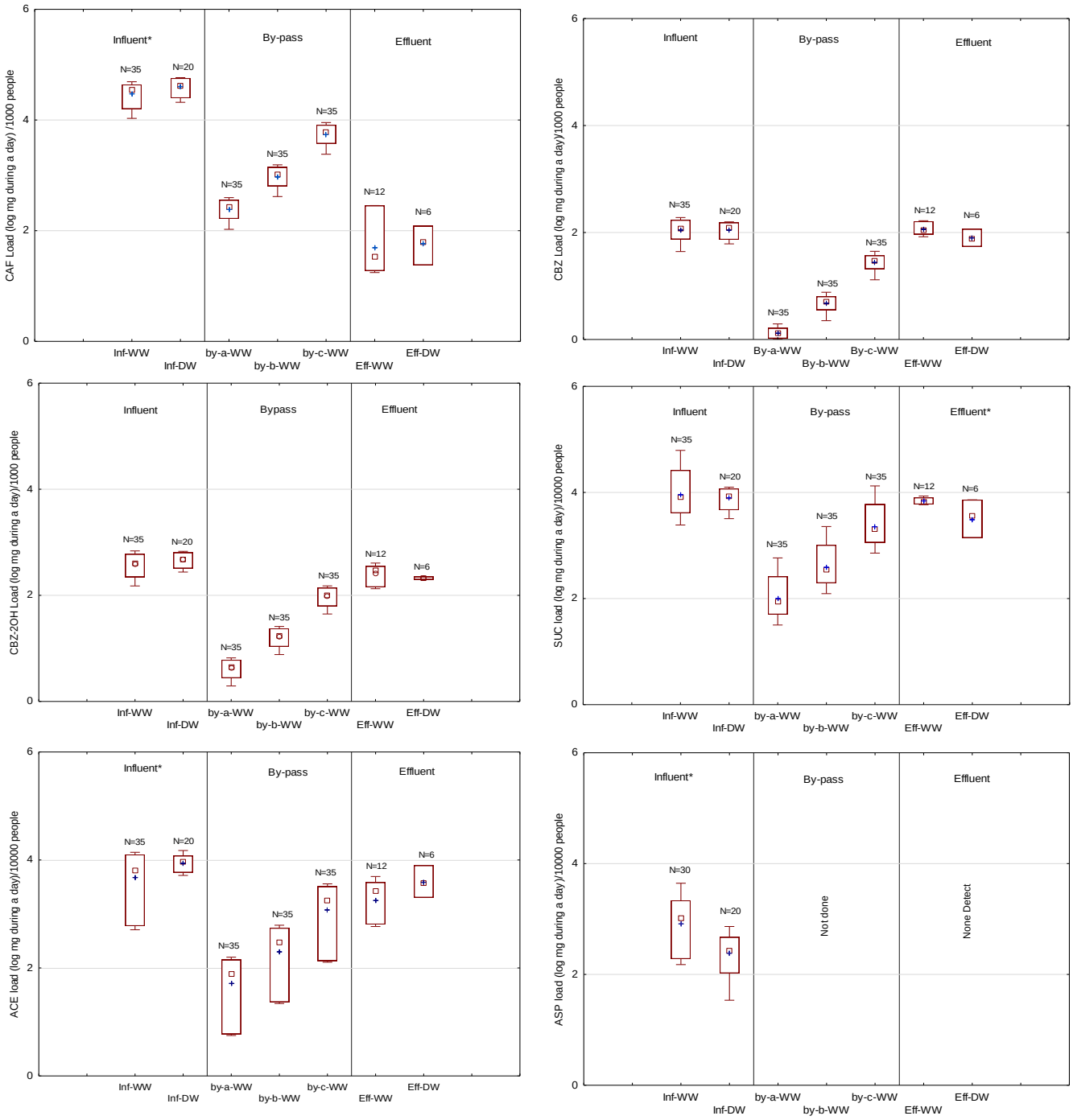

Figure 5. 4: Observed WWMPs mass loadings from the influent and effluent and estimated mass loadings from by-pass discharges. Boxplots represent the 10th and 90th percentiles, median values (•), mean (+) and whiskers (minimum and maximum values). by-a-ww: 10^{th} percentile by-passes (Q_{by}=58 ML/day and D_{by}=3h); by-b-ww: 50^{th} percentile by-passes (Q_{by}=136ML/day and D_{by}=5h); by-c-ww: 90^{th} percentile bypasses (Q_{by}=240 ML/day and D_{by}=16.5 h) respectively. Obs: are the observed by-passes through historical data *: indicates a significant difference ($p<0.05$) between wet and dry weather conditions.

5.4.4.3 Primary effluent mass loadings under wet weather conditions (representing by-pass discharges)

Parasite, FIB, WWMPs and TSS loads from primary effluent during a by-pass discharge were estimated using assumptions adopted for the by-pass flowrate and duration (by-a (10^{th} percentile by-pass), by-b (median by-pass) and by-c (90^{th} percentile by-pass)) and are illustrated in Figures 5. 3 and 5. 4. The estimated ranges of *Giardia*, *E. coli*, *C. perfringens* and TSS daily loads from by-pass discharges per 1000 people were 3.9-7.5 log cysts/day, 8.8-11.3 log CFU *E.coli*/day, 7.6-10.4 log CFU *C. perfringens*/day and 1.9-4.7 log g TSS/day, respectively. The estimated values for *E. coli* and TSS are in agreement with the observed daily by-pass loads (using historical data from 2007-2015) which are in the range of 6.7 - 10.7 log CFU *E. coli*/1000 people and 2.5 - 4.7 log g TSS/1000 people, respectively (Figure 5. 3). Historical data were not available for other contaminants.

The fractions of mass loadings from a primary effluent following a by-pass discharge to effluent discharges during wet weather condition (F_1) were calculated and illustrated (Table 5. 3). The relative loadings from by-pass discharges were higher for the microbial contaminants as compared to those of WWMPs that are generally less efficiently removed, an observation that confirms the findings of others for steroid hormones and six WWMPs including caffeine (Phillips et al., 2012). Aukidy and Verlicchi (2017) showed that CSOs contributed to >90% of *E. coli* and >77% of *Enterococci* monthly loads in receiving waters, despite the fact that flow rates were much lower (9% in June, 17% in July, 2% in August and 5% in September) in CSO discharges than in WRRF effluents (secondary effluent + by-pass). The fractions of effluent loads during dry weather periods to effluent loads during wet weather periods (when a by-pass discharge occurs) were also evaluated (Figure 5. 5). For the studied contaminants (except ACE), the values of F_2 were generally < 1, suggesting their higher loads into Lake Ontario during wet weather periods than during dry weather period. For ACE, the amount of $F_2 \geq 1$ can be explained by its relatively higher solubility and poor removal through wastewater treatment.

At the studied WRRF, a maximum of two by-pass discharges occur yearly and they can last for up to 16.5 h, which suggests that the contribution of by-pass loads to total annual loads is insignificant. However, it should be taken into account that maximum mass loadings into Lake Ontario were observed during wet weather periods. Higher amounts of parasites and FIB loads

have been observed in drinking water reservoirs, at drinking water intakes and in the influent of a drinking water treatment plant during wet weather periods (Kistemann et al., 2002; Burnet et al., 2014; Madoux-Humery et al., 2016). In Lake Ontario, pathogens have been studied at drinking water intakes (Edge et al., 2013) and there is a need to determine the relative importance of their sources for source water protection planning. By-passes could represent critical events for drinking water treatment plants and communication of by-pass events to drinking water treatment plant operators must be ensured.

The methodology applied in this study can be used to estimate the impacts of WRRFs on drinking water sources. Drinking water treatment is more concerned with peak contamination events outside the range of normal operating conditions than average or annual loads from wastewater effluents. This study further provides data for hydrodynamic modelling of the fate and transport of pathogens for quantitative microbial risk assessment of drinking water treatment plants.

Table 5. 3: Relative median (lower limit and upper limit) loads from a by-pass discharge to effluent discharge under wet weather conditions

Parameters	Median (lower limit-upper limit)	Treatment rank
E. coli	87.4 (0.7-1989.4)	High (>99%)
CAF	28.6 (1.3-198.3)	
Giardia	0.4 (0.1-5.4)	Moderate (90%)
C. perfringens	0.3 (0.1-2.6)	
TSS	2.2 (0.1-13.4)	
CBZ	0 (0-0.3)	Poor (≤70%)
CBZ-2OH	0.1 (0-0.4)	
ACE	0.1 (0-0.8)	
SUC	0.1 (0-0.7)	

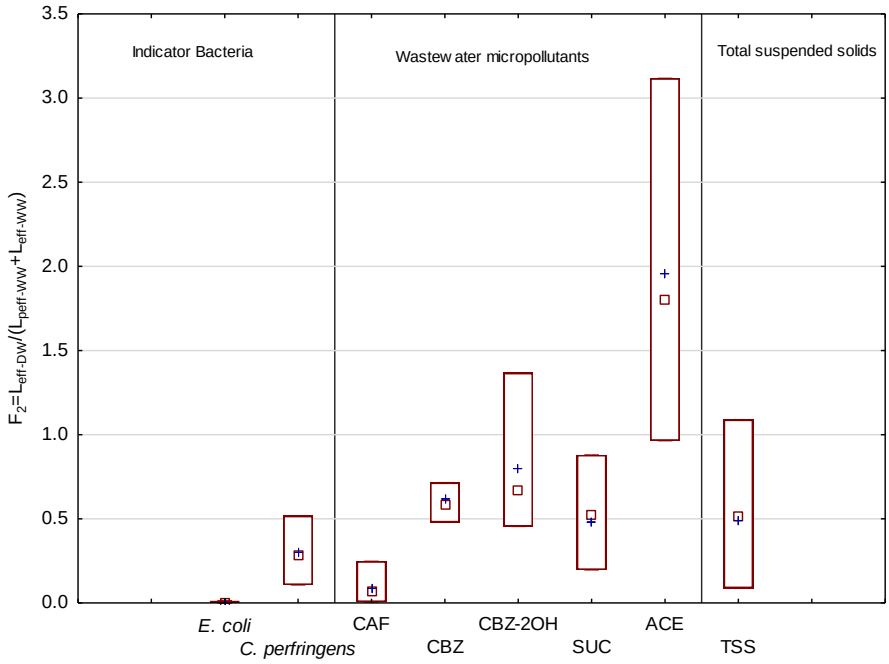

Figure 5. 5: The ratio of dry weather mass loadings to wet weather mass loadings when a by-pass occurs (F2). Boxplots demonstrate 10th and 90th percentiles, median values (•), mean (+) and whiskers (minimum and maximum values).

5.5 Conclusions

The present study provided the following key findings:

- In the influent, dilution as a result of inflow/infiltration during wet weather did not lower the loads of studied contaminants, except for ACE. In the effluent, the loads of both *E. coli* and *C. perfringens* were controlled primarily by treatment efficiency, *Giardia* and ACE by dilution processes.
- Sewer processes (deposition/resuspension and inactivation/biodegradation) are important for estimating contaminant loads under wet weather conditions. Considering all wet weather events, the increased loads as a result of sewer processes was in the range of 10%-49% and 21%-83% for *E. coli* and *C. perfringens* respectively. Among studied artificial sweeteners, the importance of sewer processes was more pronounced for ASP loads due to its lower solubility and potential for higher sorption to suspended particulate material in the sewer lines.
- Among the studied contaminants, overall removal efficiencies through wastewater treatment were generally higher for *E. coli* and CAF (>99%), moderate for *Giardia*, *C.*

perfringens and TSS (>90%) and poor for CBZ, CBZ-2OH, ACE and SUC (≤70%). The fractions of loads from primary effluent during a by-pass discharge to the final effluent were higher for microbial contaminants as compared to those of WWMPs with poor total removal efficiency rates. The relative importance of loads from a by-pass discharge depends on the removal efficiencies of contaminants through wastewater treatment. By-pass discharges are therefore more important contributors to daily loads of microbial contaminants that generally have high removal efficiencies through secondary wastewater treatment.

- For the studied FIB and WWMPs, the fractions of load during dry weather periods to load during wet weather periods (with a by-pass discharge) were generally < 1 (ACE being the exception), indicating their higher loads into Lake Ontario during wet weather periods.
- Emphasis should be placed on characterizing wet weather event discharges upstream of drinking water treatment plants as peak loads were observed during those periods.

5.6 Supplementary Information

Table 5. 4 : Literature data for the removal efficiency rates through primary treatment and disinfection processes

Parameters	Primary treatment Removal	Disinfection (Chlorine) Removal	Country	Source
Protozoa	-	0%-96.8%	-	(Jiménez et al., 2010)
Bacteria	-	99%	-	
Giardia	50.2%-65.2%	53.2%-70.8%	Italy	(Cacciò et al., 2003)
	33.9%	-	China	(Fu et al., 2010)
	24%-47%	-	Scotland	(Robertson et al., 2000)
	0-53% AND 63%-90%	-	USA	(Casson et al., 1990)
E. coli	12%	-	Montreal, Canada	(Payment et al., 2001)
	32 and 50%	-	-	(Ahammed, 2014)
C. perfringens	51%	-	Montreal, Canada	(Payment et al., 2001)
CAF	25%-30%	-	China	(Zhou et al., 2010)
	10%-15%	-	Korea	(Behera et al., 2011)

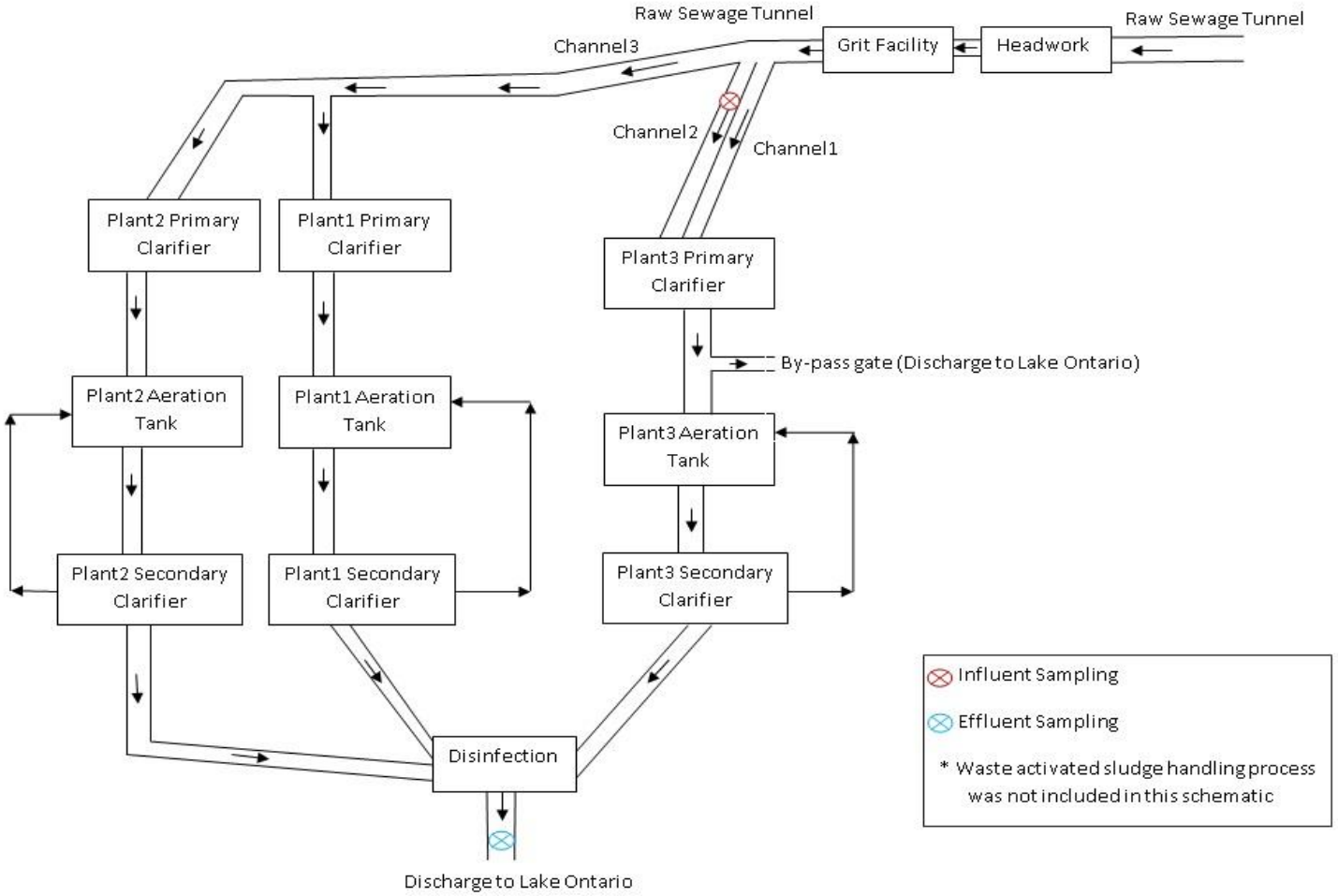

Figure 5. 6 : Schematic of treatment processes in studied water resource recovery facility

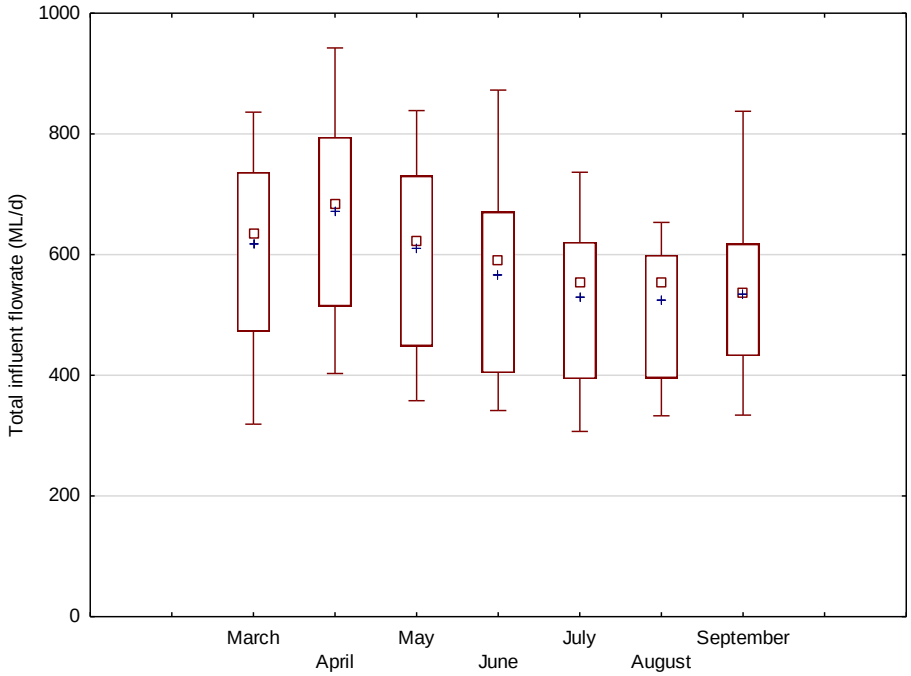

Figure 5. 7: Total influent flowrate in various months in 2014. Boxplots represent the 10th and 90th percentiles, median values (•), mean (+) and whiskers (minimum and maximum values)

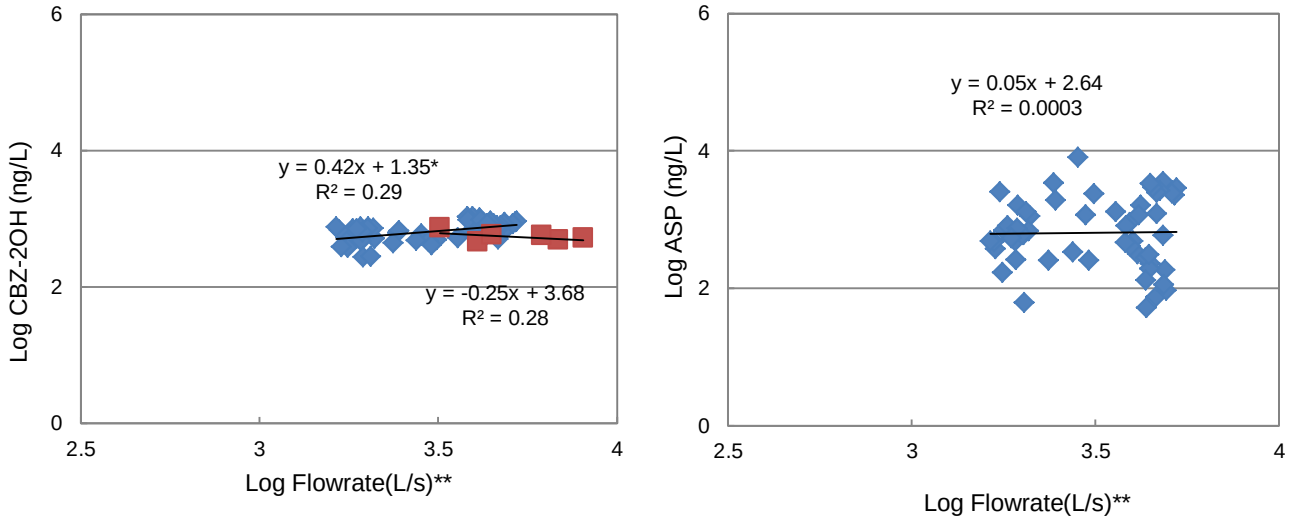

Figure 5. 8: Concentrations in the influent (diamonds) and in the effluent (squares); *: The regressions were significant at $p<0.05$;**: indicates flowrate in Channel 2; ASP data were not available in effluent

CHAPTER 6 ARTICLE 3 : ASSESSING MICROBIAL RISK THROUGH EVENT-BASED PATHOGEN LOADING AND HYDRODYNAMIC MODELLING

This chapter presents the manuscript submitted to the journal *Science of the Total Environment* in February 2019. The manuscript discusses the combined application of a discharge based pathogen hydrodynamic model with quantitative microbial risk assessment.

Assessing microbial risk through event-based pathogen loading and hydrodynamic modelling

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6.1 Abstract

The aim of this study was to assess the variability of microbial risk associated with drinking water under various contaminant loading conditions in a drinking water source. For this purpose, a probabilistic-deterministic approach was applied to estimate the loadings of *Cryptosporidium*, *Giardia and Escherichia coli* (*E. coli*) from fecal contamination sources during both dry and wet weather conditions. The relative importance of loads originating from various fecal contamination sources was also determined by a probabilistic approach, demonstrating that water resource recovery facilities were the dominant source of *Giardia*, yet rivers were more important with

regards to *Cryptosporidium*. Estimated loadings were used as input to a three-dimensional hydrodynamic model of Lake Ontario; the fate and transport of microorganisms were simulated at the influent of a drinking water intake. Discharge-based hydrodynamic modelling results were compared to observed concentrations. Simulated probability distributions of concentrations at the intake were used as an input to a Quantitative Microbial Risk Assessment (QMRA) model such that the variability of microbial risk in the context of drinking water could be examined. Depending on wind and currents, higher levels of fecal contamination reached the intake during wet weather loading scenarios. Probability distribution functions of *Cryptosporidium, Giardia* and *E. coli* concentrations at the intake were significantly higher during wet weather conditions when compared to dry conditions ($p<0.05$). For all contaminants studied, the QMRA model showed a higher risk during wet weather (0.5 log to 1 log) compared to dry weather conditions. When considering sewage by-pass scenarios, risks remained below 2.7×10^{-7} person^{-1} day^{-1} for *Giardia* and *E. coli* O157:H7. Few data were available for *Cryptosporidium* in by-pass effluents and the risk is unknown; hence it is critical to obtain reliable loading data for the riskiest scenarios, such as those associated with water resource recovery facility by-passes under wet weather.

Key words: *E. coli*, source water protection, *Cryptosporidium*, hydrodynamic modelling, quantitative microbial risk assessment, discharge-based QMRA

6.2 Introduction

The Laurentian Great Lakes, serve as important sources of drinking water in Canada and the USA, which provide about 10 million Canadians with drinking water (Environment Canada, 2016). Water for more than 100 communities around the lakes is sourced through offshore intakes located on the lake bottom within ~2km of the shoreline (Edge et al., 2013). Low vulnerability is generally assumed for the drinking water intakes (DWI) of the Great Lakes region as a result of their great depth, long distance from shore and large natural dilution potential. Yet, an important drinking water outbreak occurred in Milwaukee, Wisconsin as a result of contamination of the offshore intake by sewage-derived *Cryptosporidium* oocysts introduced into Lake Michigan (Mac Kenzie et al., 1994).

To protect public health, a multi-barrier source to tap approach is recommended to prevent waterborne pathogens from entering the drinking water supply system (WHO, 2011; Health

Canada, 2012). Quantitative microbial risk assessment (QMRA) serves as a scientific management tool for evaluating water supply systems and establishing critical goals (Petterson et al., 2016). In order to obtain reliable results, pathogen concentrations in source waters should be known in addition to their log removals through treatment (Health Canada, 2012). QMRA models have primarily focused on treatment aspects rather than source water quality and variability (Dunn et al., 2014). Microbial risk assessments have typically been performed using field monitoring data collected during routine monitoring that could be biased towards baseline conditions (i.e Ryu et al., 2008; Jaidi et al., 2009; Pintar et al., 2012; Tfaily et al., 2015). Only limited number of QMRA studies have incorporated source water monitoring data following heavy rainfall events (i.e Signor et al., 2007; Van den Akker et al., 2011), whereas source water quality depends on local hydro-meteorological conditions and varies according to (dry and wet) weather conditions (Åström et al., 2009). The majority of bacterial and parasitological loads may be associated with extreme runoff events (Kistemann et al., 2002; Signor et al., 2005; Rechenburg et al., 2006; Swaffer et al., 2014). Following a heavy rainfall event, *E. coli* concentrations as high as 10000 CFU/100 ml were reported in a creek and beach sites (Staley et al., 2018).

Both field monitoring and hydrodynamic modelling are employed to track pathogens and fecal indicator bacteria in source waters. Monitoring microorganisms in drinking water treatment plants is a common way to evaluate the microbial risk (Barbeau et al., 2000b; Jaidi et al., 2009). However, pathogen enumeration studies are relatively rare due to challenges related to sampling; hence less data are usually available to be used as an input to a QMRA model. In addition, peak concentrations of pathogens are only occasionally detected by routine monitoring processes. In order to overcome these limitations and obtain reliable results from QMRA models, the effects of upstream loadings from contamination sources can be simulated by using hydrodynamic models (to transfer contaminants to point of exposure) (McBride et al., 2012). McBride et al. (2013) performed discharge-based QMRA for estimating public health risks associated with storm water discharges in recreational waters in the United States.

Hydrodynamic models (1D, 2D, or 3D) combined with those that describe water quality loading have been commonly used to address a wide range of objectives (Hipsey et al., 2004; McCorquodale et al., 2004; Hellweger et al., 2008b; Sokolova et al., 2012; Sokolova et al., 2013; Sokolova et al., 2015; Eregno et al., 2016; Jalliffier-Verne et al., 2016a; Jalliffier-Verne et al.,

2016b); however, many do not address fundamental biological constituents (McIntyre et al., 2003) and uncertainties can be large (McIntyre et al., 2004). Probabilistic approaches are useful when considering simulation of microbial loads since uncertainty can be estimated using probability distribution functions and varying drivers of the system can be incorporated (Dorner et al., 2004; Dorner et al., 2006).

Concentrations at drinking water intakes can be influenced by short duration events that rapidly increase loads by several orders of magnitude, including those associated with stormwater discharges, water resource recovery facility (WRRF, also known as wastewater treatment plant) by-passes, combined and sanitary sewer overflows that occur following heavy rainfall events and disappear quickly. *E. coli* concentrations at a DWI of a large river (median annual flowrate>1000 m^3/s) located downstream of CSO discharges increased by approximately 1.5 log during wet weather when compared to dry weather conditions (Madoux-Humery et al., 2016). The impact of short-duration events on DWIs has to be evaluated to identify priority actions for source water protection given the association between extreme precipitation events and the higher risk of gastrointestinal illnesses for consumers whose drinking water sources have been impacted by raw sewage discharges (Jagai et al., 2015). Studies using discharge-based hydrodynamic-QMRA models in the context of drinking water are limited. Sokolova et al. (2015) examined the concentrations of norovirus in sewage discharges and employed a discharge-based QMRA-hydrodynamic model to evaluate the treatment performance of a drinking water treatment plant for various loading conditions.

The objectives of this study were to: (1) investigate the relative importance of various sources of fecal contamination that influence microbial risk at drinking water intakes; (2) quantify the variability of *Cryptosporidium*, *Giardia* and *E. coli* probability distribution functions at a drinking water intake in dry and wet weather conditions; and (3) understand the potential impact of WRRF by-pass discharges on concentrations of pathogens at a DWI and their associated microbial risk to drinking water consumers. The originality of our work lies in the unification of hydrodynamic modelling and discharge-based QMRA using probabilistic-deterministic microbial loading in the context of drinking water management practices and comparison of the importance of various contaminant sources using probabilistic approaches for improving source water protection and remediation efforts.

6.3 Material and methods

6.3.1 Study Area

Lake Ontario serves as an important source of drinking water for approximately 6 million people living in Ontario (Schiller et al., 2010). This study considers a drinking water treatment plant intake located 1.6 km from shore at a depth of 18 m, which supplies 615,000 m^3/d and serves 3.2 million residences in the city of Toronto and southern portion of York region. The intake is subject to fecal contamination from the Humber River, Credit River, Etobicoke Creek, Mimico Creek, Cooksville Creek, two secondary treated effluent discharges and WRRF by-pass discharges (Figure 6. 1b). $WRRF_1$ and $WRRF_2$ (with daily treatment capacities of 518000 m^3 and 473000 m^3 respectively) treat raw sewage using conventional activated sludge process with phosphorus removal, disinfection and dechlorination prior to discharge into Lake Ontario. A portion of $WRRF_2$ sewershed consists of combined sanitary and storm sewers. Discharge of untreated or partially treated wastewater (by-pass discharges) occurs following heavy precipitation or snowmelt events when the flowrate surpasses the design capacity of the treatment facilities. In the period of 2007-2014, $WRRF_1$ and $WRRF_2$ experienced almost 11 and 203 by-pass events respectively, primarily following heavy rainfall events (>70% of by-pass events). During these periods, 7 by-pass events occurred in both $WRRF_1$ and $WRRF_2$ simultaneously.

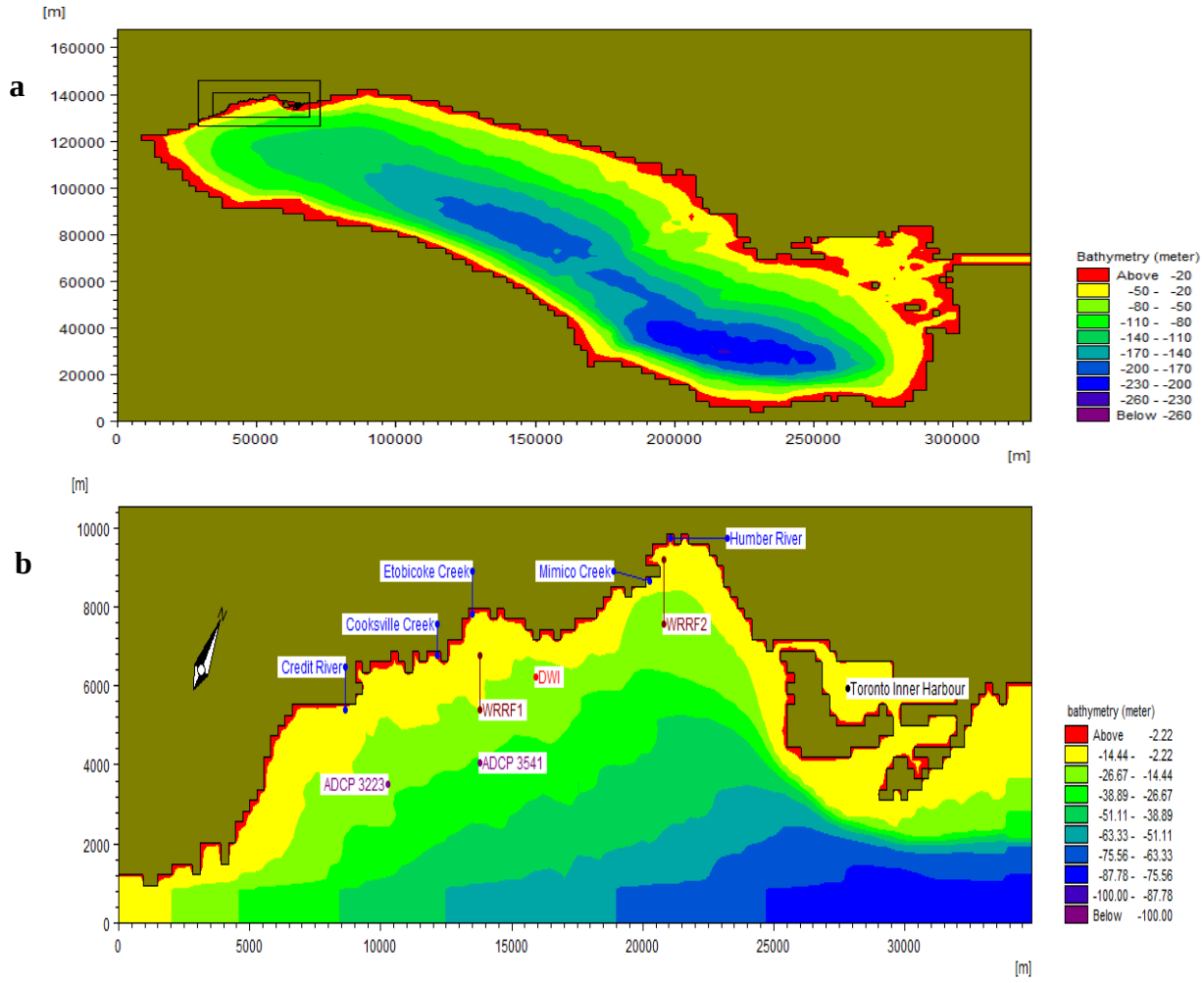


Figure 6. 1: a. Whole Lake model grid (Bay of Quinte has been removed and St. Lawrence River simplified), b. Study area model grid

6.3.2 Fecal contamination load

A probabilistic approach was used to identify the relative importance of fecal contamination sources (regardless of weather conditions) with respect to their respective loads on Lake Ontario; whereas a probabilistic-deterministic approach was used to consider the impact on the intake. Concentrations of *Cryptosporidium*, *Giardia* and *E. coli* were measured at the mouth of Humber and Credit Rivers and in the effluent discharges of a $WRRF_1$ during a monitoring campaign in 2007-2010 (Edge et al., 2013). Concentrations were also examined at the influent and treated effluent discharge of $WRRF_1$ between April 2014 and September 2014 (Tolouei et al., 2019). Since the concentrations of microorganisms at the effluent discharge of $WRRF_1$ from both studies

were at the same order of magnitude, both data sets were used for analysis. To account for the impact of Etobicoke Creek, Mimico Creek and Cooksville Creeks (for which detailed parasite data were not available), ratios based on mean *E. coli* concentrations (Wallace, 2011) were used to simulate loadings (for detailed information refer to Table 6. 3). Microbial concentrations at $WRRF_2$ were assumed to be similar to those observed concentrations at $WRRF_1$ (given similar treatment processes at both plants). Observed concentrations at the influent of $WRRF_1$ were used to estimate the concentrations at the by-pass discharge. Log removals through primary treatment (with disinfection) were assumed to be $0.7\log_{10}$ and $2\log_{10}$ for *Giardia* and *E. coli* respectively (based on the scientific literature (Casson et al., 1990; Robertson et al., 2000; Payment et al., 2001; Cacciò et al., 2003; Fu et al., 2010; Zhou et al., 2010; Behera et al., 2011) and the observed total removal efficiency rates in $WRRF_1$ and observed *E. coli* concentrations in by-pass discharges). Historical flowrate data was obtained from hydrometric stations located near the point of discharge of rivers and creeks to Lake Ontario and from treated effluent and by-pass discharges of WRRFs (Tables 6. 3, 6. 4).

Cryptosporidium, *Giardia* and *E. coli* probabilistic loadings from rivers (Humber River and Credit River), Creeks (Etobicoke Creek, Mimico Creek, Cooksville Creek) and treated effluent discharges of water resource recovery facilities ($WRRF_1$ and $WRRF_2$) were estimated regardless of weather conditions using the Latin Hypercube Sampling (LHS) method (with 1000 trials). In comparison to Monte Carlo the LHS method avoids sampling repeatedly in the distribution (Vose, 2008). Potential daily pathogen loadings were estimated by fitting log-normal and log-normal (3P) density functions to microbial and daily flowrate data respectively. Blum et al. (2017) modeled flow duration curves of nearly 400 perennial streams in the US and showed that a log-normal (3P) density function provides a good approximation of daily flowrate.

Microbial data, serving as input to the hydrodynamic model under various loading conditions were divided into two data sets of wet weather (WW) and dry weather (DW) conditions according to rainfall data at the Toronto Lester B. Pearson INT'L A Ontario and Toronto INTL A Ontario stations (Climate ID:6158733 and 6158731). These were defined as events in which the 2-day cumulative rainfall prior to sample collection was > 10 mm and 0mm respectively. Log-normal probability distribution functions were fit to microbial data to determine mean and standard deviations. Based on the fitted log-normal distributions and observed minimum and maximum of

the concentrations at the fecal contamination sources, random numbers were generated (8 time series with hourly data over the course of a day (24h)) using MATLAB. Historical hourly flow rate data (8 time series with hourly data over the course of a 24h) were also used as input to the hydrodynamic model. Hourly flowrate data were selected according to rainfall data (2-day cumulative rainfall prior to sample collection>10 mm for WW conditions and 2-day cumulative rainfall=0mm for DW conditions) and based on the dates of available data at the DWI under study. For both $WRRF_1$ and $WRRF_2$, by-pass flowrate and duration was assumed to be 3.1 m^3/s and 12h respectively.

Several loading scenarios were examined in order to determine concentration variability at the DWI:

I. Baseline loading scenario

SC1: Loadings from two rivers, three creeks and treated effluent discharges of $WRRF_1$ and $WRRF_2$ under dry weather conditions.

II. Precipitation-driven loading event scenarios

SC2: Loadings from two rivers, three creeks and treated effluent discharges of $WRRF_1$ and $WRRF_2$ under wet weather conditions.

SC3: Loadings from two rivers, three creeks, treated effluent and by-pass discharges from the both $WRRF_1$ & $WRRF_2$ under wet weather conditions.

6.3.3 Hydrodynamic and microbiological model of Lake Ontario

A three-dimensional MIKE-3 package developed by the Danish Hydraulic Institute (DHI) was used to simulate pathogen transport in Lake Ontario. The model was initially developed to assess the impact of wet weather flows on water quality (*E. coli* concentrations) along the north shore of Lake Ontario and the Toronto Inner Harbour, as well as to determine the impact of spill scenarios on the water quality (*E. coli*, tritium, benzene and total suspended solids) at municipal DWIs (Dewey, 2003 and 2011 and 2012). In this study the model was modified to simulate the variation of pathogen concentrations (*Cryptosporidium, Giardia and E. coli*) for the DWI examined.

The whole lake resolution was selected as 2430m with nested grids of 810m and 270m, vertical resolution as 40 vertical layers with 2m layer thickness and a time step of 60 seconds (Figure 6. 1).

Lake Ontario water level fluctuations were provided by the Environment Canada Kingston Gauge (daily data). The major tributary to Lake Ontario, the Niagara River daily inflow (Water Survey Canada) for 2008 was discharged at the river mouth. The Acoustic Doppler Current Profilers (ADCP) data from the Ontario Ministry of Environment database, hourly meteorological data including 2D wind field and air temperatures from National Oceanographic and Atmospheric Administration (NOAA), relative humidity and cloud cover from Toronto's Pearson station (Climate ID:6158733 and 6158731) were used in the hydrodynamic modelling process. The model accounted for the speed and directions of currents and winds on the lake surface, temperature conditions at the lake surface and bottom, water withdrawal from a DWI located at the lake bottom (18 m), the inflow to the lake from two rivers, three creeks and two WRRFs and heat exchange between the lake and atmosphere. The temperature data for the tributaries were simulated using HSPF models of watersheds. Temperature of WRRF effluent and by-pass discharges were set constant at 10°C, typical for the time of the year under study.

Transport of fecal bacteria and pathogens in Lake Ontario was simulated using an advection-dispersion module linked to the lake hydrodynamic module. Previous modelling experience regarding Lake Ontario indicated that the *E. coli* decay rate was spatially variable around Toronto Inner Harbour; thus, calibrating the model for the *E. coli* decay rate was difficult as it improved the model accuracy for some locations but not for others (Dewey, 2012). This is likely the result of other effects including various currents and additional sources such as waterfowl. Given that only short-term events were simulated all fecal organisms were assumed to be conservative (persistent and non-reactive); settling of microbial contaminants in Lake Ontario was not considered.

Model accuracy was measured by evaluating the Fourier Norm (F_N) scores and root mean square error (RMSE) (Equations 6.1 and 6.2). F_N is based on the average difference between the two vector components of velocity and RMSE on each velocity vector. V represent the onshore-offshore (north-south) direction whereas U is in the alongshore (east-west) direction.

$$F_N = \frac{\left\|\vec{v}_o, \vec{v}_c\right\|}{\left\|\vec{v}_o, 0\right\|} \text{ where } \left\|\vec{v}_o, \vec{v}_c\right\| = \left\langle \left\langle \frac{1}{N} \right\rangle \sum_{t=1}^{N\Delta t} \left|\vec{v}_o - \vec{v}_c\right|^2 \right\rangle^{\frac{1}{2}} \quad (6.1)$$

$$RMSE(v) = \left\|v_o, v_c\right\| \quad (6.2)$$

v_o : observed data and v_c : computed data

In this study, most of the parameters were set as per previous calibrated models (Dewey, 2011 and 2012); the hydrodynamic model was calibrated using the measured currents and temperature by the Ontario Ministry of Environment in Lake Ontario. The Ministry of Environment deployed Acoustic Doppler Current Profilers (ADCP 3223 and 3541) in 2004, for the period May through October. Speed and direction were averaged at 30 minute intervals. The locations of aforementioned ADCPs relative to the other fecal contamination sources are presented in Figure 6. 1. Detailed information regarding calibration is presented in Section 6.7.1.

The calibrated hydrodynamic model was used to simulate the concentrations of fecal organisms at the DWI following various loading scenarios (8 time series for each scenario). Log-normal distributions were fit to the simulated concentrations at the intake; averaged log-normal distribution of the concentrations were estimated using the Latin Hypercube Sampling (LHS) method (with 1000 trials) for each loading scenario. Results were compared with observations and used for risk analysis. Observed concentrations were also divided into DW and WW data sets, based on the rainfall data and considering travel time between point of discharges and the drinking water intake.

6.3.4 Quantitative microbial risk analysis (QMRA)

Health Canada has developed a user-friendly QMRA model (HC QMRA) that may be used by municipal engineers and local decision makers for estimating health risks, associated with five reference pathogens *Cryptosporidium parvum, Giardia duodenalis,* Rotavirus, *Campylobacter and E. coli* O157:H7. Daily probabilities of infection for *Cryptosporidium* and *Giardia* can be estimated by using an exponential model (Equation 6.3) and for *E. coli* O157:H7 by beta Poisson dose-response models (Equations 6.4).

$$P_{\inf} = 1 - e^{rD} \quad (6.3)$$

$$P_{\inf} = 1 - (1 + \frac{D}{\beta})^{-\alpha} \quad (6.4)$$

$$D = C_{sw} \times (1/R) \times I_{fraction} \times 10^{-\text{logremoval}} \times V \quad (6.5)$$

P_{inf} is the daily probability of infection for a person per exposure, r, α, β are the parameters of dose response models. D is the dose of ingested organisms, C_{sw} the microorganisms' concentration in the source water, R the recovery rate of the analytical method, $I_{fraction}$ the infectious fraction and V the consumed unboiled water volume.

The simulated data at the DWI were recovery corrected before entering them into model. Recoveries of *Cryptosporidium* and *Giardia* were assumed to be 40% and 69% respectively (Jaidi et al., 2009). Most of the *Cryptosporidium* oocysts genotypes were not associated with human disease in the study area; while human-infectious forms of *Giardia* were widespread in Lake Ontario (Edge et al., 2013); thus the fraction of infectious *Cryptosporidium* was assumed to be 8% (considering *C. hominis* and *C. parvum* as dominant human infectious form of *Cryptosporidium*) (Pintar et al., 2012) and *Giardia* 80% (Zmirou-Navier et al., 2006). *E. coli* simulations were used to estimate the concentrations of *E. coli* O157:H7*;* the fraction of *E. coli* O157:H7 was assumed to represent 3.49% of the total *E. coli* (Martins et al., 1992). It is assumed that all *E. coli* O157:H7 are infectious and that 1L of unboiled water is consumed per person per day (Health Canada, 2012). Dose response were assumed to be as follows: r= 0.018 for *Cryptosporidium* (Messner et al., 2001); r=0.01982 for *Giardia* (Rose et al., 1991), α=0.0571 and β=2.2183 for *E. coli O157:H7* (Strachan et al., 2005).

The performance of the water treatment plant was assessed based on the monitoring data and reported literature values included as part of the QMRA model. For chemical disinfection, pathogen inactivation is estimated through a continuous-stirred tank reactor in-series (N-CSTR) calculation module that provides more reliable risk estimation (when compared to the use of CT10 or CT50) because it is less sensitive to high inactivation conditions (Tfaily et al., 2015). Since log removals were not significantly different for the water treatment plant under dry and wet weather conditions (Andrews 2018, personal communication), the same values were used for both dry and

wet weather loading conditions. The assigned overall log removals and inactivation for *Cryptosporidium*, *Giardia* and *E. coli* O157:H7 using the site specific data were 4.3, 5.5 and 10.4 respectively. The risk of probability of infection for various loading scenarios was quantified and compared with the 2.7×10^{-7} risk of infection per person per day (Signor et al., 2009) which is the equivalent of a 10^{-4} risk of infection per person per year (Regli et al., 1991).

6.3.5 Statistical analysis

Statistical analyses were performed using STATISTICA software (Version 12). EPA's ProUCL software was used to impute values below the limit of detection (Singh et al., 2013). Easy Fit Professional software (Version 5.6) was used to fit log-normal probability distributions to the concentrations of fecal organisms. Given that concentrations were lognormally distributed, t-tests were performed to assess differences between concentrations under dry and wet weather conditions. A confidence level of 5% (α=0.05) was applied to assess significance. In order to compare the means of two log-normal distributions a likelihood-based test was used as a powerful test in terms of the type I error when data follow a log-normal distribution (Zhou et al., 1997). An Oracle Crystal Ball spreadsheet-based application was also used to estimate probabilistic microbial loadings from various fecal contamination sources.

6.4 Results and discussion

Cryptosporidium, *Giardia* and *E. coli* loadings from rivers, creeks and treated effluent discharges of water resource recovery facilities were estimated regardless of weather condition to identify their relative importance. In addition, the concentrations of *Cryptosporidium*, *Giardia* and *E. coli* were simulated at the drinking water intake using various loading scenarios and hydrodynamic modelling. Finally, the QMRA model was performed to understand the impact of source water quality variability on the drinking water intake.

6.4.1 Concentrations and loadings of pathogens and *E. coli* associated with contamination sources

Cryptosporidium, *Giardia* and *E. coli* concentrations at the outlet of the Humber and Credit Rivers and $WRRF_1$ effluent discharges were described by log-normal distributions (Table 6. 1). The observed mean concentrations of *Cryptosporidium* and *Giardia* at the mouth of the Humber and

Credit Rivers and treated effluent discharge of $WRRF_1$ were not significantly different when comparing dry and wet weather conditions ($p>0.05$). However, the mean concentration of *E. coli* was significantly higher during wet conditions at the mouth of the Humber and Credit Rivers. Mean concentrations of *Giardia* and *E. coli* were also significantly higher in by-pass discharges when compared with treated effluent discharges ($p<0.05$ in t-test). The mean concentration of *Giardia* was significantly higher and *E. coli* lower at the effluent discharge of $WRRF_1$ (compared to the Humber and Credit Rivers). The mean concentration of *E. coli* was also higher at the mouth of Humber River (compared to the Credit River and $WRRF_1$ effluent) although this difference was not statistically significant between the Humber and Credit Rivers. The higher concentration of *E. coli* in lower the Humber River has been attributed to the impact of CSO_S and stormwater systems with sewage cross-connections (Staley et al., 2016b).

The probabilistic microbial (*Cryptosporidium, Giardia* and *E. coli*) loadings to Lake Ontario from the rivers (Humber River and Credit River), creeks (Etobicoke Creek, Mimico Creek, Cooksville Creek) and water resource recovery facility effluent discharges ($WRRF_1$ and $WRRF_2$) were estimated during the period of 2008-2010 regardless of weather conditions (Figures 6. 7, 6. 8 and 6. 9). The mean of the estimated probability distribution functions of each microbial loadings were significantly different among various sources ($p<0.05$ in likelihood-based test). Considering all sources, they ranged from 6.0 to 8.2 log oocysts/d, 6.3 to 9.4 log cysts/d and 10.4 to 12.8 log CFU/d. These are similar to the mean loads of *Cryptosporidium, Giardia* and *E. coli* reported from the Sûre River and effluent discharges of a WRRF in Luxembourg (Burnet et al., 2014). The relative contribution of each of the contaminant sources to the mean environmental loadings of *Cryptosporidium, Giardia* and *E. coli* into Lake Ontario is shown in Figure 6. 2. The Humber and Credit Rivers were the most important contributor of *E. coli* and *Cryptosporidium* loadings into Lake Ontario; while the contribution of WRRF effluent discharge for the loadings of *Giardia* were significant. Mölndalsån River and on-site sewer discharges were also identified as the most important source of *E. coli* loads into Lake Rådasjön (Sokolova et al., 2013).

Table 6. 1: Log-normal probability distribution function (mean, standard deviation), minimum, maximum and number of microbial data in fecal contamination sources

	Contamination sources	Weather	Mean	SD	Min	Max	N of data
Cryptosporidium (oocysts/L)	Humber River	All data	0.2	1.9	<0.01	1.2	38
		DW	0.1	0.3	<0.01	0.2	16
		WW	0.5	19.9	<0.01	0.9	8
	Credit River	All Data	0.2	0.3	<0.01	0.6	34
		DW	0.1	0.2	<0.01	0.5	17
		WW	0.3	0.3	0.05	0.6	8
	$WRRF_1$-effluent discharge	All data	0.1	0.2	<0.01	0.4	50
		DW	0.1	0.1	0.02	0.4	23
		WW	0.1	0.2	<0.01	0.2	16
Giardia (cysts/L)	Humber River	All data	0.9	2.3	0.02	5.4	38
		DW	0.5	0.9	0.02	1.8	16
		WW	1.2	1.5	0.18	5.4	8
	Credit River	All data	0.2	0.4	0.01	0.9	34
		DW	0.2	0.4	0.01	0.8	17
		WW	0.3	0.4	0.03	0.9	8
	$WRRF_1$-effluent discharge	All data	6.1	11.7	0.1	28.3	51
		DW	5.1	6.5	0.6	17.3	24
		WW	5.6	16.2	0.1	17.3	16
E. coli (CFU/100ml)	Humber River	All Data	1268	2665	15	7270	79
		DW	504	582	42	1800	37
		WW	2103	2983	124	5200	17
	Credit River	All Data	590	1457	14	8400	89
		DW	226	405	14	1550	36
		WW	2165	5346	95	8400	21
	$WRRF_1$-effluent discharge	All Data	117	821	<1	2960	108
		DW	74.1	444.2	<1	2730	46
		WW	257	2881	<1	2960	33

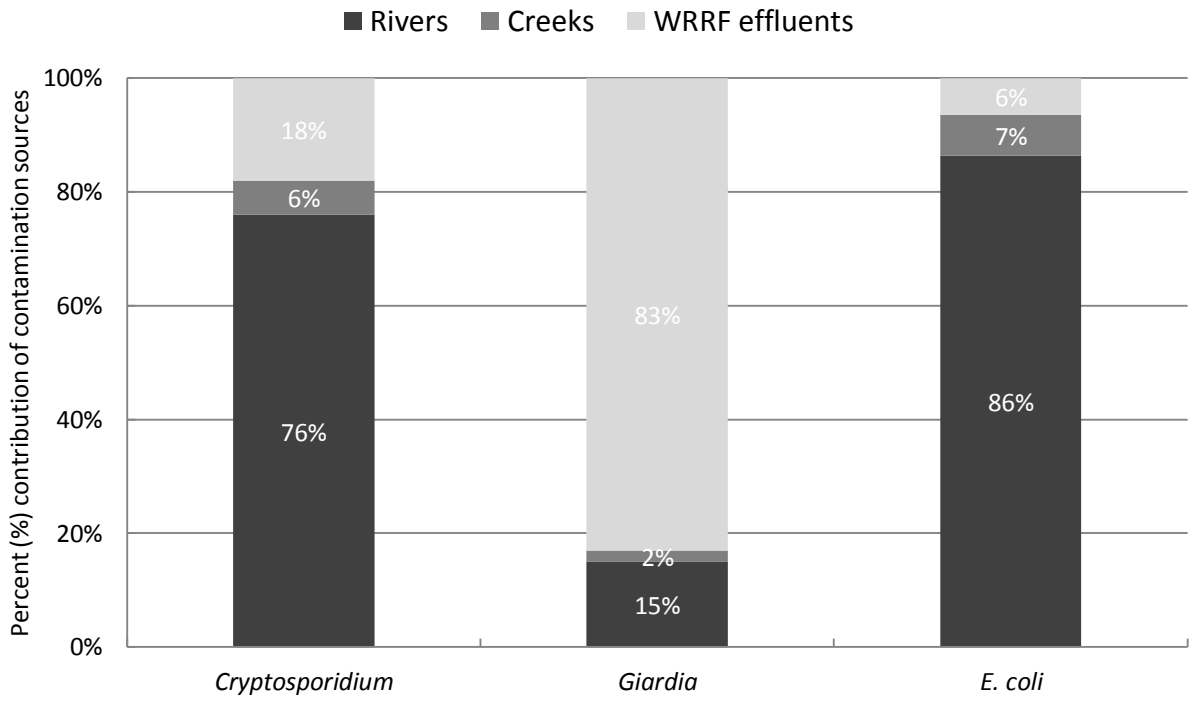


Figure 6. 2: Relative contribution of each fecal contamination source to the total loads of *Cryptosporidium*, *Giardia* and *E. coli* during dry and wet weather conditions

6.4.2 Simulated concentrations at the studied drinking water treatment plant intake

Concentrations of *Cryptosporidium*, *Giardia* and *E. coli* at intake were simulated in response to various loading scenarios by using three-dimensional hydrodynamic model and compared with observations (Table 6. 2). For *Cryptosporidium,* SC3 was not run due to lack of sufficient data (prevalence rate of 8.6% in raw sewage samples of $WRRF_1$) and the simulation results and observations were not compared. The prevalence rate of *Cryptosporidium* in water samples collected from the influent of the water treatment plant was 13% (Edge et al., 2013). In addition, *Cryptosporidium* was only detected in 6 out of total 33 samples collected in the influent of same water treatment plant (February 2008 to October 2010) with a maximum value of 6 oocysts/10L (City of Toronto, personal communication). Thus, we were unable to fit probability distribution functions to *Cryptosporidium* observations as >80% of data were below the limit of detection and data imputation was statistically meaningless (Helsel, 2011). Fitted log-normal distributions for *Giardia* and *E. coli* simulated concentrations following dry and wet weather conditions (SC1 and SC2) were compared with the log-normal distributions fitted to the observed concentrations at the

intake. Overall, the means of the simulated concentrations were not significantly different from the ones observed. However, simulated mean concentrations at the intake were slightly lower than those observed. In addition, simulated concentrations at the intake arising from the identified fecal contamination sources do not describe some of the observed peak values. The maximum simulated concentrations of *Cryptosporidium*, *Giardia* and *E. coli* at the drinking water intake were 0.1 oocysts/L, 0.6 cysts/L and 60 CFU/100ml whereas the maximum measured concentrations were 0.4 oocysts/L, 0.7 cysts/L and 66 CFU/100ml under both dry and wet weather conditions (Edge et al., 2013). These results indicate that the amount of fecal contamination entering Lake Ontario has been underestimated. This could be due to the existence of unquantified fecal contamination sources, such as inputs from other smaller watersheds like Etobicoke Creek (Staley et al., 2018), unrecognized sewage discharges into the stormwater infrastructure (Staley et al., 2016b) and birds and other animals. Lu et al. (2011) found fecal pollution from gulls to be widespread in urban coastal and riverine areas in southern Ontario, including across the Toronto waterfront. Gull fecal pollution, in addition to a variety of sewage sources, are also known to be contributors to fecal pollution in the Humber River discharging into our study area (Edge et al., 2010; Staley et al., 2016a).

Modelling results indicate that the log-normal distributions of concentrations at the intake resulting from baseline loading (SC1) and precipitation-driven event loading scenarios (SC2 and SC3) are significantly different. According to the fitted log-normal distributions to the simulated concentrations (Figure 6. 3), the mean of concentrations following dry weather conditions (SC1) was significantly lower than for wet weather conditions (SC2 and SC3). In addition, the mean of concentrations following by-pass events from both water resource recovery facilities (SC3) was significantly higher than for the condition where no by-pass occurred from both plants (SC2) following wet weather conditions. Peak concentrations of fecal organisms in raw sewage were observed during wet weather periods (Tolouei et al., 2019). Our results indicate that by-pass discharges following wet weather periods represent critical times with respect to contamination of the DWIs and hence risk analysis should consider these short and infrequent events. Microbial loads substantially increase from all types of wastewater discharge points including secondary effluent, combined and sanitary sewer overflows following wet weather conditions (Åström et al., 2007; Åström et al., 2009). In addition, seasonal phenomena such as increased river flows in spring during runoff periods influence the fate and transport of pathogens entering the Great lakes

(Edge et al., 2013). During this period, pathogens usually survive longer due to colder temperature and the isothermal water column facilitates mixing throughout. Therefore, contamination that is discharged to the lake surface may find its way more easily to drinking water intakes.

We observed that hydrodynamic events mostly depend on wind and currents as well as transient wind-driven up-welling or down-welling of the thermocline. Simulation of nearshore waters of western Lake Ontario and north-shore hydrodynamics and contaminant transport also showed that up-welling and down-welling events play an important role in the variability of water quality (Rao et al., 2012; Paturi et al., 2014). Similarly, Sokolova et al. (2013)) confirmed that wind and the vertical temperature distribution in Lake Rådasjön were the major drivers of *E. coli* concentrations at the water intake.

Table 6. 2: Mean and standard deviation of the simulated and observed concentrations of microorganisms at the drinking water intake for various loading conditions

Microorganisms	SC1	SC2	SC3	Observations DW	Observations WW
Cryptosporidium (oocysts/L)[a]	0.002(0.001)	0.01(0.01)	-	-	-
Giardia (cysts/L)	0.01 (0.01)[b]	0.03 (0.04)[b]	0.06 (0.08)	0.02(0.3)	0.07(0.91)
E. coli (CFU/100ml)	0.83 (0.49)[b]	4.65 (6.1)[b]	11.73 (24.42)	0.9 (2.2)	8.7(81.4)

SC1: Baseline loading scenario (loadings from two rivers, three creeks and treated effluent discharges of $WRRF_1$ and $WRRF_2$ under dry weather condition); SC2: Precipitation-driven loading scenario (loadings from two rivers, three creeks and treated effluent discharges of $WRRF_1$ and $WRRF_2$ under wet weather condition); SC3: Precipitation-driven loading scenario (loadings from two rivers, three creeks, treated effluent and by-pass discharges of $WRRF_1$ and $WRRF_2$ under wet weather condition); [a]: At the drinking water intake, the prevalence rate and concentration of *Cryptosporidium* were 13% and BLD-0.4 oocysts/L respectively; [b]: The simulations were not significantly different from the observations ($p<0.05$ in a likelihood-based test); DW:Dry weather; WW: Wet weather

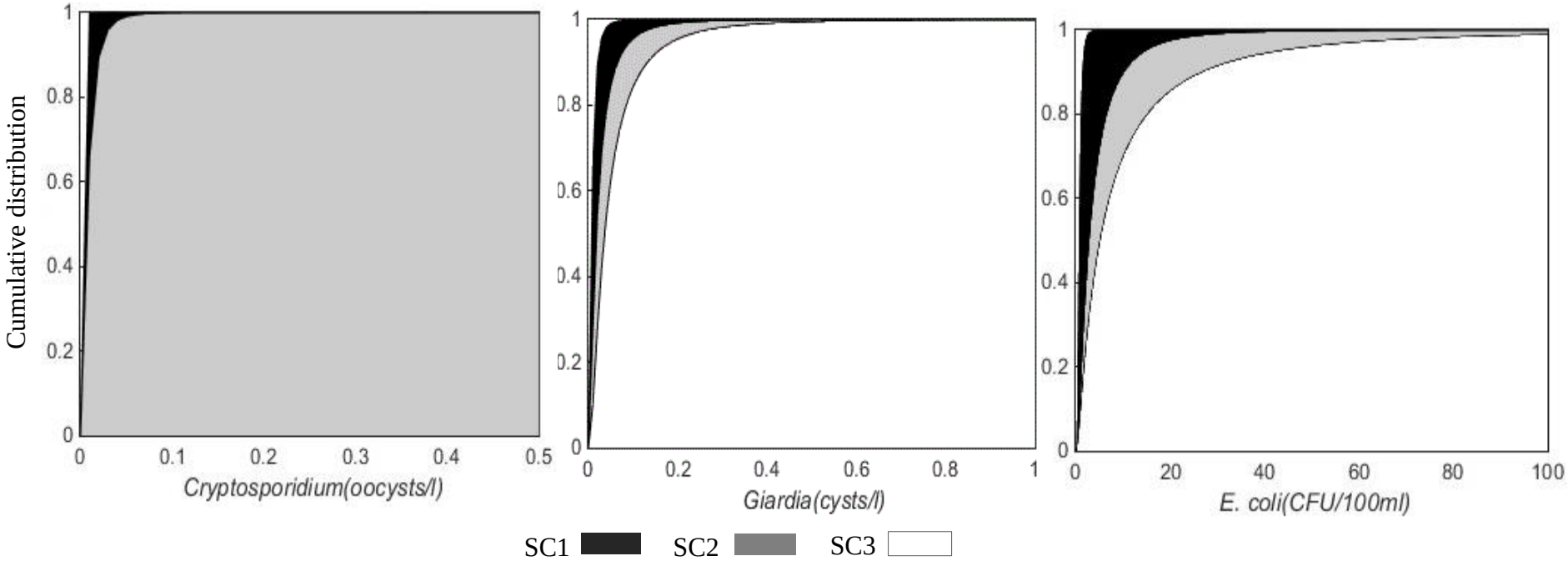


Figure 6. 3: Simulated concentrations of *Cryptosporidium*, *Giardia* and *E. coli* at the drinking water intake under baseline (SC1) and precipitation-driven loading scenarios (SC2 and SC3)

6.4.3 Risk estimates predicted following various loading scenarios

Average risk probability estimated for *Cryptosporidium* and *Giardia* under baseline (SC1) and precipitation-driven loading events (SC2 and SC3) are shown in Figure 6. 4. A higher risk following precipitation-driven loading events is evident when compared to baseline conditions, as expected. However, the estimated daily probability of infection (2.7×10^{-13}-4.1×10^{-9} person^{-1} day^{-1}) for the reference pathogens studied was well below the risk benchmark of 2.7×10^{-7} person^{-1} day^{-1}. The highest pathogen risk was primarily driven by *Giardia* and *Cryptosporidium.* Although the risk was higher following SC3 (compared to SC2), it didn't substantially change following by-pass discharges from both WRRFs. However, in the event of reduced treatment effectiveness, water treatment plants could be at risk. When applying the QMRA model, *Cryptosporidium*, *Giardia* and *E. coli* O157:H7 inactivation via chlorine disinfection was assumed to be 0.02 log, 1.97 log and 8 log, respectively; as such inadequate chlorination could lead to increased microbial risk associated with *Giardia* and *E. coli* O157:H7. It should be noted that the QMRA model was also tested for the observed mean and maximum concentrations at the intake as the simulated concentrations were slightly underestimated; the risks were also less than the risk benchmark of 2.7×10^{-7} person^{-1} day^{-1}. Effluent discharges from WRRFs, the Humber and Credit rivers were the most important contributor of *Giardia* and *Cryptosporidium* loadings into Lake Ontario and the highest pathogen risk was associated with *Giardia* and *Cryptosporidium;* it suggests the importance of sewage discharge and rivers discharge on the contamination of DWI studied.

The HC QMRA model is a useful systematic evaluation tool to assess the impact of various loading scenarios from alternative locations of contamination sources under different hydro-meteorological conditions on the microbial quality of drinking water intakes. Many assumptions and sources of uncertainties exist in the HC QMRA model which suggest that results should not be considered as an exact risk, but rather one that is useful for comparative purposes and scenario evaluation (Tfaily et al., 2015). For instance, pathogen risks associated with distribution systems were neglected in the model. Despite these limitations, the HC QMRA model can be easily used by water utilities with regards to source water protection and planning as well as treatment performances.

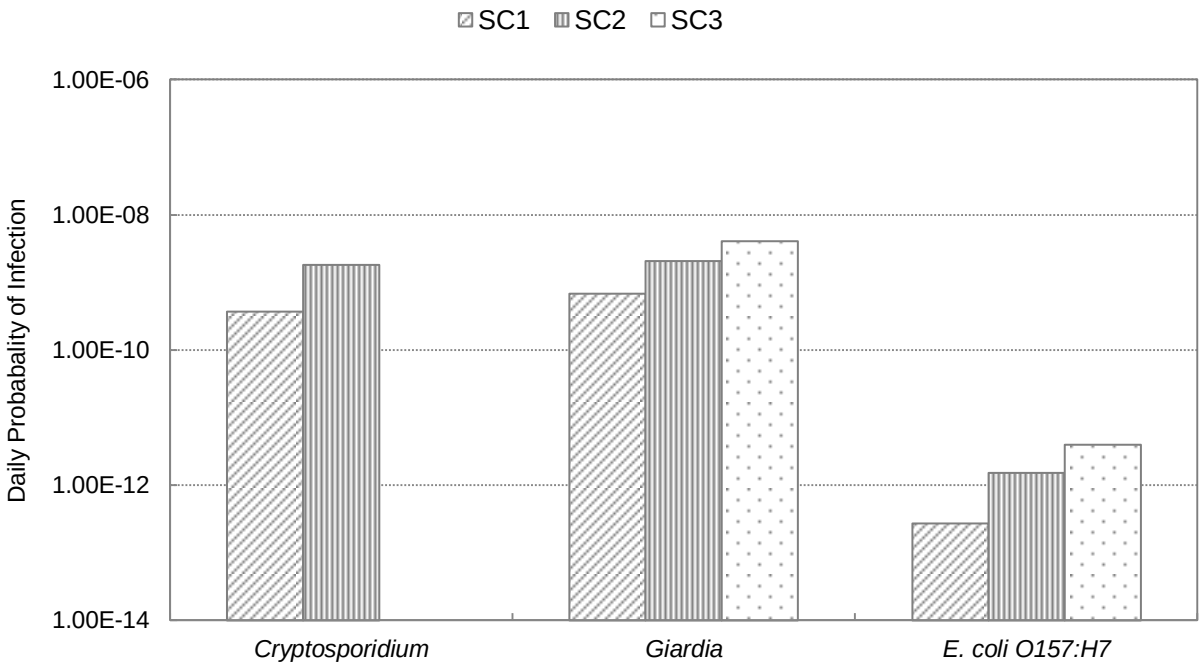


Figure 6. 4: Daily probability of infection for *Cryptosporidium, Giardia* and *E. coli* O157:H7 under various loading conditions; SC3 was not run for Cryptosporidium due to lack of data

6.5 Conclusions

The present study provided the following key findings regarding understanding and managing waterborne pathogens risk to drinking water with respect to source water quality variability:

- Hydrodynamic modelling to compare baseline loadings with precipitation-driven loading conditions with by-pass events demonstrated that unquantified fecal sources remain important for estimating water quality.
- In the study area, the rivers (Humber and Credit Rivers) were the most important contributors of *E. coli* and *Cryptosporidium* loadings to Lake Ontario, whereas the water resource recovery facility effluent discharges served as the dominant source of *Giardia* to Lake Ontario.
- *Giardia* and *Cryptosporidium* estimated through simulation were both drivers of microbial risks, demonstrating the impact of sewage and river discharges on the drinking water intake studied

- The microbial risk remained within acceptable boundaries for all tested scenarios even though the risk increased 0.5 log to 1.2 log during precipitation-driven loading condition (with/without by-pass events) compared to baseline loading condition for all reference pathogens studied
- Coupled hydrodynamic modelling and QMRA serve as useful tools for risk mitigation and management.

6.6 Supplementary Information

Table 6. 3: Microbial and flowrate data for study area

Site	Microbial data	Hourly Flowrate data
Humber River (HR)	Edge et al. (2013)	Station 02HC003
Credit River (CR)	Edge et al. (2013)	Station 02HB029
Etobicoke Creek (EC)	a*× concentration at the mouth of Humber River	Station 02HC030
Mimico Creek (MC)		Station 02HC033
Cooksville Creek (CC)		0.35**× Q_{MC}
$WRRF_1$- effluent	Edge et al. (2013) AND Tolouei et al. (2019)	1.2***× Q_{WRRF2}
$WRRF_2$- effluent	Assumed to be as same as in $WRRF_1$	Historical data
$WRRF_1$- by-pass	Tolouei et al. (2019)	Historical data
$WRRF_2$- by-pass	Assumed to be as same as in $WRRF_1$	Historical data
DWI	Edge et al. (2013)	-

*: α=0.3, 0.2 and 0.1 for Etobicoke Creek, Mimico creek and Cooksville Creek, respectively; **: based on daily data in the creeks (2006-2010); ***: based on annual average flowrate data in the water resource recovery facilities (2006-2010)

Table 6. 4: Descriptive statistics for daily flowrate (m3/s) (2008-2010) at the mouth of rivers, creeks and treated effluent discharges of WRRF1 and WRRF2

Site	Mean±SD	Min-Max	Lower (10%)	Median (50%)	Upper (90%)
Humber River	8.5±12.8	1.6-175.0	2.2	4.5	18.1
Credit River	10.2±10.4	3.3-111.0	4.5	6.8	17.8
Etobicoke Creek	3.5±6.5	0.2-80.0	0.5	1.2	8.5
Mimico Creek	1.1±2.1	0.1-26	0.1	0.3	2.9
Cooksville Creek	0.4±0.8	0.03-7.5	0.05	0.1	0.9
$WRRF_1$*	4.6±1.1	2.02-11.8	3.6	4.3	5.9
$WRRF_2$	3.8±0.9	1.7-9.8	2.9	3.6	5

*: based on daily data from $WWRF_2$

6.6.1 Calibration of hydrodynamic model

Most of the parameters of previous calibrated models (Dewey, 2011 and 2012) were used in the calibration of the current model (Table 6. 5). The model also calibrated based on the Acoustic Doppler Current Profilers (ADCP) of the Ministry of Environment deployed in 2004. For instance, temperature, surface speed and direction time series for ADCP 3223 are presented in Figures. 6. 5 and 6. 6, respectively. The overall Fourier Norm scores for ADCPs were poor, with values above 1.0. Basically it was difficult to achieve low scores and high accuracy in this area of the lake, for instance the scores for ADCP 3223 are listed in Table 6. 6. As shown in this table, the Fnorm scores and RMS improve with depth. The poor performance may be due to the 270 m resolution which does not delineate the shoreline features in the area. In another earlier study that had a 90m grid, the Fnorm scores were a bit better but not less than one. In other words, there are significant shoreline “obstacles” that can affect the currents and those are not present in the 270m grid.

Table 6. 5: Model calibration parameters (Adapted from (Dewey, 2012))

	Parameter type	Value
Hydrodynamic model	Time step	60 s
	Transport scheme	Quickest-Sharp
	Turbulence model	Mixed κ-ε Smagorinsky
	Eddy Viscosity Coefficient	0.4 m2/s (default)
	Temperature Dispersion Coefficients	Horizontal 0.1 m^2/s , Vertical 0.001 m^2/s
	Temperature Dispersion Scheme	Eddy velocity relationship
Heat Exchange Coefficients	Dalton's Law constant	0.5 (default)
	Dalton's Law wind constant	0.9 (default)
	Sun constant a	0.395 (default=0.295)
	Sun constant b	0.691 (default =0.371)
	Displacement (Day light saving)	-1 hour
	Standard Meridian	-75 degree west
	Beta in Beer's Law	0.3 (default)
	Light Extinction	1/m default
	Runge-Kutta integration order	2^{nd} order
	Bed roughness	0.05 m (default)
Advection –Dispersion Module	*Cryptospridium, Giardia, E. coli*	Conservative
	Initial ambient conditions	0 #/100mL
	Decay rates	0/s
	Dispersion Coefficients	1 m^2/s (default)
	Dispersion scheme	Eddy viscosity relationship

Table 6. 6: FNorme and RMSE scores for ADCP3223

Depth	FNorm	U RMSE (m/s)	V RMSE (m/s)
Surface	2.50	0.13	0.11
5m	1.89	0.10	0.09
10m	1.41	0.09	0.09
bottom	1.11	0.09	0.08

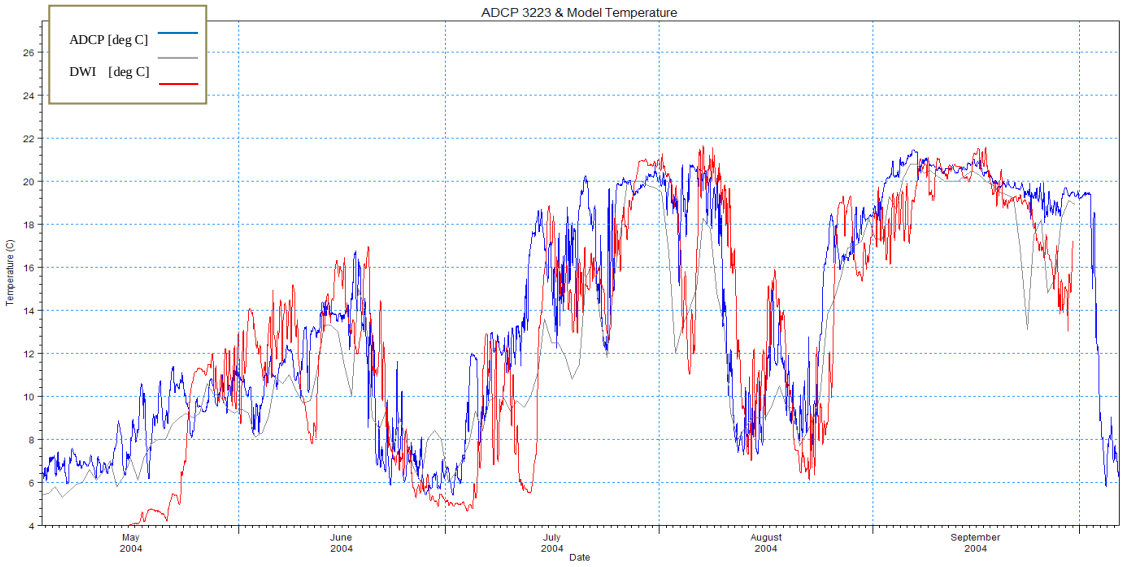
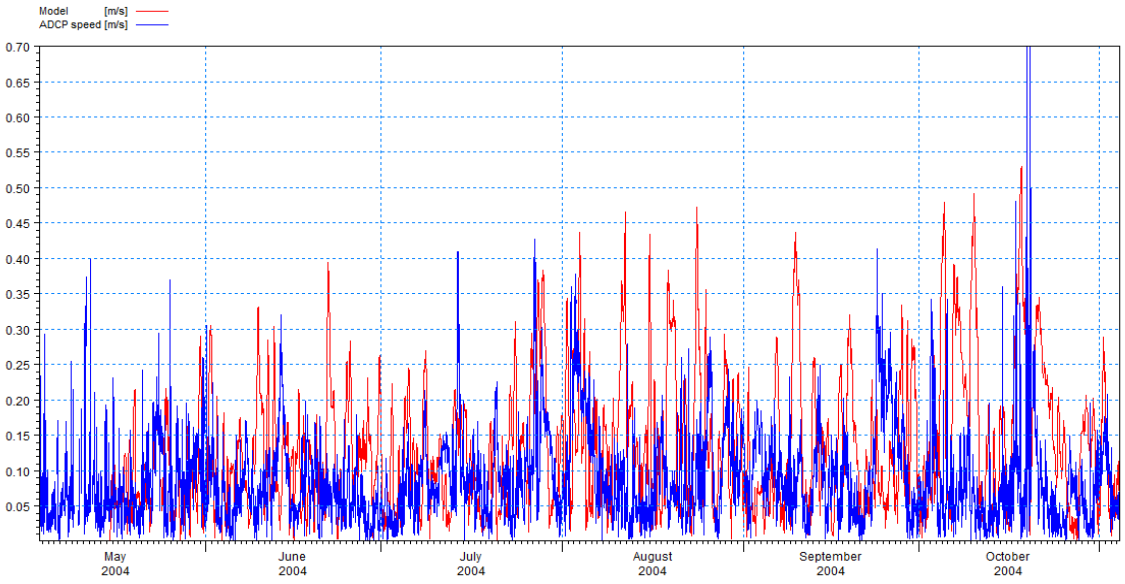


Figure 6. 5: Temperature time series for ADCP 3223

a



b

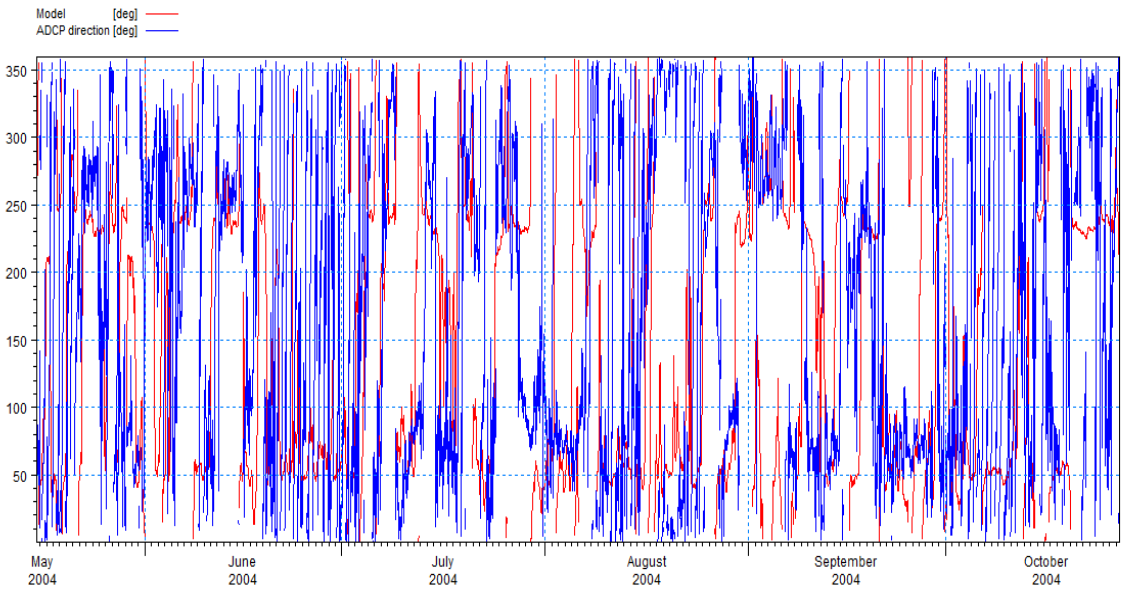


Figure 6. 6: Time series of a. surface speed and b. surface direction for ADCP 3223

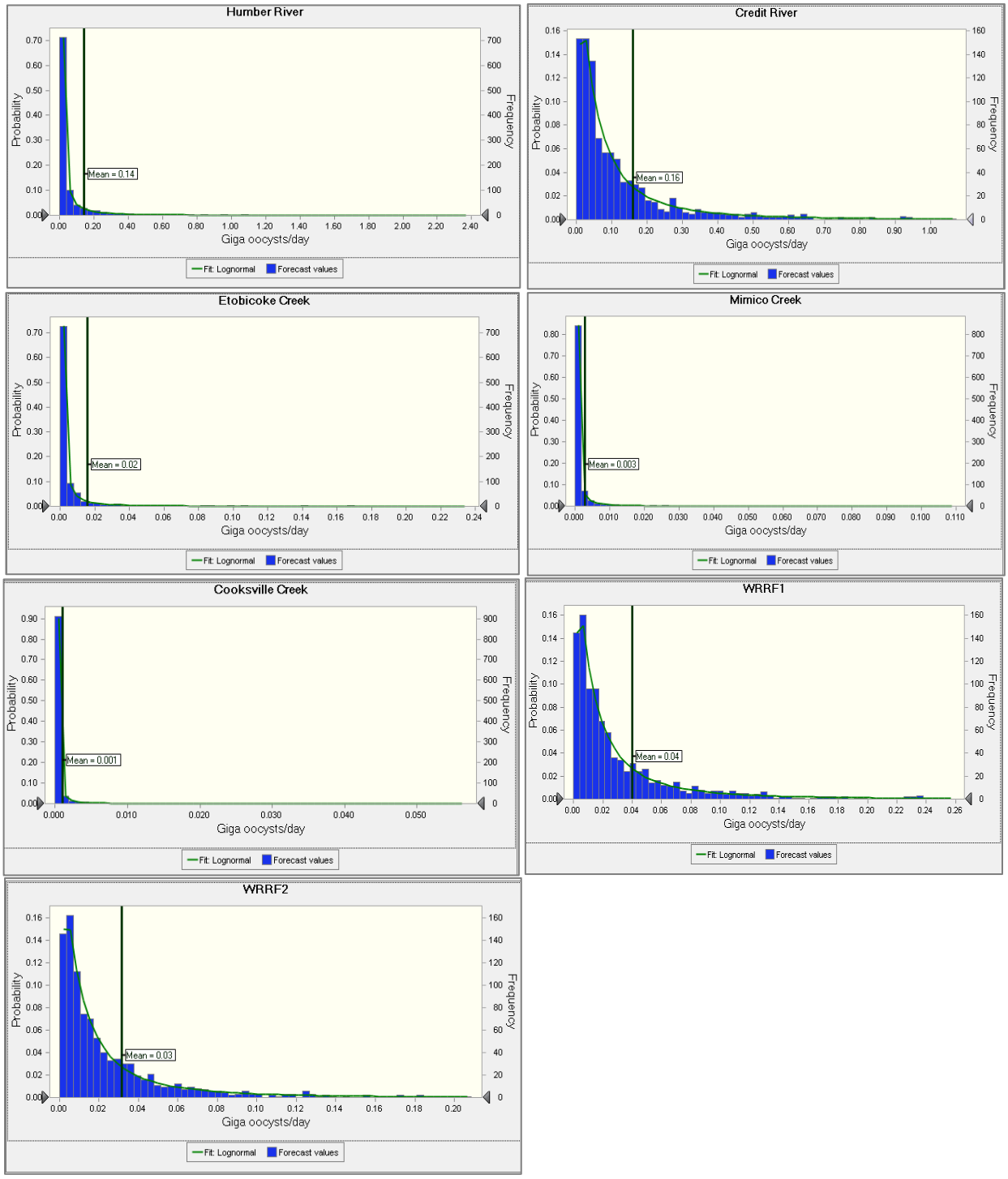


Figure 6. 7: Probability density function of *Cryptosporidium* loading (Giga-oocysts/day) for rivers (Humber and Credit Rivers), creeks (Etobicoke, Mimico and Cooksville Creeks) and effluent discharge of WRRF1 and WRRF2 irrespective of weather condition

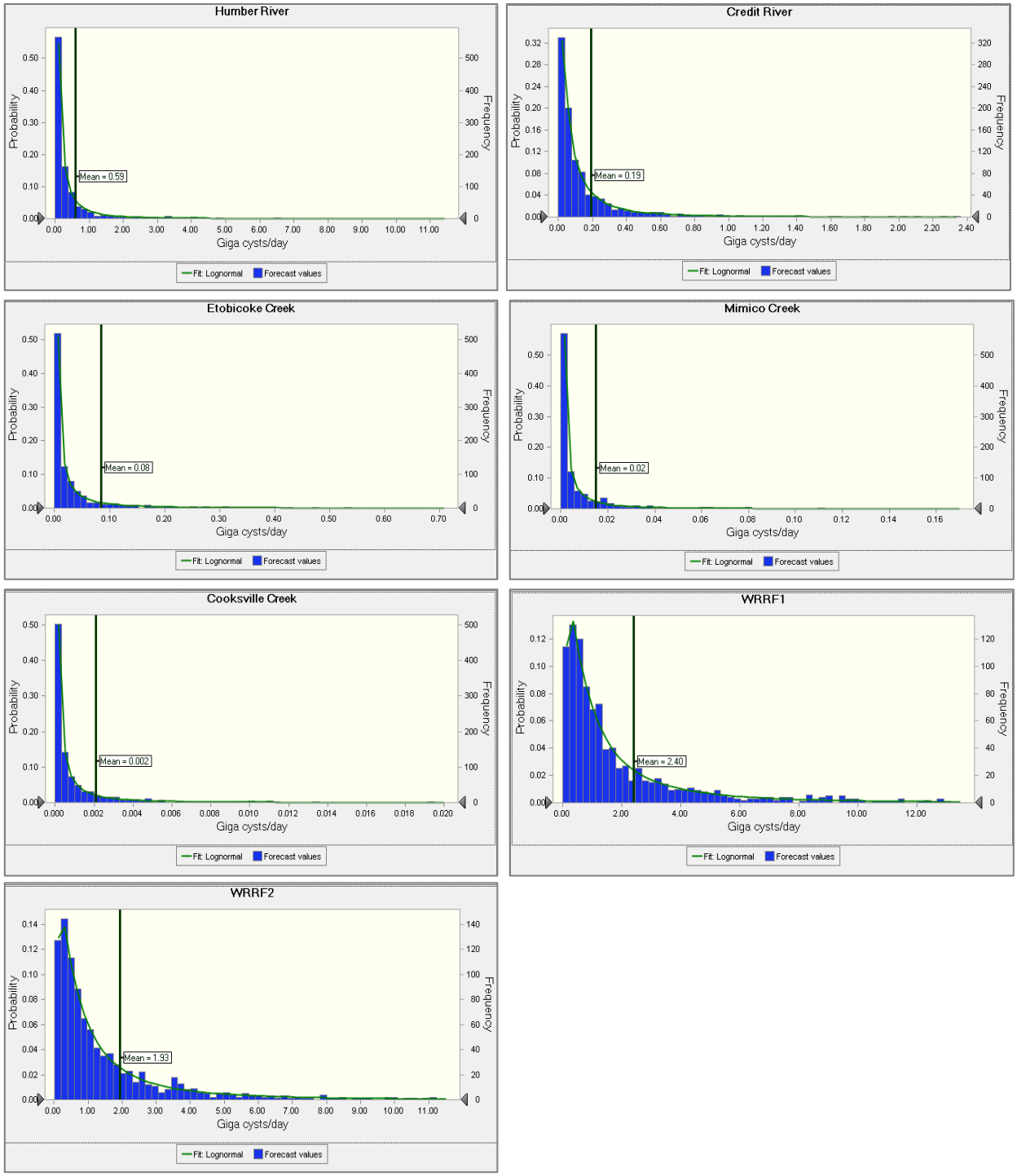


Figure 6. 8: Probability density function of *Giardia* loading (Giga-cysts/day) for rivers (Humber and Credit Rivers), creeks (Etobicoke, Mimico and Cooksville Creeks) and effluent discharge of WRRF1 and WRRF2 irrespective of weather condition

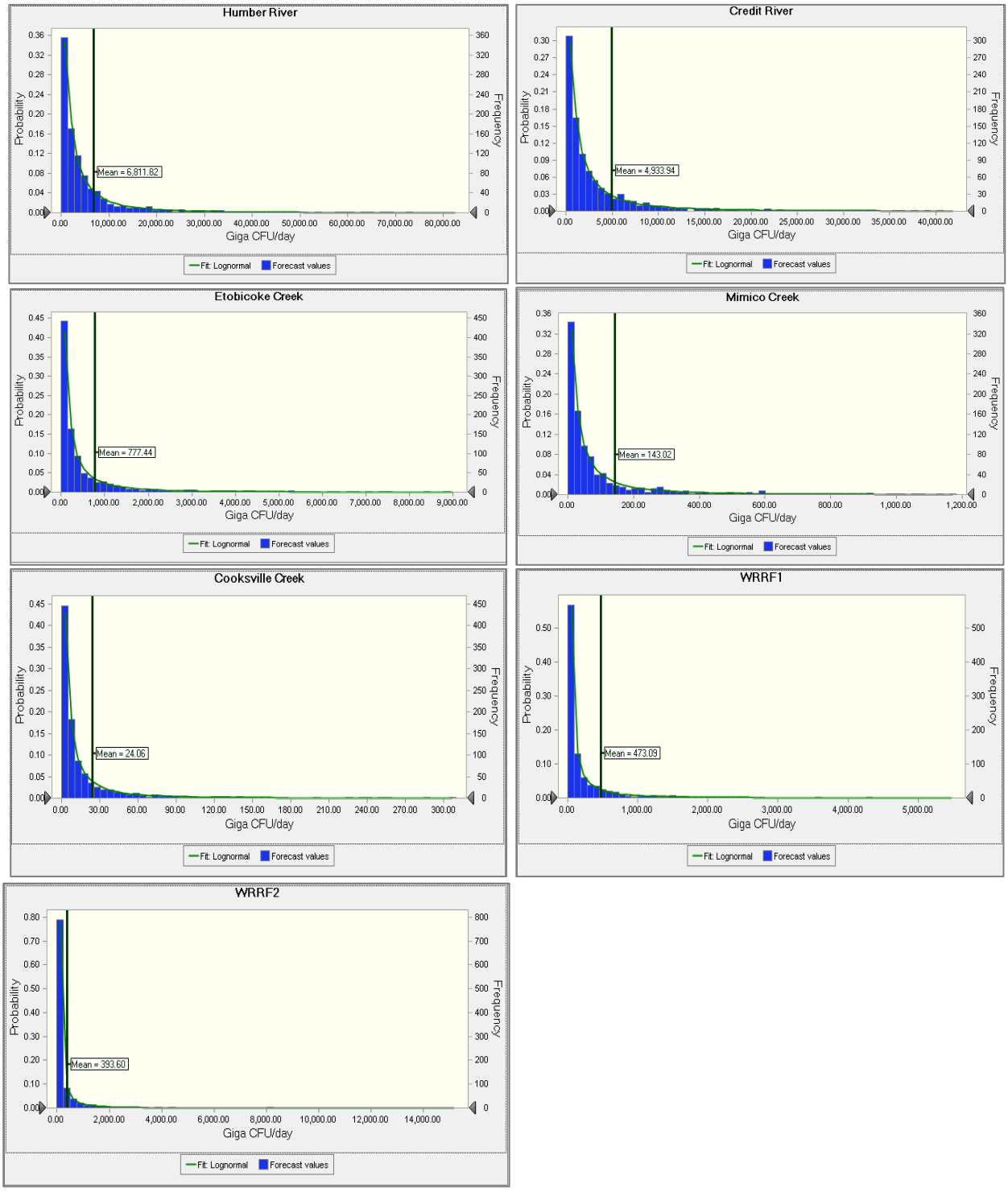


Figure 6. 9: Probability density function of *E. coli* loadings (Giga-CFU/day) for rivers (Humber and Credit Rivers), creeks (Etobicoke, Mimico and Cooksville Creeks) and effluent discharge of WRRF1 and WRRF2 irrespective of weather condition

CHAPTER7 SUMMARY AND GENERAL DISCUSSION

This chapter highlights the main findings of this research project. The general objective was to present a risk-based management framework for source water protection planning. To achieve this goal: 1) fecal contamination sources that may possibly have an impact on a DWI of a Laurentian Great Lake were identified; 2) the fecal sources were ranked using probabilistic approaches; 3) the influent and effluent of a WRRF (collecting only sanitary flows with inflow and infiltration) was characterized for pathogenic protozoa, fecal indicator bacteria, wastewater micro-pollutants and total suspended solids under dry and wet weather conditions; 4) microbial loadings from the studied WRRF and other sources were quantified during normal/baseline and heavy rainfall events; 5) excess loads from by-pass discharges were quantified relative to effluent discharges under wet weather conditions 6) the most important factors governing the loadings from the influent and effluent were determined 7) the contribution of sewer process to mass loadings from the influent were evaluated 8) probability distribution functions of concentrations were estimated at a drinking water intake 9) combined discharged based hydrodynamic model with QMRA was performed to assess microbial risk associated with drinking water following various loading scenarios; 10) finally, best management practices for risk mitigation and/or elimination were suggested.

The most important research questions that were addressed through this research project are listed below.

I. How the dynamic behaviour of target parasites, fecal indicator bacteria, WWMPs and TSS in raw sewage change under dry and wet weather conditions in relation to flowrate? What is the range of concentration variation within an event and between events? What is the relationship between the variability pattern of parasites, FIB, WWMPs and TSS concentrations with the peak flowrate during wet weather events? Can WWMPs be used as markers of waterborne pathogens in surface waters impacted by untreated sewage discharges?

II. At a WRRF served only by sanitary flows with high level of infiltration/inflow, how is the variability of parasite, FIB, WWMPs and TSS mass loadings under various conditions (dry weather *versus* wet weather, normal operating conditions *versus* incomplete

treatment and final effluent discharges *versus* by-pass discharges)? What are the most important environmental factors controlling the concentrations at the influent and effluent? What is the contribution of sewer process to the contaminant loadings arriving in to WRRF?

III. How do the environmental loadings under dry and wet weather conditions change microbial risk at a DWI? How is the impact of short-term events such as WRRF by-pass discharges on a downstream DWI?

As mentioned before, this dissertation contains two main themes: (1) event-based monitoring of a sanitary WRRF and (2) assessing the variability of microbial risk at a DWI following various loading conditions. The discussion is presented according to the two themes. The variability of contaminants between and within events is, first of all, discussed in terms of concentrations (Article 1) and loads (Article 2). In addition, appropriate indicators of fecal pollution in by-pass discharges (Article 1) as well as the major controlling factors of contaminant concentration in influent and effluent (Article 2) were discussed. Furthermore, the variability of contaminant concentrations with peak flowrate (Article 1) and the contribution of sewer process for the loadings of contaminants into a studied WRRF (Article 2) were demonstrated. Finally, we identified the relative importance of fecal contamination sources (rivers, creeks, WRRFs) with respect to *Cryptosporidium*, *Giardia* and *E. coli* loads and evaluated the variability of microbial risk at a DWI under baseline/normal loading and precipitation driven loading events, particularly by-pass discharges (Article 3).

7.1 Event-based monitoring of a water resource recovery facility

7.1.1 The influence of infiltration and inflow on the flowrate

The flowrate of a WRRF served by separate sewer system depends on human activity (such as time of day and/or day *versus* night) as well as inflow and infiltration (as a result of groundwater table depths, precipitation and snowmelt). In order to show how each event was influenced by precipitation or snowmelt, ΔQ (Channel 2's event flowrates - the average flowrate for its corresponding month) and historical hourly flowrates for the corresponding months in Channel 2 are shown in Figure 4. 1a. Regardless of the time of day the events occurred, ΔQ was generally

higher for the events with 2-day cumulative rainfall > 15 mm prior to sample collection (Ev1, Ev3 and Ev5); suggesting a link between rainfall and flowrate in those events. Among the events monitored, ΔQ was highest for Ev3 (June 12th, daytime, 2-day cumulative rainfall of 24 mm) and lowest for Ev6 (September 10th, night time, trace precipitation) ($p < 0.05$), further demonstrating the effect of precipitation on flowrates. Historical data analysis indicates that the flowrate was usually higher in Channel 2 during the spring months, particularly in April following the snowmelt period (Figure 4. 1b).

7.1.2 Prevalence and removal rate of contaminants

We monitored the influent and effluent of a WRRF served only by a sanitary sewer system with high level of infiltration/inflow for pathogenic parasites (*Cryptosporidium* and *Giardia*), FIB (*E. coli* and *C. perfringens*), WWMPs (CAF, CBZ, CBZ-2OH, ACE, SUC and ASP) and TSS under varying weather conditions ranging from trace to intense rainfall as high as 32mm.

The prevalence of *Giardia* was higher than *Cryptosporidium* in raw sewage (88.6% *versus* 8.6%) and in treated effluent (100% *versus* 30%). Bacterial indicators including *E. coli* and *C. perfringens* were observed in 100% of the influent and effluent samples. In another study of the same WRRF, 40% and 90% of samples were positive for the *Cryptosporidium* and *Giardia* in the treated effleunt (Edge et al., 2013). The occurrence of both *Cryptosporidium* and *Giardia* in treated effluent was higher than in raw sewage as expected due to the complex matrix of raw sewage and the resulting lower recovery efficiency. This has already reported by other studies (e.g. Rose et al., 2004; Lalancette et al., 2012). For both *Cryptosporidium* and *Giardia*, higher recovery rates were detected in treated effluent than in raw sewage. For *Cryptosporidium*, the recovery rate was in the range of 9.1% to 76.8% and 0% to 44.7% in treated effluent and raw sewage, respectively. For *Giardia*, it also ranged from 32.3% to 66.3% in treated effluent and 0% to 91.9% in raw sewage. Highly variable *Cryptosporidium* recoveries were also reported in raw sewage (0% to 83.8%) and in secondary treated effluent (0% to 62.6%) of ten WRRFs across the US (McCuin et al., 2006).

The concentrations of *Cryptosporidium*, *Giardia*, *E. coli* and *C. perfringens* in raw sewage were in the range of below the limit of detection (LOD) to 10 oocysts/L, below the LOD to 1010

cysts/L, 2.6×10^5-2.7×10^7 CFU *E. coli*/100 mL and 2.4×10^3- 3.2×10^5 CFU *C. perfringens* /100 mL. Their concentrations in treated effluent also varied from below the LOD to 0.2 oocysts/L, 0.1 to 11.1 cysts/L, 2-1000 CFU *E. coli*/100 mL and 225-750 CFU *C. perfringens* /100 mL. The measured concentrations of *Giardia* and FIB were lower in treated effluent than in raw sewage. Total removal efficiency of *Giardia, E. coli* and *C. perfringens* were in the range of 72.6% to 99.9%, 99.9% to 99.99% and 98.2% to 99.7%, respectively. Depending on the plant sizes and treatment conditions, various values have been reported for the removal efficiencies of fecal contaminants in WRRFs in literature (Kistemann et al., 2008; Fu et al., 2010). The observed concentrations in raw sewage and treated effluent as well as total removal efficiencies fall within the ranges documented in the literature (Tables 4. 1, 4. 10, 4.7. 11).

Micropollutants including CAF, CBZ, CBZ-2OH, ACE and SUC in raw sewage and treated effluents were observed in 100% of samples, while ASP was detected in 92.7% of raw sewage samples and was absent in the treated effluents. ASP was not also detected in treated effluents of two plants in Tjanjin, China (Gan et al., 2013). The absence of ASP in effluent samples is attributed to the its higher degradability (Kokotou et al., 2012; Lange et al., 2012). Among three studied artificial sweeteners (ACE, SUC and ASP), the median concentration of ASP was significantly lower than others ($p < 0.05$ in Mann-Whitney U test). It suggests different consumption patterns of ASP compared to ACE and SUC in addition to its higher degradation rate. The elimination rate of ASP from the human body is larger compared to other studied artificial sweeteners (Nabors, 2001), while plenty of ACE and SUC find their ways into receiving waters in an unchanged form (Buerge et al., 2009).

Detected concentrations of WWMPs were lower in treated effluent than in raw sewage, CBZ being an exception (Table 4. 1). In our study, the CBZ concentration demonstrated an average increase of 26% during the treatment process. CBZ is not easily degraded or adsorbed during treatment processes as it is more persistent (Tran et al., 2018). An increase by as much as 100% in treated effluents has previously been reported for CBZ (Miao et al., 2003; Clara et al., 2004; Gao et al., 2012; Bahlmann et al., 2014). This behaviour is possibly related to the partial cleavage of N-glucuronide conjugates during treatment processes. In raw sewage and treated effluent, the concentration of CBZ-2OH was usually higher than its parent CBZ and could be due to the different nature of their glucuronides; CBZ forms N-glucuronides while CBZ-2OH is excreted as

O-glucuronides like all hydroxylated metabolites of CBZ. The studied WRRF could effectively remove CAF and TSS, while poor removal and even negative removals were observed for CBZ, CBZ-2OH, ACE and SUC (Table 5. 2). These observations are also consistent with other Canadian and non-Canadian WRRFs (Miao et al., 2003; Miao et al., 2005; Buerge et al., 2009; Lee et al., 2011; Scheurer et al., 2011; Sim et al., 2011; Gao et al., 2012; Lee et al., 2013; Hoque et al., 2014; Subedi et al., 2014b).

7.1.3 Characterizing temporal variability of contaminant concentrations in raw sewage

7.1.3.1 Between and within events fluctuations

The contaminant concentrations that were monitored under various rainfall and flowrate concentrations were evaluated between and within events. Concentration variations were higher for microorganisms than for WWMPs (up to 2 orders of magnitude) (Figures 4. 2, 4. 3 and 4. 4) and the variation of CBZ and its metabolite CBZ-2OH was lower among studied WWMPs (Figures 4. 3, 4. 4 and 4.7. 1).

Within events, the peak value of *Giardia* (1010 cysts/L) was observed during the first June wet weather event with the highest ΔQ (as compared to other events). The median concentration of *E. coli* was higher during wet weather events than during dry weather events ($P<0.05$ in Mann-Whitney U test). The median concentrations of *C. perfringens* were significantly higher in the spring weather events (April and May weather events) as compared to other monitored events (Figure 4. 2). Sewer processes including sewer deposit resuspension and shorter travel times as a result of the addition of wastewater flow and inflow from stormwater are dominant drivers of the contaminant concentrations in the sewer lines. Sewer deposit resuspension depends on the type and configuration of the sewershed, rainfall intensity and antecedent dry period (Madoux-Humery et al., 2015). The association of *C. perfringens* with settleable particles and their long survival in surface waters and sediments has been discussed by others (Edwards et al., 1998; Lisle et al., 2004; Krometis et al., 2007; Mueller-Spitz et al., 2010). The flowrate is generally higher at the studied WRRF in spring as a result of higher infiltration/inflow; hence *C. perfringens* was more likely to accumulate in sewer sediments during low flow conditions and

were mobilized by higher flows following snowmelt period. The results demonstrate the greater impact of untreated or partially treated sewage discharges on Lake Ontario during wet weather and snowmelt periods. Higher flows do not necessarily lead to greater dilution of wastewater effluents.

The temporal variability of contaminant concentrations over the course of a day was evaluated (Figure 4. 4) and its relationship with flowrate was discussed (refer to section 7.1.3. 2). Over the course of a day, *E. coli* and *C. perfringens* concentrations decreased overnight (0:00 to 7:00 AM), then they increased and reached their peak values in the early afternoon during wet weather events. For *E. coli*, the peak value was observed later during dry weather periods (around 3:00 PM) as compared to wet weather periods (around 11:30). Flowrate, defecation patterns and residence time in sewer system are drivers of the concentrations in raw sewage. During wet weather periods, the inflow enhances the flowrate and velocities within the sewer network and consequently makes the travel time of the fecal contamination shorter (Madoux-Humery et al. 2013). No temporal trend for *Giardia* over the course of a day was observed due to uncertainties related to the methods and their sporadic spatial occurrence in a sewershed. Since fewer samples were positive for *Cryptosporidium* in raw sewage (prevalence rate of 8.6%), temporal patters of *Cryptosporidium* in raw sewage samples were not observed.

A clear trend was observed for CAF, SUC and CBZ over the course of a day when considering all monitored events. Their concentration decreased overnight (0:00 AM to 7:00 AM) and then started to increase from 7:00 AM and reached their peak values at 6:00 PM for both CAF and SUC and 3:30 PM for CBZ (Figure 4.4). The consumption patterns of WWMPs, their half-lives in the body, their excretion pathways and retention time in the sewer system were identified as the most important factors that control their concentrations in untreated sewage (Madoux-Humery et al. 2013). Clear temporal trends were not observed for CBZ-2OH, ACE, ASP and TSS over the course of a day (Figure 4. 6).Our study shows that the afternoon and/or early evening are critical times with regards to discharge of untreated or partially treated sewage discharges into Lake Ontario because that is when the concentrations and flowrates were the highest.

7.1.3.2 Relationship between concentrations and flowrate

The concept of mass-limited and flow-limited contaminants (e.g. Cristina et al. (2003)) was used to describe the effect of flowrate on the microorganisms, WWMPs and TSS concentrations as well as the relationships between the variability pattern of contaminant concentrations and peak flows. With mass limited contaminants, the source of the contaminants is exhausted (or diluted) and hence mass is the governor of the pollution concentrations and loads; while with flow limited concentrations, the source of the contaminants remains; thus the concentration will increase as flow increases (Piro et al., 2014). We identified the major environmental factors that control the contaminants' concentrations in raw sewage and treated effluent of a WRRF (served only by sanitary flow and impacted by a high level of infiltration/inflow during wet weather periods) by the log concentration-log flowrate (log C- log Q) plots (Figure 5. 1). This trend was previously evaluated for hormones, WWMPs and indicator bacteria in CSOs, raw sewage and treated effluent of WRRFs severed by combined sewer systems (Phillips et al., 2012; Madoux-Humery et al., 2015). The slope in Log C- Log Q plots shows the significance of dilution on contaminant concentrations. The slopes greater than -0.7, indicates that the decrease of concentrations is in lower rate than the increase in flowrates.

In raw sewage, *Giardia, E. coli* and *C. perfringens* concentrations increased with increasing flowrate (Figure 5. 1). It indicates that the fate and transport dynamics of microbial contaminants in the sewer networks were similar. In addition, the concentrations of fecal microorganisms were not strongly mass limited and hence were not strongly affected by dilution processes (as a result on infiltration/inflow). This could be partly due to greater resuspension of sewer sediments, less deposition as a result of high flows, less die-off due to shorter travel time, less degradation and higher flows associated with higher fecal loads. In the effluent, the concentration of *Giardia* decreased as flowrate increased, while the concentrations of *E. coli* and *C. perfringens* increased with increasing flowrate (Figure 5. 1). The observed trends indicate that decrease of treatment efficiency during wet weather periods due to the decrease of hydraulic retention time influences the concentrations of *E. coli* and *C. perfringens* in treated effluent. Given that *Giardia* is environmentally resistant, treatment efficiency and degradation processes were not strongly

influencing *Giardia* concentrations in treated effluent. Dilution processes may be the more relevant environmental factor that controls their loads.

The slope of log C- Log Q plots in raw sewage samples for CAF, CBZ, CBZ-2OH and SUC ranged between 0.4-0.44, while the slope of ACE and TSS were -1.1 and -0.6 respectively (Figure 5. 1). The correlations were not statistically significant for SUC and TSS ($p>0.05$). It indicates that the ACE concentration was strongly mass limited and hence the dilution process controls its load in raw sewage. This behaviour can be partly explained by its higher solubility compared to other studied WWMPs (Table 4. 7). For TSS, the slope remained above -0.7, which reflects that non-wastewater sources such as sewer deposit resuspension may contribute to the loads of TSS and reduces the effect of dilution.

In the effluent, the slope of all observed WWMPs decreased with increasing flowrate (Figure 5. 1). However, it should be noted that the correlations were not statistically significant ($p>0.05$) and were based on only 6 data points. Biodegradation seems to be the governor of CAF loads in effluent due to its much shorter half-life (0.8-5 h) as observed in wastewater with biological processes (Buerge et al., 2003). Dilution might be a primary factor that influences CBZ, CBZ-2OH, ACE and SUC loads in the effluent as they are less biodegradable and more persistent in WRRFs (Miao et al., 2003; Lange et al., 2012; Sauvé et al., 2012; Subedi et al., 2014a; Tran et al., 2018).

The variability of contaminant concentrations in relation to peak flowrates were examined as by-pass discharges usually occur following high flow conditions and sampling is rarely conducted during by-pass discharges due to the difficulties related to sampling and prediction (Figure 4. 5). From a drinking water perspective, peak contaminant concentrations in source waters need to be addressed to ensure the appropriateness of downstream drinking water treatment plant processes. The concentration variations (C/C_{peak}) for the last decile of normalized flowrate ($0.9<Q/Q_{peak}<1$) were higher for *Giardia, E. coli, C. perfringens* and TSS (with the median values in the range of 0.3-0.73) and lower for CAF, CBZ, CBZ-2OH and SUC (with the median values in the range of 0.79-0.9). This indicates that the peak concentrations of microorganisms may not be observed during high flow conditions and hence they are more mass limited than flow limited with regards to their sources in sewer networks.

7.1.4 Selection of appropriate markers of fecal pollution in surface waters impacted by WRRF by-pass discharges

Sources, fate and transport characteristics of contaminants, which are variable in sewer systems, play an important role on the selection of appropriate indicators. Hence, the correlation among pathogens, fecal and chemical indicators should be assessed and should also consider the effects of weather conditions that can influence sewer processes governing their fate.

In order to determine a suite of suitable indicators of fecal contamination in a by-pass discharge and/or failed WRRF, the correlations among parasites, FIB and WWMP concentrations in raw sewage were assessed following trace precipitation and during wet weather conditions (Table 4. 2). In wet weather, *Giardia, E. coli* and *C. perfringens* were positively and significantly correlated with CBZ, CBZ-2OH and CAF ($R \geq 0.4$, $p < 0.05$), whereas *Giardia* correlations with *E. coli* and *C. perfringens* were insignificant. Significant correlations were also observed between *C. perfringens* and *E. coli* during wet weather conditions ($R = 0.76$, $p < 0.05$). CAF is biodegradable and can easily be removed through activated sludge processes (Kbiol>10 L/gMLSS d in activated sludge) (Xue et al., 2010), while CBZ is persistent (kbiol=0.005-0.389 L/gMLSS d in activated sludge) and its removal is usually less than 20% during biological wastewater treatment (Tran et al., 2018). Given the positive correlations between CBZ and the studied microorganisms, the refractory behaviour of CBZ and the environmental persistence of *Giardia*, CBZ appears to be the most suitable marker of fecal contamination in by-pass discharges and/or failed treatment following wet weather conditions. The presence of CBZ in source waters indicates a potential to detect *Giardia*.

In trace precipitation weather conditions, *Giardia* and *E. coli* were significantly correlated ($R = 0.79$, $p < 0.05$); suggesting *E. coli* as suitable indicators of *Giardia* in WRRF effluent during a treatment failure. However, further investigation is needed as this finding is based on a small data set (N=7).

7.1.5 Mass loadings variability from studied WRRF

In order to assess the variable impact of the studied WRRF on Lake Ontario and consequently on a DWI, mass loadings were evaluated from influent (representative of failed treatment), primary

effluent (representative of by-pass discharge) and effluent (representative of normal operation conditions) under trace precipitation and wet weather conditions (Figures 5. 3 and 5. 4).

The average mass loadings of fecal pollution per 1000 people were 6.5 log oocysts/d, 7.9 log cysts/d and 13.2 log CFU *E. coli*/d and 11.4 log CFU *C. perfringen*s/d from the influent; and were 4.5 log oocysts/d, 6.2 log cysts/d, 7.9 log CFU *E. coli*/d and 9.3 log CFU *C. perfringen*s/d from the effluent under dry and wet weather conditions. The median load of *E. coli* from the influent and *C. perfringens* from the effluent were significantly higher during wet weather ($p<0.05$ in Mann-Whitney U test test). Maximum mass loading of *Giardia* and *C. perfringens* from the influent (8.8 log cysts/d/1000 people and 12.5 log CFU/d/1000 people) and *E. coli* and *C. perfringens* from the effluent (9.8 log CFU *E. coli*/d/1000 people and 9.7 log CFU *C. perfringen*s/d/1000 people) were also observed during wet weather. For all studied WWMPs, the maximum mass loadings from the influent and effluent were generally observed during wet weather. However, the maximum mass loadings of CAF from the influent and ACE from the influent and effluent occurred during dry weather period maybe as a result of the higher solubility of ACE as compared to other studied WWMPs (see Table 4. 7).

Mass loadings from the primary effluent during a by-pass discharge were estimated based on an assumption for the by-pass flowrate and duration (Figures 5. 3 and 5. 4). Two fractions were calculated: i) the fraction of mass loadings from primary effluent following a by-pass discharge to effluent discharges during wet weather (F_1) and ii) the fraction of effluent loadings during dry weather to effluent loadings during wet weather period when a by-pass occurs (effluent and by-pass discharges, F_2). We found that excessive loads of *Giardia*, *E. coli*, *C. perfringens*, CAF and TSS are being discharged into Lake Ontario following by-pass discharges in addition to effluent discharges; hence the downstream drinking water treatment plant must be able to handle these extra loads (Table 5. 3). Interestingly, the contribution of loads of contaminants with high removal efficiencies from by-pass discharge is more important than that of parameters with poor removal efficiency rates. With the exception of ACE, the values of F_2 were <1 for all studied contaminants, suggesting higher contaminant loadings into Lake Ontario during wet weather periods (when a by-pass occurs) (Figure 5. 5). The value of F_2 remains ≥1 for ACE and could be partly explained by the higher solubility of ACE as compared to other studied WWMPs. It seems that the concentration of ACE is sensitive to dilution processes.

In summary, these findings indicate that routine monitoring following regular sampling dates is not adequately representative; hence meteorological conditions should be considered in source monitoring for the better quantification of risk at DWIs.

7.1.5.1 Sewer process contribution to the mass loadings arriving at WRRF

The contribution of sewer processes to contaminant loadings into a WRRF (served only by sanitary sewer and impacted by a high level of infiltration/inflow during wet weather periods) was evaluated in relation to flowrate (Figure 5. 2). As expected, the sewer process contribution varied extensively among studied contaminants and events depending on flowrate. As mentioned, the flowrate of the WRRF was usually higher during the spring and daytime (see section 7.1.1). The contributions of sewer processes (deposition and resuspension, shortened travel times) for the contaminant loadings were generally higher during the April and the first June wet weather events (events that occurred in spring and day time, respectively). This could be partly related to more resuspension of sewer sediments, less deposition with higher flows and less degradation in addition to higher fecal loads in tandem with higher flows.

The contribution of sewer processes to the mass loadings of *E. coli*, *C. perfringens* and TSS was in the range of 10%-83%. Sewer sediment resuspension contribution for the loadings of FIB including *E. coli*, *Enterococci*,and TSS was demonstrated in CSOs (Chebbo et al., 2001; Gasperi et al., 2010; Passerat et al., 2011; Madoux-Humery et al., 2015). Depending on rain events, sewer deposit resuspension contribution to the TSS load from CSO discharges has been shown to be extensively variable; 10%-70% for low-intensity events and >60% for the high intensity events (Gasperi et al., 2010).

For the studied WWMPs, the contribution of sewer processes for the loadings of CBZ, SUC and ASP was higher. In contrast, its contribution to ACE loadings was limited. Sorption of WWMPs to sludge/biosolids depends on their physiochemical properties and molecular structures (Tran et al., 2018). CBZ with log K_{ow} < 3 and partition coefficient (K_d) of 17-66 L/kgMLSS in lab-scale (Jones et al., 2002; Urase et al., 2005; Wick et al., 2009) and <8-314 L/kgMLSS in full scale sewage sludge (Abegglen et al., 2009; Radjenović et al., 2009; Stevens-Garmon et al., 2011; Yan et al., 2014) seems to exhibit low sorption potential. However, Hajj-Mohamad et al. (2017)

showed that the desorption constants of CBZ from the native suspended and settled sediments of a combined sewer system was higher as compared to CAF even though CAF had a higher sorption coefficient. Thus, the contribution of sewer processes for the loadings of CBZ might be partly explained by its lower biodegradability (k_{biol}=0.005-0.389 L/gMLSS d in sludge (Plósz et al., 2009; Fernandez-Fontaina et al., 2013; Casas et al., 2015; Alvarino et al., 2016)) and its desorption characteristics. Madoux-Humery et al. (2015) identified the sewer sediments as a reservoir for CBZ in the majority of monitored CSO events and showed that the contribution of the mixture of run-off and sewer sediments for the CBZ load reached as high as 86% in the first 50% of the total discharged volume, which discharged the largest fraction of WWMPs. For the studied artificial sweeteners (including ACE, SUC and ASP) this result can also be explained by their physicochemical properties (refer to Table 4. 7). The sorption of ASP to sewer sediments appears to be higher (with lower water solubility and relatively higher log K_{ow}) as compared to ACE and SUC. The fraction of total ASP sorbed to suspended particulate matter was reported to be 50.4% and higher than that of ACE and SUC (Subedi et al., 2014a).

7.2 Event-based microbial risk at a drinking water intake

Microbial loadings (*Cryptosporidium*, *Giardia* and *E. coli*) from two rivers, three creeks and effluent discharges of two water resource recovery facilities were estimated by probabilistic approaches. For the baseline and precipitation driven loading events, the concentrations of target microorganisms were also estimated at the studied DWI from the identified fecal contamination sources by a three-dimensional hydrodynamic model (Figure 6. 1). Finally, the impact of source water quality variability on the studied drinking water intake was investigated by applying the HC QMRA model (Douglas et al., 2015)

7.2.1 Microbial loadings from various fecal sources under dry and wet weather conditions

We estimated *Cryptosporidium*, *Giardia* and *E. coli* loadings from two rivers (Humber and Credit rivers), three creeks (Etobicoke reek, Mimico and Cooksville creeks) and two WRRFs ($WRRF_1$ and $WRRF_2$) using probabilistic approaches during dry and wet weather periods (2008-2010). Considering all sources, the mean of the estimated probability distribution functions of

Cryptosporidium, *Giardia* and *E. coli* loadings was in the range of 6.0-8.2 log oocysts/d, 6.3-9.4 log cysts/d and 10.4-12.8 log CFU/d, respectively. There were significant differences in loadings among the various sources (rivers, creeks and WRRF effluent discharges) ($p<0.05$ in likelihood-based test). The estimated loadings for the *Cryptosporidium*, *Giardia* and *E. coli* are in the ranges of reported values from the Sûre River and effluent discharges of a WRRF in Luxembourg (Burnet et al., 2014).

The contributions of various sources including rivers, creeks and WRRFs for the total microbial loadings into Lake Ontario were quantified in order to determine the relative importance of fecal contamination sources (Figure 6. 2). The results indicate that the rivers were the most important contributor of *Cryptosporidium* and *E. coli* loadings into Lake Ontario as compared to creeks and WRRFs; while the contribution of WRRF effluent discharges for the loadings of *Giardia* were most important.

7.2.2 Event based hydrodynamic model linked to probabilistic microbial loadings

The generated time series of *Cryptosporidium*, *Giardia* and *E. coli* from the probability distribution functions of identified fecal contamination sources and deterministic flowrate data from the point of discharge (8 time series with hourly data over the course of a day (24h)) were used as inputs to a hydrodynamic model under various loading scenarios (SC1 (dry weather scenario), SC2 (wet weather scenario without any by-pass discharges) and SC3 (wet weather scenario with by-pass discharges from both $WRRF_1$ and $WRRF_2$). The log-normal distributions were fitted to the simulated concentrations at the intake and were compared with the probability distribution functions of the observations (Figure 6. 3, Table 6. 2). For *Cryptosporidium*, we were unable to run SC3 and compare the modelling results with the observations due to a lack of sufficient data.

According to the modelling results, the differences between the observed and simulated concentrations were insignificant ($p>0.05$ in likelihood-based test), but the estimated concentrations were slightly lower than observations and peak observations were not well simulated. The underestimation could be explained by the unquantified fecal contamination

sources such as birds, gulls, dogs and other animals. In southern Ontario, non-point sources such as gulls contribute to fecal pollution loads in urban coastal and riverine areas (Lu et al., 2011).

The results show that the differences among simulated concentrations under various loading events (SC1, SC2 and SC3) were statistically significant ($p<0.05$ in likelihood-based test). They were in the rank order of SC3>SC2>SC1. It confirms the increase of up to 1log fecal loads at the DWI following wet weather conditions compared to dry weather conditions. In addition, it suggests that higher fecal loads are delivered to the DWI during infrequent by-pass events. We also found that the fate and transport of target microorganisms in Lake Ontario is mostly controlled by the wind, currents, up-welling and/or down-welling events in addition to inflows as also suggested by other studies conducted in Lake Ontario (Rao et al., 2012; Edge et al., 2013; Paturi et al., 2014).

7.2.3 Risk assessment in a drinking water intake under various loading scenarios

The HC QMRA model is an effective tool for source water protection and planning even with its many simplifications. The relative increase and decrease of risk at a DWI following baseline and precipitation driven loading events were evaluated. According to modelling results, the estimated daily probability of infection was in the range of 2.7×10^{-13}-4.1×10^{-9} person^{-1} day^{-1} for the reference pathogens studied including *Cryptosporidium*, *Giardia* and *E. coli* O157:H7 following all tested scenarios. As expected, the modelling results indicate that the level of risk was higher during wet weather periods (SC3 and SC2) than during dry weather periods (SC1) and following by-pass event from both WRRFs (SC3) compared to situation when no by-passes occur (SC2) (Figure 6. 4). In addition, it suggests that the level of risk from *Giardia* and *Cryptosporidium* was higher as compared to *E. coli* O157:H7. Although the risk level was higher following wet weather periods, particularly SC3, it was lower than the risk benchmark of 2.7×10^{-7} person^{-1} day^{-1}. However, it should be noted that, for SC3 we couldn't estimate the microbial risk associated with *Cryptosporidium* and it might be higher than other scenarios tested. In addition, inadequate physical removal and chemical inactivation in water treatment plant could increase the risk level.

CHAPTER8 CONCLUSIONS AND RECOMMENDATIONS

In order to fulfill the main and specific objectives of this research project, influent and effluent of a WRRF were monitored for pathogenic parasites, FIB, WWMPs and TSS under weather conditions with varying precipitation patterns. For source water protection and planning, a discharged based hydrodynamic model coupled with deterministic-probabilistic loading and QMRA models was applied to assess microbial risk at a drinking water intake. The following conclusions were derived from the monitoring and modelling parts of this research project. As in previous sections, the conclusions of this study are also presented according to the two main themes.

I. Event-based monitoring of a water resource recovery facility

- Investigated pathogenic parasites, indicator bacteria and wastewater micro-pollutants were detected in raw sewage and treated effluents. *Cryptosporidium* and *Giardia* were detected in 88.6% and 8.6% in raw sewage samples and 100% and 30% in treated effluents, respectively. The occurrence was higher in effluents due to the higher recovery efficiencies from reduced water matrix effects.
- The concentrations of all contaminants decreased in treated effluent, CBZ being an exception. CBZ increased 26% in treated effluent possibly due to the cleavage of N-glucuronide conjugates during wastewater treatment.
- In this conventional WRRF, *E. coli* and CAF (with removal efficiency rates >99%), *Giardia, C. perfringens* and TSS (with removal efficiency rates >90%) were reduced through wastewater treatment; while CBZ, CBZ-2OH, ACE and SUC (with removal effeciency rates <70%) persisted and found their way into Lake Ontario.
- In raw sewage, inter-event and intra-event variability of microorganisms were higher as compared to WWMPs (up to 2 orders of magnitude). The variability of CBZ and CBZ-2OH was the lowest. Greater variability was also observed following wet weather periods.
- Daily patterns related to human behaviour mostly influenced the contaminant concentrations in raw sewage. Precipitation events only moderately influenced the concentrations. Afternoon and early evening as well as the snowmelt and wet weather

periods were identified as potential critical times with respect to the impact of untreated and/or partially treated wastewater discharges into Lake Ontario.

- In raw sewage, *Giardia, E. coli and C. perfringens* are weakly and positively correlated with flowrate; it suggests fecal microorganisms are not strongly mass limited. Thus, dilution processes (as a result of infiltration/inflow) are not strongly reducing the concentrations of fecal microorganisms in by-pass discharges.
- Given the lower biodegradability of CBZ in activated sludge processes and its refractory behaviour which is similar to that of *Giardia* (due to being environmentally resistant), CBZ was identified as a suitable marker of fecal pollution in untreated and/or partially treated sewage such as by-pass discharges.
- At the WRRF influent, ACE was the only contaminant that was affected by dilution processes as a result of infiltration/inflow into sewer lines during wet weather conditions.
- With the exception of ACE, the relative contribution of sewer processes to the total loads of the all studied contaminants differed depending on the type of event and increased with increasing flowrate. Among studied artificial sweeteners, sewer process contribution was higher for the ASP load as a result of its lower solubility and higher potential to be sorbed to the sewer sediments as compared to ACE and SUC.
- The fractions of mass loadings from WRRF by-pass discharges relative to mass loadings from treated effluent discharges were higher for microbial parameters with higher total removal efficiency rates as compared to those of WWMPs with lower total removal efficiency rates during wet weather conditions.
- It is recommended to assess *Cryptosporidium* and *Giardia* recovery efficiency rates in every single sewage sample as they are highly variable in raw sewage and treated effluents over various time scales and between the *Cryptosporidium* and *Giardia*.

II . Event-based microbial risk at a drinking water intake

- The most important contributor of *Cryptosporidium* and *E. coli* loads into Lake Ontario was the riverine load. WRRFs were most important for *Giardia*.

- For all tested scenarios, the level of risk associated with *Cryptosporidium, Giardia and E. coli* O157:H7 (2.7×10^{-13}-4.1×10^{-9} person^{-1} day^{-1}) was lower than risk benchmark of 2.7×10^{-7} person^{-1} day^{-1} even though the risk level increased 0.5 log to 1.2 log during precipitation-driven loading conditions.
- The risk associated with *Giardia* and *Cryptosporidium* was higher, highlighting the impact of both sewage and rivers discharge on downstream drinking water intake studied.
- Coupled hydrodynamic modelling and QMRA is an effective tool for risk mitigation and management with respect to source water protection and planning.

This work also highlighted new ideas for future research. It would be interesting to:

- Collect samples from the mouth of rivers and creeks and the point of discharge of WRRFs including by-pass discharges and treated effluents into Lake Ontario at the same time and DWI (considering time lag in Lake), enumerate those samples to infectious parasites and viruses and perform combined discharge-based hydrodynamic model with QMRA using infectious data rather than raw concentrations.
- Examine the feces of animals from riverine and coastal area to parasites, estimate their loadings into Lake Ontario and finally evaluate their impact on a downstream DWI.
- .Monitor the dynamic behaviour of microorganisms in a drinking water intake under various conditions.
- The impact of urban fecal sources on downstream drinking water intakes is important and it is necessary to identify the sources of fecal contamination in the Credit and Humber Rivers.
- The impact of sewage discharges on downstream drinking water intake was pronounced and hence the advanced methods for the removal of waterborne pathogens from sewage discharges (including WWRF by-pass and effluent discharges, sanitary sewer overflows and combined sewer overflows) should be considered.

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