Assessing quality of source water on Six Nations Reserve

Assessing potability of drinking-water sources and quality of surface water on the Reserve of the Six Nations of the Grand River, Ontario (Canada)

By

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General Abstract

Although water covers 70% of the earth's surface, less than 1% of it is freshwater that can be used for drinking. Even in Canada, where there is an abundance of freshwater in groundwater and in rivers and lakes, there are many indigenous communities that lack a sustainable source of drinking water. Such is the case for the Six Nations of the Grand River, the largest indigenous Reserve in Canada, located within an hour drive from major urban centers in southern Ontario and where less than 9% of the residents have access to safe, treated potable water. The major tributaries that drain the Six Nations reserve are part of the McKenzie Creek Watershed, which has been characterized as having the highest loading of sediments and nutrients to the lower Grand River, which eventually drains into the eastern basin of Lake Erie. This research project was initiated by the Six Nations community, who wanted an update on the prevalence of fecal contamination in their drinking water sources (wells, cisterns). Secondly, the community wanted to know the ecosystem health status of tributaries flowing through the Six Nations Reserve (McKenzie and Boston Creeks), and to determine if land uses in the watershed were negatively affecting the health of these streams. A study conducted in the summer of 2018 confirmed that 29% of the tap water tested in 75 households were contaminated with *E. coli;* 40% of the wells and 15% of the cisterns were contaminated and these were distributed throughout the Reserve with no apparent pattern. A study conducted in the summer of 2019 found that the McKenzie Creek was highly polluted with total phosphorus (P), total suspended solids, turbidity and total-ammonia nitrogen (N), while Boston Creek was highly polluted with soluble reactive P and *E. coli* as well as total-nitrate N. Nitrogen concentrations at 14 stations were highly and significantly related to percentage of agricultural land in catchments. Elevated levels of pollutants have been observed in the two creeks for three decades, indicating that conditions will not improve without remedial actions.

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LIST OF ALL ABBREVIATIONS AND SYMBOLS

°C	Degree celsius
%AG	%Agricultural land
%CL	%Clay substrate
%FO	%Forested land
%GR	%Gravel
%HE	%Hedgerow
%MR	%Marsh
%OD	%Organic deposit
%PA	%Paleozoic bedrock
%SA	%Sand
%SI	%Silt
%SW	%Swamp
%TI	%Till
%UR	%Urban land
%WT	%Wetland
BldgD	Building density
BWA	Boil Water Advisory
CFU	Colony forming unit
Cond	Specific conductance
Cr	Creek
DO	Dissolved oxygen
DO % Sat	Percent saturation dissolved oxygen
DWAs	Drinking water advisory
E. coli	*Escherichia coli*
FN	First Nation
GIS	Geographic Information System
GRCA	Grand River Conservation Authority
GRW	Grand River Watershed
HABs	Harmful algal blooms
IK	Indigenous knowledge

INAC	Indigenous and Northern Affairs Canada
km^2	Square kilometer
L.	Lake
LULC	Land use-land cover
MCW	McKenzie Creek watershed
N	Nitrogen
NTU	Nephelometric Turbidity Unit
OHN	Ontario Hydro Network
OMNRF	Ontario Ministry of Natural Resources and Forestry
ORN	Ontario Road Network
P	Phosphorus
PCA	Principal Components Analysis
PDEM	Provincial Digital Elevation Model
SN	Six Nations
SNWTP	Six Nations Water Treatment Plant
SOLRIS	Southern Ontario Land Resource Information System
SRP	Soluble reactive phosphorus
TAN	Total-ammonia nitrogen
TD	Tile drainage area
Temp	Temperature
TN	Total nitrogen
TNN	Total-nitrate nitrogen
TP	Total phosphorus
TSS	Total suspended solids
Turb	Turbidity
WS	Western Science
µg/L	Microgram per liter
µS/cm	MicroSiemens per centimeter
USEPA	U.S. Environmental Protection Agency

Chapter 1: GENERAL INTRODUCTION

Healthy Aquatic Ecosystems

Water covers 70% of the earth's surface, but less than 0.5% of the total water on earth is available for human use (Baker et al. 2016). Canada is fortunate in having an abundance of freshwater; in fact, 20% of the world's total freshwater is found in this country (Molles & Cahill, 2011). Freshwater is important for supporting terrestrial and aquatic biodiversity; it is also required for human survival, for sanitation, navigation and other economic benefits, including commercial and recreational fishing (Metcalfe, 2013; Lui, 2015). Ecosystem health is required to maintain structure (e.g. species composition) and function (e.g. nutrient cycles) of communities, keeping them resilient to stress and ensuring they will be sustainable over time (Karr, 1999). A healthy aquatic ecosystem is free of algal blooms and has high biodiversity, and provides safe drinking water (O'Brien et al., 2016). To monitor health of ecosystems, many researchers have used physical, chemical and biological indicators (Metcalfe et al., 2013; O'Brien et al., 2016).

There is more pressure on freshwater ecosystems than ever before, because of increased anthropogenic pollution sources (O'Brien, 2016) from agricultural and industrial activities (Environment Canada et al., 2006) Agricultural runoff and septic systems contain microbial pathogens associated with intestinal tracts of farm animals and human wastes, and if these enter surface or ground water, they can render the water unpotable (Swain et al., 2006). In addition, surface water gets contaminated likely due to point sources (e.g. wastewater plants, sewage lagoon, industrial discharge, manure) and non-point sources of pollution (e.g. runoff of agricultural and urban areas). Increased nutrients loads and fecal pollution in surface water often lead to eutrophication that result in low dissolved oxygen and eventually to dead zones in lakes (ECCC, 2018).

Consumption and use of water contaminated with fecal coliform (*E. coli*) can cause gastrointestinal illnesses (Health Canada, 2020). Groundwater that is polluted with excess nitrate can lead to acute and sub-lethal conditions in babies (Swain et al., 2006). Surface water that is contaminated with fecal bacteria can also lead to severe gastro-intestinal illnesses if people use these in recreational contact (swimming), while surface waters with high pH and turbidity can increase eye irritation and reduce visibility in the water (Health Canada, 2012). Therefore, impaired water quality is a huge concern because of health consequences to people and negative impacts on aquatic organisms, fisheries, and recreational uses (ECCC, 2020).

<u>Poor Water Quality in Canada</u>

Canada has an abundance of water in streams but 20% of the Canadian rivers were in marginal to poor water quality condition from 2016 to 2018 (ECCC, 2020). Groundwater has frequently been contaminated with *Escherichia coli* (*E. coli*), a gram negative bacterium that is commonly used to indicate presence of fecal pathogens that are known to cause gastrointestinal illnesses and in severe cases, death (Swain et al., 2006; Health Canada, 2020). An example of this is the outbreak that occurred in Walkerton, Ontario, where groundwater wells were polluted with *E. coli* from manure. Environment and Climate Change Canada (2020) showed that approximately 25% of the Great Lakes Basin had marginal or poor water quality, especially lands associated with highly urbanized and agricultural activities.

Water is sacred and has cultural significance for the FN communities (Boyd, 2011). Indigenous communities have linked their health to water, and from a spiritual perspective, water is considered their first medicine (Sanderson, 2008). Many FN reserves do not have access to clean safe drinking water; as of 2015, 169 drinking water advisories (DWAs) were in effect for indigenous communities, 79 of these being in the province of Ontario (Lui, 2015). After the outbreak in

Walkerton in 2000, attention was focused on the safety of drinking water in Canada, particularly in indigenous communities; even so, there are still many indigenous communities that have no access to water treatment or wastewater treatment plants in Canada (O'Connor, 2002; Black & McBean, 2017). For example, the Batchawana First Nation, located in northern Ontario had well water concerns that ranged from high turbidity to uranium contamination, while the Grassy Narrows First Nation in western Ontario have been under "do not consume" order and had a cancer-causing chemical in their water system (Human Rights Watch, 2016).

The federal government spent millions and millions over the years to improve water and wastewater infrastructure for First Nations communities, but the financial support alone is not enough to solve the water crisis on FN reserves (Boyd, 2011; Human Rights Watch, 2016). Part of the problem is that responsibility for the welfare of FN rests with the federal government, while the provincial and territorial governments look after infrastructure such as water and wastewater treatment facilities. Funding is rarely coordinated at the federal and provincial levels. Often, funds for operation and maintenance of public water and wastewater systems are only funded up to 80%, leaving the FN to raise funds for the remaining 20%. In addition, there is lack of source water protection and government support for private water and wastewater systems (Boyd, 2011; Lui, 2015; Human Rights Watch, 2016; Lucier et al., 2020).

The Six Nations Reserve of the Grand River and MCW

The Six Nations of the Grand River, Ontario, Canada is the largest urbanized indigenous reserve with population of 12,892 residents living on the reserve (Groat, 2020). It is located within an hour's drive from major urban centers in southern Ontario, all of which are served by publicly funded water treatment plants. The only source of treated drinking water comes from the Six Nations Water Treatment plant (SNWTP), which was built in 2013 and distributes treated water to only 9% of the households in the town of Ohsweken (Baird et al, 2012; Human Rights Watch, 2016; Collins et al., 2017). For the overwhelming majority of the community, however, the source of drinking water is from groundwater via private wells (drilled or bored) that are pumped directly into taps, or stored in cisterns and then pumped into taps (Collins et al., 2017; Dyck et al., 2015). To an unknown extent, some households purchase water from the SNWTP that is either poured into the well or in cisterns. In all cases, the home owners bear the entire cost of managing, cleaning and treating these sources and all risk to their health from drinking contaminated water (Duncan & Bowden, 2009; Levangie, 2009; Human Rights Watch, 2016). Previous studies indicated that the quality of the groundwater at the Six Nations reserve was poor, with many pathogen and pollutants, as well as 27% and 19% of the groundwater wells being contaminated with *E. coli* in 2003 and 2004, respectively (Jamieson et al., 2003; Neegan Burnside, 2005).

The McKenzie Creek Watershed, which flows through the Six Nations Reserve has two major tributaries, the McKenzie and Boston Creeks. Despite their impaired condition, the creeks provide habitat for many threatened species of plants and animals, with a total of 59 significant species; 22 and 19 of them were considered endangered species provincially and federally, respectively. MacVeigh et al. (2016) indicated that the McKenzie provides fish habitat for 56 species within the watershed. As well, water from the upper reaches of McKenzie and Boston Creeks are used for irrigation, since the major land use within the watershed is agricultural lands.

In this thesis, I will evaluate the freshwater ecosystem health of creeks in the Six Nations Reserve to gain a better understanding of water related concerns in the Six Nations Reserve that helps in developing management plans and to plan for future investigations. In the first chapter, my objectives were to evaluate the extent of fecal contamination among different tap water sources in the Six Nations reserve, and to understand how residents of the Six Nations are using the tap-water sources for drinking, cooking, hygiene and other domestic purposes. The second chapter was designed to survey the quality of surface water of McKenzie and Boston creeks to determine the exceedances from water-quality objectives and to investigate the relationship between pollutant concentrations (nutrients) and land use-land cover (LULC) classes and tile drainage area (TD) within the McKenzie creek watershed.

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Chapter 2: Prevalence and use of *E. coli*-contaminated tap water in the Six Nations Reserve of the Grand River, Ontario

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Keywords:

Six Nations reserve, Grand River, indigenous communities, *E. coli*, drinking water security

Abstract

Drinking water security is only available to a small percentage of the Six Nations of the Grand River in Ontario. As of 2019, only 9% of the 12,892 living on the reserve had access to uncontaminated tap water from the Six Nations Water Treatment plant (SNWTP); tap-water sources for the overwhelming majority are currently from wells and cisterns. Studies completed in 2003 (312 households) and in 2004 (104 households) showed that 27% and 19%, respectively, of tap-water sources were contaminated (at least 1 colony of *E. coli* per 100 mL). A follow-up study in 2018 showed similarly high prevalence (29% of 72 households) of *E. coli* contamination but unlike earlier studies, only 23.6% of the contaminated water sources came from wells, while 5.6% came from cisterns; none of the households served by the SNWTP had contaminated samples. Seventeen of 42 (40%) well sources were contaminated while only 4 of 27 (15%) cistern sources were contaminated. In 2019, 60 of the households were formally surveyed to determine what percentage of the households consumed or otherwise used their tap water. Only a third of the households (18.3% tap water from cisterns; 16.7% tap water from wells) actually used tap water as a drinking water source; 51.7% drank bottled water, 6.7% drank water picked up from the SNWTP, 5% used combined tap and bottled water, while only 1.7% used water piped from the SNWTP. We found that 8.3% of the households drank contaminated tap water, while 2% used tainted water for powdered drink, 4% for coffee and tea, 9% for food preparation, 15% for washing produce, 17% for brushing teeth, 22% for washing dishes and laundry, and 26% for showering. *E. coli* contamination in tap water was widespread across the reserve and not restricted geographically. This high prevalence of fecal contamination has serious health implications for residents living on the Six Nations Reserve.

Introduction

Access to safe drinking water supplies is an essential human need whether it is consumed for drinking, cooking, domestic, or recreational uses. Everyone has the right to have adequate, constant, affordable, and potable water sources (WHO, 2017b). Yet a large number of people around the world do not have access to a safe and clean drinking water supply. More than 785 million people in 2017 do not have access to safe drinking water, making this a major global health issue (WHO, 2019). Furthermore, the world health organization (WHO, 2019) estimates about two billion people around the world are using a source of drinking water that is contaminated with feces.

Fecal Indicator Bacteria and Waterborne Illnesses

Drinking water sources need to be monitored regularly for fecal indicator bacteria like *Escherichia coli* (*E. coli*), a member of the family Enterobacteriaceae; it is a coliform, characterized as a gram-negative, facultative anaerobic, non-spore-forming and rod-shaped bacterium, and depends on some physical and biological factors (temperature, pH, solar radiation, presence of other microorganisms, nutrients and the ability to persist in biofilms) to survive in waterways (Ishii & Sadowsky, 2008; WHO, 2017b; Health Canada, 2020). They are present in the intestinal tract of warm-blooded animals and in human feces; the majority of them are harmless bacteria but their presence in water sources indicate a recent fecal contamination, and so potential pathogens may be present in the environment which can pose a health risk to the public (Ishii & Sadowsky, 2008; Health Canada, 2020). The Canadian drinking water guideline is that no *E. coli* colony-forming units (CFU) should be detected per 100 mL of water to ensure it is safe for consumption (Health Canada, 2020).

A lack of access to safe water can be the cause of many waterborne bacterial infection. Indeed, good quality water is a vital deciding factor for human health and mortality. So exposure to these water contaminants (*E. coli*) in polluted drinking water can have health impacts that range from gastrointestinal illness (mild to bloody diarrhea) to kidney failure and death in severe cases (Federal-Provincial-Territorial Committee on Drinking Water & CCME, 2004; Health Canada, 2013a). Fortunately, the symptoms of gastrointestinal illnesses (nausea, headache, fever, diarrhea, vomiting, abdominal pain) are usually mild and only last a few days to a week, and just a small percentage of those affected need to consult a physician (Health Canada, 2013a; Public Health Agency of Canada, 2017).

In Canada, the federal government acknowledges that risks of waterborne diseases across the First Nations communities are 26 times higher than that of the Canadian population (Boyd, 2011; Galway, 2016) with severe effects on children, older adults, and people with disabilities (Human Rights Watch, 2016). In a systematic review of Canadian waterborne outbreaks between 1974 and 2001, of the 288 waterborne infections reported, the most influential factors were water-treatment failure, contamination from wildlife, and extreme precipitation events (rain/ snowmelt) (Schuster et al., 2005). In May 2000, more than 2,300 people became ill, and 7 deaths resulted from contaminated drinking water system with *E. coli O157:H7* in Walkerton, Ontario (O'Connor, 2002). Another tragedy related to contaminated water happened in 2005 in Kashechewan reserve, Ontario. As a result, the Ontario provincial Minister of Aboriginal Affairs demanded to evacuate ~ 1,000 residents (Human Rights Watch, 2016).

Other infectious and non-communicable diseases are also linked to insufficient access to water. For example, a survey conducted by Human Rights Watch (2016) reported some skin issues such as infections, eczema or other skin problems that were believed to be related to their poor water condition. Therefore, to improve public health, sanitation of drinking and wastewater is used to reduce infectious disease morbidity and mortality (Boyd, 2011). On the other hand, up to half a million deaths globally in 2016 were related to inadequate drinking water and over 800,000 annual deaths (more than 500,000 kids under 5 years) of diarrheal diseases were due to poor water supply, sanitation and hygiene (Prüss-Ustün et al, 2019; WHO, 2017a; WHO, 2019). Several waterborne pathogens, such as *E. coli O157:H7*, have unacceptably high mortality rates (Hunter, 1997).

E. coli is the most commonly used microbial indicator for testing and monitoring drinking water supplies and used as a measurement of the prevalence of fecal contamination in different water supplies. Use of *E. coli* as an indicator has led to significant progress in the safety of drinking water worldwide, allowing governments to establish drinking water quality guidelines and standards (WHO & OECD 2003).

First Nations Communities and Drinking Water Advisories

While indigenous people account for up to 4.9 % of Canadian population (First Nations, Métis and Inuit), 2.8% of them are First Nations in Canada and 1.8% of the First Nations are located in Ontario (Statistics Canada, 2017). Water plays a vital role in the culture, spirituality and way of life of First Nations communities (Plummer et al, 2009). Although Canada is one of the most water-rich countries and the vast majority of the Canadian citizens have access to clean and safe drinking water, this is not true for many indigenous communities, as they experience challenges to accessing safe drinking water (Lui, 2015; Human Rights Watch, 2016). In fact, some communities like Little Buffalo, Alberta did not have running water as recently as 2018; local sources are contaminated and unsafe to drink, and they must purchase bottled water in stores located an hour from their community (Boyd, 2011).

In Canada, provinces regulate safety of drinking water sources while the welfare of First Nations communities fall under the jurisdiction of the federal government (Indigenous and Northern Affairs Canada INAC). For many decades, the federal government has not provided appropriate infrastructure to ensure the availability of clean drinking water to indigenous communities (Human Rights Watch , 2016). Between 2009-2011, 73% of water systems in the First Nations communities were considered to be at medium to high-risk of producing contaminated drinking water (Neegan Burnside Ltd., 2011). Therefore, when water is suspected or confirmed to be fecally contaminated and unsafe for human consumption, communities are placed under Drinking Water Advisory (DWA). a public announcement issued by the responsible authority to protect public health from waterborne contaminants such as bacteria, viruses or parasites that can be present in drinking water (Health Canada, 2015). Health Canada issues three types of DWA: "boil water", "do not consume", and "do not use" advisories. Majority of the DWA in Canada are "boil water" advisories (BWA), which require the water to be boiled for at least one minute before it can be used for drinking or for making ice cubes, baby formula, food preparation, washing produce and dental hygiene (Health Canada, 2015; Galway, 2016).

Drinking water advisories are a strong indicator of the water quality issues faced by Canada’s First Nations communities. The data show that ~70% of all on-reserve communities in Ontario were under at least one DWA from 2004-2013, 14% of them being long-term advisories that stayed in effect for more than one year (Galway, 2016). Some First Nations communities such as Neskantaga and Shoal Lake 40, have been under DWA for ~ 20 years (Human Rights Watch, 2016). Over the past five years, the government of Canada funded programs to improve water infrastructure on reserves, to remove long-term DWA and to stop short-term DWA from becoming long-term (INAC, 2021). Still, the 78 long-term DWA among First Nations reserves in 2016 (ECCC, 2018) had only been reduced to 67 long-term DWA by 2018 (Indigenous Service Canada, 2018). To date, there are still 51 long-term DWA in 32 indigenous communities (INAC, 2021).

Six Nations of the Grand River in Ontario

A significant proportion (~ 43%) of impaired drinking water quality on the First Nations reserves is due to presence of fecal bacteria (Farenhorst et al., 2017). Therefore, this chapter intends to evaluate the bacterial contamination condition at the largest populated First Nations reserve, the Six Nations (SN) of the Grand River, Canada. The SN is the largest urbanized indigenous reserve with a population of 12,892 residents living on the reserve (Groat, 2020). A primary source of drinking water comes from treated water Six Nations Water Treatment plant (SNWTP), which was built in 2013 and distributes treated water to only 9% of the households in the town of Ohsweken (Baird et al, 2012; Human Rights Watch, 2016; Collins et al., 2017). For the overwhelming majority of the community, however, the source of drinking water is from groundwater via private wells (drilled or bored) that are pumped directly into taps, or stored in cisterns and then pumped into taps (Collins et al., 2017; Dyck et al., 2015). To an unknown extent, some households purchase water from the SNWTP that is either poured into the well or in cisterns. In all cases, the home owners bear the entire cost of managing, cleaning and treating these sources and all risk to their health from drinking contaminated water (Duncan & Bowden, 2009; Levangie, 2009; Human Rights Watch, 2016).

Poor water quality has been a major issue for the community for decades. Residents have complained about unpleasant odor and taste of water from their wells and cisterns, as well as biological contamination with *E. coli*. The first formal study of the groundwater of the Six Nations reserve was conducted in 2003 with 312 wells (bored or dug/ drilled) being tested (Jamieson et al., 2003). A follow-up investigation with sampling of 104 groundwater wells in 2004 assessed the quality of the wells and the potential sources of contamination across the reserve. The overall assessment from these studies was that there was widespread fecal contamination across the community, with 19% of their wells contaminated with *E. coli* in 2004 (Neegan Burnside, 2005).

There has been no follow-up of the 2004 study, despite there being continued challenges to accessing uncontaminated drinking water over the past 15 years. Therefore, the SN community requested an update of water testing as part of the Co-Creation of Water Quality Tools Project funded by the Global Water Futures program. The aim was to investigate the current status of fecal contamination in different tap-water sources (WTP, wells, cisterns) and to understand how residents of the Six Nations are using the tap-water sources for drinking, cooking, hygiene and other domestic purposes. This information will inform the local health unit of the current health risks to the indigenous community.

Methods

Description of study area

The Six Nations of The Grand River is the largest urban First Nations reserve in Canada located within the Grand River watershed in Sothern Ontario. The Six Nations Reserve is about 18,000 hectares of land located nearly 25 km southwest of the city of Hamilton city and situated between the cities of Brantford, Caledonia, and Hagersville (**Figure 1**), with 27,559 of total Six Nations' memberships (12,892 residents living on reserve) (Groat, 2020).

Historic Data

The SN Band office (Clynt King) provided us with data from a previous study that had been conducted in 2003 and 2004 (Neegan Burnside, 2005). The 2003 study is considered the first formal study at the Six Nations to evaluate the bacteriological condition of groundwater wells. A stratified random sampling process was used to sub-sample 312 wells (56 drilled wells and 256 dug/bored wells) to ensure that all six administrative units of the Six Nations Reserve were represented. In 2004, a subset of 104 groundwater wells (27 drilled wells and 77 dug/bored wells) were re-sampled to confirm the potential fecal contamination sources.

2018 Sampling

Due to constraints imposed by the Six Nations community, it was not possible for us to survey households on a random basis or for us to re-sample the same households that had been sampled in 2003-2004. Instead, a Community Navigator, Ms. Denise McQueen contacted interested members and arranged a time for us to enter their homes to sample the tap water. Each visit to a household required the presence of Ms. McQueen or one of her assistants. As a result of these differences in sampling approaches, there was no overlap in sites between the 2003-4 and 2018 studies. We tested water samples in 75 households, and for three of these, we re-tested the samples because of inconclusive results.

Tap-Water Sampling Procedure

Ethics approvals were obtained from the Hamilton Integrated Research Ethics Board and the Ethics Committees of the Indigenous communities. After that, group meetings, elder guidance, and workshops took place at the Six Nations Reserve to engage the community members and to understand their lifestyle and what water sources were used. Using a mixed method of Indigenous Knowledge (IK) and Western Science (WS), we designed a sampling procedure that would satisfy the requirements of both approaches.

Before collecting the sample, we let the tap water run at least two minutes. This is standard practice to ensure that any water sitting in the pipes have been emptied and that we are sampling the tap-water source. Then, we filled a sterile Whirl-Pak bag about 3/4 full with tap water and closed the bag tightly by twirling it at least twice and placing it in a cooler with freezer packs. In all cases, a sodium thiosulfate tablet had also been added to the bag beforehand to neutralize any chlorine that had been added to the tap water to ensure that the results of microbial tests reflect the actual water quality at the time of collection (Clesceri et al., 1998; WHO & OECD 2003; Murray et al., 2018).

We recorded the site's location with a GPS unit, the date of collection, and source of the tap water (i.e. cistern, well, water treatment plant). All samples were returned to the lab within 6 hours of collection. They were then analyzed with the Pathogen Detection System TECTA™ B-16, an automated, USEPA-approved microbiological system that can yield rapid results (within 18 hours). It was approved for USEPA use following a verification study (James et al., 2007). We received on-site training after the machine was installed at McMaster by a representative of the Pathogen Detection System. We followed the weekly cleaning and validation protocols created by the manufacturer throughout the study period. Prior to testing samples for this study, we ran positive and negative controls in triplicate for quality assurance. Bramburger et al. 2015 evaluated the performance of the TECTA™ B-16 by comparing its detection time and accuracy to those of two common culture-based methods, which are widely used for recreational water quality monitoring in Canada. Their results demonstrated that *E. coli* densities inferred by the TECTA™ B-16 method were generally in agreement with those generated by standard culture methods.

In turn, whirl-pak samples were retrieved from the cooler. After thoroughly mixing the sample in the whirl-pak, we poured 100 mL into a TECTA™ B-16 EC/TC cartridge, and then tightly closed the cap and labelled it with the site name or ID. The cartridges were swirled for about 2 minutes until the growth medium was completely dissolved. We used the "Standard Mode

Operation" setting to conduct all tests. The maximum incubation period was 18 hours for uncontaminated samples, but highly contaminated samples could be completed within 2 hours.

Human Health Survey

For 60 of the 75 households included in the fecal bacteria survey, members of the Human Health Team of the Co-Creation of Water Quality Tools project conducted a human-health survey. In this survey, one representative of each household specified the drinking water source, the tap-water source, and how the household members used their tap water (see **Table 1**). Additional information was obtained that are being analyzed and published elsewhere but will not be used or reported in this study.

Statistical Analysis and Geographic Information System

We used SAS JMP v.14 (SAS Institute, Cary, North Carolina, U.S.A.) to assemble data and to create graphs. We used ArcGIS Pro (v.2.7.0, ESRI, 2020) to delineate the McKenzie and Boston watersheds from a digital elevation model and to produce maps to compare the distribution of contaminated and uncontaminated tap-water sources in the Six Nations Reserve in 2003-4 and in 2018.

Results

Although we sampled 75 households, results from 3 were inconclusive, even after we collected additional samples and analyzed them again. We have assumed that some constituent in the tap water of these three households had interfered with the method we used to analyze for *E. coli* and have therefore removed them from further consideration. The remaining 72 households yielded conclusive results (**Figure 2**). Of these, 29% of the households had contaminated tap-water sources (i.e. with at least 1 cfu/100 mL of *E. coli*). By comparison, 27% and 19% of the wells tested in 2003 and 2004, respectively, were contaminated with *E. coli* (**Table 2**). In the 2003-4 study, the authors reported their results according to the type of well (drilled or bored/dug) that had been used. They found that tap-water sources from drilled wells tended to be lower (18% in 2003 and 15% in 2004) than that from of bored/dug wells (29% in 2003 and 21% in 2004). In 2018, only 58.3% (42 of 72) of the tap-water sources came from wells (type unknown), while 37.5% (27 of 72) were from cisterns and the remaining 4.2% (3 of 72) were from SNWTP. While 23.6% of the contaminated water sources came from wells, only 5.6% came from cisterns, and none of the samples from the SNWTP were contaminated (**Figure 3**). Well source water was associated with 40% (17 of 42) contamination compared with cisterns at only 15% (4 of 27).

To discern spatial trends in location of contaminated households, we overlaid the households sampled in 2003-4 (**Figure 4**). Generally, we found more of the highly contaminated sites within the McKenzie Creek subwatershed than in the Boston Creek subwatershed, but it is important to note that fewer sites were located in the Boston than in the McKenzie Creek catchments. Similarly, we found that a larger number of highly contaminated sites were located in the McKenzie Creek catchment in the 2018 study, and again fewer sites surveyed were located in the Boston Creek catchment (**Figure 5**).

The location of 60 households that were surveyed regarding how they used their water sources are shown in **Figure 6**. For majority of the surveyed households (51.7%), their source of drinking water was purchased bottled water, while a third (35%) used tap water from cisterns (18.3%) and from wells (16.7%). Of the remainder, 6.7% drank treated water that they picked up from the SNWTP, 5% drank a combination of tap water and bottled water and 1.7% used piped-in water from the SNWTP (**Figure 7a**). Of the households that used tap water as a source of drinking water, 8.3% drank water that was tested and found to be contaminated (**Figure 7b**).

Besides using tap water as their drinking water, the surveyed households also used the tap water to prepare powdered drink, strawberry juice and coffee or tea (**Figure 8**). Of the approximately 27% that used tap water to prepare powdered drink, about 2% of these were from contaminated sources. Although none of the water sources used for preparation of strawberry were tested positive for *E. coli*, 4% of tap water used for coffee or tea were contaminated with fecal bacteria. There were other uses of contaminated tap water that could pose a health risk to the SN community. This included food preparation (9% contaminated), washing produce (15%), brushing teeth (17%) and washing dishes (22%). Not surprisingly, a large proportion of the households used tap water for showering and for laundry, and approximately 26% and 22% of these, respectively, were tested positive for *E. coli*. These percentages do not add up to 100% in all cases, because some participants did not provide a response to some questions.

Discussion

During the summer of 2018, we investigated the quality of different sources (SNWTP, wells, cistern) of the tap-water samples at the Six Nations community. The results showed that 29% of the tap-water samples were contaminated with *E. coli* (i.e. with at least 1 cfu/ 100 mL), which means that they exceeded the Canadian guideline for drinking water quality and should not have been consumed without first boiling the water for at least one minute.

Over half (58%) of the households surveyed in this study used well water as their tap-water source, and 40% of the wells were contaminated with *E. coli*. This problem is not unique to First Nations. Many reports have confirmed the contamination of *E. coli* bacteria in groundwater wells, with the earliest survey conducted between 1950 and 1954, showing that ~15% of wells in Ontario were contaminated with *E. coli*. In another survey conducted in Ontario in 1998, 34% of the tested wells were contaminated with bacteria (*E. coli* or faecal coliforms or total coliforms) (Goss et al., 1998).

In Canada, 4.1 million households depend on a constant supply of groundwater, with about 1.5 million of them in Ontario (Felleiter et al., 2020; Latchmore et al., 2020). According to Human Rights Watch (2016), it is estimated that one of five households in First Nations reserves depends on private groundwater wells. The responsibility for cleaning, treating, and monitoring the water quality of these groundwater supplies (in wells or cisterns) rests solely with the households, and lack of knowledge on how to maintain these can lead to further contamination (Latchmore et al., 2020).

There are many contributing factors that can lead to contaminated well water. Neegan Burnside (2005) noted that in the case of the Six Nations reserve, some of the wells were improperly sited or were poorly constructed. Placement of the well too close to septic systems or agricultural wastes could expose the well to fecal contamination during rain events (Levangie, 2009; Baird et al, 2012; Allevi et al., 2013; Dyck et al., 2015; Health Canada, 2020). In fact, improperly maintained septic systems led to groundwater contamination, and has been cited as the reason of several outbreaks of waterborne diseases in the United States (Goss et al., 1998). In the study of 2005 at the Six Nations, 97% of the tested wells had vermin because they did not have vermin-proof lids. So proper well sealing is important to ensure good water quality and avoid bacterial contamination (Allevi et al., 2013; Health Canada, 2013b). Application of manure too close to the well or allowing livestock to get too close to poorly maintained/constructed wells could also lead to fecal contamination (Goss et al., 1998; Schuster et al., 2005; Neegan Burnside, 2005).

Another factor that may lead to further bacterial contamination is well characteristics. Many studies found a correlation between bacterial contamination and (well type, depth and age), as shallow depth in dug/bored wells were more often contaminated than drilled wells. Failure of the well-head and well-casing when the well is old may also lead to bacterial contamination (Goss et al., 1998; Federal-Provincial-Territorial Committee on Drinking Water & CCME, 2004; Owusu et al., 2021). In the previous studies, dug/ bored wells were 1.7 and 1.4 times more contaminated with *E. coli* in 2003 and 2004, respectively.

Cisterns are used throughout Canada on and off reserves for storing water for long time periods. On the Six Nations Reserve, a little over a third (37.5%) of the households surveyed in 2018 used water from cisterns as their tap-water source. The source of the water for cisterns is from the SNWTP, and this was usually delivered by a private service (Baird et al., 2013; Bradford et al., 2018). In a previous survey conducted at the Six Nations in 2010, a similar number of households (35%) relied on trucked water from water treatment plant to their cisterns (General, 2010). This percentage is high relative to the mean of 10% for other First Nations communities in Ontario, and the mean of 21% in Saskatchewan, but about the same as the mean of 31% for communities in Alberta and Manitoba (Neegan Burnside Ltd., 2011).

In our study, 15% of the tap water from cisterns were contaminated with fecal bacteria. Many studies have reported high occurrence of *E. coli* contamination in cisterns (Farenhorst et al., 2017). For example, ~39% of the cisterns in the Beardy's and Okemasis Reserves in Saskatchewan were contaminated with total coliforms (Bradford et al., 2018), and ~74% of surveyed cisterns were contaminated with *E. coli* in Brazil (Alves et al., 2014). *E. coli* and other fecal bacteria can enter cisterns that are poorly constructed and improperly maintained, or that have not been disinfected adequately (Levangie, 2009; Baird et al., 2013; Bradford et al., 2018). Bacterial contamination can also occur during loading, transporting and filling of cistern, because of the large number of individuals handling the hoses that are used to siphon the water (for example, when they touch the ground or when they are not properly disinfected and stored (Duncan & Bowden, 2009; Lebel &

Reed, 2010; Baird et al., 2013; Bradford et al., 2018). Cracks in the cistern tank or lids, or unlocked lids are also contamination pathway for vermin or bugs or small animals or pathogen during or after heavy rainfall (Lebel & Reed, 2010; Baird et al., 2013; Alves et al., 2014; Bradford et al., 2018). Having the water in the cistern stored for a long time without adding more disinfectant could promote growth of bacteria (Copeland et al., 2009; Baird et al., 2013). Therefore, regular cleaning of the cistern is necessary to ensure the water is potable (Duncan & Bowden, 2009; Health Canada, 2013b).

According to Neegan Burnside (2005), abandoned wells were present at the Six Nations community and many households have more than one well on their properties. Some of the households we visited recognized that one of their wells were no longer functioning properly but did not take steps to permanently close them off properly to avoid spread of bacterial contamination. To save on cost of trucking water to the cistern or well, some people have piped water from their eavestroughs to their cistern or well and this can transfer contaminants such as bird and animal feces from rooftops into the well or cistern (Neegan Burnside, 2005).

The distribution of 2018 households with contaminated tap water was widespread and not necessarily restricted to one geographic region, and this was similar to that of the 2003-4 study. In both studies, there were more contaminated households located in the McKenzie Creek catchment than in the Boston catchment. Even after adjusting for the total number of households sampled in each drainage basin, there was a higher proportion of households in the McKenzie Creek subwatershed contaminated compared with that in the Boston Creek subwatershed. As well, the level of contamination within the McKenzie Creek catchment was higher.

The current pattern of water use by the Six Nations community is heavily influenced by past contamination history. In 2013, the Six Nations community had been under a Boil Water Advisory (BWA) for more than 10 years, with 86% of their groundwater wells contaminated and up to 300 houses lacking access to safe and treated water supply (Dupont et al., 2014). As a result of the history of poor water quality and lack of access to potable water sources, the Six Nations community began to rely on alternate sources of drinking water, which was primarily purchased bottled water. This reliance on bottled water is reflected in our survey on how households used their water. We found that over half (51.7%) of the surveyed households paid for their primary drinking water, regardless of the potability of their tap water sources. Even for the 4.5% of the households that had access to piped-in water from the SNWTP that was not contaminated, none of the participants surveyed in this group used their tap water as drinking water, and instead consumed purchased bottled water. This high percentage is not surprising among First Nations residents, who are not used to access to safe water supply and do not trust their tap water. In the most extreme situation, such as that of Little Buffalo, Alberta, 100% of the First Nations residents must drive an hour to purchase bottled water to consume (Boyd, 2011). In addition to the half that used purchased bottled water for drinking water supply in our survey, only about a third (18.3% tap-water came from cisterns; 16.7% tap-water came from wells) used their tap water as a drinking water source, with 6.7% picking up water directly from the SNWTP (available without cost), and 5% using both tap water and bottled water.

The high percentage (52%) of surveyed households that relied on purchased bottled water for their primary drinking water supply is high compared with non-indigenous Canadians that have access to water from a treatment plant. In a survey of households in Toronto and across Canada, only ~23% and 25%, respectively, consumed bottled water as their drinking water source (Auslander & Langlois, 1993; Roche et al, 2012). Dupont et al., (2014) surveyed four Canadian Frist Nations communities which included the Six Nations Reserve. 76% of surveyed households in the Six Nations Reserve believed that bottled water was safer than tap water and a high proportion of participants (69%) totally depended on drinking bottled water. This is much higher than the ~43% of

Canadian residents across Canada who believed that bottled water was safer than the tap water, and only 8% of participants relied on drinking bottled water.

In our study, of the 35% of the respondents that used tap water as their drinking water source, only one household tested positive for *E. coli.* Subsequent to our test results, we discovered that the homeowner had installed in-line UV treatment; hence, none of the households we surveyed actually used contaminated water for their drinking water source. Nevertheless, the residents also used tap water for other purposes that could pose health risks. These included using tap water to prepare food, washing produce, brushing teeth etc. Accordingly, many participants of the survey at the Six Nations (2% and 4%) were using contaminated tap-water for powdered drinks and to prepare coffee and tea, respectively. And 9% for preparing food, 15% for washing produce and 22% for washing dishes. They also used contaminated tap water for hygienic purposes; about 17% were brushing their teeth with the contaminated water, and 22% and 26% were using it for laundry and showering, respectively. So even though the residents did not drink their tap water because they were afraid of health risks, they were still unwittingly consuming tap water through powdered drinks and washing their produce. In a survey conducted in Wauzhushk Onigum First Nation, 70% of the surveyed households used the tap water for brushing their teeth while more than half of the surveyed households (58%) used their tap-water for cleaning their teeth when they were under a BWA (Lucier et al., 2020). To reduce the risk of being infected by *E. coli* or any other pathogen that are present or suspected to be present in the tap-water sources, water should have been boiled before use for drinking, preparing food, preparing hot or cold beverages, washing produce like fruits and vegetables, and teeth cleaning.

Drinking, washing produce and brushing teeth are considered to be a high-risk activities and are potential transmission pathways for waterborne diseases because people may ingest the bacterial pathogens during these activities (Lucier et al., 2020). Other practices such as washing dishes and laundry may be considered to be a lower risk activity because there is no risk of ingesting the contaminated tap water during the activity, and the presence of soap or detergent would kill the bacteria if ingested. In the case of showering, however, residents have to avoid swallowing the water during the shower, and this may be difficult when babies and children are involved. Therefore, during BWA, babies and children and elderly should only have sponge baths to avoid inadvertently ingesting contaminated tap water.

During this study, we did not have time to collect any information on the characteristics of the well (e.g. well type, depth or age). Knowing how the well had been constructed (drilled or bored) would have provided the information we needed to compare with the 2003-4 study. We did not have any information on the location of the septic systems, and this may have offered some insight on the why certain wells/cisterns had been contaminated. According to Neegan Burnside (2005), of the 15 septic systems that they inspected at the Six Nations, most were in poor health, with half of them showing signs of failure or improper siting. Almost half of them did not meet the minimum separation distance from the wells, which is at least 100 m (Federal-Provincial-Territorial Committee on Drinking Water & CCME, 2004). The discharge or leakage from the septic systems could have caused the groundwater to be polluted. For this reason, investigating the septic systems while collecting the water samples is important and would help determine the sources of *E. coli* contamination.

We only tested the tap water during dry conditions. There is a significant relationship between extreme weather events like heavy rains or snow melt, and *E. coli* presence in private water supplies (Health Canada, 2020), and high percentage of waterborne outbreaks in Canada (1974- 2001) and the United States (1948- 1994) was associated with extreme weather events (Curriero et al., 2001;

Schuster et al., 2005). Therefore, collecting water samples during both dry and wet conditions is necessary to obtain a complete picture of fecal contamination. All else being equal, proper maintenance of wells and cisterns is probably key to preventing contamination, and this should include regular inspections and cleaning. It is important for households to regularly test the wells twice a year for presence of coliform bacteria, and to time one of the test after storms, heavy rains or during snow melt, and to test the cisterns at least four times a year (Health Canada, 2013b; Health Canada, 2018). The lid of the well or cistern should be locked properly, and if the lid is damaged or cracked, it should be repaired or replaced immediately. All old or unused wells should be abandoned properly. When tap water is tested positive for *E. coli*, it should not be used for high-risk activities without being boiled first for at least one minute. And finally, there should be better community awareness of sources of fecal contamination, how infrastructure should be properly maintained, and how contaminated water should be rendered safe to use.

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Table 1: Tap-water uses identified by members of the Six Nations in each household during a 2019 survey conducted by the Human Health Team of the Co-Creation of Indigenous Water Quality Tools Project (C. Moffatt, Team Leader)

Use of water	Relative impact on human health
Source of drinking water	High
Making strawberry drink*	High
Coffee or tea	Low
Powdered drinks	High
Food preparation	Low
Washing produce	High
Washing dishes	Low
Brushing teeth	High
Showering for healthy adults	Low
Bathing for babies, young children, and compromised individuals	High
Laundry	Low

* common drink consumed by First Nations communities

Table 2: Tap water samples that were tested positive with *E. coli* (+ve) in 2003 and 2004 compared with those in 2018. The source of tap water in the 2003 and 2004 studies only included wells, whereas that in 2018 included wells (type unknown), cistern and the Six Nations Water Treatment Plant (Treatment).

	Type of well									
	Drilled		Bored/Dug		All wells		Cistern		Treatment	
Year	+ve	total	+ve	total	+ve	total	+ve	total	+ve	total
2003	**10** (18%)	**56**	**75** (29%)	**256**	**85** (27%)	**312**	**--**	**--**	**--**	**--**
2004	**4** (15%)	**27**	**16** (21%)	**77**	**20** (19%)	**104**	--	--	--	--
2018	--	--	--	--	**17** (23.6%)	**42**	**4** (5.6%)	**27**	**0** (0%)	**3**

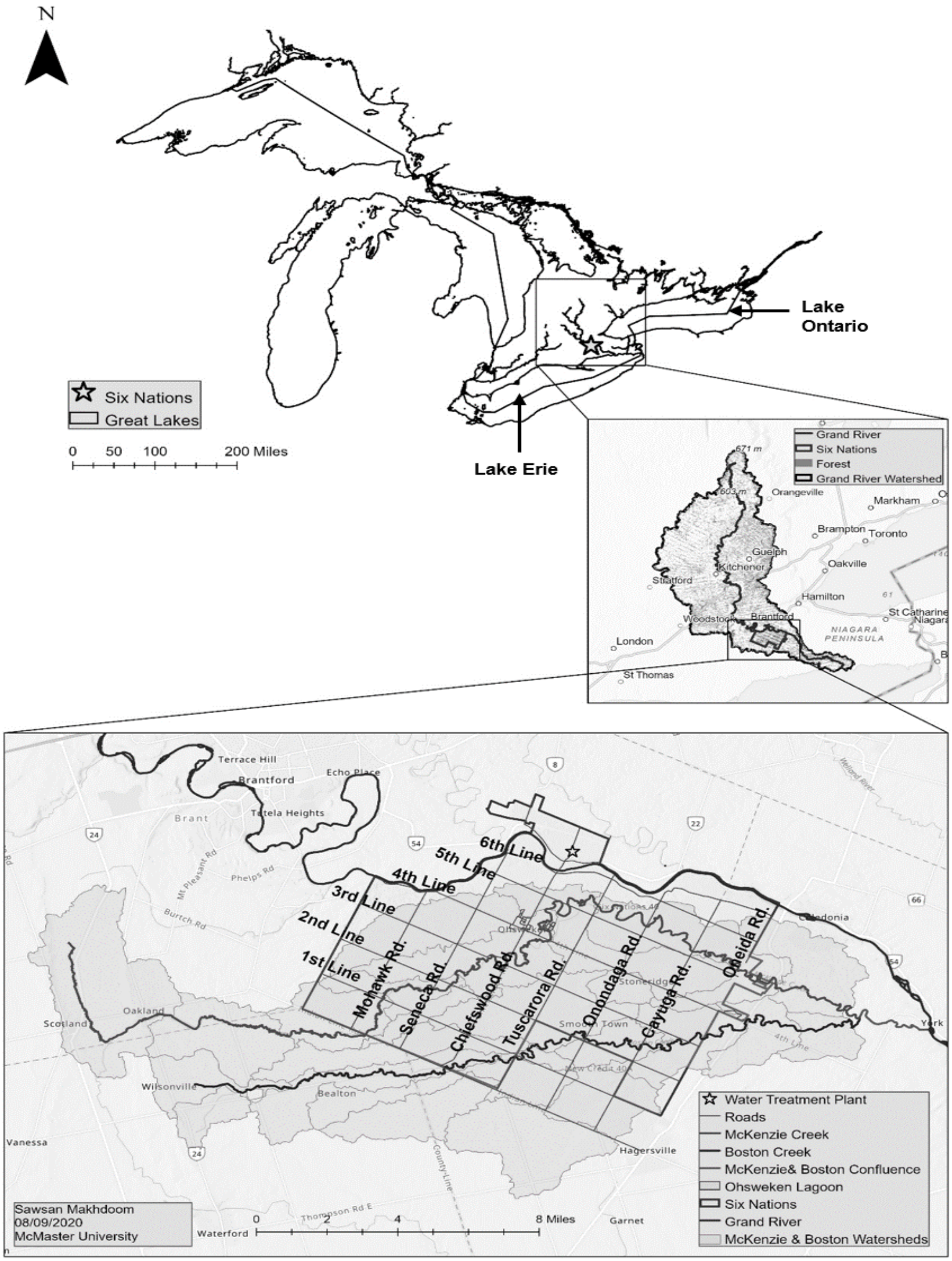


Figure 1: Location of the Six Nations Reserve in the Grand River watershed, north of Lake Erie.

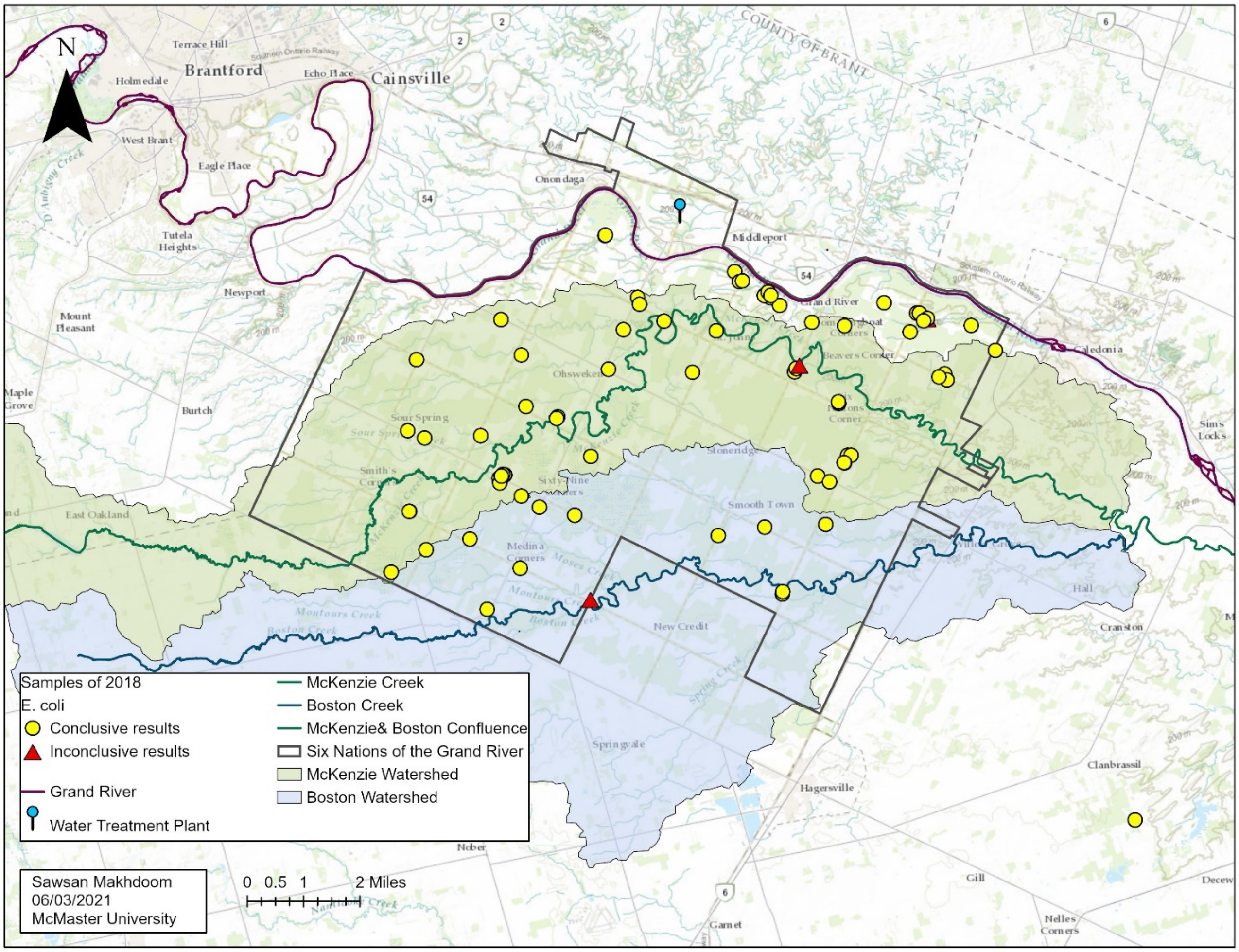


Figure 2: Location of all households sampled in 2018. Households sampled that had inconclusive results are indicated.

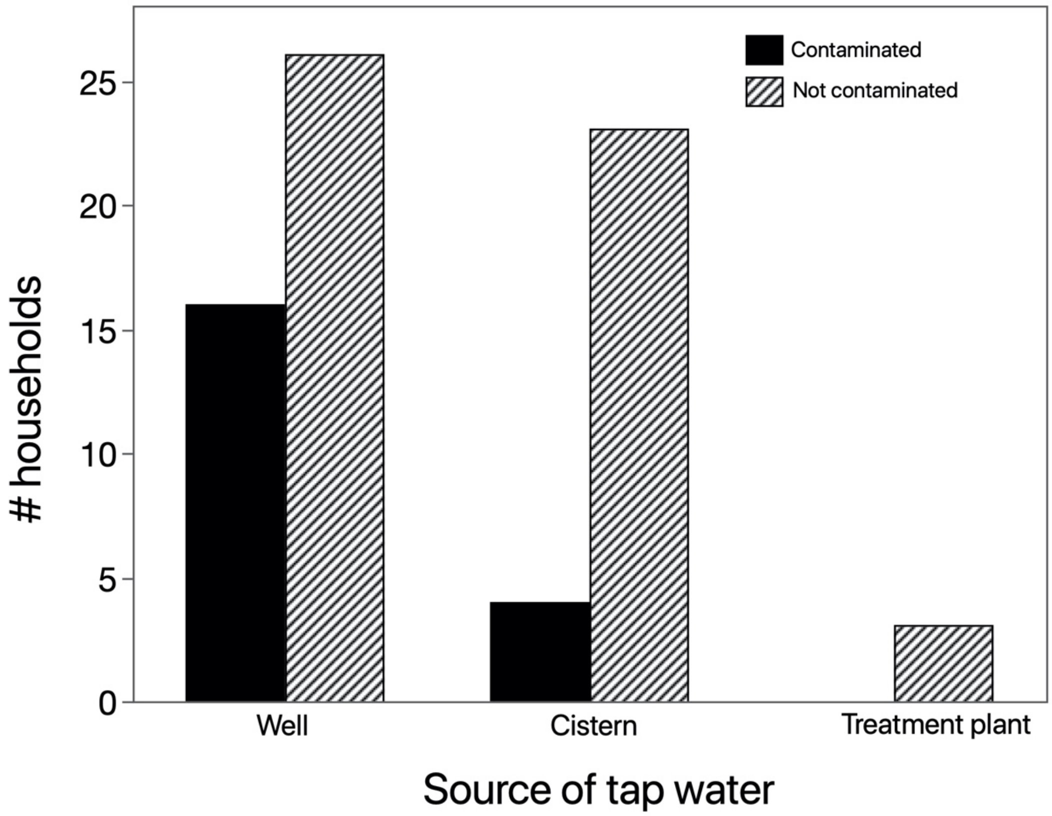


Figure 3: Summary of tap-water sources sampled in 2018, and the number of households that tested positive for *E. coli* (at least 1 CFU/100 mL).

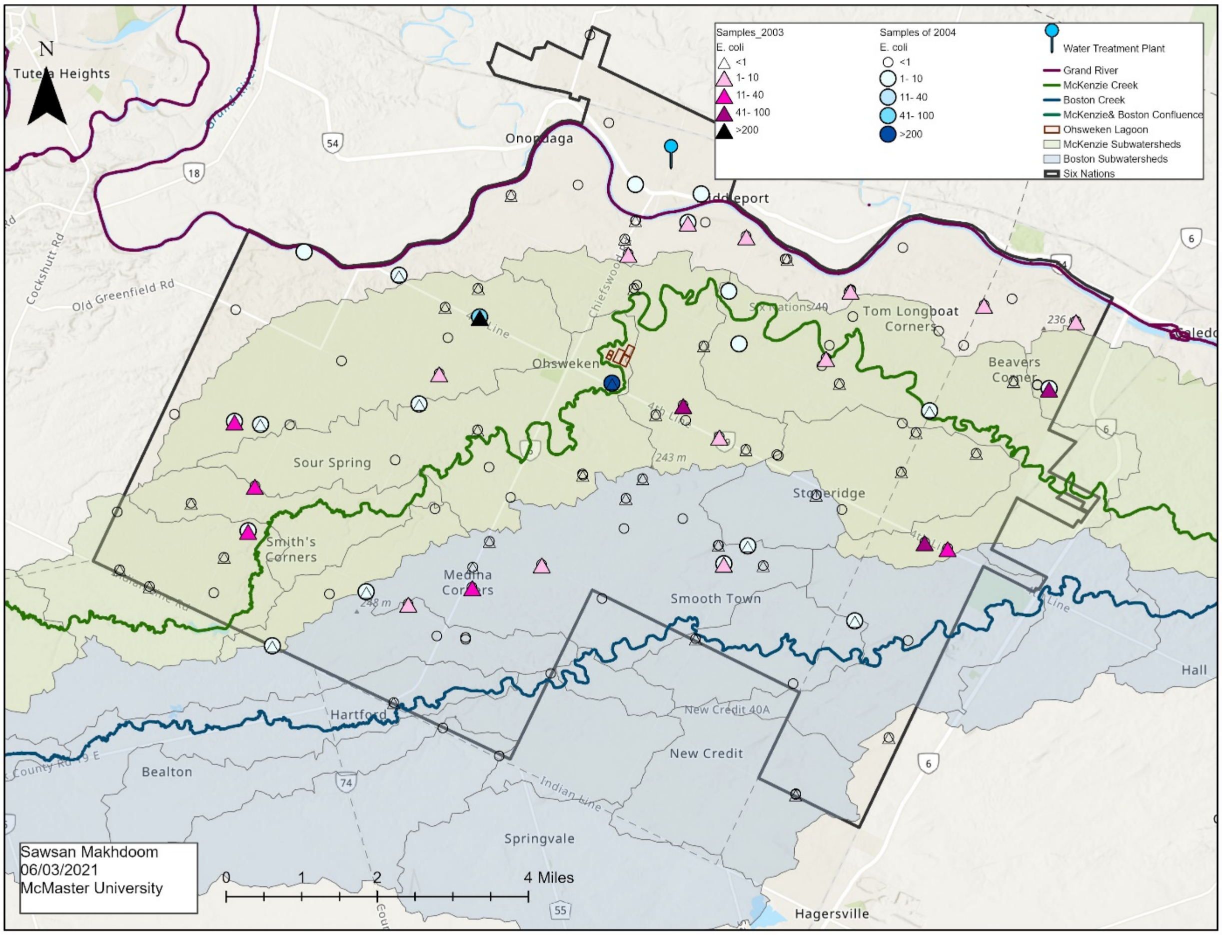


Figure 4: Locations of households in which tap water had been contaminated with *E. coli* (minimum 1 CFU/100 mL) in 2003 and 2004.

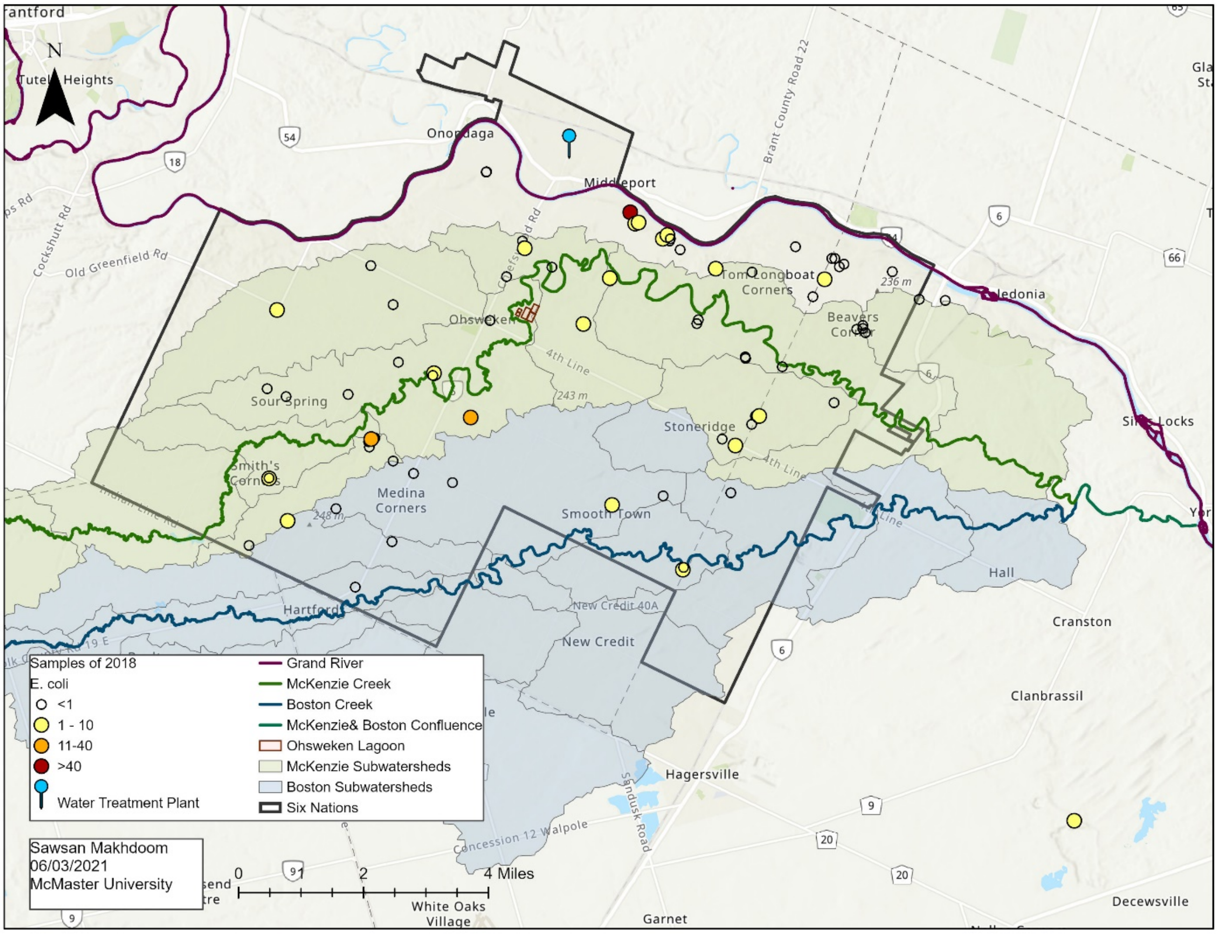


Figure 5: Locations of households in which tap water had been contaminated with *E. coli* (minimum 1 CFU/100 mL) in 2018.

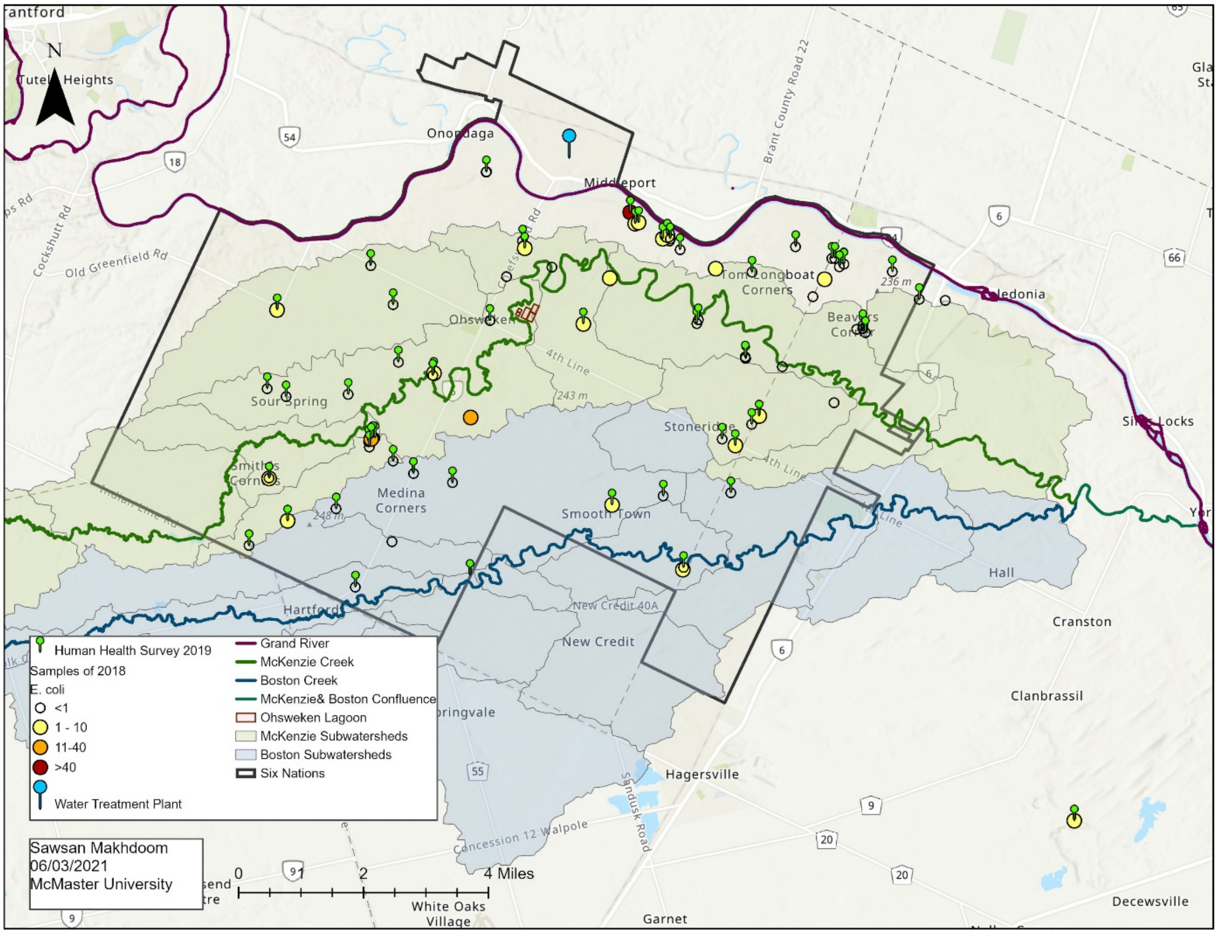
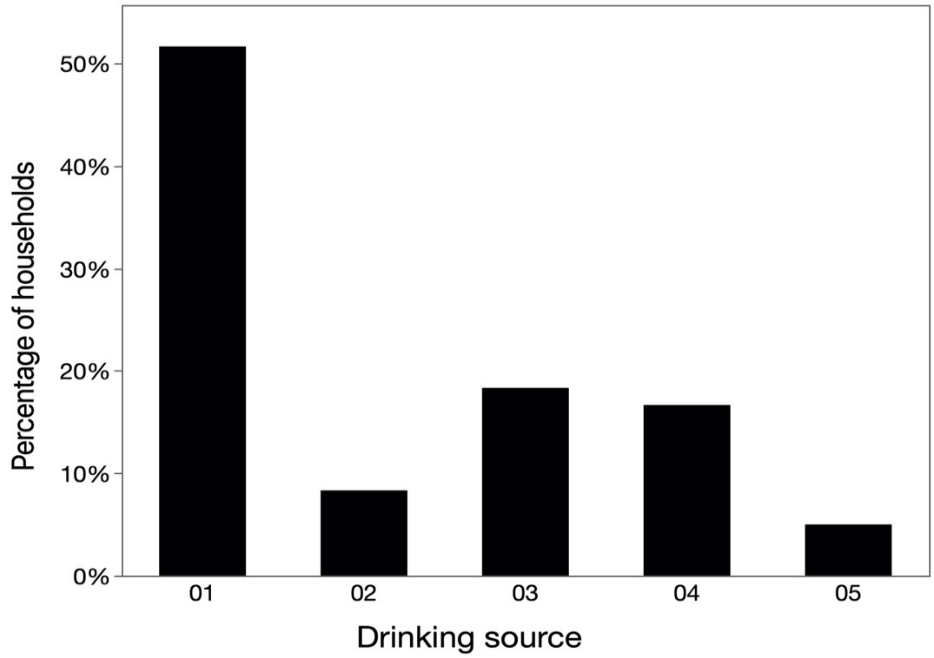


Figure 6: Distribution of households that participated in the formal survey of water usage in 2019.

a)



b)

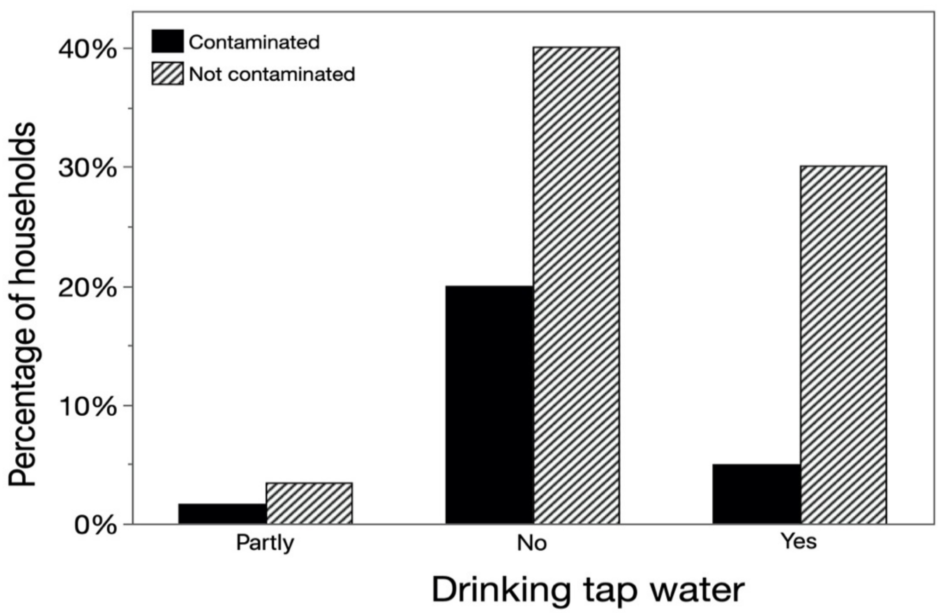


Figure 7: a) Percentage of households tested for *E. coli* that were formally surveyed for source of drinking water (01=bottled water; 02=water from SNWTP either picked up in bulk or piped into home; 03=tap water from cisterns; 04=tap water from wells; 05=both tap water from cisterns/wells & bottled water. b) Percentage of households with that used tap water as source of drinking water that were either contaminated or not.

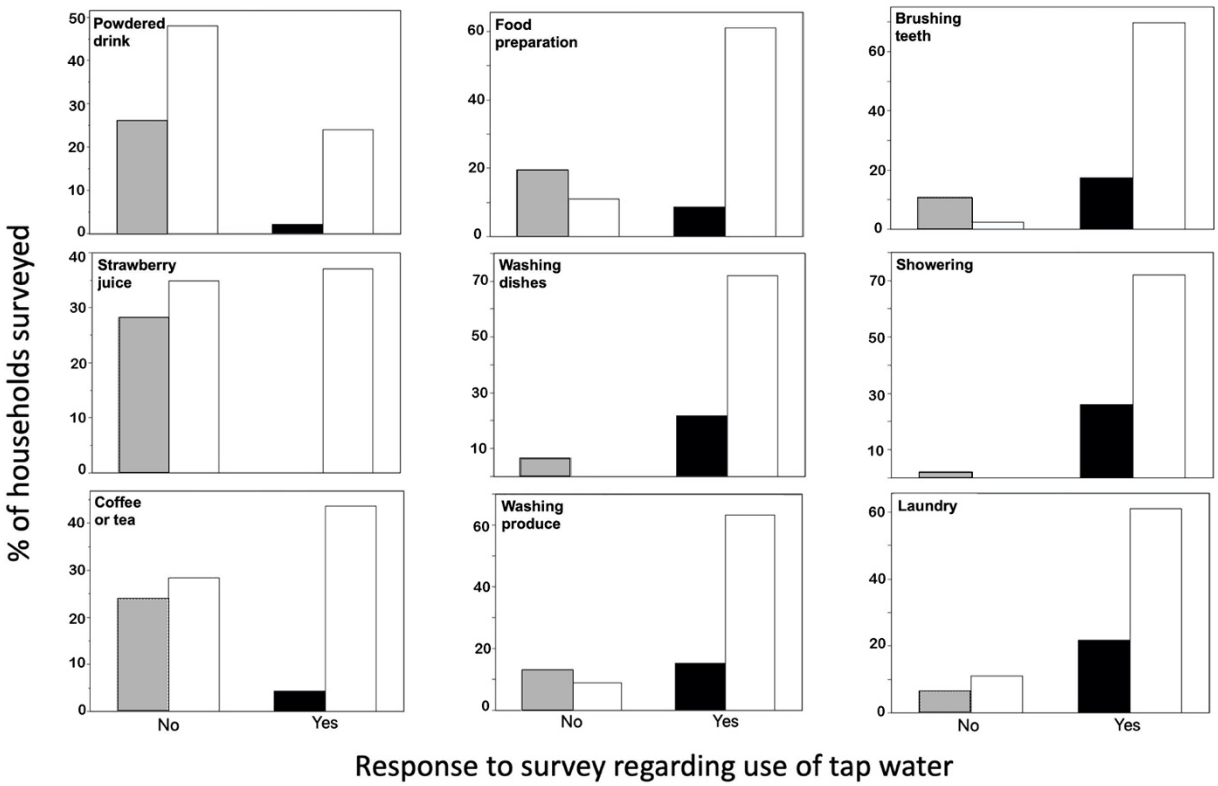


Figure 8: Percentages of households surveyed that did not use water (No) and those that did use water (Yes) for various purposes. Grey bars correspond to percentage of households that did not use water when the tap water was contaminated; black bars correspond to percentage of households that used water that was contaminated. Not all participants responded to questions.

Chapter 3: Relating tributary pollutant concentrations to land-use and land-cover characteristics of the McKenzie Creek Watershed of the Grand River, Ontario, Canada

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Keywords:

Land-use impacts, fecal bacteria, nutrients, water quality, McKenzie Creek, Boston Creek, Grand River, land cover

Abstract

The Grand River in Ontario is the largest contributor of nutrients and suspended solids to the eastern basin of Lake Erie. The purpose of this study is to characterize the nutrient and suspended sediment concentrations from the McKenzie Creek Watershed (MCW), one of the most understudied catchments of the Grand River Watershed (GRW), and to relate phosphorus, nitrogen, fecal coliform (*E. coli*) and other physicochemical characteristics to land-use and land-cover (LULC) categories and geological classes. Since a large portion of the MCW belongs to the Six Nations (SN) of the Grand, we co-designed a sampling program with the Six Nations band council specifically to address their concerns. Between April and November 2019, we sampled 15 stations in the two main tributaries, McKenzie and Boston Creeks, during base-flow conditions. Seasonal mean [TP] exceeded the provincial objective at all stations, while seasonal mean [TSS] exceeded the provincial objective at all McKenzie Creek sites but only four in Boston Creek. The seasonal geometric mean density of *E. coli* exceeded the Canadian guideline for recreational contact (200 CFU/100 mL) at only 2 stations in each of McKenzie and Boston Creeks that occur within the Reserve. Variations in water turbidity, Total Ammonia-N (TAN), Total P (TP) and Total Suspended Solids (TSS) were significantly correlated. Generally, McKenzie Creek was more polluted with TSS, TP and TAN, while Boston Creek was more polluted with fecal coliform and the soluble reactive form of P (SRP). We also found a significant positive relationship between mean monthly Total Nitrate-N (TNN) and %agricultural land and tile drainage areas within catchment of sampling stations. Based on historic data, these elevated levels of nutrients and fecal coliform have been observed in the two creeks for at least three decades; therefore, tributary conditions will probably not return to a healthy state on their own without remedial actions.

Introduction

Home to 95 million people, the Laurentian Great Lakes and their surrounding region represent 15% of the contiguous U.S. land area and 26% of Canada, with one of the largest economies in the world totalling $4.1 trillion USD in gross regional product (Campbell et al. 2015). At least part of this economy is dependent on good water quality that allows people to use the water as a source of drinking water and to support recreation, tourism, and fishing. Unfortunately, changing agricultural practices combined with a changing climate has resulted in a disproportionate loading of soluble phosphorus (P) in Lake Erie's western basin that have led to increased harmful algal blooms (HABs) that are threatening both the economy and ecology of the region (Steinman et al. 2017). These blooms can lead to a shutdown of the public drinking water supply (Steinman et al. 2017), a decline in the economic value of waterfront properties (Bechard 2021), as well as degradation of fish and aquatic habitat (Anderson et al. 2012).

The appearance of HABs in Lake Erie's western basin has been very well studied (Michalak et al. 2013; Schmale et al. 2019); by comparison, less attention has been paid to the eutrophication problem in the eastern basin, which is characterized as being deep and oligotrophic (Dove and Chapra 2015). Despite this characterization, severe blooms of the macroalga (*Cladophora*) that once fouled beaches in the eastern basin in the 1950s to the 1970s, have returned since the mid-1990s (Higgins et al. 2005; Joosse and Baker 2011). The occurrence of this nuisance alga along the Canadian shoreline of the eastern basin has emerged as a serious concern for lake managers because of perceived loss of ecosystem services, particularly with respect to recreational use, loss of property values and aquatic habitat (ECCC, 2018).

The most significant contributor of nutrients and sediments to the eastern basin of L. Erie is the Grand River Watershed (ECCC, 2018), which is located on the northeastern shoreline of Lake

Erie, comprising an area of 7,000 km^2. The dominant land-use in the GRW is agricultural (AG; 76%), with a smaller percentage of forested land (FO; 17%) and only a small percentage urban land (UR; 5%) (see **Table 1** for explanation of all acronyms and abbreviations). Twenty-six sewage treatment plants serve 80% of the residents of the GRW, while the remaining 20% depend on septic systems (Loomer and Cooke, 2011). These point source (sewage plants) and non-point sources (agricultural runoff) accumulate in the lower Grand River, making the concentration of suspended sediment four times higher than the provincial water quality objective (25 mg/L) about 81% of the time between 2000 to 2004 (Cooke, 2006).

The major subbasin that contribute high suspended solids, and phosphorus loads to GRW is the McKenzie Creek Watershed (MCW) (Cooke 2006; Holeton, 2013; MacVeigh et al., 2016), occupying an area of 358 km^2 and containing drainage areas of two main tributaries, the McKenzie and Boston Creeks. Although the MCW accounts for only 7% of the Grand River Watershed by area, it contributed more than 20% of suspended solids loads, making it a major pollutant of concern (MacVeigh et al. 2016). Historic data showed that loading of suspended solids is higher from McKenzie than from Boston Cr, with 84% exceedance of the benchmark in McKenzie compared to 27% exceedance in Boston Cr (MacVeigh et al., 2016). Both creeks are also known to have a history of high nutrient and *E. coli* contamination in its groundwater; in addition, TP concentrations have also exceeded the provincial objective 71% and > 98% of the time in Boston and McKenzie Cr, respectively between 2000 and 2006 (MacVeigh et al., 2016).

The MCW is currently managed by multiple jurisdictions, including the Grand River Conservation Authority (GRCA), Ontario Ministry of Natural Resources and Forestry (OMNRF) and the Six Nations of the Grand River that lives on the Six Nations Reserve, with an area approximately 53% (190 km^2) of the MCW (**Figure 1**). Both creeks have been monitored downstream of the Six

Nations Reserve by the Grand River Conservation Authority between 2003 and 2008 (Cooke 2006; Loomer and Cooke 2011). The Six Nations Band Council also carried out an assessment of fish habitat in the McKenzie and Boston Creeks within the Six Nations Reserve more than 30 years ago (Whitlow 1989, 1990). The variables monitored at 11 stations in 1989 and 1990 included only physical parameters such as dissolved oxygen (DO), pH, specific conductance, water temperature and turbidity, and no nutrient data. Besides DO concentrations being lower than the Canadian guideline at one station, the biggest problem at that time was exceedances in water turbidity for fish and recreational use 64% and 73% of the time in 1989 and 1990, respectively (Whitlow 1989, 1990).

As mentioned, the dominant land use class in the MCW is agricultural land, with most of it occurring either upstream of the Six Nations Reserve in the Norfolk Sand Plain or downstream of the Reserve near the outlet to the Grand River (see Figure 1). The McKenzie Creek catchment has 64% agricultural land while the slightly smaller Boston Cr catchment has 72% agricultural land (see **Table 2**). Since the agricultural crops include mostly cash crops (tobacco, potatoes, ginseng and root vegetable crops), there is intensive irrigation with Boston and McKenzie Creek water.

The soils in the upper subwatershed are poorly drained and many farmers use artificial drainage systems, which consist of tiles placed subsurface to reroute or store water (Kokulan 2019). Such systems are classified as "Tile Drainage Areas" (TD) by the Province of Ontario. In North America, approximately 14% of Canada's croplands (9.46 million ha) are artificially drained (ICID 2018). In general, tile drains reduce waterlogged conditions and occurrence of overland flow by removing excess water and improving soil aeration (Kokulan 2019); however, tile drainage tends to increase the risk of downstream flooding because of "edge of field" runoff (Rahman et al. 2014) and can also lead to increased loading of nutrients, especially nitrates (Coelho et al. 2020).

We initiated this study specifically to fill a knowledge gap with respect to current water-quality conditions of the McKenzie Creek Watershed, given that there has been no follow-up study within the Six Nations Reserve for three decades, and no comprehensive study throughout the MCW to reflect simultaneous conditions both upstream and downstream of the Six Nations Reserve. Specifically, we wanted to determine the exceedances from water-quality objectives for both McKenzie and Boston Creeks, and investigate the relationship between pollutant concentrations (nutrients) and land use-land cover (LULC) classes and tile drainage area (TD) within the McKenzie creek watershed.

We targeted water-quality variables that included both soluble and total forms of phosphorus and nitrogen and total suspended solids because elevated levels of these pollutants have been reported previously (MacVeigh et al., 2016). We also included *E. coli* because it is an indicator of fecal contamination. Makhdoom and Chow-Fraser (2021; Chapter 2) found that 29% of tap water sources tested in 2018 were contaminated with *E. coli*, and we wanted to determine if spillage from the sewage lagoon within the reserve may be a continual source of fecal contamination at downstream sites. This was a request from community members of the Six Nations of the Grand. We also included physico-chemical variables measured in Whitlow's 1989 and 1990 studies so that we could conduct a comparison through time for temperature, pH, turbidity and specific conductance.

Residents of the Six Nations Reserve have had many questions about the state of their creeks, and whether or not the sewage lagoon has been a source of fecal contamination. The community is also worried about the negative effect of agricultural runoff upstream of the Reserve that may be degrading the water quality of their creeks. This study was co-designed with members of the Six Nations community to primarily provide answers to their questions and secondly, to provide needed data to other jurisdictions so that proper abatement strategies can be developed to manage nutrient and suspended sediment loading in the McKenzie Creek Watershed.

Methods

Location of study area

The McKenzie Creek Watershed (MCW) is composed of McKenzie Creek catchment, which drains an area of 183.5 km^2 and the Boston Creek catchment, which drains an area of 174.9 km (**Figure 1; Table 2**). The two Creeks flow eastward towards the Grand River, merging with it just below the town of York. A large portion of the MCW is owned by the Six Nations of the Grand, the largest First Nations Reserve in Canada, which is home to all six Haudenosaunee nations (Seneca, Cayuga, Onondaga, Oneida, Mohawk and Tuscarora). A smaller portion of the MCW is owned by the Mississaugas of the New Credit First Nation. Two sewage lagoons, located on the Six Nations Reserve (town of Ohsweken) and the Mississaugas of the New Credit First Nation Reserve discharge effluent every spring and fall into the McKenzie and Boston Creeks, respectively.

Geographic Information System (GIS) data sources

GIS data layers were provided from the Six Nations Band office in late 2018. These files included the Reserve boundary, locations of named roads, streams and other water bodies. We used the Ontario GeoHub website to obtain the most recent version of land use layer of Southern Ontario Land Resource Information System (SOLRIS 3.0). We also downloaded the most recent Provincial Digital Elevation Model (PDEM), and used the Scholar GeoPortal website to download the surficial geology layer, tile drainage area and Ontario Hydro Network (OHN) Watercourse layer and imported them into ArcGIS Pro (v.2.7.0, ESRI, 2020).

Site selection process

Prior to choosing, we had to first determine stream order for all tributaries within the MCW as per Strahler's (1957) method. By definition, all headwater streams were defined as first order streams. Second-order streams were defined as the convergence of two first-order streams, third-order streams defined as the convergence of two second-order streams, and so on. All second- and third-order streams within the Ontario Hydro Network (OHN) Watercourse layer produced by Ontario Ministry of Natural Resources (2010) were then intersected with the Ontario Road Network (ORN) to identify easy road access points to the tributary. These intersection points were deemed potential sampling points. We used the MCW shapefile to clip out land-use land-cover (LULC) classes from the SOLRIS 3.0 database. Based on the type of LULC within 100-m buffers of the potential sampling points, and verification on Google Earth that the Creek was clearly visible, we chose 30 points that represented a range of LULC. At that point, we consulted Mr. Clynt King (the environmental technician of the Six Nations Band office) to finalize site selection. Based on sampling budget and available resources, we determined that it was feasible to sample 15 sites on a monthly basis. Therefore, we chose 7 sites each on McKenzie and Boston Creeks, and one at the confluence of these two creeks before the McKenzie Creek merged with the Grand River (**Figure 2; Table 3**).

Field sampling

We sampled McKenzie and Boston Creeks between April and November in 2019. Due to road closures and inclement weather conditions, some variables were only available between April and September. Due to a rainstorm in October, we were unable to collect all variables at all sites. Therefore, we have opted to exclude October data except for Total Nitrogen (TN), which were only collected and analyzed in April, July, September, and October for seasonal means. The day prior to each sampling trip, we ensured that all collection bottles and caps were acid-washed and dried, and that all field equipment were calibrated. The sampling usually took place between 09:00 and 15:00 on two consecutive days. Sites off the reserve were usually sampled on the same day, while sites on the Six Nations Reserve were sampled on the following day. Mr. Clynt King or Mr. Rod Whitlow (residents on the Reserve) accompanied the sampling team for all sites on the Reserve.

We used a telescopic pole sampler (maximum length of 14-ft) to collect water from the middle of creeks (if possible). The pole was extended either from the stream bank or from a bridge over the creek. Samples were collected in 500-mL sterile Whirl-Paks for *E. coli*. For all nutrients and total suspended solids (TSS), we collected water samples in acid-washed Snap ‘n Seal jars. For soluble reactive phosphorus (SRP), we filtered the sample with a syringe filter (pore size 0.45µm) and collected the filtrate in Snap 'n Seal jar. All water samples were immediately placed on ice packs inside a cooler. We also used the *In Situ* Aqua Troll 500 sonde to measure ambient pH, specific conductance (Cond), water temperature (Temp), dissolved oxygen (DO) concentration and DO saturation (% Sat). We also used a Hach Portalab Turbidimeter to measure water turbidity (Turb) in triplicate.

After our return to McMaster, we analyzed *E. coli* and TAN samples within 4-6 hours of collection. *E. coli* were analyzed with the TECTA™ B-16 microbiological analyzer (Pathogen Detection System) while the TAN samples were analyzed with the Hach DR 890 with Hach reagents and protocols. All other nutrient samples were placed in a -20 C freezer for storage until it was convenient to process the samples (usually within 6 months). TSS samples were filtered through pre-weighed GF/C filters (pore size 0.45µm); filters were folded, placed in small petri plates and then stored in the freezer. All variables were analyzed in triplicate, except for TN samples, which were only analyzed in duplicate because of the relatively high cost and labour entailed and values between replicates usually varied by no more than 15%. All procedures we used to analyze water samples for nutrients and suspended solids have been provided in detail elsewhere (Chow-Fraser 2006.; DeCatanzaro et al., 2009).

Determining LULC in catchments

To determine individual catchments of the 15 sampling stations, we imported the PDEM (Ontario Open Government, 2021) into ArcGIS Pro and used the Fill tool to eliminate sinks in the DEM and then created the Flow Direction and Flow Accumulation raster layers consecutively. We used the Snap Pour Point (Watershed) tool to create pour points at the 15 sampling sites to delineate all catchment areas. After that, we dissolved all sub-catchment boundaries using the Dissolve tool. We did this to eliminate any overlapping areas between catchments so that the sum of all catchment areas equaled that of the McKenzie Creek Watershed.

To produce the LULC layer for the whole sub-watershed, we imported SOLRIS 3.0 into ArcGIS Pro and converted the raster layer to vector layer and clipped it against all catchment layers. We merged some of the original LULC classes to form 7 as follows: Forest, Swamp, Marsh, Open Water, Agriculture, Hedgerows and Urban. We also clipped the surficial geology layer against the MCW and tributary catchments and reduced them to 7 geological classes: Paleozoic Bedrock, Clay, Sand, Till, Gravel, Organic Deposits and Silt (see **Table 2**).

To calculate the percentage LULC and geological classes within catchments of sampling stations, we exported the attribute table for these layers to Excel and used PivotTable in Microsoft Excel (v. 2106) to calculate percentages. To determine building densities, we downloaded World Imagery basemap (Wayback 2019-06-26) from the ArcGIS website and imported it into the GIS. We manually digitized all buildings visible in the images and did not distinguish building type. The individual catchments were used to clip the building layer to provide an estimate of total number of buildings for each catchment. We calculated the density of buildings by dividing the number of buildings in the catchment by total area of the catchment.

Statistical Analysis

We used SAS JMP v. 14 (SAS Institute, Cary, North Carolina, U.S.A.) to conduct all statistical tests and to produce graphs. Statistical tests included a correlation analysis and linear regression analysis. Because of the large number of variables in our study, we also ran a Principal Components Analysis to explore linear combinations of variables, particularly with respect to co-variates of pollutants and LULC classes. We calculated the seasonal mean for all variables (physico-chemical variables and nutrients); however, for *E. coli*, we calculated the geometric mean as recommended by Health Canada (2012). Some variables had missing values because of malfunctioning equipment, or unexpected road closure on the Reserve that prevented us from accessing the sampling site.

A non-parametric Spearman's Rank Analysis was used to determine the relationships among LULC, geological classes and water quality variables for 14 stations in Boston and McKenzie Creeks; we excluded site MB, which is at the confluence of both creeks. To reduce the chance of committing a Type 1 error due to the multiple comparisons, we applied a Bonferroni correction; consequently, instead of alpha of 0.05, we used alpha of 0.0036. We also ran a Principal Components Analysis (PCA) to reduce dimensionality of the large dataset and to explore linear combinations of water-quality variables that may be significantly correlated with LULC and geological classes. We regressed response variables (nutrients, suspended solids and physico-chemical variables) against TD to determine a relationship between pollutants and this agricultural practice. We also carried out a Mann-Whitney test to determine if there is a significant difference between creeks with respect to seasonal mean concentrations of nutrients, suspended solids and other physico-chemical variables.

Results

LULC characteristics

The MCW contains intensively agricultural land that make up 68% of the drainage basin; however, it also contains two of the largest forest blocks that remain within the GRW, with 26% of the drainage basin being forests and wetlands that occur primarily within the Six Nations Reserve (**Figure 3a**). Only a small percentage (4.5%) of the drainage basin is classified as urban; within the Reserve, there is the town of Ohsweken, and upstream of the Reserve, there is the town of Oakland. Tile drainage, which is associated with high nutrient runoff, occurs in only 13.4% of the MCW, but is concentrated in Boston Creek (69% of the catchment), mostly upstream of the Six Nations Reserve (**Figure 3b**).

The topography of the MCW consists mostly of gently rolling hills and low relief with highest elevation being 250 m above sea level (asl) that occurs in the western portion of the watershed; the eastern portion is relatively flat and at the confluence of the McKenzie and Boston Creeks, the elevation is 190 m asl. Although there are four dominant surficial geology classes (Haldimand Clay Plain, Norfolk Sand Plain, Wentworth Till and Exposed Bedrock), the Haldimand Clay Plain (73.7%) make up the vast majority of surficial geology within the MCW; this landform is characterized by deep waters and dominated by surface runoff with low infiltration (**Figure 4**). The Norfolk Sand Plain (16.9%), characterized by shallow waters, is the dominant feature in the western portion of the MCW. It contains coarse sands and silts that are associated with high infiltration and low surface runoff. An example is the Oakland Swamp, located in the headwater of McKenzie Creek, which is a groundwater storage zone.

There are relatively large stands of forests and swamps within the Six Nations Reserve, (**Figure 5a and 5b**, respectively), whereas the predominant land use outside the Reserve is agricultural (**Figure 5c**), majority of which is located upstream of the Reserve. Relative to the Boston Creek catchment, the McKenzie Creek catchment had 1.3 times greater %swamp, %forest and %urban areas (**Table 3**). Similarly, there was 2.6 and 3.9 times greater %sand and %gravel in the McKenzie Creek catchment, respectively. On the other hand, Boston Creek had a higher percentage of agricultural lands, 1.3 times more % hedgerow, and 2.2 times greater %tile drainage areas. There were also 1.4 and 1.5 times greater %silt and %till substrate in the Boston Creek catchment compared with the McKenzie Creek catchment. The Boston Creek catchment contained no organic deposit while the McKenzie Creek catchment contained no Paleozoic bedrock (**Table 3**).

We examined variation in %LULC and substrate type among the catchments of the 14 sampling stations in McKenzie and Boston Creeks (**Table 4**). As noted earlier, wetlands and forests were more prevalent within the Reserve than outside the Reserve; hence, the highest %swamp was in the M04 catchment (29%) while the highest %forest was in the M07 catchment (23%). Station B02, which is located upstream of the Reserve had the lowest %swamp (1.5%) and %forest (2.5%) and the highest %agricultural lands (90%). The M07 catchment also had the highest %clay (97.4%) whereas the M01 catchment had the lowest (6%). Given its location, the M01 catchment had the highest %sand (78.5%) while the B05 catchment had the lowest (0.7%).

Water quality characteristics

Water turbidity measured at the McKenzie Cr stations ranged from 29 to 124 NTU whereas those measured at the Boston Cr stations were much lower, ranging from 5 to 34 NTU (**Table 5**). TSS concentrations measured at McKenzie Cr were similarly high, varying from 31 to 122 mg/L, whereas those measured at Boston Cr were substantially lower with a narrower range (12 to 44 mg/L)(**Table 5**). When we pooled the data by creek and compared them statistically, we found that both turbidity levels and TSS concentrations were significantly higher in McKenzie relative to

Boston Creeks (NTU of 61 vs 21 NTU, respectively; $P<0.0001$; TSS of 63 vs 26 mg/L, respectively; $P<0.0001$; **Table 6**).

Range in mean seasonal TP concentrations for the two creeks were similar, with slightly larger range for McKenzie Cr compared with Boston Cr (78 to162 µg/L for McKenzie Cr sites, vs 53 to 132 µg/L for Boston Cr sites) (**Table 5**). Even though the mean value for McKenzie Cr was 1.4 times greater than that for Boston Cr, there was no statistically significant difference between creeks when we pooled data ($P=0.0649$; **Table 6**). For the soluble form of P, however, concentrations of SRP ranged from 19 to 72 µg/L for Boston Cr sites and only from 10 to 36 µg/L for McKenzie Cr sites (**Table 5**). When we combined the data by creek, we found significantly higher mean SRP concentrations for Boston Cr that was twice that for McKenzie Cr (44±5.6 vs 21±2.5 µg/L for Boston and McKenzie Creeks, respectively; $P=0.0002$; **Table 6**).

Seasonal variation in TN and TNN did not vary greatly across sites within McKenzie Creek (1.5 to 2.2 mg/L and 0.38 to 0.79 mg/L, respectively; **Table 5**). The range in seasonal mean concentrations of these nitrogen fractions was slightly larger for Boston Cr; concentrations ranged from 1.3 to 3.92 mg/L and from 0.24 to 2.18 mg/L, for TN and TNN, respectively (**Table 5**). There were no significant differences between creeks for TN and TNN (**Table 6**). For TAN, seasonal mean concentrations at the Boston Cr sites ranged from 0.09 to 0.13 mg/L while those at the McKenzie Cr sites ranged from 0.13 to 0.20 mg/L (**Table 5**). The mean TAN concentration for McKenzie Cr was 1.4 times higher than that for Boston Cr (0.15±0.11 mg/L vs 0.11±0.009 mg/L; $P=0.0051$; **Table 6**).

Seasonal mean pH did not vary a great deal across sites, varying from a pH of 7.89 to 8.14 in Boston Cr and from 8.00 to 8.31 in McKenzie Cr (**Table 5**). Mean pH for Boston Cr was 8.00 vs 8.14 for McKenzie Cr, but these were not significantly different ($P=0.0774$; **Table 6**). Generally, both

Boston and McKenzie Creeks were relatively well oxygenated, ranging from 7.67 mg/L at site B04 to a high of 9.11 mg/L at site B07. Based on thermal characteristics at each site, these concentrations corresponded to a minimum of 80% saturation at all sites. Although the mean DO concentration measured at Boston Cr was slightly higher than that at McKenzie Cr (8.25 vs 8.16 mg/L, respectively), these means were not significantly different (P=0.9072; **Table 6**). Specific conductance values in Boston Cr ranged from 816 to 1659 and were generally much higher than those for McKenzie Cr, which ranged from 657 to 702 (**Table 5**). Mean conductivity of all Boston Cr sites was significantly higher than that for McKenzie Cr (1156 vs 685 μS/cm, respectively; P=0.0022; **Table 6**).

E. coli densities varied greatly across sampling sites in both creeks. We calculated geometric means for each site using the monthly measurements from April to November. For Boston Creek, densities ranged from 3.89 at B07 to $6.1 \bullet 10^4$ at B05 (**Table 5**). For McKenzie Creek, the lowest value was 31 at M06, and the highest was 233 at M05. On review of the monthly data, we noted that densities in April at all sites were relatively low (**Figure 6**). Densities rose in June, especially at B05, and peaked in July and August. Unfortunately, access to B03 was blocked in September and October and we were unable to collect any samples during these months. By November, *E. coli* densities at B05 dropped by several orders of magnitude compared to the previous months but were still the highest amongst all sampling sites. Seasonal maxima were also observed at B02, M03 and M05 with more gradual increase at McKenzie Cr sites (**Figure 6**).

Water-quality objectives and guidelines

Many jurisdictions have published water-quality objectives and guidelines for flowing water to protect people from water-borne pathogens when they use streams recreationally. They are also designed to protect aquatic life such as fish, shellfish, and wildlife. These objectives/guidelines vary across jurisdictions and for some parameters, no objectives/guidelines have been proposed (i.e. specific conductance and dissolved P). In this paper, we consulted Health Canada's (2012) guideline for recreational water quality and Ontario's (1994) water quality objectives, as well as the standard for turbidity developed by the Minnesota Pollution Control Agency (**Table 7**) since neither of the federal or provincial documents recommended a guideline for aquatic life.

Turbidity levels at all sites in McKenzie Cr exceeded the guideline of 25 NTU for fish protection and recreational use while only three stations in Boston Cr had turbidity that exceeded this guideline (**Table 5**). With respect to aesthetic considerations, only three of the McKenzie sites exceeded the guideline, whereas none of the Boston sites exceeded the guideline of 50 NTU. Since TSS are highly correlated with turbidity, not surprisingly, all McKenzie Cr and four in Boston Cr exceeded the Canadian guideline of 25 mg/L for TSS. Generally, the entire McKenzie Cr can be said to be too polluted with suspended sediment for recreational use or fish habitat, whereas portions of Boston Cr are still in relatively good condition (i.e. B01, B03 and B05).

Phosphorus is considered the most limiting nutrient in freshwater ecosystems (Schindler et al. 2008). For rivers and streams, Ontario recommends maintaining phosphorus concentrations below 30 μg/L, regardless of the form measured (i.e. TP or SRP). TP concentrations at all stations in McKenzie and Boston Creeks exceeded 30 μg/L and in the case of M03, mean seasonal concentration of 162 μg/L was over 5 times higher than the objective (**Table 5**). Even for SRP, which is normally much lower than TP, concentrations exceeded 30 μg/L at 4 sites in Boston Cr and at 3 sites in McKenzie Cr. It appears that TP concentrations were generally higher in McKenzie Cr than in Boston Cr, while the reverse is true for SRP (**Table 5**).

The TN guideline for recreational water quality in streams of the MixedWood Plains zone is 1.07 mg/L (**Table** 7). This guideline was easily exceeded at all sites we sampled (**Table 5**). TN concentrations were generally higher in Boston than in McKenzie Cr (seasonal mean of 2.24 vs 1.81 mg/L, respectively) and these mirrored the higher TNN concentrations in Boston Cr vs those in McKenzie Cr (seasonal mean of 0.86 vs 0.57 mg/L, respectively; **Table 6**). It is notable, however, that even the high concentration of 2.18 mg/L at B01 did not exceed the guideline of 3.00 mg/L. The guideline for ammonia requires calculating the concentration of the un-ionized form based on ambient pH and temperature. For protection of aquatic life in freshwater, the guideline value is 0.019 mg/L. Given the range of pH and temperatures experienced at our sites, TAN concentrations would have to exceed 0.5 mg/L for the ammonia guideline to be violated. Therefore, all our sites met the water-quality guideline (**Table 5**).

Only a few guidelines exist for physical parameters in stream environments. DO concentrations at all sites were above 7 mg/L, and since the most conservative guideline for minimum DO concentrations is 5.5 mg/L, de-oxygenation is not a threat to aquatic life during daylight hours (**Table 7**). Given the guideline for pH covers a wide range (5.0 to 9.0), and that pH values measured at all our sites ranged from 7.89 to 8.31, extreme pH is not a concern for the tributaries in the MCW. Specific conductance is affected by the ions such as chloride, phosphates, and nitrates. Since de-icing salt is a source of Cl^{-1}, urbanized streams with many bridges and road crossings usually have elevated Cond values. At the same time, phosphates and nitrates from agricultural and urban runoff will also lead to elevated Conductance values. USEPA indicated that streams supporting good mixed fisheries have a range between 150 and 500 µS/cm (U.S. Environmental Protection Agency 2012). Therefore, seasonal mean Conductance values ranging from 657 to 1659 µS/cm indicate that Boston and McKenzie Creeks have higher values those in streams that support good fish habitat (**Table 5**).

The presence of *E. coli* in water courses is not usually a concern for aquatic life, although it is a public health concern because it is an indicator of fecal pathogens and may cause gastrointestinal illnesses if ingested. For recreational contact (secondary contact), the guideline in Canada is a geometric mean of 200 CFU/100 mL for a minimum of 5 samples over a season (**Table 7**). Only 2 sites each on McKenzie (M03 and M05) and Boston Creeks (B02 and B05) exceeded this guideline (**Table 5**).

Comparison across the McKenzie Creek Watershed

We mapped mean seasonal concentrations of TP, SRP, TSS and Turb at the 14 stations in Boston and McKenzie Creeks to examine the distribution of pollutants along the two tributaries (**Figure 7**). Turbidity and TSS concentrations within the Six Nations Reserve were generally higher than upstream and downstream sites. It is noteworthy that B01 had relatively clear water (seasonal mean of 9.30 NTU and 13.85 mg/L of TSS) but by the time water flowed to station B02, both Turb and TSS had exceeded guidelines for fishery and recreational use. Generally, TP concentrations at the McKenzie Cr sites were uniformly high and exceeded water-quality objectives; similarly, TP concentrations exceeded water-quality objectives in the Boston Cr even though values were not as high as those in McKenzie Cr. By contrast, the SRP concentrations tended to be much higher at the Boston Cr sites within the Reserve. SRP concentrations at M05, M06 and M07, which are located downstream of the sewage lagoon (see Figure 2) were all higher than those measured at the upstream sites. Station M03 (Seneca Rd crossing) had maximum values for TP, TSS and Turb, while B03 (at the 1st Line crossing) had minimum values of TSS and Turb.

Generally, TAN concentrations did not vary very much within the two tributaries and were well below the guidelines; nevertheless, values were generally higher within the Reserve than outside (**Figure 8**). Within the McKenzie Cr catchment, TNN concentrations outside the Reserve were lower than those within the Reserve whereas within the Boston Cr catchment, the opposite was true. Most of the sites outside of the Reserve (M01, M02, M07, B01, B07) had very low *E. coli* densities (**Figure 9**). Within the Reserve, B05 had extremely elevated densities compared to the other sites. Because there was no road crossing on Boston Cr at 2nd Line, Clynt King recommended we move the sampling location to Spring Creek, a tributary of Boston Creek. The elevated *E. coli* densities at B05 points to a probable source of contamination. The other sites that had high *E. coli* counts were B02, M03 and M05, with densities that exceeded the guideline for recreational use.

Relating water quality to LULC

Nutrients and suspended solids tend to have similar anthropogenic sources stemming from various land uses and are therefore spatially correlated. For example, Station M03 was the most polluted with respect to Turb, TSS and TAN, and had levels of *E. coli* that exceeded the Health Canada guideline for secondary contact use. In addition, Tile Drainage Areas (TD) are known to be sources of nutrients and suspended solids (Coelho et al. 2020) and would therefore be spatially correlated with land-use and land cover categories. We wanted to first explore the correlation between TD and LULC classes and between water-quality parameters, before determining significant relationship between pollutants (nutrients and sediments) and LULC categories within the Boston Cr and McKenzie Cr catchments.

As expected, Tile Drainage Areas (TD) was positively correlated with % Agricultural land (AG; $\rho = 0.8721;\ P < 0.0001$) and negatively correlated with % Forested land (FO; $\rho = -0.7744;\ P < 0.0011$). There was also a positive correlation between % TD and % Marsh (MA; $\rho = 0.7729;\ P < 0.0012$)) and % Silt (SI; $\rho = 0.7744;\ P < 0.0011$). With respect to water-quality variables, we found a highly significant correlation between TSS and Turb ($\rho = 0.9604;\ P <$

0.0001, between TNN and TN ($\rho = 0.9385;\ P < 0.0001$, as well as between TAN and Turb ($\rho = 0.7407;\ P < 0.0024$).

When we regressed pollutants against %TD, the only relationship that was significant were those involving TNN and TN (**Figure 10a and b**, respectively). Since %TD was significantly correlated with %AG, we also found a significant regression between TNN and %AG (r^2=0.32; P=0.0347) and between TN and % AG (r^2=0.31; P=0.0391). Given the negative correlation between % FO and % AG, we also found a significant negative relationship between TN and %FO (r^2=0.295; P=0.0447). There was also a significant positive relationship between TNN and % UR, and between TN and % UR (**Figure 11a and b**, respectively). We found no other significant relationship between a pollutant and a LULC class.

The first two axes of the Principal Components Analysis explained almost 52% of the variation in the dataset (**Figure 12**). The first axis separated out sampling stations located upstream of the Six Nations Reserve (M01, M02, B01 and B02; right quadrants) from downstream sites (left quadrants) while the second axis separated out sampling stations according to Creeks (McKenzie Cr top quadrants and Boston Cr bottom quadrants). Catchments of sites upstream of McKenzie Cr (top right quadrant) had a relatively large percentage of Gravel and Sand, while those upstream of Boston Cr (bottom right quadrant) had a larger percentage of Tile Drainage area and Agriculture. Accordingly, the highly tiled B01 and B02 were associated with high TN and TNN. The McKenzie Cr stations located on the Reserve (M03, M04, M05 and M06) were associated with relatively high concentrations of TP, TAN and TSS as well as Turbidity. These sites, particularly M03 was associated with higher percentage Swamp. B04, B06 and B07 were clustered close to the origin, indicating that they are very similar to each other and to M05, M06 and M07. By contrast, B05 appears to be an outlier, with high water temperatures, high SRP, high COND and high *E. coli* densities.

Discussion

A picture is emerging that shows not only major differences in geological substrates and land cover between the catchments of McKenzie and Boston Creeks, but also stark contrast between sites upstream of the Six Nations Reserve and those within the Reserve. First, there is dense cropland and a high proportion of tiled drainage areas (especially in the Boston Creek catchment) upstream of the Reserve, whereas within the Reserve, there is a higher proportion of natural land cover (forest and swamp). Secondly, upstream portions of both creeks had a much higher proportion of sand, till, silt and gravel, whereas within the Six Nations Reserve, clay is the dominant geological substrate class, forming ~74% of the MCW. These variations appear to be the basis of differences in pollutant loading at various sampling stations across McKenzie and Boston Creeks.

Nutrients and sediments

Total phosphorus has been considered a nutrient of concern in the MCW for many years (MacVeigh et al., 2016); we confirmed that all stations in 2019 uniformly exceeded the Canadian water quality objective. Many sources contribute to a high loading of phosphorus, including effluent from the sewage lagoon that is discharged seasonally into McKenzie and Boston Creeks, runoff from improperly maintained septic systems, urban and agricultural runoff, and in-stream bank erosion (MacVeigh et al., 2016). Some sources like the sewage lagoon, manure and fertilizers are high sources of SRP (ECCC, 2018). In addition, surficial geology plays an important role in nutrient loading; for example, the portion of the watershed located on Clay plain would have higher runoff and a higher loading of P and sediments. Therefore, the higher percentage of clay plain and agricultural lands in McKenzie Cr should be associated with a higher concentration of TP.

SRP readily adsorbs to suspended sediment particles in the water to form TP in water bodies. Hence, there is generally a lower ratio of SRP to TP when TSS concentrations are high. This explains why we observed comparatively higher SRP concentrations in Boston Cr that has very low TSS concentrations. By contrast, both TSS and TP are proportionately higher in McKenzie Creek, and SRP concentrations were generally low, except at sites downstream of the sewage lagoon (between M04 and M05; Figure 7). This suggests the sewage lagoon is a source of nutrient contamination (Holeton, 2013) and should be further investigated. To improve water quality in the GRW, total and dissolved P must be reduced especially from the non-point pollution source, since 71% of the Canadian SRP load to L. Erie is contributed from non-point sources between 2003 to 2013 (ECCC, 2018).

The significant relationship between TD and TNN is supported by literature showing that tile drainage resulted in increased loading of nitrates in downstream water bodies, particularly during spring and summer storms after fertilization application (Kokulan et al. 2019, 2021). TD is used in 14% of Canadian agricultural lands, and more specifically about 45% of the agricultural lands in southern Ontario have been tiled to decrease problems with waterlogged soils and to increase plant growth (Kokulan 2019). Since high concentrations of nitrates in streams can pose a threat to ecosystem health (Pease et al. 2018; Wherry et al. 2021), these negative effects of high nitrate loss from agricultural land may offset any benefits of TD. This has become a problem in agricultural catchments in Quebec, where 50% of TD accounted for most of the nitrate loading (Kokulan, 2019). Williams et al. (2015) also showed that TD is the primary factor controlling watershed discharge of N, especially nitrate, in agricultural headwater watersheds.

In the MCW, the vast majority of TD and cropland areas are located upstream of the watershed (69% in Boston Cr catchment), and this explains the higher concentrations of TN and TNN in upstream stations (B01 and B02) within the watershed. During the summer of 2019, all stations increased in TN concentration above the Provincial Water Quality Objectives for protecting aquatic life, and this is a contribution to nutrient enrichment of the lower Grand River, and ultimately a contributor to problems in Lake Erie including increased primary production, turbidity, sediments and lower DO levels (Metcalf, 2013). Although TN and TNN were higher in Boston Cr, TAN was higher in McKenzie Cr (especially inside the Six Nations Reserve). Ammonia is commonly released from the effluent of industrial and fertilizer plants, agricultural, residential and municipal releases such as spills of ammonia-rich fertilizer (CCME, 2010). The Canadian guideline for protecting aquatic life uses the un-ionized form of ammonia, which is highly toxic to aquatic organisms because it can diffuse across biological membranes more readily than the other forms and lead to acute, chronic, and sub-fatal effects to the fish community (CCME, 2010). None of our sites in this study exceeded the Canadian water quality guideline for un-ionized ammonia, although there were slightly higher concentrations at M03, which is inside the Six Nations Reserve on McKenzie Cr. It is noteworthy that TNN between 2000 to 2008 in MCW were also below the Canadian water quality guideline.

Increases in suspended solids can reduce the penetration of sunlight in a stream and negatively affect productivity of aquatic ecosystems. Sediment in the water column can also affect fish movements and behaviour as well as reproductive success (Whitlow 1990; Metcalf et al. 2013; Holeton 2013). It can also affect recreational activities (e.g. swimming) and water with turbidity greater than 50 NTU is deemed to be unsuitable for secondary contact (Health Canada, 2012). High levels of TSS in the Southern Grand River has been related to the geology of the region (i.e. Clay plain) (Loomer and Cooke, 2011); likewise, high TSS concentrations in McKenzie Cr has been reported previously during the summer in 2003 (MacDougall and Ryan, 2012). In 2019, we found that Turb concentrations were still quite high in McKenzie Cr, especially within the Six Nations Reserve, and that these levels were higher than those in Boston Cr. Given the strong positive correlation between TSS and Turb, and Turb with TP, we recommend installing continuous monitoring devices with Turb sensors to save on processing costs of TSS and TP wherever we have elevated levels of nutrients and sediments.

We could not find a significant relationship between elevated TP, TSS, TAN and Turb with LULC classes. Nevertheless, the highest levels recorded at M03 suggest that there may be a point source. Besides exceeding guidelines for Turb, TP, TSS and TAN, the M03 station also had the highest densities of *E. coli*, which exceeded the water quality guideline for recreational use. M03 is situated downstream of the Victoria Mills dam (M02) and Victoria Lake, which is a large turbid reservoir (Holeton, 2013; Metcalf, 2013). Reservoirs are generally built to increase water flow during low flow times, and to mitigate flooding during high flow times (Loomer and Cooke, 2011). In the case of Victoria Mills dam, it is no longer operational, but it still funnels water and its content into McKenzie Cr upstream of M03. Victoria Lake may be a point source of pollution with greatest effect during the summer season when the water flow is low (MacVeigh et al., 2016), and this could explain why the highest pollutant concentrations were recorded at M03 station.

<u>Fecal Coliforms (*E. coli*)</u>

E. coli is a bacterium that lives in the intestinal tracts of mammals and is a good indicator of fecal contamination in water. When ingested, water contaminated with *E. coli* may contain pathogens that are responsible for gastrointestinal illnesses. Between 1992 to 2002, 25% of all gastrointestinal illness outbreaks in U.S. were due to *E. coli* contamination in surface waters (Health Canada 2012).

There are several sources of fecal contamination in surface water including sewage lagoon and septic systems as well as episodic runoff from agricultural and urban areas during storms (Health Canada, 2012). Levels of *E. coli* in surface water has not been well documented within the Grand River Watershed; however, some samples had been collected at the Elora Gorge Conservation Area during the summer from 2000 to 2004, and concentrations varied from 10 to 4000 CFU/100 mL) (Cooke, 2006). Within McKenzie and Boston Creeks, a 2004 study found that levels were below the Canadian water quality guideline (MacDougall and Ryan, 2012). During 2019, *E. coli* densities were relatively low in April and May throughout the creeks, but rose in June 2019, probably because of increased temperature and nutrient concentrations (Rosen, 2000; Ishii & Sadowsky, 2008; Health Canada, 2012). The geometric mean *E. coli* densities exceeded the Canadian guideline for recreational water during the summer at only four stations (B02, B05, M03 and M05) during 2019.

Physical variables

Dissolved oxygen is produced when plants (vascular and non-vascular plants and algae) photosynthesize; it can also be replenished in streams from rainwater or by turbulence. Oxygen depletion is primarily through decomposition of organic matter, respiration by organisms living in the water, and when there is an increase in temperature. A low DO concentration can have lethal and sub-lethal effects on fish, especially juveniles (CCME, 1999; Holeton, 2013). In this study, water in both creeks were well oxygenated throughout the day. That there is high DO concentration during the daylight hours, however, does not mean that anoxic conditions do not develop during the nighttime. In streams with high nutrient loads during the summer, there can be diurnal fluctuations of DO (Cooke 2006; Seilheimer et al. 2007); therefore, future studies should determine diurnal trends in DO by installing continuous DO loggers in McKenzie and Boston Creeks to ensure that DO levels do not dip below water-quality objectives during the night. Algal respiration during the night likely reduces

DO to levels not tolerable by fish. The problem could be corrected with proper fencing to limit access by cattle into the stream.

Specific conductance levels at our sites were generally much higher than the guideline set by the USEPA (2012), especially those in Boston Cr. Over 30 years, conductivity concentrations were high in the Six Nations reserve, with the highest values in Spring Cr (the largest catchment contribute to Boston Cr) near the gypsum plant in both years of 1989 and 1990 (Whitlow 1989, 1990). The high conductance values are high within the watershed, with highest values at downstream stations (B05, B06, B07). Therefore, we suggest that the gypsum plant and de-icing salts from urbanized runoff off the bridges may be the source of the high conductivity in Boston Cr.

<u>Comparison with historic data</u>

During the summer of 1989, Whitlow conducted stream habitat assessments for 11 km (~28% of its length on the Six Nations Reserve) on the main channel of McKenzie Creek and for 15.3 km (100% of the flowing portion through the Six Nations Reserve) of the main channel of Boston Creek. During 1990, he also conducted similar surveys for 14.3 km (~37% of its length on the Reserve) of McKenzie Cr in 1990. In these assessments, Whitlow documented physical, chemical and biological conditions of the aquatic environment and determined factors that were limiting fish production and recommended fisheries management alternatives. He focused on physical parameters such as turbidity, dissolved oxygen concentrations, temperature, pH and specific conductance, as well as the type and amount of riparian vegetation and bank characteristics. Eleven stations were established within the Six Nations for weekly monitoring (see Table A1 and Figure A1 in Appendix).

Seven of the eleven stations sampled by Whitlow in 1989 and 1990 corresponded to sites that we sampled in 2019 (**Table 8; Figure 13**). Similar to what we found in 2019, Whitlow (1990) reported the highest level of *E. coli* at B05, a station located on Spring Cr near the gypsum plant. In August 2019, we saw excessive algal growth in the water, which was a sign of nutrient enrichment, and this increased in September 2019. By October, the creek surface was fully covered with algal growth, and we recorded high levels of TP, SRP, TN and *E. coli.* Since pollution at this site appears to have been on-going for at least 30 years, we recommend more extensive surveys of the catchment to identify the source of contamination. Another site where we found high levels of *E. coli* and nutrients in 2019 is Station B02. Whitlow's (1989) description of the surrounding land at this site was open pasture where cattle could enter the creek unimpeded. This is probably still occurring and may explain the high density of *E. coli* and nutrients. Livestock along streams has been known to increase bank erosion and increase concentrations of nutrients (P and N) from cattle excrement and trampling (Scrimgeour & Kendall, 2002; O'Callaghan et al., 2019). Thus, Whitlow's observations, made 30 years ago that the most limiting factors for Boston Cr in terms of fish habitat is dense instream vegetation and fecal pollution, appears unchanged today.

Station M05, located on McKenzie Cr, is downstream of the outflow from the sewage lagoon. Nutrient and sediment levels as well as *E. coli* exceeded federal and provincial guidelines and objectives. We found inflated levels of nutrients and sediments at Station M03, and Whitlow (1989) described sewage seeping into the creek from the school in 1989; he also reported finding domestic garbage at that site (Whitlow, 1989). Generally, Whitlow found that the most limiting factor for fish habitat in McKenzie Cr 20 years ago was high turbidity and suspended solids, and these conditions are still in effect today. The DO concentrations reported by Whitlow ranged from 7.7-9.9 mg/L in 1989 and from 7.3 to 11 mg/L in 1990, whereas the range in 2019 was slightly narrower (7.8 -8.5 mg/L). According to Whitlow (1989 & 1990), turbidity levels at the 7 overlapping stations between 1989/1990 and 2019 exceeded the Canadian guideline of fishery and recreation (Table 8); therefore turbidity was the most limiting factor in McKenzie Cr and the suspended solids in some stations (Whitlow, 1989).

Conclusion

Despite the relatively high percentage of forest fragments inside the Six Nations Reserve, there is a surprising amount of water-quality problems and nutrient hotspots (M03, M05, B03 and B05). More detailed investigations are required during base-flow condition and after storm events to better assess water quality of the watershed. In general, McKenzie Cr is more polluted with TP, ammonia and suspended solids, while Boston Cr has a greater concentration of SRP and higher conductivity. Some land use practices along the Boston Creek resulted in deterioration of stream. For example, cattle manure in the stream has led to enrichment, which has encouraged heavy algal growth along the substrate, but this is a problem that may be corrected by fencing and some public education. A coordinated program with appropriate monitoring and remedial actions should be developed before there is another 30 years of inaction.

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Table 1: List of abbreviations/acronyms and their meaning. LULC=land-use land-cover category.

Abbreviation or acronym	Meaning	Notes
GRW	Grand River Watershed	All land drained by the Grand River and its tributaries
MCW	McKenzie Creek Watershed	All land drained by the McKenzie Creek and tributaries, including the McKenzie and Boston Creek catchments
EC	*Escherichia coli*	Fecal indicator; measured in Colony-Forming Units (CFU)·100 mL^{-1}
% FO	Forested land	LULC category
% HE	Hedgerow	LULC category
% MR	Marsh	LULC category
% WT	Water	LULC category
% SW	Swamp (treed wetland)	LULC category
% AG	Agricultural land	LULC category
% UR	Urban land	LULC category
% TD	Tile drainage	Type of agricultural practice
% CL	Clay substrate	Type of substrate
% GR	Gravel	Type of substrate
% OD	Organic deposit	Type of substrate
% PA	Paleozoic bedrock	Type of substrate
% SA	Sand	Type of substrate
% SI	Silt	Type of substrate
% TI	Till	Type of substrate
BldgD	Building density	# buildings per unit area (#/km^2)
Temp	Water Temperature	Water-quality variable ; °C
Turb	Water Turbidity	Water-quality variable; NTU
Cond	Specific conductance	measure of the ability of water to pass an electric current; µS/cm
DO	Dissolved Oxygen concentration	Water-quality variable; mg/L
DO %Sat	Dissolved Oxygen in percent saturation	Water-quality variable; standardized measure of dissolved oxygen in water at a given temperature and elevation
TP	Total Phosphorus	Water-quality variable; µg/L
SRP	Soluble Reactive Phosphorus	Water-quality variable; µg/L
TAN	Total Ammonia Nitrogen	Water-quality variable; mg/L
TNN	Total Nitrate Nitrogen	Water-quality variable; mg/L
TN	Total Nitrogen	Water-quality variable; mg/L
TSS	Total Suspended Solids	Water-quality variable; mg/L

Table 2: Comparison of land-use, land-cover categories and geological classes associated with Boston and McKenzie Creeks. Abbreviations of variables are explained in Table 1.

Variable	Boston	McKenzie
Total drainage area	174.9	183.5
% MR	0.13	0.15
% SW	13.36	17.47
% WA	0.11	0.41
% FO	9.28	12.15
% HE	0.85	0.62
% UR	3.9	5.18
% AG	72.35	64.01
% PA	3.72	0.0
% CL	79.29	68.27
% SA	9.23	24.28
% TI	3.87	2.62
% GR	0.42	1.68
% OD	0	0.48
% SI	3.72	2.67
% TD	69.18	30.82

Table 3: Coordinates of 15 sampling stations of the McKenzie and Boston Creeks and associated main road crossings.

Site ID	Main road crossing associated with station	Creek	Latitude	Longitude
B01	Norfolk County Rd 19	Boston	42.99392	-80.24936
B02	Indian Line	Boston	43.00330	-80.16373
B03	1st Line	Boston	43.00769	-80.11998
B04	Onondaga Rd	Boston	43.01672	-80.08482
B05	2nd Line & Spring Cr	Boston	43.00295	-80.05134
B06	3rd Line	Boston	43.01450	-80.02687
B07	Highway 6	Boston	43.01991	-79.99985
M01	Cockshutt Rd	McKenzie	43.02491	-80.28305
M02	Indian Line	McKenzie	43.01952	-80.20874
M03	Seneca Rd	McKenzie	43.04112	-80.16950
M04	Chiefswood Rd	McKenzie	43.05507	-80.12884
M05	Tuscarora Rd	McKenzie	43.08001	-80.07697
M06	Cayuga Rd	McKenzie	43.05775	-80.02425
M07	Highway 6	McKenzie	43.04413	-79.97832
MB	Haldimand Rd	Confluence of McKenzie and Boston	43.02351	-79.91505

Table 4: Land-use, land-cover and substrate type in catchments of 14 sampling sites established in the McKenzie and Boston Creeks for this study. Site IDs beginning with "B" and "M" refer to sites in Boston and McKenzie Creeks, respectively (see Figure 1). Bldg=building.

Site ID	size (km^2)	# Bldgs	Bldg density	% SW	% MR	% WT	% FO	% HE	% UR	% AG	% PA	% CL	% SA	% TI	% GR	% OR	% SI	% TD
B01	21.3	510	24.0	4.5	0.1	0.0	7.0	1.5	7.1	80.0	0.0	8.2	48.8	18.1	2.7	0.0	22.2	37.4
B02	7.9	161	20.4	1.5	1.2	0.0	2.5	0.4	4.7	89.7	0.0	76.0	15.4	6.0	0.0	0.0	2.6	52.1
B03	31.7	317	10.0	7.9	0.0	0.0	5.9	0.7	2.5	83.1	4.6	89.5	4.6	1.0	0.4	0.0	0.0	32.9
B04	41.5	552	13.3	28.3	0.2	0.0	13.9	0.3	3.1	54.2	0.0	95.7	1.5	0.2	0.0	0.0	2.6	13.7
B05	35.7	473	13.2	9.0	0.0	0.4	6.9	1.5	2.9	79.3	14.1	84.4	0.7	0.9	0.0	0.0	0.0	10.7
B06	11.9	159	13.4	24.6	0.0	0.4	20.1	0.6	1.7	52.6	0.0	86.5	6.6	6.9	0.0	0.0	0.0	0.0
B07	3.1	63	20.2	24.6	0.0	0.4	20.1	0.6	1.7	52.6	0.9	91.6	3.1	4.3	0.0	0.0	0.0	0.0
M01	47.5	1214	25.5	16.7	0.5	1.1	4.8	0.4	6.4	70.1	0.0	6.0	78.5	6.7	6.5	1.7	0.6	11.7
M02	15.5	151	9.7	5.8	0.1	0.8	7.4	1.1	2.7	82.6	0.0	52.4	9.9	8.0	0.0	0.0	29.8	48.7
M03	16.0	319	19.9	23.8	0.0	0.0	11.7	0.8	2.3	61.8	0.0	91.6	8.4	0.0	0.0	0.0	0.0	1.3
M04	14.9	371	24.9	29.1	0.0	0.0	17.7	0.0	2.8	50.1	0.0	93.0	6.7	0.0	0.0	0.4	0.0	0.0
M05	38.8	913	23.6	19.0	0.0	0.2	14.5	0.2	5.4	60.4	0.0	95.0	4.9	0.0	0.0	0.0	0.0	0.7
M06	12.2	258	21.2	23.0	0.2	0.0	22.3	0.0	2.2	52.0	0.0	91.4	8.6	0.0	0.0	0.0	0.0	1.1
M07	17.3	277	16.0	18.9	0.0	0.0	23.2	0.0	2.2	54.2	0.0	97.4	2.2	0.5	0.0	0.0	0.0	0.0

Table 5: Monthly means of water-quality variables collected at 15 sampling stations of the McKenzie and Boston Creeks from April to November in 2019 during base-flow conditions. See Table 1 for explanation of abbreviations of variable labels and Table 2 for coordinates of sampling sites. TN means correspond to only 4 months (April, July, September and October). Mean for all variables are arithmetic means, whereas those for EC are geometric means. Highest mean values across sites for each variable are bolded. Values that exceed water-quality guidelines/objectives appear in red.

Site ID	TURB (NTU)	TSS (mg/L)	TP (µg/L)	SRP (µg/L)	TN (mg/L)	TNN (mg/L)	TAN (mg/L)	DO (mg/L)	pH	Cond (µS/cm)	DO (mg/L)	Temp (°C)	EC #/100 mL
B01	9.30	13.85	76.23	28.90	**3.92**	**2.18**	0.09	8.57	8.14	1061	8.57	16.23	57.21
B02	28.02	30.29	52.61	19.23	2.65	1.35	0.11	8.34	7.89	1018	8.34	19.10	273.36
B03	5.32	12.52	132.32	**69.50**	2.31	0.84	0.09	8.25	7.99	816	8.25	16.37	25.17
B04	34.16	34.39	127.78	62.08	2.00	0.48	0.12	7.80	8.03	869	7.80	17.77	93.75
B05	11.21	20.00	122.02	72.41	1.30	0.24	0.13	7.67	7.94	1539	7.67	19.04	**61010**
B06	29.22	43.67	107.69	45.09	1.67	0.45	0.12	8.10	8.01	1134	8.10	18.11	13.61
B07	22.75	25.72	53.07	22.77	1.70	0.51	0.10	**9.11**	7.99	**1659**	**9.11**	17.97	3.89
M01	36.32	48.13	113.39	14.36	2.18	0.57	0.13	8.46	8.25	696	8.46	16.82	49.73
M02	64.50	58.28	134.15	10.46	2.20	0.79	0.16	8.55	**8.31**	657	8.55	16.14	67.85
M03	**124.32**	**122.20**	**162.35**	16.05	1.83	0.75	**0.20**	8.47	8.05	680	8.47	16.41	216.57
M04	58.67	63.41	124.17	18.68	1.78	0.59	0.12	8.31	8.17	693	8.31	16.13	110.21
M05	44.98	48.38	134.39	32.99	1.51	0.45	0.12	7.94	8.00	702	7.94	16.77	232.72
M06	29.42	30.82	108.47	36.23	1.68	0.40	0.11	8.44	8.03	688	8.44	17.73	31.00
M07	40.52	41.51	96.22	26.79	1.50	0.38	0.13	7.90	8.10	677	7.90	18.26	43.86
MB	32.02	37.00	78.17	23.11	1.68	0.42	0.11	7.85	8.09	1061	7.85	**19.51**	24.34

Table 6: Monthly mean (±SE) water-quality variables in McKenzie and Boston Creeks during summer 2019. See Table 1 for explanation of abbreviations of variable labels. Means for TN corresponded only to 4 months (April, July, September, and October). Mean for all variables are arithmetic means, whereas those for EC are geometric means. P-values correspond to Mann-Whitney tests comparing means between creeks.

Variable	Boston	McKenzie	P-value
Turb (NTU)	20.73 (±2.647)	61.16 (±8.524)	**<0.0001**
TSS (mg/L)	26.07 (±2.679)	63.00 (±7.478)	**<0.0001**
TP (µg/L)	94.14 (±10.88)	127.81 (±12.92)	0.0649
SRP (µg/L)	44.52 (±5.675)	20.64 (±2.539)	**0.0002**
TN (mg/L)	2.24 (±0.317)	1.81 (±0.108)	0.4062
TNN (mg/L)	0.86 (±0.127)	0.57 (±0.066)	0.2917
TAN (mg/L)	0.11 (±0.009)	0.15 (±0.111)	**0.0051**
pH	8.00 (±0.060)	8.14 (±0.052)	0.0774
Cond (µS/cm)	1156 (±122)	685 (±5.61)	**0.0022**
DO (mg/L)	8.26 (±0.217)	8.16 (±0.219)	0.9072
Temp ($°C$)	17.86 (±1.011)	16.43 (±0.978)	0.7305
EC (CFU/ 100 mL)	108.86	73.5	not applicable

Table 7: Canadian water quality guideline for protecting aquatic life/ recreational waters for several variables in this study.

Variable	Guideline	Source
TSS	25 mg/L	• Guidelines for Canadian Recreational Water Quality[1]
Turb	25 NTU for fishery/recreation 50 NTU for aesthetic	• Minnesota Pollution Control Agency[2] • Guidelines for Canadian Recreational Water Quality[1]
TP, SRP	<30 µg/L	• Provincial Water Quality Objectives for protecting aquatic life[3]
TN	1.07 mg/L	• Provincial Water Quality Objectives for protecting aquatic life[3] (Ontario--Mixedwood Plains)
TNN	3.0 mg/L	• Guidelines for Canadian Recreational Water Quality[1]
TAN	In freshwater, equivalent to 0.019 mg/L of un-ionized ammonia	• Guidelines for Canadian Recreational Water Quality[4]
DO	- Early life stages 6 mg/L - Other life stages 5.5 mg/L	• Guidelines for Canadian Recreational Water Quality[1]
pH	5.0- 9.0 6.5- 8.5	• Guidelines for Canadian Recreational Water Quality[1] • Provincial Water Quality Objectives for protecting aquatic life[3]
E. coli	Geometric mean of minimum 5 samples ≤ 200 CFU/100 mL	• Guidelines for Canadian Recreational Water Quality[1]

[1]Health Canada. 2012. Guidelines for Canadian Recreational Water Quality. Third Edition. Downloaded from http://www.healthcanada.gc.ca/
[2]Minnesota Pollution Control Agency, March 2008. Turbidity: description, impact on water quality, sources, measures. Downloaded from https://www.pca.state.mn.us/sites/default/files/wq-iw3-21.pdf
[3]Ontario Ministry of Environment and Energy. 1994. Provincial Water Quality Objectives Appendix A.
[4]Canadian Council of Ministers of the Environment, 2010. Canadian Water Quality Guidelines for the Protection of Aquatic Life.

Table 8: Seasonal means of water-quality variables collected at 7 sampling stations of the McKenzie and Boston Creeks in 1989, 1990 and 2019 during base-flow conditions (see location of sites in **Figure 13; coordinates in Table 3**). See **Table 1** for explanation of abbreviations of variable labels. Values that exceed water-quality guidelines/objectives appear in red.

Site ID	1989					1990					2019				
	Turb	pH	Cond	DO	Temp	Turb	pH	Cond	DO	Temp	Turb	pH	Cond	DO	Temp
B02	108.0	8.00	688	9.67	22.00	28.3	8.00	745	9.85	25.57	28.0	7.89	1018	8.34	19.10
B04	100.0	8.50	810	8.67	22.33	29.5	7.75	718	7.30	23.23	34.2	8.03	869	7.80	17.77
B06	10.0	8.50	1642	8.13	23.33	26.7	8.00	1533	11.05	23.70	29.2	8.01	1134	8.10	18.11
M02	85.0	8.00	563	9.47	22.00	39.3	8.00	530	9.65	22.90	64.5	8.31	657	8.55	16.14
M03	90.0	7.75	525	7.73	21.50	65.3	7.75	563	9.15	22.35	124.3	8.05	680	8.47	16.41
M04	82.0	8.00	559	9.93	20.67	69.3	8.00	518	9.55	22.73	58.7	8.17	693	8.31	16.13
M06	55.0	8.00	553	9.00	20.00	56.6	7.88	573	8.40	26.13	29.4	8.03	688	8.44	17.73

a)
b)
c)

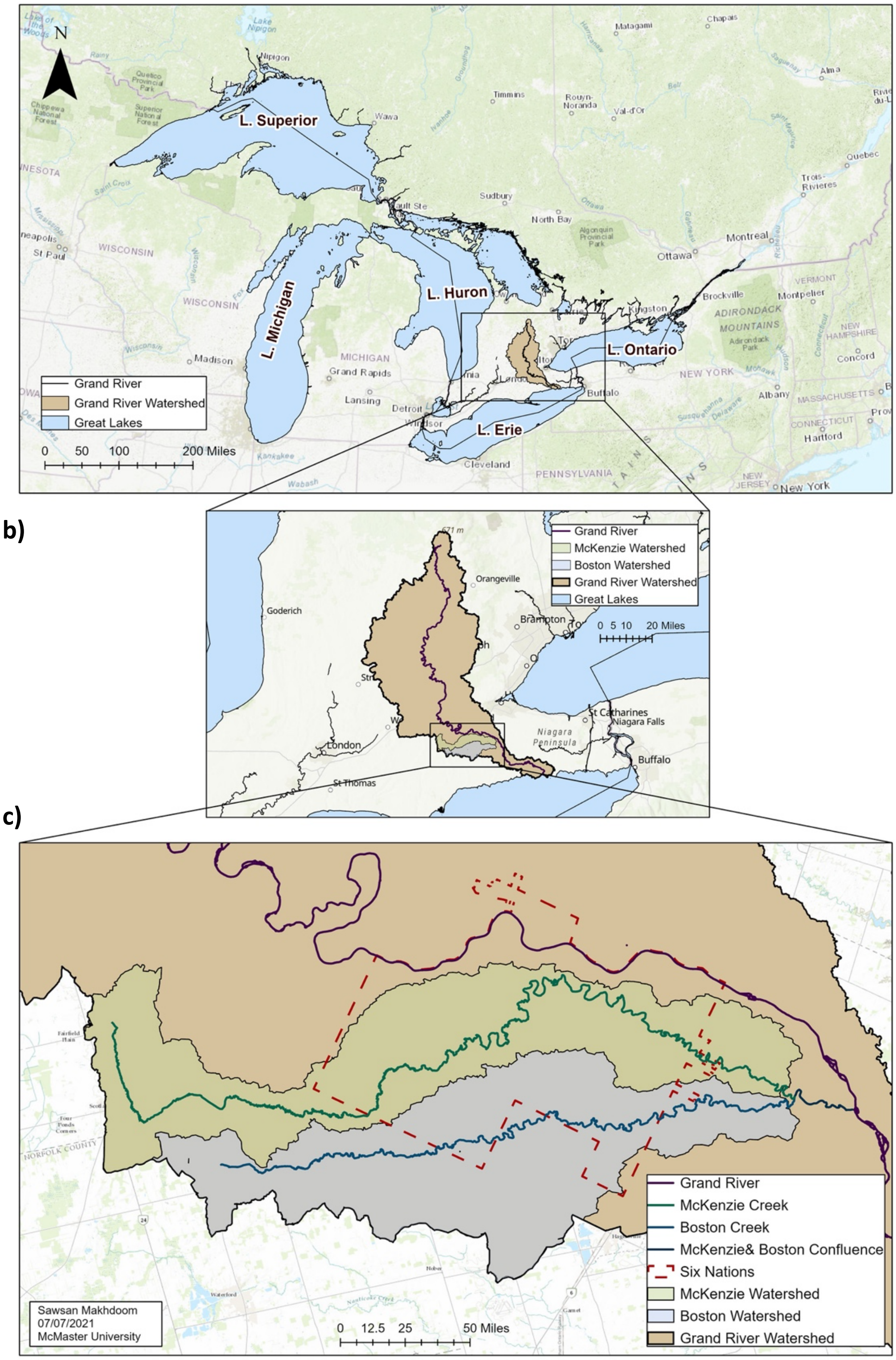


Figure 1: a) The Grand River Watershed (GRW) located north of Lake Erie's east basin. b) The McKenzie Creek Watershed (MCW) within the GRW. c) Location of the Six Nations Reserve within MCW. The MCW consists of catchments of two main tributaries, McKenzie and Boston Creeks.

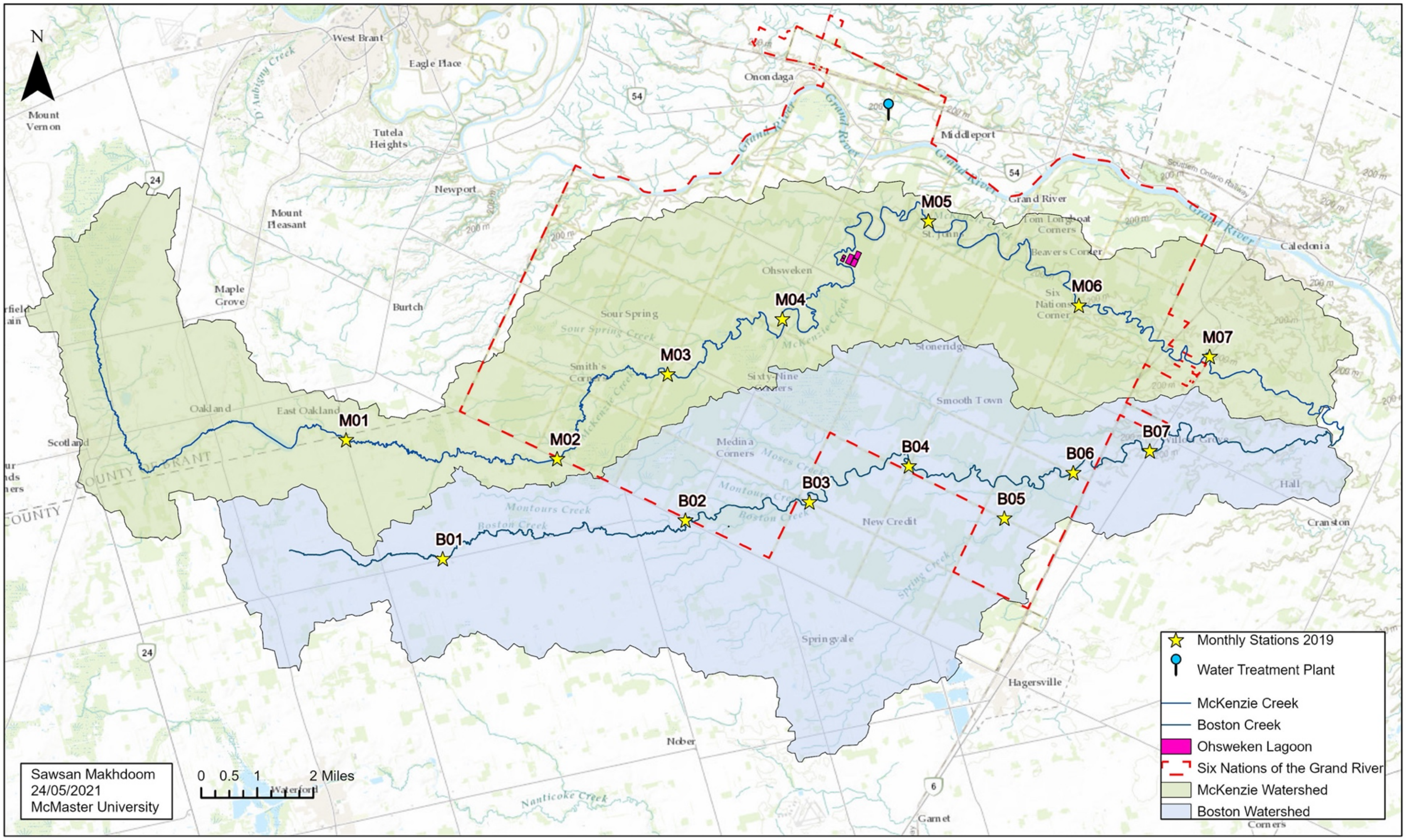
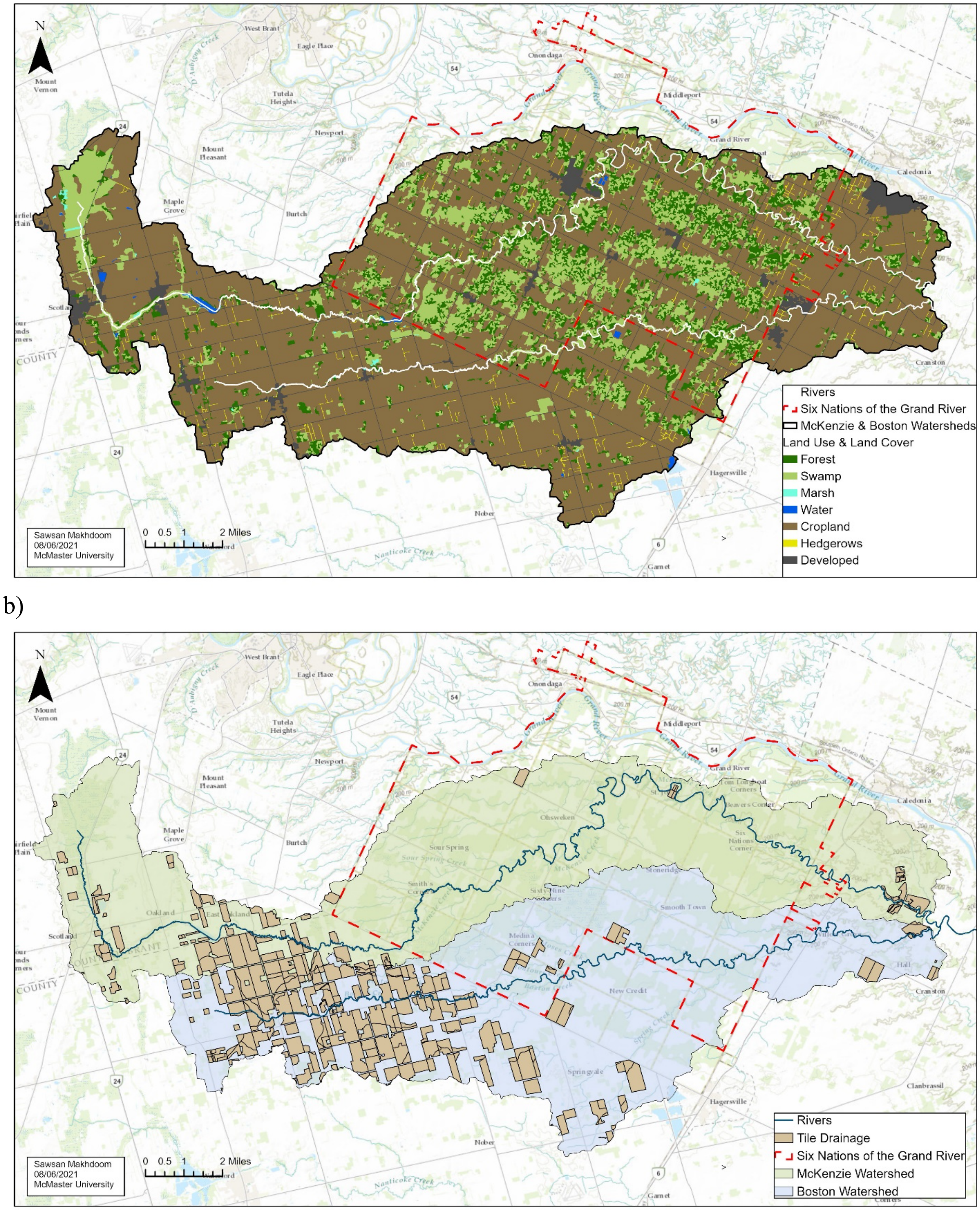


Figure 2: Stations in the McKenzie and Boston Creek catchments sampled monthly in 2019 from April to November (except October) during base flow conditions.

a)



b)

Figure 3: a) Land-use categories in the McKenzie Creek Watershed. Data are from Southern Ontario Land Resource Information Systems v. 3.0. b) Location of tile drainage areas in the McKenzie and Boston Creek catchment.

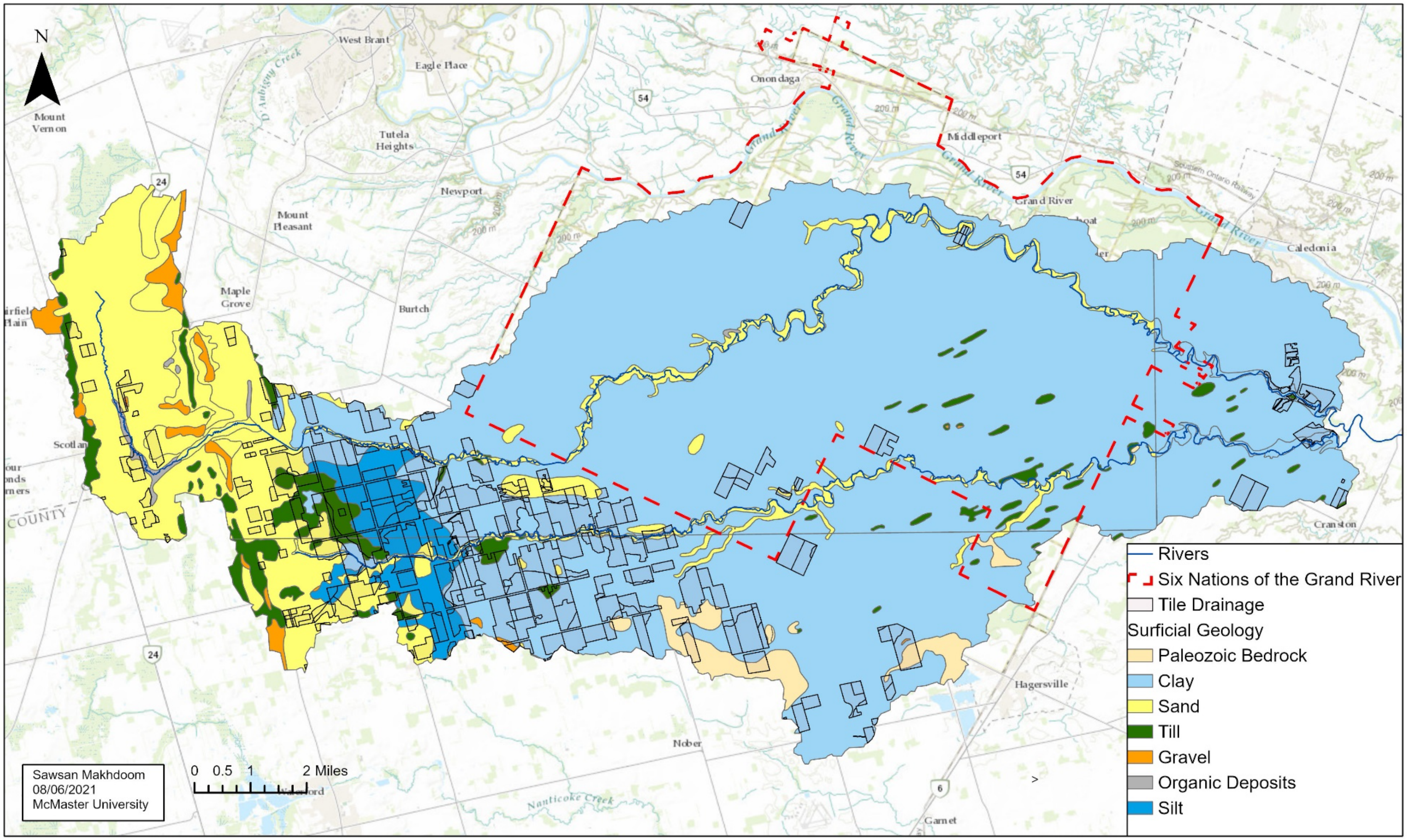


Figure 4: Distribution of 7 geological classes in the McKenzie and Boston Creek catchments. Superimposed are location of the tile drainage areas.

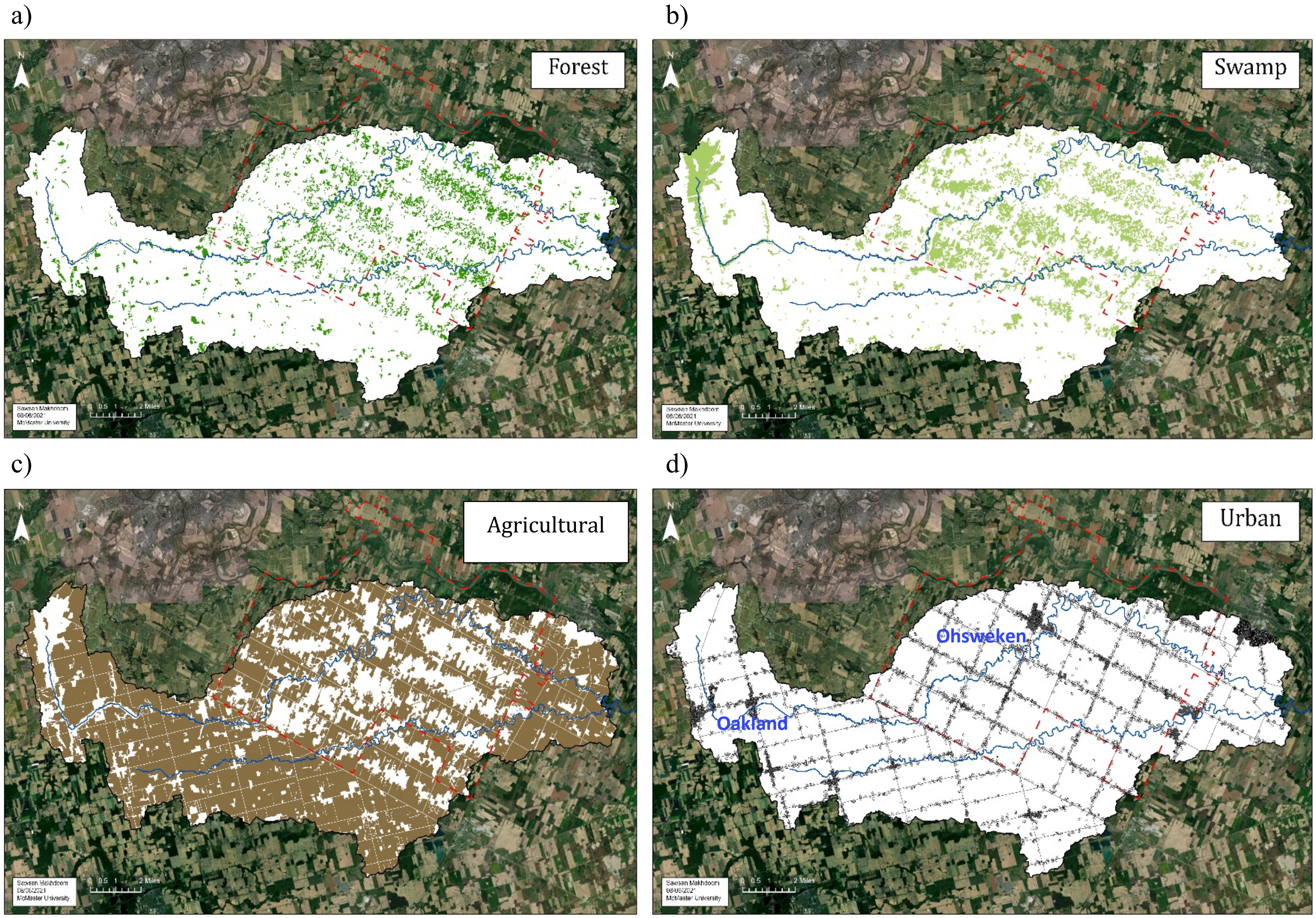


Figure 5: Land-use and land-cover classes within McKenzie and Boston Creek catchments showing the distribution of a) forests, b) swamps, c) agricultural land and d)urban areas, along with location of buildings.

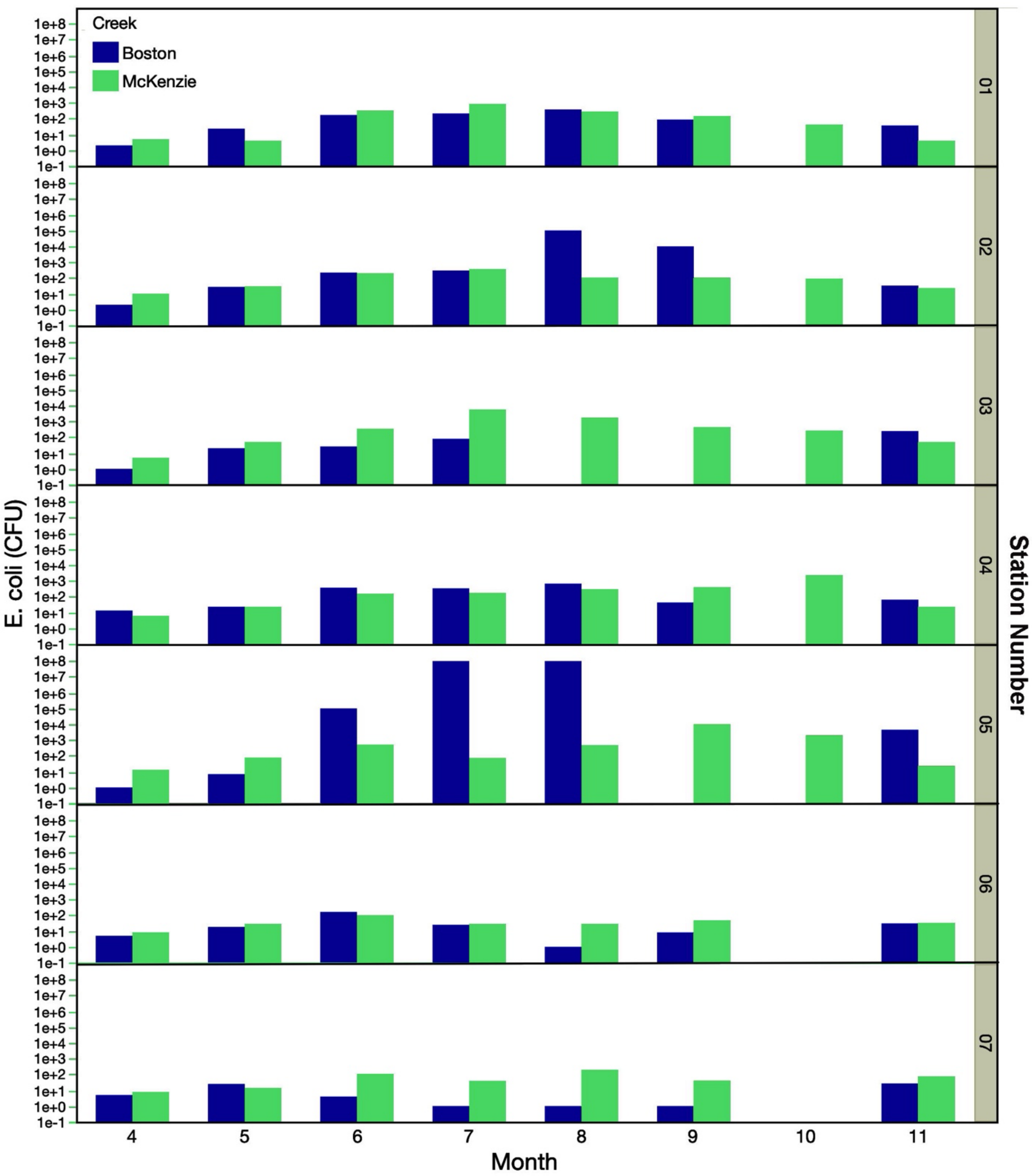


Figure 6: *E. coli* densities (CFU; #•100 mL^{-1}) measured monthly at 14 stations in Boston and McKenzie Creeks.

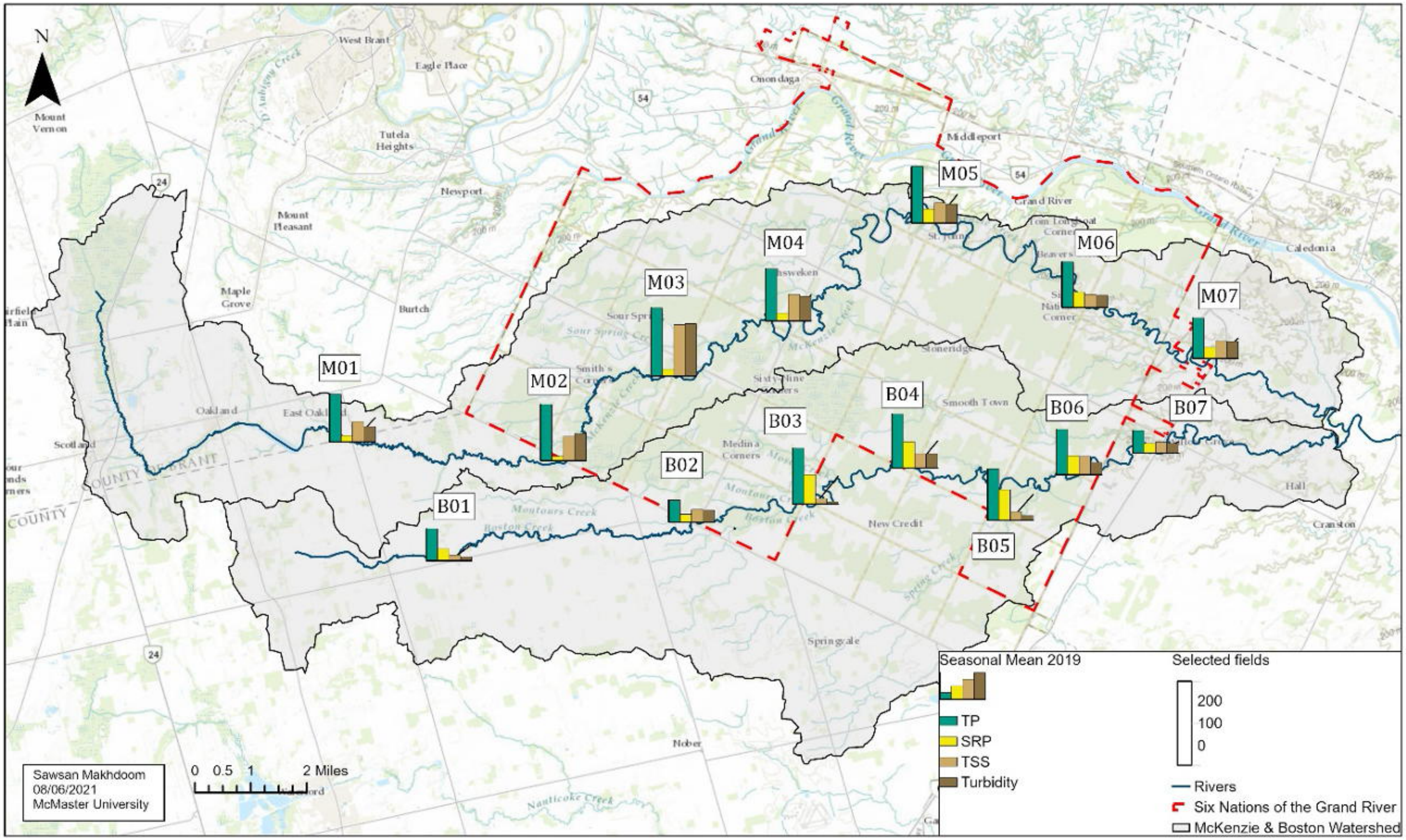


Figure 7: _Mean seasonal concentrations of total P (TP; µg/L), soluble reactive P (SRP; µg/L), total suspended solids (TSS; mg/L) and turbidity (NTU) at 14 sampling stations in McKenzie (M) and Boston (B) Creeks.

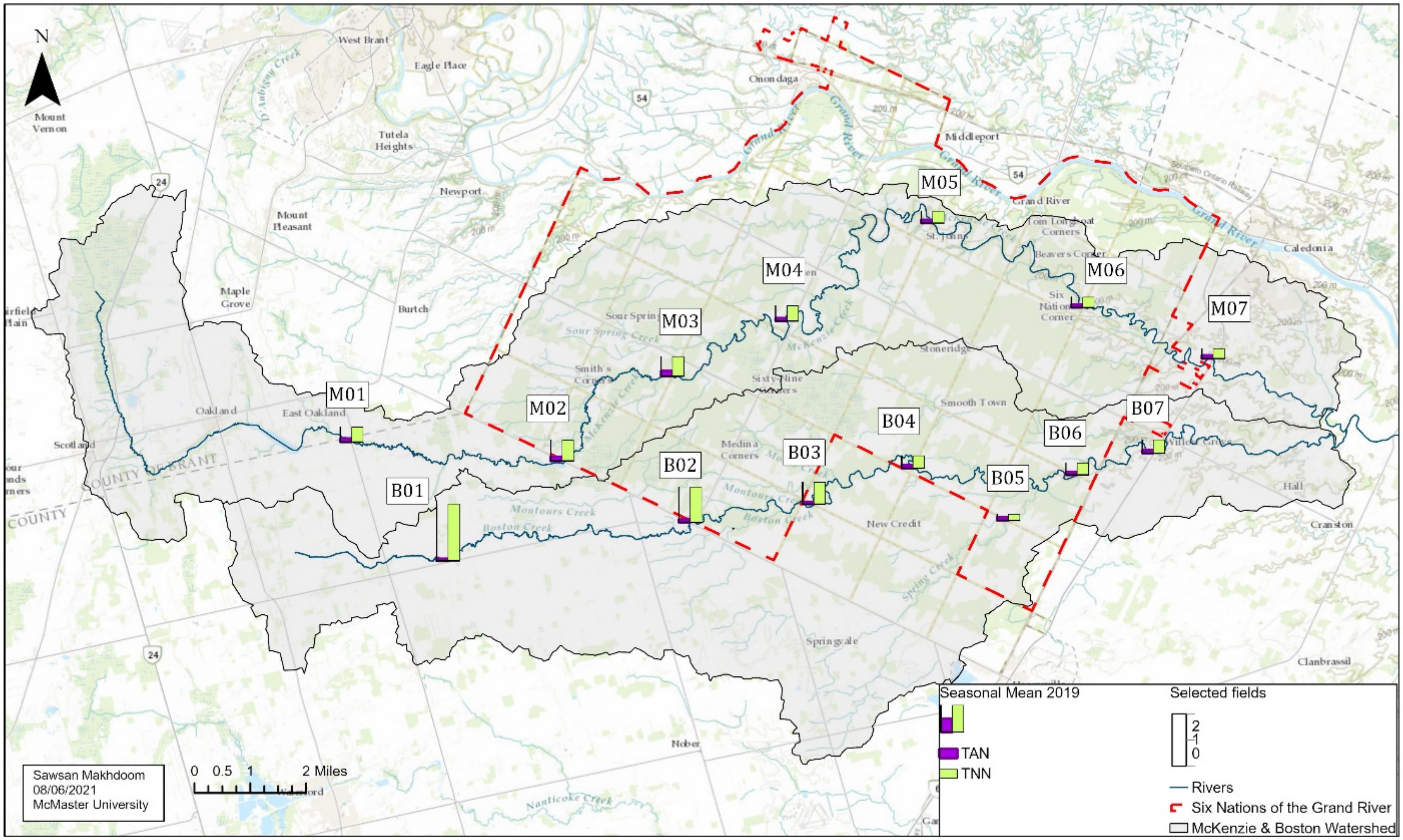


Figure 8: Mean seasonal concentration of total ammonia N (TAN; mg/L), total nitrate N (TNN; mg/L) at 14 sampling stations in McKenzie (M) and Boston (B) Creeks

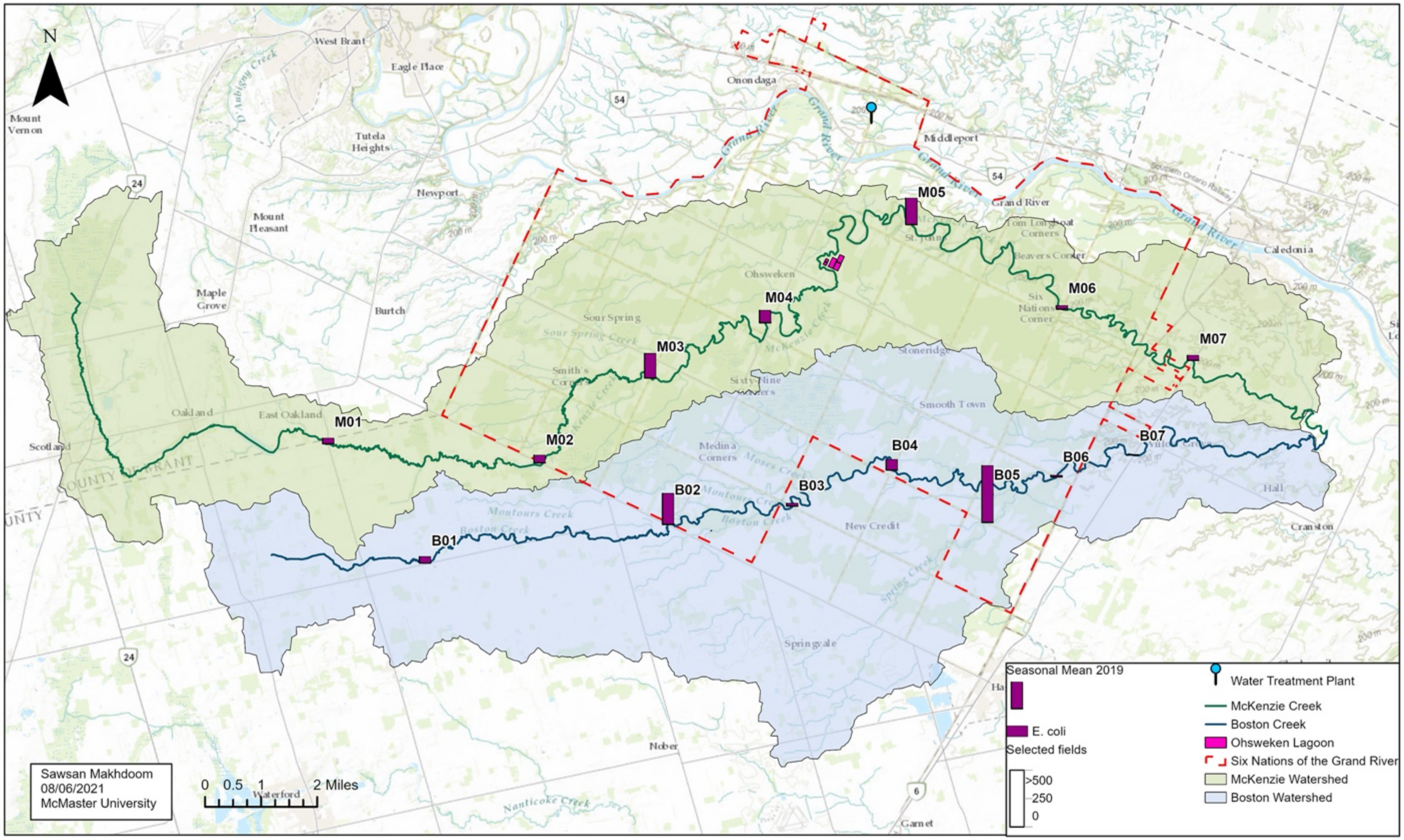


Figure 9: Mean seasonal concentrations of *Escherichia coli* (*E. coli*; CFU) measured at 14 stations on McKenzie (M) Cr and Boston Cr.

a)

b)

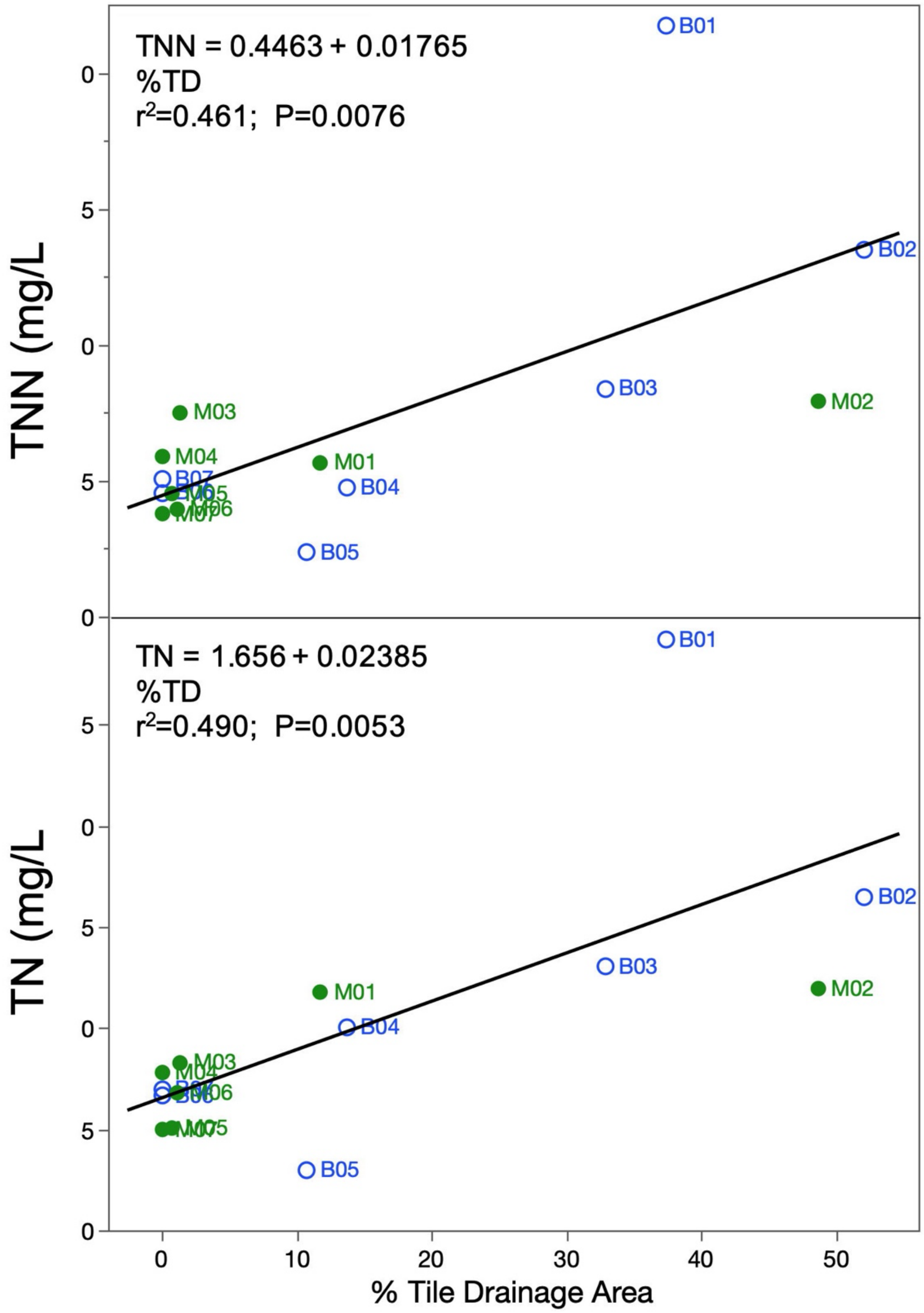


Figure 10: Linear regression of a) TNN and b) TN against % Tile Drainage Area (TD). Data are for 14 stations in McKenzie (solid symbols) and Boston (open symbols) Creeks.

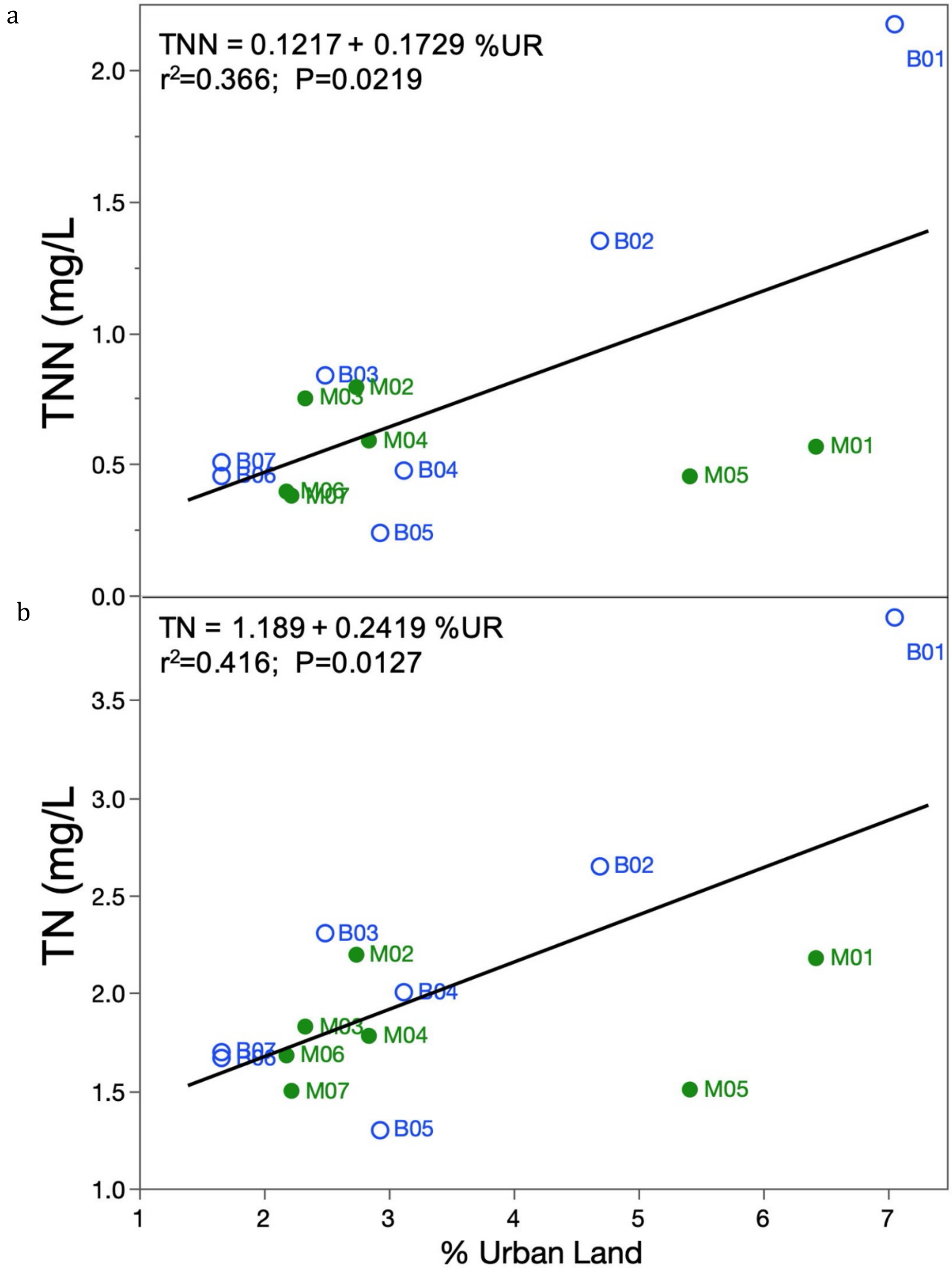


Figure 11: Linear regression of a) TNN and b) TN against % Urban Land (UR). Data are for 14 stations in McKenzie (solid symbols) and Boston (open symbols) Creeks.

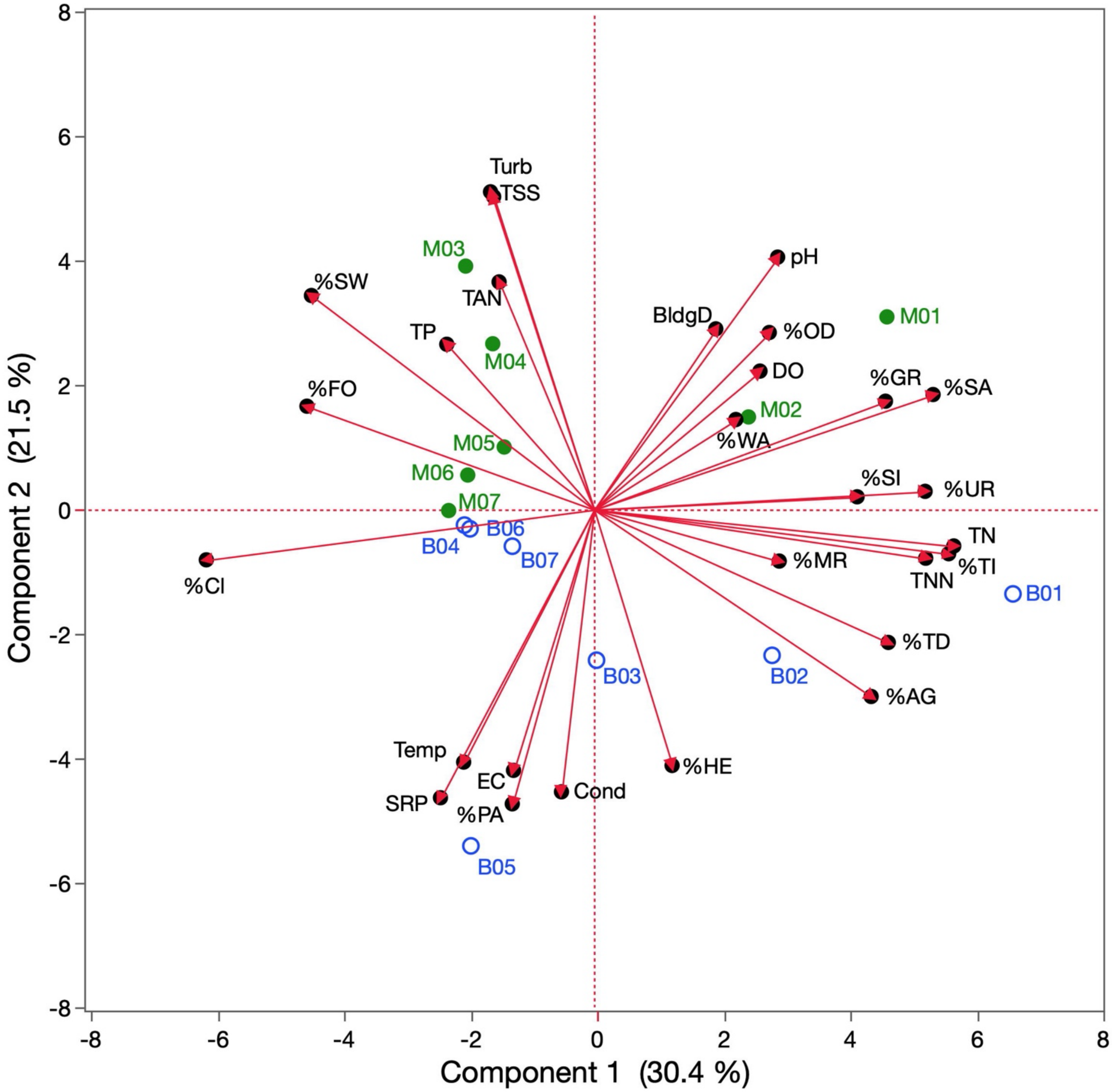


Figure 12: Bi-plot of Principal Components 2 vs Principal Components 1. See Table 1 for explanation of abbreviations for water-quality variables and land-use land-cover categories. Stations M01 to M07 are those located on McKenzie Creek and B01 to B07 refer to those on Boston Creek.

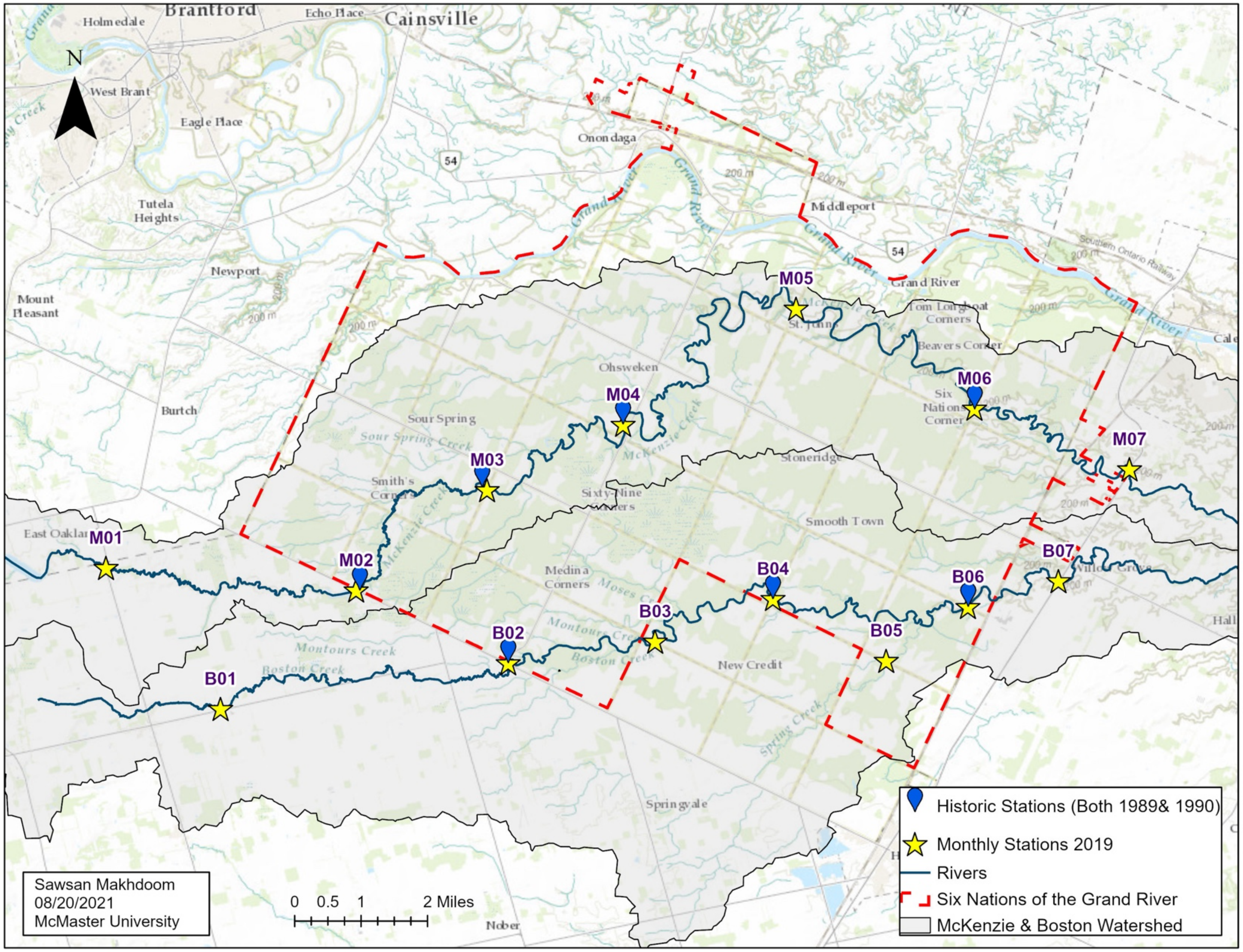


Figure 13: Location of historic sampling locations sampled by Whitlow (1989, 1990) relative to those in this study.

Appendix

Table A1: Description of sampling stations in the 1989 study (see Appendix A1).

Station	Creek/River	Location
1	McKenzie Creek	Below the dam at Victoria Mills, Townline between Mohawk and Seneca Roads
2	McKenzie Creek	VanEvery property, Seneca Road between 1st and 2nd Line (pike spawning area)
3	McKenzie Creek (Frog Pond)	3rd Line between Chiefswood Road and Seneca Road
4	Williams/Styres Creek	Chiefswood Road between 4th and 5th line
5	Lower McKenzie Creek	Cayuga Road between 5th and 6th Line
6	Boston Creek	Near Little Buffalo, Town Line between Chiefswood and Tuscarora Road
7	Boston Creek	2nd Line between Tuscarora and Onondaga Roads
8	Spring Creek	2nd Line between Cayuga Road and CN Railroad
9	Lower Boston Creek	3rd Line between Cayuga Road and CN Railroad
10	Grand River	Under Chiefswood Bridge
11	Fishes Creek	3rd Line between Bateman Line and Mohawk Road

Table A2: Location of sampling stations established for the 1990 season.

Station	Creek/River	Location
1	McKenzie Creek	Below the dam at Victoria Mills, Townline between Mohawk and Seneca Roads
2	McKenzie Creek	VanEvery property, Seneca Road between 1st and 2nd Line (pike spawning area)
3	McKenzie Creek (Frog Pond)	3rd Line between Chiefswood Road and Seneca Road
4	McKenzie Creek	Cayuga Road between 5th and 6th Line
5	Lower McKenzie Creek	5th Line between Cayuga Road and CN Railroad; (pike spawning area)
6	Boston Creek	Near Little Buffalo, Town Line between Chiefswood and Tuscarora Roads
7	Boston Creek	2nd Line between Tuscarora and Onondaga Roads
8	Spring Creek	Cayuga Road between 1st and 2nd Line
9	Lower Boston Creek	3rd Line between Cayuga Road and CN Railroad
10	Boston Creek	Tuscarora Road Between 2nd and 3rd Line
11	Fishes Creek	3rd Line between Bateman Line and Mohawk Road

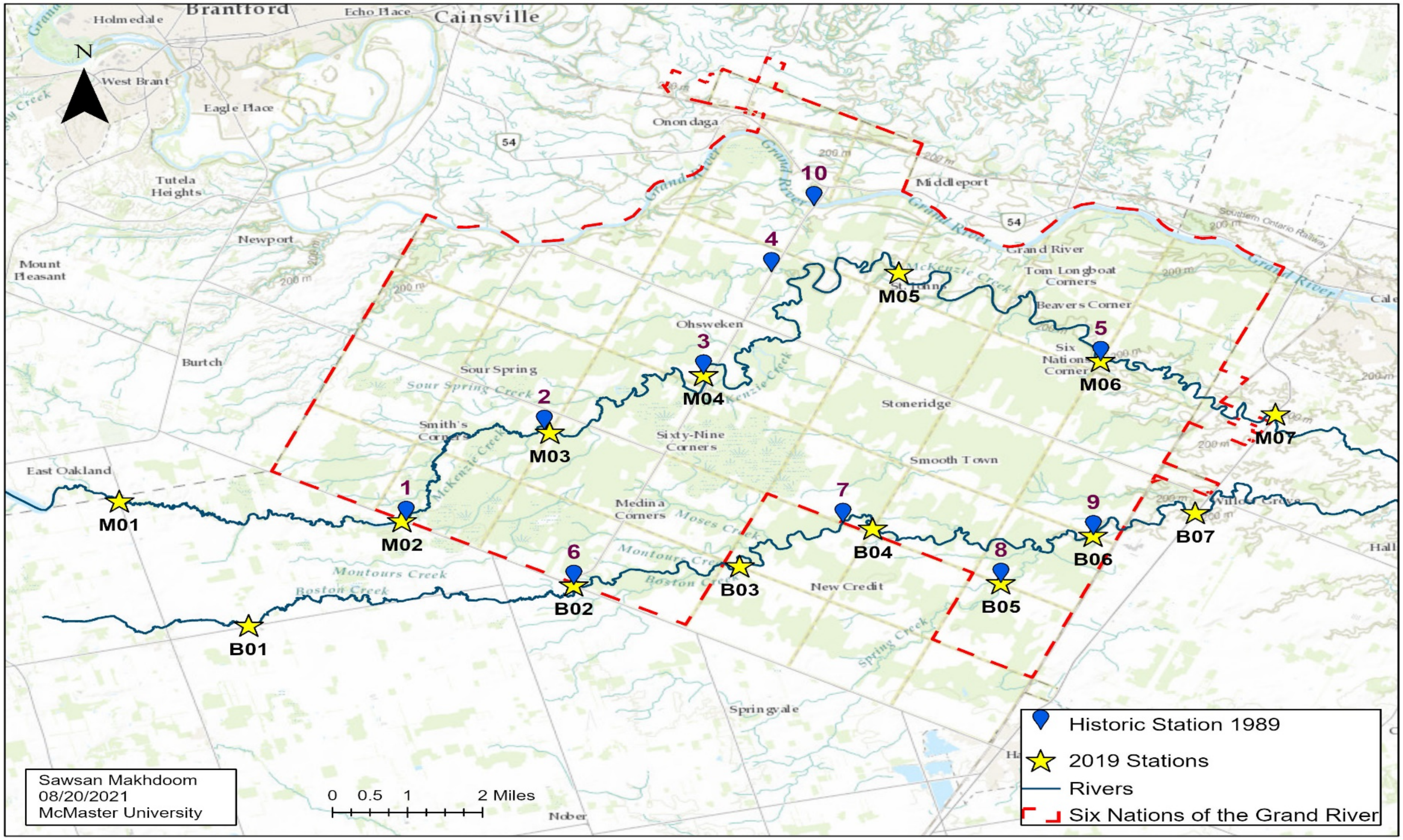


Figure A1: Location of sampling sites on the McKenzie Creek (M1 to M7) and Boston Creek (B1 to B7) as well as the site after the confluence of these two creeks (MB) during the 2019 monitoring program. Some of these stations were also sampled during 1989 in McKenzie Creek (#1 to #5) and Boston Creek (#6 to #9) and on the Grand River (#10).

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