# Insights into water insecurity in Indigenous communities in Canada: assessing microbial risks and innovative solutions, a multifaceted review (#99300)



First submission

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- Clear, unambiguous, professional English language used throughout.
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- Is the review of broad and cross-disciplinary interest and within the scope of the journal?
- Has field been reviewed recently. It there a good reason for this review (different viewpoint, audience etc.)?
- Introduction adequately introduces the subject and makes audience and motivation clear.

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- Are sources adequately cited? Quoted or paraphrased as appropriate?
- Is the review organized logically into coherent paragraphs/subsections?

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# Insights into water insecurity in Indigenous communities in Canada: assessing microbial risks and innovative solutions, a multifaceted review

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While most Canadians have access to high-quality drinking water, several Indigenous reserves face water insecurity. Drinking water systems (DWS) on reserves face limitations ranging from aging infrastructure and shared administration of water regulations. When potential hazards are identified in source waters, local environmental authorities may issue “water advisories”. Up to date, more than 20 long-term water advisories remain unresolved in Indigenous reserves in Canada. The risks associated with water insecurity include the presence of pathogenic microorganisms (i.e. *Escherichia coli* and total coliforms) and the reaction of natural organic matter (NOM) with disinfection chemicals in the DWS potentially forming disinfection by-products (DBPs). We revised the challenges and the potential use of different methods to remove NOM from water including coagulation, high- and low-pressure membrane filtration procedures, ozone, Ion exchange (IEX), and Biological Ion exchange (BIEX). Moreover, we reviewed the benefits and drawbacks that high throughput tools such as metagenomics, culturomics, and microfluidics devices could represent for water monitoring in Indigenous reserves. This review pursues a better understanding of the microbiological and chemical risks that water insecurity causes in Indigenous reserves in Canada. Additionally, we evaluate the potential implications of the potential technical and microbiological solutions that can be used to prevent the effect of pathogens in water and protect public health in Indigenous reserves.

# "Insights into water insecurity in Indigenous communities in Canada: assessing microbial risks and innovative solutions, a multifaceted review."

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# Abstract

While most Canadians have access to high-quality drinking water, several Indigenous reserves face water insecurity. Drinking water systems (DWS) on reserves face limitations ranging from aging infrastructure and shared administration of water regulations. When potential hazards are identified in source waters, local environmental authorities may issue “water advisories”. Up to date, more than 20 long-term water advisories remain unresolved in Indigenous reserves in Canada. The risks associated with water insecurity include the presence of pathogenic microorganisms (i.e. *Escherichia coli* and total coliforms) and the reaction of natural organic matter (NOM) with disinfection chemicals in the DWS potentially forming disinfection by-products (DBPs). We revised the challenges and the potential use of different methods to remove NOM from water including coagulation, high- and low-pressure membrane filtration procedures, ozone, Ion exchange (IEX), and Biological Ion exchange (BIEX). Moreover, we reviewed the benefits and drawbacks that high throughput tools such as metagenomics, culturomics, and microfluidics devices could represent for water monitoring in Indigenous reserves. This review pursues a better understanding of the microbiological and chemical risks that water insecurity causes in Indigenous reserves in Canada. Additionally, we evaluate the potential implications of the potential technical and microbiological solutions that can be used to prevent the effect of pathogens in water and protect public health in Indigenous reserves.

## Nomenclature

DBPs: Disinfection by-products

BAFs: Biological activated filters

DWS: Drinking water system

NOM: Natural organic matter

## Introduction

Canada, the second largest country in the globe, harbors less than 1% of the total world´s population but possesses 2,850 billion cubic meters of renewable water. This number represents the fourth-largest renewable supply of freshwater on the planet after Brazil, Russia, and the United States.(Natural Resources Canada, 2017; Desforges et al., 2022). While the vast majority of Canadians have access to high-quality drinking water emerging from the tap, numerous Indigenous reserves face water insecurity (Basdeo & Bharadwaj, 2013a; Brown et al., 2016; Waldner et al., 2017; Indigenous Services Canada, 2020; Wu et al., 2020; Lucier et al., 2020). The drinking water systems (DWS) located in First Nation (FN) communities generally lack new infrastructure or have become obsolete(O'Connor, 2002). In addition, factors such as location (being remote), restricted access to many services(Statistics Canada, 2022, 2023), a limited number of qualified personnel on site, as well as dependence on sporadic water operation and maintenance are factors that contribute to Indigenous reserves experiencing water insecurity (Zimmermann et al., 2020a; Lucier et al., 2020) . Water insecurity on reserves represents a health threat and could be one of the causes of FN populations having the lowest projected life expectancies across Canada(Statistics Canada, 2017). In Canada, most water-related activities are the responsibility of provincial and territorial governments, and the obligations regarding monitoring water contaminants differ greatly from province to province(Hill, Carey, et al., 2006; Environment and Climate Change Canada, 2015, 2017). In the case of Indigenous reserves, water delivery, and maintenance responsibilities are shared between the Federal Government, specifically Indigenous Services Canada (ISC) and

Health Canada (HC) with FN communities leadership groups (Boyd, 2011; Bradford et al., 2016; Lucier et al., 2020). In some provinces of the country, when potential hazards are identified in the water sources of public water systems, local environmental authorities may issue alerts to the public known as “water advisories” to warn about water consumption or ban completely its use (Environment and Climate Change Canada, 2020). The length of the advisory could be less than a year (short-term advisory) or longer than 12 months (long-term advisory) (Government of Canada, 2021; Province of Manitoba, 2021). Depending on the results of water quality and a risk evaluation approach to the conditions of the place of the drinking water system, the local authorities (Environment and Climate Change Canada, 2020; Government of Canada, 2021; Conservation and Climate Province of Manitoba) may or may not issue different water warnings. The water precautionary warnings recommended by Environment and Climate Change Canada include but are not limited to Boiling water advisories (BWA), Do not consume (DNC), and Do not use (DNU)(Environment and Climate Change Canada, 2022). Since 2015, there have been more than 100 long-term water advisories lifted on reserves around the country, however, there are more than 20 long-term drinking water advisories remaining in Indigenous reserves as of March 2024(Indigenous Services Canada, 2024a). Another factor that challenges DWS in Indigenous reserves could be associated with the high levels of Natural organic matter (NOM) found in water sources around these areas in some provinces of Canada (Sadrnourmohamadi, Goss & Gorczyca, 2013). NOM is a particularly complex mixture of organic compounds and directly interferes with the efficacy of drinking water treatments (Bhatnagar & Sillanpää, 2017). Even though NOM is generally found in all groundwater and surface waters (Bhatnagar & Sillanpää, 2017; Health Canada, 2019), high levels of NOM are of concern because of their indirect effects on public health. Some of these indirect effects include negatively affecting the inactivation of pathogens (Dai & Hozalski, 2002; Templeton, Andrews & Hofmann, 2005; Pham, Mintz & Nguyen, 2009; DiCesare, Hargreaves & Jellison, 2012a; Jacquin et al., 2020a) contributing to biofilm development(Dai & Hozalski, 2002), and reaction with chemicals used in the drinking water treatment forming DBPs that contain potential organic genotoxic compounds (McKie et al., 2015; Wu et al., 2019a). In this context, many efforts and resources are expected to be applied to eliminate NOM in drinking water treatments all over Canada, especially in Indigenous communities. The plan of this paper is as follows: First, we review the water advisories remaining in Indigenous reserves in the country. We analyze additional factors that contribute to water insecurity in FN communities such as cisterns and wells and the microorganisms and other genetic elements that have been found in some water locations of Indigenous reserves. Moreover, we also review the negative effects of NOM in DWS, and some methods available to remove NOM such as coagulation, high- and low-pressure membrane filtration procedures, ozone, Ion exchange (IEX) and Biological Ion exchange (BIEX). Besides, we emphasize the challenges of applying each of these methods in Indigenous reserves. Finally, we review high throughput tools such as metagenomics, culturomics, and microfluidics devices that can provide important information regarding microbial communities and microbial pollutants present in source and DWS in Indigenous reserves. Despite the alleged efforts from the federal government to eliminate all long-term drinking water advisories in Canada by March 2021(Office of the Auditor General of Canada, 2021), three years after the deadline, the issue persists. Our review synthetizes existing literature and Canadian public records concerning the prolonged state of water insecurity in Indigenous reserves to promote comprehension of the microbiological and chemical risks that this represents for public health. While we restricted the scope of this review to technical and microbiological issues, we acknowledge that water insecurity in Indigenous reserves is a multidimensional matter that started with the colonial past and continued with the deterioration of water, the environment, and other complex socio-economic structures existing in Canada (Sarkar, Hanrahan & Hudson, 2015). In this multifaceted review, we discuss technical and cutting-edge molecular alternatives so they can be considered to overcome long-term drinking water advisories. Presently, water quality monitoring practices assess fecal contamination by looking for indicator bacteria such as *Escherichia coli* (*E. coli*). These indicator microorganisms were established more than a century ago, and they have been proven ineffective in assessing water for other microorganisms such as viral, bacterial, or protozoan pathogens (Ramírez-Castillo et al., 2015). Advanced molecular tests reviewed in this article can complement the assessment of other pathogens and indicator microorganisms present in drinking water to help ensure safe water in Indigenous reserves. This article is one of the few that reviews both technical and advanced molecular tools that could be applied in remote Indigenous communities and is useful for researchers involved in the fields of environmental sciences, microbiology, water treatment engineering, and Indigenous studies.

## Methods

## Database search and literature screening

Ten federal and provincial government portals were used to review the status of water advisories in Indigenous communities of Canada as well as the recommended Guidelines for NOM and drinking water in the country. The federal government web pages included in this review are “Indigenous Services Canada”, ‘Environment and Climate Change Canada”, “Health Canada”, “Statistics Canada”, “Natural Resources Canada”, “Canada’s Chief Public Health Officer”, “Office of the Auditor General of Canada”, “Standards Council of Canada”, “Province of Manitoba” and “Government of Ontario”. Furthermore, PubMed and Google Scholar databases were used to identify relevant literature. The strings (Indigenous communities OR First Nations of Canada OR Indigenous reserves) AND (NOM) AND (water or DWA or BWA or Water Systems) AND (Microbiology or Uncultivable bacteria or Metagenomic or DNA sequencing) were used. We kept peer review publications assessing NOM, microorganisms, metagenomics, and/or other technologies such as culturomics and microfluidics that could be applied in DWS and Indigenous communities**.** Studies assessing microbiological threats in DWS outside Canada were excluded. A total of **157** scientific articles and 14 government web pages are included in our final review.

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## Water advisories in Canada

In Canada, the drinking water systems (DWS) located in indigenous communities are generally in remote locations(Statistics Canada, 2022), therefore are susceptible to irregular connectivity, limited number of qualified personnel on site, and generally depend on sporadic water operation and maintenance (Basdeo & Bharadwaj, 2013b;

Islam & Yuan, 2018; Lucier et al., 2020). Moreover, several DWS on reserves have been reported to lack new infrastructure and need upgrades(Zimmermann et al., 2020; Lucier et al., 2020). Additionally, the shared administration of water delivery and maintenance between the Federal Government, specifically Indigenous Services Canada (ISC) and Health Canada (HC) with Indigenous communities' leadership groups has led to divergences in drinking water regulations in indigenous reserves (Boyd, 2011; Bradford et al., 2016). All these factors contribute to Indigenous reserves experiencing water insecurity (Lucier et al., 2020). Water insecurity on reserves represents a health threat and could be one of the causes of FN populations having the lowest projected life expectancies across Canada (Statistics Canada, 2017). In the country, most water-related activities are the responsibility of the corresponding authority of that province or territory, and the responsibilities regarding monitoring water contaminants differ vastly from province to province(Hill, Carey, et al., 2006; Environment and Climate Change Canada, 2015, 2017). In some provinces of the country, when potential hazards are identified in the water sources of public water systems (i.e. *E. coli*, Cyanobacteria blooms, or DBPs), local environmental authorities may issue alerts to the public known as "water advisories" to warn about the water consumption or ban completely its use (Environment and Climate Change Canada, 2020). The length of the advisory could be less than a year (short-term advisory) or longer (long-term advisory) (Environment and Climate Change Canada, 2020; Province of Manitoba, 2021; Indigenous Services Canada, 2024a). Depending on the results of water quality, the nature of the water issue encountered, and a risk evaluation approach to the conditions of the place of the drinking water system, different warnings may be issued by the responsible environmental public health officer(Environment and Climate Change Canada, 2020; Government of Canada, 2021; Conservation & Manitoba). Boiling water advisories (BWA), Do not consume (DNC), and do not use (DNU) are the type of advisories recommended by Environment and Climate Change Canada (Environment and Climate Change Canada, 2020, 2022). BWA, the most common type of advisories, are most of the times precautionary and generally issued when poor water disinfection, deficient filtration, pressure loss in the distribution system, or inadequate maintenance of the equipment used to treat water is recognized(Health Canada, 2015). Similarly, DNC (also referred to as “do not drink”) and DNU advisories are recommended during emergencies(Health Canada, 2009a). For example, catastrophic events, chemical spills, or other pollutants that affect human health after short-term exposure, unexpected changes in the physical characteristics of water, or invasion of undetermined contaminants through cross-connection problems (Health Canada, 2009a). When the contaminant present in water can alter human health only through ingestion, the advisory issued corresponds to DNC(Health Canada, 2015). On the other hand, DNU recommendations are communicated when the existing pollutant has an effect through dermal and/or inhalation contact (fig 1) (Health Canada, 2009a, 2015). Yet, every province and territory uses its own terminology to issue its water quality recommendations and some regions (such as Ontario, Alberta, and some provinces of the Arctic) do not report water advisories in minor drinking water systems (Ministry of Health Ontario, 2011; Government of Canada, 2021; Water today Canada, 2021). Since 2015, there have been more than 100 long-term water advisories lifted on reserves around the country(Government of Canada, 2021; Indigenous Services Canada, 2024).

**Fig 1. Drinking water advisories (DWA).** Types of Drinking water advisories (DWA) issued in Canada. Boil Water Advisories (BWA), Do not consume (DNC), and Do not use (DNU). BWA represents the majority of DWA. Most of them are precautionary and are related to planned maintenance work, line breaks or pressure losses, poor water disinfection, or inadequate maintenance of the instruments used to treat water. Likewise, DNC and DNU are issued in atypical situations. For example, when a chemical substance or another pollutant is suspected or confirmed in the drinking water system (DWS). When the contaminant can alter human health through ingestion DNC is recommended. Whereas, if the substance of concern in the DWS affects the nose, skin, and/or eyes the advisory issued corresponds to DNU.

Nevertheless, as of March 2024, there are approximately 26 First Nations with long-term DWA on public systems on reserves(Indigenous Services Canada, 2024b). Astonishingly some of them have been dealing with advisories for more than 10 years (fig 2) (Indigenous Services Canada, 2024b). Regarding short-term BWA, official sources only report the ones located south of the 60 parallel(Indigenous Services Canada, 2023). However, when contemplating all provinces and territories of Canada the estimated number of BWA advisories to be solved might exceed 1000 cases. (Canada's Chief Public Health Officer, 2013; Government of Canada, 2021; Water today Canada, 2021; Indigenous Services Canada, 2024b).

**Fig 2. Remaining long-term drinking water advisories (LTDWA) in Canada in 2024.** (Top) Map based on the number of unresolved, long-term drinking water advisories (LT-DWA) issued around Canada: Saskatchewan=3, Manitoba=3, Ontario =18. Note: provinces and territories colored with light blue have no LT-DWA to address or the information is unavailable. (Bottom) Timeline displaying the start year of the current unresolved LT-DWA across the country. Last updated: March 2024.

## Microbiological and chemical risks of cisterns and wells in Indigenous Reserves

Another factor that contributes to water insecurity in indigenous reserves is the materials used in cisterns and wells when a potable source of water is not found (Neegan Burside, 2011; Murdock et al., 2024). The construction materials accepted by the Canadian Standards Association include steel, stainless steel, concrete, reinforced concrete, fiberglass, and polymers (i.e. polyethylene) (Standards Council of Canada, 2020). The materials and components used in water storage tanks are critical to maintaining water quality(World Health Organization, 2014). One example of this is the use of corrosive materials that harbor the development of iron and iron/manganese-oxidizing bacteria such as *Gallionella spp*. and members of the Siderocapsa genus (Kobrin & Lamb, 1997a; Slavik et al., 2020). Generally, iron-oxidizing (i.e., *Gallionella spp*.) and sulfur-reducing bacteria such as *Desulfovibrio spp*. are responsible for microbial-induced corrosion and biofilm development on unveiled metal surfaces(Annuk & Moran, 2010). Although biofilm development in these types of water storages might represent a class of “blockade” to corrosion, it can also increase the risk of pathogen development that can potentially affect human health (Slavik et al., 2020). The complex heterogenous microorganisms present in biofilms can communicate through chemical interactions (known as “quorum sensing”) to facilitate multicellular activities, exchange nutrients, and transfer hereditary material (Wirthlin, Marshall & Rowland, 2003; Flemming, 2008). In terms of biofilm growth, particularly, iron water storages have been found to harbor higher total bacteria counts than their counterparts made of plastic (Rilstone et al., 2021). Nevertheless, plastic cisterns have been associated with the presence of unacceptable levels of metals such as lead, aluminum, copper, among others, and even carcinogenic compounds (i.e. benzene) in potable water (Moura et al., 2019). Moreover, high temperatures, changes in pH, and the presence of chloramines can influence the degradation of the polymeric matrix of these types of cisterns and favor the transference of the toxic compounds to stored water(Moura et al., 2019). Equally important is to ensure proper closure of the cisterns and avoid leakages(Artiola, Rock & Hix, 2012; Slavik et al., 2020). Algae, fungi, protozoa, bacteria, and viruses for example, can enter from windblown dust, debris, and rainwater if the water storage tank is not properly sealed (Benskin et al., 2009; Artiola, Rock & Hix, 2012). Additionally, the presence of leakages in the storage unit could allow the introduction of bird feces which are known to carry harmful bacteria such as *Salmonella spp*. and *Campylobacter spp*. (Benskin et al., 2009b; Artiola, Rock & Hix, 2012a; Slavik et al., 2020). The recommendations for these water storage tanks state that testing the water at least once per year is advisable to validate the presence/ absence of microbiological pathogens (i.e. total coliforms and *E. coli*) (Health Canada, 2009; Green communities Canada, 2017). Nonetheless, some studies have confirmed that the water quality in these water storage tanks is below the stipulated standards (Fernando et al., 2016; Farenhorst et al., 2017; Murdock et al., 2024). The guidelines for Canadian drinking water quality state that the maximum contaminant level (MCL) for *E. coli* and total coliforms is 0 CFU/100 mL of water (Health Canada, 2013; Conservation and Water Stewardship Manitoba, 2014). However, *E. coli* counts higher than 60,000 CFU/100 mL were detected in drinking water distribution systems in a fly-in First Nation community in the Island Lake region in the province of Manitoba (Farenhorst et al., 2017). Likewise, unacceptable levels of *E. coli* and total coliforms (>800 CFU/100 mL and >900 CFU/100 mL respectively) were found in piped water in 2 out of 10 sampling events performed during 2023 and early 2024 in a First Nation community located in Manitoba.(Moniruzzaman & Uyaguari-Diaz, 2024). Moreover, in the same community, high heterotrophic counts in piped water (>5000 CFU/100 mL) and in a fiberglass tank ( >200 CFU/100 mL) used in the oxidation lagoon facility for wastewater treatment, were also reported (Moniruzzaman & Uyaguari-Diaz, 2024) ( Fig. 3). Even though heterotrophic bacteria do not represent a direct threat to public health and the counts obtained did not exceed the maximum acceptable concentrations (500 CFU/ mL), these high counts can interfere with *E. coli* and total coliforms recovery methods (Health Canada, 2013). Additionally, lower heterotrophic counts have been associated with better maintenance of the water facilities (Chowdhury, 2012; Health Canada, 2013b). Furthermore, the presence of antibiotic resistance genes (ARGs) such as *ampC* (β-lactam resistance), *mecA* (methicillin resistance), and *sul1* (sulfonamides resistance) in both, source, and drinking water in reserves of First Nation Communities have been reported(Fernando et al., 2016; Farenhorst et al., 2017; Mi et al., 2019). Moreover, the isolation of pathogenic bacteria such as *Legionella pneumophila* (MacMartin et al., 2021), responsible for Legionnaires' disease, associated with high-risk pneumonia (Kooij et al., 2017) has also been found. These microorganisms or genetic elements (i.e. ARGs, plasmids, and integrons)(Jin et al., 2020) may spread into the water system through wrecked plumbing or cisterns that are in contact with contaminated water or by natural formation of biofilms which start building up within the first week on surfaces underwater (Artiola et al., 2013).

**Fig 3. Microbiological counts observed per 100 ml of drinking water samples from a First Nation community in Manitoba, Canada.**

*E. coli*, total coliforms (TC), and heterotrophic plate counts (HC) from tap water collected from a community centre (piped water) and oxidation lagoon facility (fiberglass tank) in a First Nation community of Manitoba. Duplicate counts were assessed during 10 different time periods during 2023 and early 2024. Unacceptable levels of *E. coli* and TC appear in both locations in 2 sampling events (for piped water) and 1 sampling event for fiberglass tank.

## The negative effects of Natural organic matter (NOM) in DWS

In Canada, all public, semi-public, and private drinking water systems (DWS) are subject to provincial and territorial regulations and are specific to the type of raw water supply a drinking-water system will be relying upon (Manitoba, 2007, 2020; Bhatnagar & Sillanpää, 2017; Environment and Climate Change Canada, 2017). In potable water sources in the province of Manitoba, for example, the high levels of dissolved organic carbon (a component of NOM) often exceed 20 mg/L (Sadrnourmohamadi, Goss & Gorczyca, 2013). NOM is generally contained in natural aquatic sources because of the interaction between the disruption of plants habiting in the geosphere and the by-product of bacteria, and eukaryotes (i.e. algae)(Bhatnagar & Sillanpää, 2017). Seasonal changes, runoff of dissolved organic carbon from the land to the source of water (i.e. in storms) can also trigger the levels of NOM in aquatic environments (Findlay & Sinsabaugh, 2002; Saraceno et al., 2009; Winter et al., 2018).  The presence of NOM in drinking water treatment has been associated with bacterial resurface and less efficiency in the inactivation of other microorganisms [e.g. bacteriophages (Templeton, Andrews & Hofmann, 2005b; Pham, Mintz & Nguyen, 2009b; Jacquin et al., 2020), *Cryptosporidium* (Dai & Hozalski, 2002a; DiCesare, Hargreaves & Jellison, 2012) among others. Organic and inorganic complexes represent a source of energy for either heterotrophic or chemoautotrophic bacteria, respectively (Kooij, 1992). This event allows transportation of both: the hydrophobic organic compounds present in NOM

(Bhatnagar & Sillanpää, 2017; Winter et al., 2018; Wu et al., 2019b; Dixit et al., 2021) and toxic heavy metals such as copper (Cu), arsenic (As), lead (Pb), mercury (Hg), cobalt (Co), iron (Fe) and chromium (Cr) (Liu et al., 2018; Rilstone et al., 2021). When NOM interacts with chlorine used for water disinfection, different halogenations and oxidations result in the formation of DBPs which contain potential organic genotoxic compounds(McKie et al., 2015; Wu et al., 2019b) . For instance, trihalomethanes (THMs), and haloacetic acids (HAAs) are the DBPs regulated in the Guidelines for Canadian Drinking Water Quality. The maximum acceptable concentration within Canada for these compounds is 100 µg/L for THMs (Health Canada, 2013a) and 80 ug/L for HAAs (Health Canada, 2010). However, it has been documented that more than 300 water systems in Canada with populations of less than 5000 people have exceeded the maximum concentration of HAAs permitted (Table 1).

**Table 1. Number of small drinking water systems (DWS) that exceeded haloacetic acid (HAA5) levels in Canada.**

Moreover, the additional costs associated with high levels of NOM are generally because an augmentation of the dissolved organic carbon produces an increase in the coagulation and filtration processes that drinking water treatments execute (Eikebrokk, Vogt & Liltved, 2004; Soros et al., 2019a). This stands for a significant demand in coagulants such as aluminum sulfate ($Al_2(SO_4)_3$), polymerized ferrous sulfate (PFS), poly-aluminum chloride (PAC) or chitosan to limit biological growth, and chemicals to adjust the pH of water (Eikebrokk, Vogt & Liltved, 2004; Yang et al., 2016a; Soros et al., 2019a). In urban settings of Canada such as Toronto, Winnipeg, Edmonton, and Regina the drinking water processes to remove NOM and other water contaminants may include the phases of: A) coagulation/flocculation: to aggregate and grow the size of the particles present and NOM;B) Sedimentation to remove the suspended solids from water; C) Ozonation: to decompose NOM into low molecular weight fractions, and chemically destroy microbial cells (Zouboulis et al., 2007a; Hammes et al., 2008; Epcor, 2021; City of Winnipeg, 2021; Ontario, 2021). (Zouboulis et al., 2007a; Hammes et al., 2008a; Epcor, 2021; Ontario, 2021; Winnipeg); D) Rapid sand filtration: commonly used to remove biodegradable organic matter, ammonium and other organic micropollutants. ; E) Addition of chlorine:  to inactivate remaining pathogenic and non-pathogenic microorganisms present; F) UV disinfection: to induce biochemical inactivation of waterborne parasites(Li et al., 2019; Epcor, 2021; City of Winnipeg, 2021).

## Other methods for NOM removal and their potential limitations in Indigenous Reserves

As mentioned above, **coagulation** is the most used method in Canada for NOM removal(Epcor, 2021; City of Winnipeg, 2021; Ontario, 2021). Coagulation reduces the repulsion forces and aims to transform dissolved organic matter into neutral particles by adsorption onto aluminum or iron-based coagulants (Edzwald, 1993). The particles efficiently accumulate through flocculation to be afterward removed by clarification (Edzwald, 1993; IWA publishing). The downsides of applying coagulation in remote drinking water systems include high costs and high doses of anticoagulants and other chemicals needed for pH modification (Eikebrokk, Vogt & Liltved, 2004; Soros et al., 2019a).

**High-pressure membrane filtration** such as reverse osmosis (RO) and Nanofiltration (NF). RO is a pressure-driven technology that uses a semi-permeable membrane typically made of cellulose and polyamide enabling the passing of water size particles but blocking all the solids, dissolved matters, colloids, salts, and organic matters with a molecular weight greater than 50 to 100 Da(Zhao, 2018a; Prasad, 2020). Likewise, NF uses mostly polymer membranes with larger pore sizes (100-1 nm) than the ones used in RO(Nguyen, Roddick & Harris, 2010; Zhao, 2018a). The pressure used in NF is lower and thus consumes less energy than RO (Zhao, 2018b). The limitations of RO and NF are the high cost of the membrane, maintenance, and biofouling(Follet & Hatfield, 2001; Nguyen, Roddick & Harris, 2010; Zhao, 2018b). This method is not easy to implement in indigenous reserves due to high energy demand (which is commonly not available in remote communities), costs associated with membrane replacement, and highly trained operator requirements (Follet & Hatfield, 2001; Zimmermann et al., 2020b).

**Low-pressure membranes**. Microfiltration (MF) and ultrafiltration (UF) membranes are commonly used after the employment of coagulants. This improves the permeate flux and helps to avoid membrane fouling(Nguyen, Roddick & Harris, 2010). Notably, MF and UF are effective for particle and microbial removal, the drawback is that the membranes used for these methods are recognized to be fragile and costly (Hoslett et al., 2018). Additionally, in most cases, only a fragment of the organic components is removed (Hoslett et al., 2018). MF and UF membrane treatments fail to remove significant levels of NOM (Jacangelo, Trussell & Watson, 1997; Hoslett et al., 2018). Consequently, low-pressure membrane filtration systems are contemplated as pre-treatments of other sophisticated methods such as NF or RO and the cost outweighs their implementation in the drinking water systems of Indigenous communities (Hoslett et al., 2018).

**Ozone in combination with activated carbon adsorption.** Ozone is an advanced oxidation process that is useful for breaking organic chains and detaching aromatic rings in recalcitrant organic complexes along with efficient microorganism inactivation (Zouboulis et al., 2007a; Loh et al., 2021). Biological activated carbon (BAC) filters used after ozonation have demonstrated substantial removal of NOM, ozonation transformation products, DBPs precursors as well as taste and odor compounds(Simpson, 2008; Hamid et al., 2019). There are more than 800 water facilities using ozone as part of their water purification system across Canada (Health Canada, 2016). Some disadvantages of ozone treatment, depending on the water matrix (surface water or wastewater), and/or residual pollutants, are the incomplete degradation of organic substances which results in the generation of pathogenic by-products (i.e. brominated organics, aldehydes, and carboxylic compounds)(Huang, Cheng & Cheng, 2007). Furthermore, as a result of ozonation, dissolved organic carbon levels could increase because of the liberation of extracellular organic matter and other proteins and polysaccharides (Huang, Cheng & Cheng, 2007). Moreover, high ozone doses are required for the successful inactivation of microorganisms(Zouboulis et al., 2007b; Manasfi, 2021). Lastly, the lifetime of ozone is brief and needs on-site production which is not an option for water systems on Indigenous reserves(Zimmermann et al., 2020b). Regarding the limitations of BAC systems, their gradually decreasing absorption capacity over time and low dissolved organic carbon removal should be noted (Zimmermann et al., 2020b). When dissolved organic matter and other complex compounds are not adequately removed during the water treatment, pathogenic microorganisms can proliferate inside the water distribution system (Okabe, Kokazi & Watanabe, 2002; Simpson, 2008; Hamid et al., 2019) [84,85,90]. Without proper maintenance, this method represents a health risk for the consumers in addition to poor water quality(Okabe, Kokazi & Watanabe, 2002).

**Ion exchange** (IEX) is an electrochemical method where ions from the water body are swapped with ions within resins that contain active centers with acidic or basic groups (electrically charged)(Gefter, 1962; Keller, 2005; Drioli, Giorno & Fontananova, 2017; Al-Asheh & Aidan, 2021). The most common materials used for IEX resins include methacrylic acid, sulfonated styrene, and divinyl benzene (DVB) (Brady et al., 2017). Cation exchangers carry sulfate or carboxyl groups and use sodium, potassium, and hydrogen as counterions (Brady et al., 2017). Whereas anionic ion exchange resins contain quaternary ammonium groups with chlorine as a counterion (Brady et al., 2017). During water treatment for dissolved organic carbon removal, anionic IEX takes place when the negatively charged dissolved organic matter in waters with pH values ranging from 6 to 8 (which is the case of most drinking water sources) has a higher attraction for the ion exchange resin than the ion being traded (Arias-Paic et al., 2016). The removal of anions releases a $Cl^-$ ion from the anionic resin (Arias-Paic et al., 2016). Anionic IEX can eliminate up to 90 percent of dissolved organic carbon (Dixit, Barbeau & Mohseni, 2020; Zimmermann et al., 2020b). Additionally, the cost of a traditional IEX system fluctuates around \$0.1–\$0.2 per 1,000 liters of purified water, which is significantly lower than other membrane-based methods (Dixit, Barbeau & Mohseni, 2020). Removing dissolved organic carbon from water through IEX may be considered a viable, efficient, and affordable alternative for rural systems as it does not require continuous operational capacity or personnel to operate other treatment processes. The drawbacks of IEX implementations include the saturations of resins with chloride counter-ions after 3 to 8 weeks of performance (depending on dissolved organic carbon levels, conditions of operation, and IEX capacity), which requires frequent regeneration (Amini et al., 2018a; Winter et al., 2018; Zimmermann et al., 2020b). The regenerations produce elevated concentrations of sodium chloride and NOM that demand careful disposal since it can negatively affect the aquatic ecosystem and the plumbing structure of the area if disposed directly into the sewer sheds (Rokicki & Boyer, 2011; Liu et al., 2020). Thus, the constant transportation of regenerants (10-12% NaCl solution) represents a clear disadvantage for remote Indigenous Communities (Rokicki & Boyer, 2011; Liu et al., 2020). In IEX, the favorable conditions of the macro-porous anatomy of the resin and the presence of high sources of carbon, benefit biofilm development in the membrane in the absence of resin regeneration (Flemming, 1987; Schulz et al., 2017).

The establishment of biofilm was considered undesirable, nevertheless, several recent studies have demonstrated that biological activity found in the IEX resins contribute to the removal of NOM (Schulz et al., 2017; Amini et al., 2018a; Winter et al., 2018; Liu et al., 2020; Zimmermann et al., 2021). This high-performance method, now called Biological Ion Exchange (BIEX) enables the saturated membrane of IEX to extend its lifetime without filter maintenance, minimal waste discharge, and low operational cost(Amini et al., 2018b; Liu et al., 2020; Zimmermann et al., 2021). BIEX has recently been applied in the drinking water system used by the Middle River community located in central British Columbia. This indigenous reserve, which is part of the Tl'azt'en Nation, has been under drinking water advisory for over a decade(Zimmermann et al., 2020b). The implementation of BIEX in this community resulted in high dissolved organic carbon removal, with no need for replacement of the filter in more than 12 months (Zimmermann et al., 2020b). Remarkably, after the implementation of BIEX in this community, the drinking water advisory was lifted (Zimmermann et al., 2020b). While there is not yet a consensus established about the percentage of dissolved organic carbon removal exerted by the biological activity of the resins associated with this method, there are strong suggestions that biodegradation might directly impact the concentrations of dissolved organic carbon (Zimmermann et al., 2021). This method can be applied in complementation with UV disinfection, an advanced oxidation process, and chlorine for the elimination of NOM (Zimmermann et al., 2020b). Further research is required to elucidate the dynamics of biofiltration in NOM removal through IEX. It is critical to identify accessible technologies capable of removing NOM in DWS of indigenous communities, above all the ones with long-term drinking water advisories to comply with water quality standards and guarantee the health of all users.

## Importance of Determining the Microbiome Inhabiting Drinking Water Treatments

One of the main goals of drinking water treatments (DWT) is to remove NOM since its presence is problematic because of the potential formation of DBPs (McKie et al., 2015; Wu et al., 2019b), contribution to biofilm growth (Templeton, Andrews & Hofmann, 2005b; Pham, Mintz & Nguyen, 2009b; Jacquin et al., 2020), and high operation costs (Eikebrokk, Vogt & Liltved, 2004; Yang et al., 2016b; Soros et al., 2019b) as previously reviewed. The use of biological-activated methods in drinking water treatment plants has demonstrated a positive impact on the degradation of a fraction of NOM (Zhang et al., 2017). Even though some micropollutants need specialized electrochemical degradation (Asadi Zeidabadi et al., 2023), the effective depletion of contaminants including pharmaceuticals, personal care products, endocrine disrupting compounds, arsenic, manganese, and ammonium, has been well reported (Zhang et al., 2017; Wagner et al., 2018; Kirisits, Emelko & Pinto, 2019; Environmental Protection Agency USA, 2020). In this context, biofilters assist in the reduction of membrane fouling, color and odor constituents, disinfectant doses, and DBPs precursors (Cuthbertson et al., 2019; Kirisits, Emelko & Pinto, 2019; Verdugo et al., 2020). A better understanding of the physiological, metabolic, biochemical, and ecological characteristics of the microbial networks found in DWS can be achieved by the identification of their genome (Willey, Sherwood & Woolverton, 2011; Bruno et al., 2018a). Understanding the growth conditions, symbiotic relationships, pathogenicity risks, and habitat preferences of the microorganisms present in drinking water systems and raw water is critical for making water safe. Besides, the aforementioned factors provide insights to alter the conditions surrounding the microbiota and modulate the development of biofilms(Bruno et al., 2018b). Regulating the microbial community established in the biologically activated filters used in DWS could contribute to elaborate cutting-edge approaches to impact positively different aspects regarding water safety. For instance, once the microbiome in water filters and source water is monitored, the prevention of high-risk pathogens could be easily achieved (Bruno et al., 2018). In addition, when using BAFs, efforts should be made to provide an ideal working environment for the microorganisms contributing to the degradation of water contaminants (Kirisits, Emelko & Pinto, 2019). These efforts will allow the recognition of the correlation between microbial community structure and biofilter function (Kirisits, Emelko & Pinto, 2019). The customization of the microbial community present in biological activated filters (BAF) according to the definitions proposed by Kiristis et al. (2019) includes 3 approaches. A) Bioaugmentation with the inoculation of pivotal endogenous or exogenous microorganisms from an enriched source to the one of interest to accelerate the normal microbial establishment process and fasten biodegradation (Lauderdale et al., 2012; Kirisits, Emelko & Pinto, 2019). B) Amendment that refers to the adjustment of exogenous compounds required for biological activity such as nutrients and oxidants (Lauderdale et al., 2012; Kirisits, Emelko & Pinto, 2019); and C) Supplementation of endogenous substances to achieve higher amounts than the ones contained naturally within the filters(Kirisits, Emelko & Pinto, 2019). Although not considered in most operative water systems using BAFs, the enhancement of biological activities has demonstrated a significant improvement in different aspects of the water filtration process(Albers et al., 2018). For instance, in an experiment of bioaugmentation conducted by Albers et al. (2018), enriched nitrifying bacteria from an operational sand filter was transferred to a novel filter, resulting in an acceleration of the development of key nitrifiers in the filters (Pinto et al., 2016; Albers et al., 2018; Kirisits, Emelko & Pinto, 2019). This approach enables speeding the oxidation of ammonium in groundwater, a process that under normal conditions would take several months (Pinto et al., 2016; Albers et al., 2018; Kirisits, Emelko & Pinto, 2019). In another study conducted by Lauderdale et al. (2012), the amendment of nutrients and peroxide resulted in filter life extension as well as a breakthrough decrease of dissolved organic carbon and other undesirable components(Lauderdale et al., 2012) . In contrast, these improvements were not observed with other BAF systems used without this enhancement (Lauderdale et al., 2012). Moreover, in research conducted by Keithley and Kirisits (2019), it was proposed that supplementation of phosphorus in the biofilters only when P levels are limited helps to ameliorate biofilter hydraulics(Keithley & Kirisits, 2019). In natural conditions, phosphorus is low, due to removal through conventional treatments (i.e., coagulation/flocculation) (Keithley & Kirisits, 2019). The deficit of P contributes to an increase in the extracellular polymeric substances of the biofilm which has been correlated with bio-clogging and less filter durability(Xia et al., 2014; Keithley & Kirisits, 2019). Therefore, more efforts should be considered to determine the microbiome established in the biofilters to increase their durability and secure correct functioning in DWS.

## Metagenomics to monitor source water, water biofilters, and drinking water systems.

Identifying the microbiome present in biological water filters represents the cornerstone for finding putative functions associated with these microorganisms. Thereby, any attempt of amendment, supplementation, and/or bioaugmentation to improve the biodegradation of any organic or inorganic component, should be complemented with the microbial fingerprints present in the biofilter to link activity and symbiosis of the microbiome(Lauderdale et al., 2012; Kirisits, Emelko & Pinto, 2019) [109]. Even though culture-dependent methods have been widely used as the technique of reference for controlling the presence of pathogenic organisms in water, these methods do not provide significant information regarding the total microbial diversity and its changes in source water and DWS(Douterelo et al., 2014; Uyaguari et al., 2016). Besides, most of bacterial cells present in the water as well as in BAFs and DWS are not culturable (Theron & Cloete, 2000; Bruno et al., 2018b; Lewis et al., 2021). To obtain information about the type, abundance, function, pathogenicity, and metabolic requirements of the drinking water microbiome, culture-independent or molecular methods are the approach of choice(Theron & Cloete, 2000; Boughner & Singh, 2016). These high-throughput methods can provide more detailed information to monitor BAFs, enable to introduce modifications, and consequently improve biologically activated filters in DWS(Theron & Cloete, 2000; Boughner & Singh, 2016; Kirisits, Emelko & Pinto, 2019). Moreover, the rapid decline in the cost of these sequencing tools makes them conveniently affordable, even for DWS in indigenous reserves(Hull et al., 2019). Up to date, amplicon-based metagenomics, or targeted metagenomics has been the procedure of choice for microbiome analysis(Kibegwa et al., 2020). One of the main reasons for these preferences is the high level of conservation and hyper-variability of the genomic marker used, which allows the identification of different species(Douterelo et al., 2014; Uyaguari et al., 2016; Kibegwa et al., 2020). The multiple sets of DNA sequences identified during high-throughput sequencing can be used to evaluate the taxonomy of the microorganisms present in drinking water and biofilter microbial communities (Kirisits, Emelko & Pinto, 2019). For instance, deep amplicon sequencing of 16SrRNA and chaperonin 60 or cpn60 (mitochondrial protein) has been used for identifying bacterial communities in source water and drinking water distribution systems (Baron et al., 2014; Uyaguari et al., 2016; Brumfield et al., 2020) . Other microorganisms such as eukaryotes and fungi have also been described in aquatic environments using 18S rRNA and Internal Transcribed Spacer (ITS) (Weber et al., 2009; Pereira et al., 2010; Schoch et al., 2012; Uyaguari et al., 2016). Similarly, characterization of the viruses found in drinking water have been attempted by studying specific viral groups using biomarkers such as RNA-dependent RNA polymerase (RdRp) for RNA virus, gene 23 (g23 ), and gene 20 (g20) for DNA virus, among others(Zheng et al., 2013; Uyaguari et al., 2016; Wang et al., 2016). Despite the virome characterization obtained using the mentioned viral biomarkers, approximately 50% of viral hits remain unknown when searched in public databases (Barrientos-Somarribas et al., 2018). The hassle relies on viruses lacking "universal" gene markers which makes the identification of abundance patterns and community structure for all viruses a challenge(Brum et al., 2015; Kulski, 2016; Uyaguari et al., 2016). Despite this, several viruses haven been associated to human fecal contamination such as: Norovirus, Enterovirus, Rotavirus, Hepatovirus A, Pepper mild mottle virus (PMMoV), crAssphage, and human adenovirus (HAdV), among others (Wong et al., 2012; Holcomb & Stewart, 2020). Additionally, depending on the source of water, animal-specific enteric viruses can also serve as fecal indicators, such as the case of porcine and bovine adenoviruses' [(PAdVs) and (BAdVs), respectively] as well as bovine enterovirus (BEV) and Bovine polyomavirus (BPyV) (Xagoraraki, Yin & Svambayev, 2014). Table 2. summarizes the different viruses and other microorganisms used to assess fecal contamination in aquatic environments. The prevalence of these microorganisms has been identified in multiple water environments (McMinn, Ashbolt & Korajkic, 2017), for this reason monitoring source water, DWT and biofilters with this technology can help ensure adequate water quality for all individuals.

**Table 2. Microorganisms used to assess fecal contamination in water. Fecal indicator bacteria (FIB), fecal indicator viruses (FIV) and common waterborne parasites.**

In addition to targeted metagenomic another culture independent approach is shotgun metagenomics, in which the contents of the complete genome are studied(Kibegwa et al., 2020). In this approach, long DNA molecules belonging to the microorganisms present in the ecosystem under study break into random fragments that are afterward sequenced(Durazzi et al., 2021).  Shotgun sequencing examines all metagenomic DNA instead of only the hypervariable regions (Kibegwa et al., 2020). When the nucleotide detection of target DNA molecules is direct, the efficacy of data analysis increases, and the coverage regions of phylogenetical relevance are extended(Sato et al., 2019). The average prices of targeted and shotgun metagenomics can be found in table 3. Even though these tools have the potential to provide important information regarding the microbial populations present in source water, DWS and biofilters, there is still evidence of a vast number of uncategorized, uncharacterized, and unclassified environmental microorganisms (Lynch & Neufeld, 2015; Bruno et al., 2018b; Lagier et al., 2018a). For this reason, complementary methods should be used to fill in the existing taxonomic “blind spots” that separate the field today from vital and novel phylogenetic information.

**Table 3.** Current prices of “Omic” technologies.

## Culturomics

Culturomics is a high-throughput culture approach that can be used to overcome the limitations of unclassified environmental bacteria that metagenomics faces (Lagier et al., 2018a; Nowrotek et al., 2019). This technique was originally introduced for the study of microbiota in the human gut (Lagier et al., 2016). This method is a combination of matrix-assisted laser desorption/ionization-time of flight (MALDI–TOF) mass spectrometry procedure and 16S rRNA sequencing for the identification of novel, living bacterial colonies(Lagier et al., 2016, 2018a). Its principle of improving culture media with the precise conditions these microorganisms require for their growth can help fill in the gaps of the so-called “uncultivable” bacteria of aquatic environments. In culturomics, diverse adjustments are applied to the incubation conditions (temperature, incubation time, media enrichment, pH, oxygen demand, and so forth) to promote the growth of otherwise uncultivable bacteria (Nowrotek et al., 2019). Consecutively, they are cleaned and prepared for the MALDI-TOF method, and if the taxonomic credentials in the database are not found, a supplementary amplification and sequencing using 16SrRNA is conducted (Nowrotek et al., 2019). The transcendental results obtained from the human gut microbiome (247 new species of bacteria and their genomes unveiled) make this method a suitable candidate to complement metagenomics for the uncategorized phyla found in DWS (Lagier et al., 2016). Conversely, the limitations of culturomics lie in the inability to identify species that do not count with any genome registration in libraries of reference (Lagier et al., 2016, 2018b). Furthermore, the sample-processing capacity is pointedly lower compared to the volume that metagenomics tools can handle in a single day(Lynch & Neufeld, 2015).

**Microfluidics or Lab-on-a-chip (LOC) technologies for water assessment**

Indigenous and remote communities would significantly benefit from fast, transportable, and on-site sensitive methods to recognize bacterial, viral, and protozoan pathogens in DWS. The term microfluidics refers to the process of small ($10^{-9}$ L to $10^{-18}$ L) fluids (Meissner, Seddon & Royall, 2019) that circulate into micrometer channels with components like microfilters, microvalves, micromixers and sensors such as detectors at the cellular and molecular level (Guo et al., 2015; Tommaso, 2019). Generally, the channel size used in the analytical devices employed in microfluidics oscillates between 10 mm to 200 mm and even 1 mm in some cases(Guo et al., 2015). Lab-on-a-chip

(LOC) employs a microsystem where the surface, gravitational, viscous, and other forces integrated with either active or passive microvalves are carefully applied to obtain a real and complete micro-laboratory (Guo et al., 2015; Tommaso, 2019). Active microvalves require external actuation (i.e., electromagnetism, thermal expansion) while passive valves base their functioning on the pressure gradient (Pan et al., 2005; Guo et al., 2015). These (passive) microvalves are generally used for micropumps (i.e. as check-valves) (Pan et al., 2005; Guo et al., 2015). Nichols et al. (2010) tested an insolation chip (ichip) composed of more than a thousand miniature diffusion chambers to inoculate microorganisms from diverse environments including aquatic settings (Nichols et al., 2010). The application of this technique has resulted in a higher recovery compared to traditional cultivation methods alone (Nichols et al., 2010). Additionally, the species found differed significantly from the ones recovered in petri dishes increasing the diversity of microbial phyla (Nichols et al., 2010).

The specific functioning, types, and components of microfluidics have been broadly studied in different areas and can be revised elsewhere (Nichols et al., 2010; Guo et al., 2015). Generally, there are more than 5 types of microfluidic platforms including linear actuated devices, microfluidic large-scale integration, centrifugal microfluidics, segmental flow microfluidics, electrokinetics, surface acoustic waves, pressure-driven laminar flow, and lateral flow tests(Mark et al., 2010; Nichols et al., 2010). For instance, centrifugal microfluidics allows the management of more sensitive liquids such as nucleic acids (Mark et al., 2010). Some examples of the application of centrifugal microfluidics include DNA extraction and nucleic acid-based assays, protein crystallization and protein-based assays, integrated plasma separation, clinical chemistry assays, and chromatography tests (Mark et al., 2010).

Similarly, lateral flow tests, have been successfully used to detect infectious agents such as *Salmonella spp.*, anthrax (*Bacillus anthracis*), viruses, and even small molecules such as antibiotics (Ho et al., 2008; Mark et al., 2010). Among the samples used in lateral flow tests are included: nucleic acids for *B. anthracis* (Carter & Cary, 2007), blood serum for *Salmonella spp.* (Ho et al., 2008), nasopharyngeal wash for respiratory syncytial virus Infection (RSV) (Mokkapati et al., 2007), milk for tetracycline detection(Chen et al., 2019) and fecal specimens for *Giardia spp.* and *Cryptosporidium spp.* (Johnston et al., 2003; Rasooly et al., 2017). In lateral flow tests, the capillary-driven process is used to absorb the sample and transport it through all over the test strip. Three types of molecules or antibodies will be present in this mechanism where: 1) Tagged antibodies for a signal-generating particle in the “conjugate pad” are hydrated with the sample and eventually bound to the antigens contained in the sample(Mark et al., 2010; Wei et al., 2023). 2) The sample continues flowing to the incubation and recognition area and meets test line antibodies that bind particles covered with antigens(Mark et al., 2010). 3) On the control line, a third class of antibodies catches the compounds that did not bind with any particle (Mark et al., 2010). This latter binding confirms a successful test performance (Mark et al., 2010). Likewise, the binding or not binding antibodies in the detection line confirm or deny the presence of the analyte of interest (Mark et al., 2010; Wei et al., 2023). To the best of our knowledge and based on current literature, these methods have not yet been used to test water microbial quality. In this context, lateral flow tests embody a cost-effective, mobile, and top-notch alternative to identify well-known pathogens in the drinking water systems of Indigenous reserves.

The mechanism of action that microfluidics has, could be replicated for the detection of fecal indicator bacteria, viruses, and protozoans in the DWS of isolated areas and indigenous communities. Nevertheless, the drawbacks of microfluidics include system blockage by small elements, as well as high contamination risks with minimal amounts, and premature absorption of the analytes of interest, among others (Guo et al., 2015). Microfluidics platforms represent low-cost, portable, high-precision [163,173], time-optimizer tools that would benefit drinking water systems with higher susceptibility to microbiological contamination as is the case of DWS located in remote indigenous reserves (Guo et al., 2015; Reboud et al., 2019).

## Conclusion

In Canada some water advisories in Indigenous reserves have not been lifted for more than 20 years (Indigenous Services Canada, 2024a). In places where traditional drinking water treatments are not available due to underfunding or lack of support from authorities or source-water quality issues (including high NOM levels), efficient and long-term alternatives should be implemented to permanently lift DWA. To improve DWS under advisories, comprehensive design studies are required in addition to provisional and permanent repairs in their infrastructure. The most common methods to remove NOM are coagulation, high- and low-pressure membrane filtration procedures, ozone, Ion exchange (IEX) and its variant Biological Ion exchange (BIEX). Every method has its advantages and limitations, however, the method to be implemented should be specific for the source water conditions of the area to be implemented.

Furthermore, source water, biofilter and drinking water monitoring is fundamental to prevent the negative health effects of pathogenic microorganisms. Metagenomics and its drop in prices represent an effective tool to monitor the water microbiome. Additionally, technologies such as culturomics can exceptionally contribute to grow and reveal the up to date “unclassified” microbial diversity in DWS. Once identifying the microbial fingerprint in source water and DWS, practical approaches such as lab-on-chip technologies can be implemented for on-site, ultra fast water microbial quality assessment. Easing water quality monitoring will only benefit the promotion of health and safe water for all consumers, especially the ones in Indigenous remote communities.

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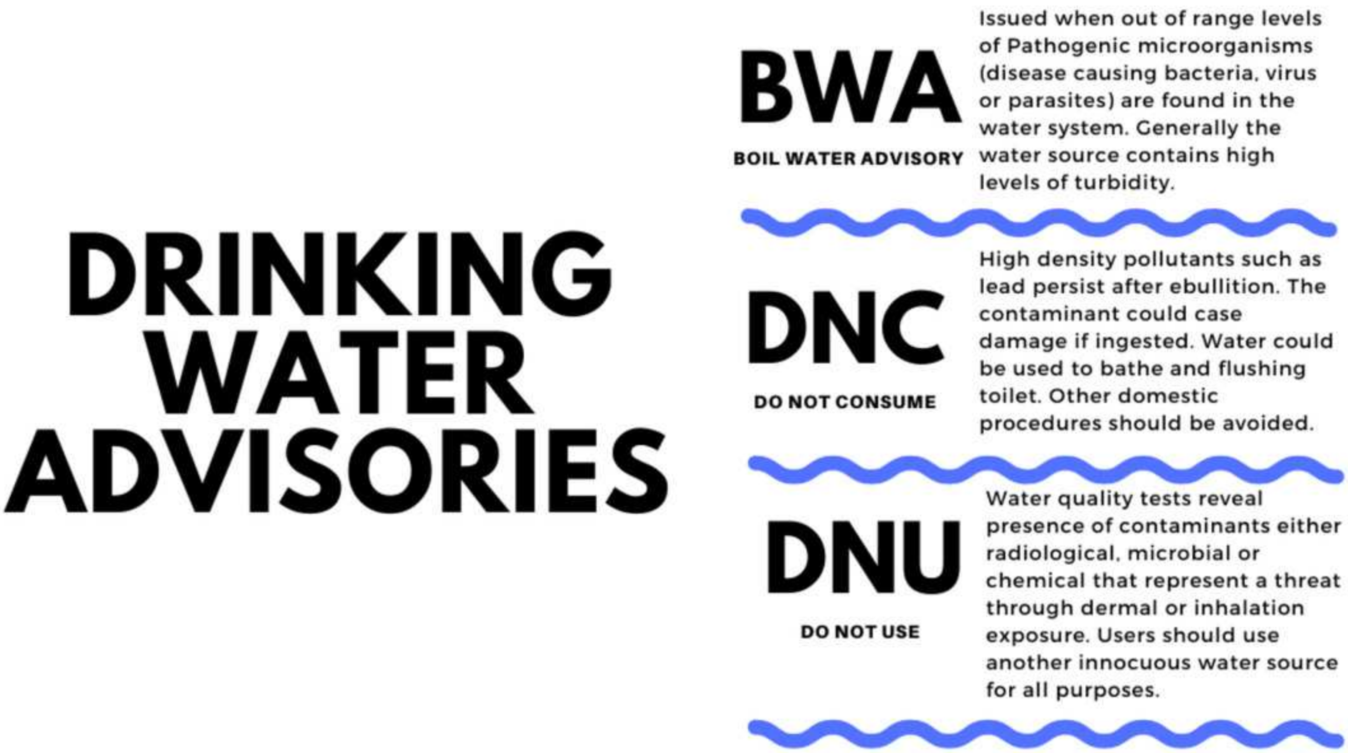
# Figure 1

Drinking water advisories (DWA)

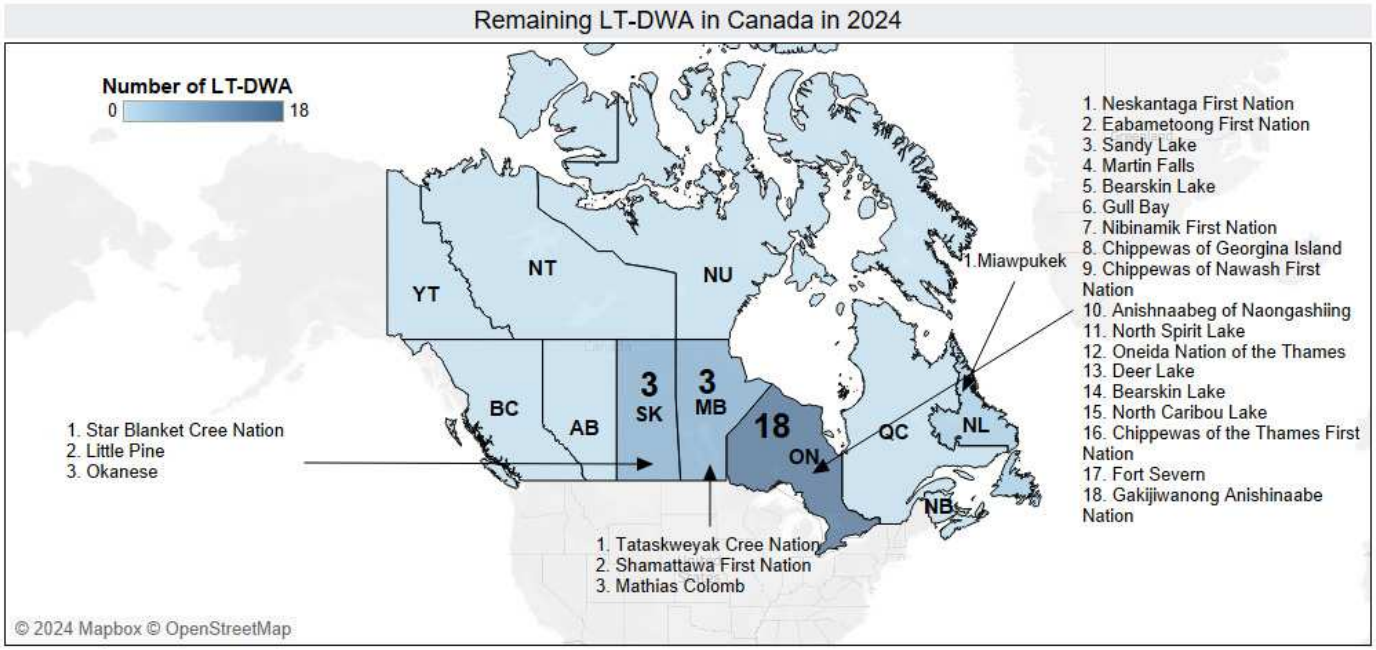
Types of Drinking water advisories (DWA) issued in Canada. Boil Water Advisories (BWA), Do not consume (DNC), and Do not use (DNU). BWA represents the majority of DWA. Most of them are precautionary and are related to planned maintenance work, line breaks or pressure losses, poor water disinfection, or inadequate maintenance of the instruments used to treat water. Likewise, DNC and DNU are issued in atypical situations. For example, when a chemical substance or another pollutant is suspected or confirmed in the drinking water system (DWS). When the contaminant can alter human health through ingestion DNC is recommended. Whereas, if the substance of concern in the DWS affects the nose, skin ,and/or eyes the advisory issued corresponds to DNU.

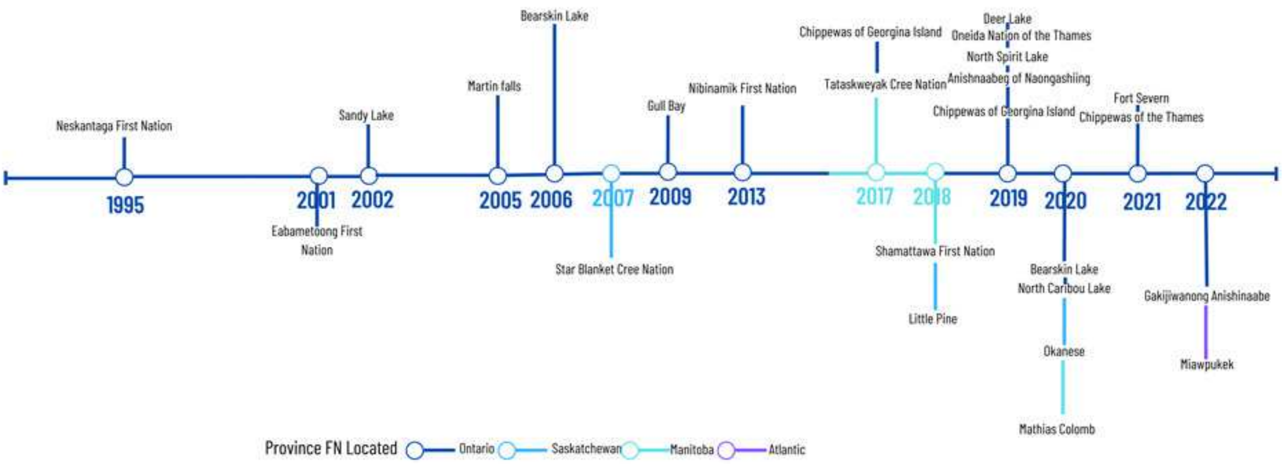

DRINKING WATER ADVISORIES
BWA
BOIL WATER ADVISORY
Issued when out of range levels of Pathogenic microorganisms (disease causing bacteria, virus or parasites) are found in the water system. Generally the water source contains high levels of turbidity.
DNC
DO NOT CONSUME
High density pollutants such as lead persist after ebullition. The contaminant could case damage if ingested. Water could be used to bathe and flushing toilet. Other domestic procedures should be avoided.
DNU
DO NOT USE
Water quality tests reveal presence of contaminants either radiological, microbial or chemical that represent a threat through dermal or inhalation exposure. Users should use another innocuous water source for all purposes.

# Figure 2

Remaining long-term drinking water advisories (LTDWA) in Canada in 2024.

(Top) Map based on the number of unresolved, long-term drinking water advisories (LT-DWA) issued around Canada: Saskatchewan=3, Manitoba=3, Ontario=18. Note: provinces and territories colored with light blue have no LT-DWA to address or the information is unavailable. (Bottom) Timeline displaying the start year of the current unresolved LT-DWA across the country. Last updated: March 2024.

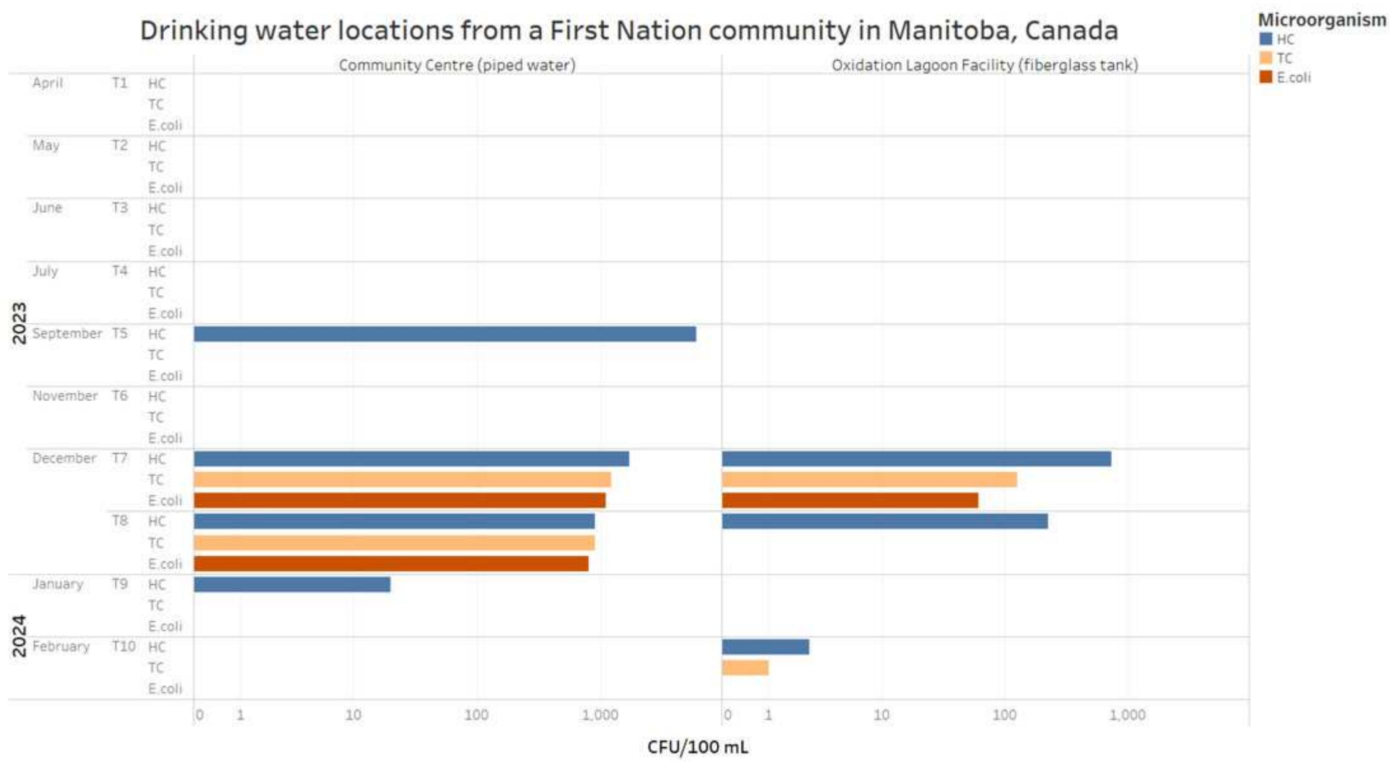

Remaining LT-DWA in Canada in 2024
Number of LT-DWA
0
18
YT
NT
NU
BC
AB
3
SK
3
MB
18
ON
QC
NL
NB
1. Star Blanket Cree Nation
2. Little Pine
3. Okanese
1. Tataskweyak Cree Nation
2. Shamattawa First Nation
3. Mathias Colomb
1.Miawpukek
1. Neskantaga First Nation
2. Eabametoong First Nation
3. Sandy Lake
4. Martin Falls
5. Bearskin Lake
6. Gull Bay
7. Nibinamik First Nation
8. Chippewas of Georgina Island
9. Chippewas of Nawash First Nation
10. Anishnaabeg of Naongashiing
11. North Spirit Lake
12. Oneida Nation of the Thames
13. Deer Lake
14. Bearskin Lake
15. North Caribou Lake
16. Chippewas of the Thames First Nation
17. Fort Severn
18. Gakijiwanong Anishinaabe Nation
© 2024 Mapbox © OpenStreetMap


1995
Neskantaga First Nation
2001
Eabametoong First Nation
2002
Sandy Lake
2005
Martin falls
2006
Bearskin Lake
2007
Star Blanket Cree Nation
2009
Gull Bay
2013
Nibinamik First Nation
2017
Chippewas of Georgina Island
Tataskweyak Cree Nation
2018
Shamattawa First Nation
Little Pine
2019
Deer Lake
Oneida Nation of the Thames
North Spirit Lake
Anishnaabeg of Naongashiing
Chippewas of Georgina Island
2020
Bearskin Lake
North Caribou Lake
Okanese
Mathias Colomb
2021
Fort Severn
Chippewas of the Thames
2022
Gakijiwanong Anishinaabe
Miawpukek
Province FN Located
Ontario
Saskatchewan
Manitoba
Atlantic

# Figure 3

Microbiological counts observed per 100 ml of drinking water samples from a First Nation community in Manitoba, Canada.

*E. coli,* total coliforms (TC) and heterotrophic plate counts (HC) from tap water collected from a community centre (piped water) and oxidation lagoon facility (fiberglass tank) in a First Nation community of Manitoba. Duplicate counts were assessed during 10 different time periods during 2023 and early 2024. Unacceptable levels of *E. coli* and TC appear in both locations in 2 sampling events (for piped water) and 1 sampling event for fiberglass tank.



## Table 1(on next page)

Number of small drinking water systems (DWS) that exceeded haloacetic acid (HAA5) levels in Canada.

Drinking water systems (DWS) with <5000 users that exceeded 80 ug/L, the level of haloacetic acids (HAA5) permitted in Canada. In Newfoundland for example, 50.91% of small DWS exceeded the level of HAAs established. Data from Health Canada last updated 2010.

| Province | No. of DWS of < 5000 users per province | No. of DWS that exceeded limit of HAA5 | Percentage of DWS that exceeded HAA5 in that region (%) |
|---|---|---|---|
| Ontario | 32 | 1 | 3.13 (%) |
| Quebec | 27 | 11 | 40.74 (%) |
| Nova Scotia | 38 | 16 | 42.11 (%) |
| Newfoundland and Labrador | 220 | 112 | 50.91 (%) |

**Table 1.** Drinking water systems (DWS) with <5000 users that exceeded 80 ug/L, the level of haloacetic acids (HAA5) permitted in Canada. In Newfoundland for example, 50.91% of small DWS exceeded the level of HAAs established.

## Table 2(on next page)

Microorganisms used to assess fecal contamination in water. Fecal indicator bacteria (FIB), fecal indicator viruses (FIV) and common waterborne parasites.

Under the FIB category, the groups, or species with an asterisk (*) represent complementary alternatives to the FIB most commonly used (E. coli, enterococcus, and total coliforms). Below the FIV section: FRNA phage= specific ribonucleic acid; PMMoV= Pepper mild mottle virus (plant virus); TMV= Tobacco mosaic virus (plant virus); AdV*= Human (HAdV), Porcine (PAdVs) and bovine (BAdVs) adenoviruses; AstVs= Astroviruses; EV*=human (HEV) and bovine (BEV) enteroviruses; HepV= Hepatitis viruses; NoV= Noroviruses; PyV*= human (HPyVs) and bovine (BPyV) polymoviruses; RV= Rotaviruses. Finally, beneath waterborne parasites, the species with an asterisk (*) are the less commonly used as a reference in this category.

| **Fecal Indicator Bacteria** | **Fecal Indicator Virus** | **Waterborne parasites** |
|---|---|---|
| *Escherichia coli* | Coliphages | *Giardia lamblia* |
| *Enterococcus* spp. | *Bacteroides*bacteriophages | *Cryptosporidium parvum* |
| Fecal *streptococci* | *Enterococci* phages | *Toxoplasma gondi** |
| Total coliforms | crAssphage | *Cyclospora cayetanensis** |
| *Bacteroides-Prevotella group** | FRNA phage | *Entamoeba histolytica** |
| *Bifidobacterium spp.** | PMMoV | *Blastocystis hominis** |
| *Clostridium perfringens** | TMV | |
| | AdV* | |
| | AstVs | |
| | EV* | |
| | HepV | |
| | NoV | |
| | PyV* | |
| | RV | |

**Table 2**. Under the FIB category, the groups, or species with an asterisk (*) represent complementary alternatives to the FIB most commonly used (*E. coli*, enterococcus, and total coliforms). Below the FIV section: FRNA phage= specific ribonucleic acid; PMMoV= Pepper mild mottle virus (plant virus); TMV= Tobacco mosaic virus (plant virus); AdV*= Human (HAdV), Porcine (PAdVs) and bovine (BAdVs) adenoviruses; AstVs= Astroviruses; EV*=human (HEV) and bovine (BEV) enteroviruses; HepV= Hepatitis viruses; NoV= Noroviruses; PyV*= human (HPyVs) and bovine (BPyV) polymoviruses; RV= Rotaviruses. Finally, beneath waterborne parasites, the species with an asterisk (*) are the less commonly used as a reference in this category.

## Table 3(on next page)

Current prices of “Omic” technologies.

Services for metagenomics are accessible through different public and private institutions around Canada and the world. The manufacturing cost of less common technologies for drinking water systems (DWS) such as culturomics and paper-based microfluidic are included. *Requires initial inversion of hardware and devices around $400,000. **Cost based on 10 million devices production.

| Technology | Commercial price range per 1 sample (CDN$) |
|---|---|
| Targeted metagenomics | $20-$50 |
| Shotgun metagenomics | $130-$340 |
| | **Manufacturing price per 1 sample (CDN$)** |
| Culturomics* | $8.00-$10.00 |
| Paper-based microfluidics ** | $0.30-$4.00 |

**Table 3.** Services for metagenomics are accessible through different public and private institutions around Canada and the world. The manufacturing cost of less common technologies for drinking water systems (DWS) such as culturomics and paper-based microfluidic are included. *Requires initial inversion of hardware and devices around $400,000. **Cost based on 10 million devices production.