Evaluating Biological and Chemical Contaminant Removal and Recovery from Water using Capacitive Deionization

by

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A Thesis
presented to
The University of Guelph

In partial fulfillment of requirements
For the degree of
Master of Science
in
Environmental Sciences

Guelph, Ontario, Canada

Abstract

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University of Guelph, 2016

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Enteric viruses and herbicides may pose a health and economic risk to populations worldwide at low environmental concentrations in source waters. Since the current concentration methods are expensive, time-consuming, and produce variable results, the efficacy of capacitive deionization (CDI) was examined as a novel method for concentrating biological and chemical contaminants from water. The ability of CDI to remove and recover a surrogate enteric virus model (MS2) and a representative herbicide 2,4-D were tested in buffered solutions, ground, surface and tap water. Both culture-based and molecular enumeration methods demonstrated the ability of CDI to remove MS2, though the removal varied based on water sample type and the enumeration method. CDI was also able to remove 2,4-D, although the removal varied based on the water type similar to MS2 results. Low recovery of both biological and chemical contaminants indicate potential degradation of both MS2 and 2,4-D by CDI.

Acknowledgements

Firstly, I would like to thank my co-advisors Dr. Marc Habash and Dr. J. Christopher Hall, and committee advisor Dr. John Motto for their continued support during my project. I especially appreciate the knowledge, training, and support Marc has provided me with over the years. I am grateful to have the kind of professor who would take the time to teach an extremely nervous undergraduate student how to pour agar plates her first time working in a lab.

I have to thank all my lab mates, past and present. I have been lucky to have lab mates who have so much knowledge, and who take time to share it. I truly have learned from each one of you. I am even luckier to have had such a nice group of people to work alongside and build friendships with- it made this process much easier. I also want to thank all of my friends outside the lab, your support has been invaluable. All of the coffees, lunches, texts, skype sessions, walks, and evening hangouts helped me push through the rough days.

Thank you to my family for their unconditional love and support, throughout everything. Thank you for always believing in me. Without you I would not be here.

Thank you to my beautiful partner, Maks. I could not have done this without your love. Every single day I am grateful to swim upstream with you by my side.

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II. List of Abbreviations

°C	Degrees Celsius
2,4-D	2,4-Dichlorophenoxyacetic acid
AGID	Acute gastrointestinal disease
ANOVA	Analysis of variance
cDNA	Complementary deoxyribonucleic acid
C_t	Cycle threshold
DNA	Deoxyribonucleic acid
ED	Electrodialysis
E. coli	Escherichia coli
E. faecalis	Enterococcus faecalis
g	Grams
GCB	Graphitized carbon blacks
HPLC	High-pressure liquid chromatography
HCl	Hydrochloric acid
H_20	Water
L	Litre
LLE	Liquid-liquid extraction
MAC	Maximum acceptable concentration
M	Molar
mg	Milligram
mL	Millilitre
mM	Millimolar
MPN	Most-probable-number
ng	Nanogram
nM	Nanomolar
PBS	Phosphate buffered saline
PS-DVB	Poly(styrene-divinyl benzene)
qPCR	Quantitative polymerase chain reaction
RO	Reverse osmosis

RNA	Ribonucleic acid
RPM	Rotations per minute
RT-qPCR	Reverse-transcription quantitative polymerase chain reaction
SPE	Solid-phase extraction
TDS	Total dissolved solids
µg	Microgram
µL	Microlitre

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1. Introduction and Literature Review

Health Canada identifies three classes of contaminants that can make drinking water unsafe for human consumption: microbiological, chemical and physical, and radiological (Health Canada, 2014a). Microbiological water contaminants include bacteria, parasitic protozoa, and enteric viruses. Chemical and physical contaminants are organic and inorganic compounds including, but not limited to, industrial byproducts, personal care products, heavy metals, and pesticides. Radiological contaminants are radionuclides, such as Radon and Iodine-131 (Health Canada, 2014a). This study will focus on microbiological and chemical contaminants of drinking water, specifically enteric viruses and pesticides, as these two classes both occur in environmental water sources at low but biologically significant concentrations. These concentrations are low enough (for viruses, estimates are 1-10,000 infectious units per 100L and for pesticides picograms to nanograms per 1L of water) that detection requires sampling large volumes of water (hundreds to thousands of litres) (Ikner et al., 2012; Kurt-Karakus et al., 2010). Sampling large volumes of water requires a concentration step to collect the contaminant of interest. Current concentration methods are expensive, time-consuming, and produce highly variable results. Additionally, concentration methods are specific to the contaminant being targeted; there is no concentration technology that is effective for concentrating different types of contaminants in one step. This work will examine the potential application of capacitive deionization (CDI), currently used for the removal of total dissolved solids (TDS) from water, as an alternative method for removing and recovering these two classes of contaminants from water. This work aims to determine if CDI has the potential to be developed as a novel concentration technology. Improving the concentration step of contaminant detection would allow for more accurate quantification of these contaminants from source waters. This literature

review will provide background information on the environmental occurrence and risks posed by enteric virus and pesticide contaminants, outline current sample concentration technologies used for their detection, and detail why capacitive deionization is being tested as a novel contaminant concentration technology.

1.1 Microbiological Contaminants

The prevention of microbiological contamination of drinking water sources is of the highest priority as per Health Canada's Guidelines for Canadian Drinking Water Quality (Health Canada, 2014a). Based on data compiled from the Centres for Disease Control's Waterborne Disease and Outbreak Surveillance System from 1971-2012, the majority of waterborne disease outbreaks in the US are caused by microbiological organisms (Figure 1.1)(Beer et al., 2015). From 1974-2001, 288 disease outbreaks were linked to drinking water in Canada, all caused by microbiological contamination (Schuster et al., 2005). Of the 288 outbreaks, 22% were attributed to bacteria, 22% to protozoa, and 8% to viruses; the remainder (48%) were due to an unidentified pathogen (Schuster et al., 2005).

Organisms which reproduce in the gastrointestinal system are referred to as enteric organisms, and often result in gastrointestinal illness. To prevent outbreaks of enteric pathogens, multi-barrier approaches to water treatment and routine screening during different stages of drinking water treatment are required by the Federal government. In order to prevent waterborne disease outbreaks, Health Canada Guidelines for Canadian Drinking Water Quality require the routine screening of treated water quality to verify treatment efficacy (Health Canada, 2013). Rather than trying to detect the absence or presence of every single waterborne pathogen, which would be extremely expensive and time consuming, treated water is sampled and screened for organisms which indicate the presence of fecal contamination and poor water quality.

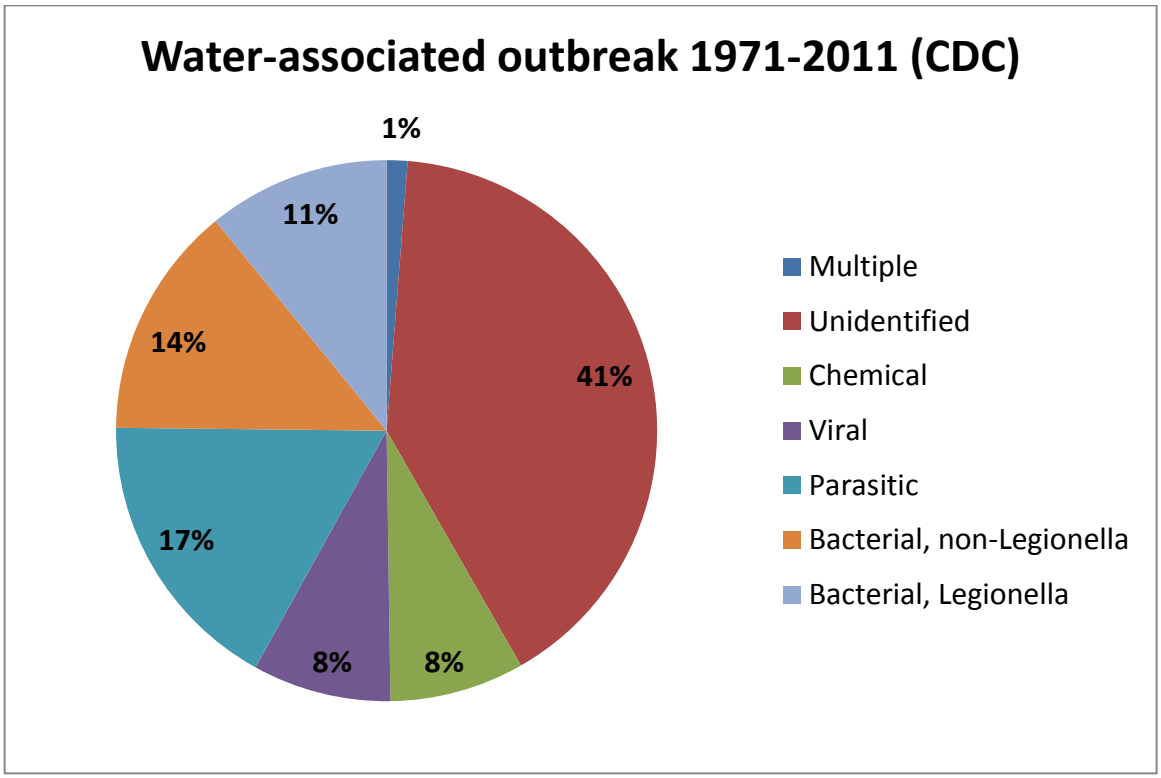


Figure 1.1 Causes of drinking water-associated outbreaks of illness in the United States from 1971-2011 (data taken from Beer et al., 2015).

If these organisms are detected in a water supply, further investigation into the cause and type of contamination can take place (Health Canada, 2013).

Health Canada requires that all jurisdictions screen for a specific species of coliform bacteria, *E. coli* (which is found in the gastrointestinal system of endotherms). It has a Maximum Acceptable Concentration (MAC) of none detectable per 100mL(Health Canada, 2014a). Monitoring of *E. coli* is used to detect recent fecal contamination (depending on conditions, it can survive between 4-12 weeks in water) of the water supply (Edberg et al., 2000). However, the use of *E. coli* as an indicator organism has come under scrutiny. One concern is that *E. coli* has been found able to propagate in waters that do not have fecal contamination (Gauthier and Archibald, 2001; Power et al., 2005). Furthermore, source waters which tested negative for *E. coli* have been found to contain enteric viruses (Locas et al., 2008; Pusch et al., 2005) or other pathogenic bacteria and protozoa (Lemarchand and Lebaron, 2003). This may be due to the fact that some enteric viruses can remain viable in the environment for longer than *E. coli* (Tree et al., 1997). Since a correlation between *E. coli* and enteric virus presence/absence often cannot be established (Horman et al., 2004; Payment and Locas, 2011; Wu et al., 2011), other methods of detecting fecal contamination are being explored, such as fecal source tracking, the use of bacteriophages as surrogates for enteric viruses, as well as the direct detection of enteric viruses.

Enteric viruses colonize the gastrointestinal system and are excreted through feces into the environment (Carter, 2005). The first waterborne virus to be identified was poliovirus in the 1940s (Paul and Trask, 1942). Currently, there are more than 140 different enteric viruses that have been identified (Taylor et al., 2001). This diverse group of viruses includes many *Enteroviruses*, hepatitis A and E, *Norovirus*, multiple adenoviruses, and rotavirus. They are known to be a common cause of gastroenteritis, with the symptoms of vomiting and diarrhea

(Carter, 2005). Health Canada Guidelines require water treatment to be capable of a minimum of 4-log removal or inactivation of viruses, but there is no routine screening for enteric viruses required as it is deemed "not practical"(Health Canada, 2014a). Routine detection is hampered, in part, because current methods for the concentration of enteric viruses from large volumes of water are expensive, inefficient, time consuming, and have widely varying results.

1.2 Viral Pathogens in Source Waters

1.2.1 Impacts on Human Health

While Health Canada does not have a program that conducts routine surveillance on waterborne disease outbreaks specifically, the Public Health Agency of Canada has two active surveillance programs to detect outbreaks of foodborne disease caused by enteric pathogens: the National Enteric Surveillance Program, which confirms the causal microbiological organisms of foodborne (including waterborne) disease cases from patient samples which have been submitted to provincial public health laboratories, and FoodNet Canada, which uses sentinel Canadian communities to gain an in-depth understanding of the epidemiology of foodborne diseases. Based on these surveillance programs, it is estimated that one in eight Canadians experience foodborne illness (usually acute gastrointestinal illness) each year (Thomas et al., 2013).

Waterborne pathogenesis often presents as acute gastrointestinal illness disease (AGID), which is estimated to have a cumulative incidence rate per year (or incidence proportion) of 0.7 in Canada (Majowicz et al., 2004) (and an incidence rate of 1.2-1.3 cases per person-year (Sargeant et al., 2008; Thomas et al., 2006)). While AGID is not usually fatal for Canadians, its high incidence means that there are societal costs due to treatment and lost productivity (Henson et al., 2008; Majowicz et al., 2006). Of the different sources of food borne AGID, it is unknown

what proportion of the cases is due to the transmission of waterborne pathogens. It is difficult to determine the extent of waterborne disease due to several factors: under-reporting of gastrointestinal illness (it has been found that for every reported case of an infectious gastrointestinal illness over three hundred went unreported in two Canadian communities (MacDougall et al., 2008; Majowicz et al., 2004)), lack of identification of the pathogen responsible (as AGID often is not serious, surveillance is passive, and often people do not visit doctors and have samples tested for diagnosis), and lack of identification of the source of transmission (Messner et al., 2006). However, an estimate for the incidence proportion of AGID due to waterborne transmission in the US is 0.06 cases per year nationally (that is, for every 100 people there are six cases of acute gastrointestinal illness due to contaminated water in one year) (Messner et al., 2006). When applied to the Canadian population of 35,749,600(Statistics Canada, 2015), this incidence translates to 2,144,976 cases per year. The estimated cost of waterborne disease (not just AGID) to Canada's economy places it at $2.7 billion, when considering both medical expenses and losses in productivity due to missed days of work(Vinson, 2012). *Noroviruses* are thought to be the single biggest source of AGID in North America (Carter, 2005).

In addition to widespread endemic AGID, some of these viruses can cause more acute illnesses that can potentially be fatal. One emerging strain of adenovirus (Ad 14) has caused outbreaks across North America and resulted in hospitalizations of about 40% of patients, even in healthy adults (Vento et al., 2011). Several *Enteroviruses*, such as strains of coxsackie and enteroviruses, can cause heart diseases, including pericarditis and myocarditis, pancreatitis, encephalitis, and paralysis (Karim et al., 2009; Mena et al., 2003). An outbreak of aseptic meningitis in Saskatchewan in 1997, which hospitalized 35 people, was caused by a strain of

echovirus (Ramsingh, 1998). Detection of enteric viruses in different sources of drinking water is essential to reducing the exposure of humans to enteric viruses.

1.2.2 Occurrence in Canadian Source Waters

Enteric viruses can be found in many aquatic environments, including groundwater, estuarine water, seawater, rivers, lakes, aerosols emitted from sewage treatment plants, insufficiently treated water, and private wells (Fong and Lipp, 2005). Drinking water sources can be polluted by both point and non-point sources of fecal contamination through natural discharge and recharge cycles, as well as increased runoff due to weather events. Though enteric viruses are usually present at low levels in the environment (estimates of 1-10,000 infectious units per 100L of water (Health Canada, 2012)), it is difficult to extrapolate generalizations from studies due to variability between viruses as well as temporal and geographic variation. For example, three types of enteric viruses were detected via cell culture at 0-59 most-probable-number(MPN)/100L in the source water for an urban centre in Wisconsin over nine years of surveillance (Sedmak et al., 2005). Another study found cultured enteric viruses were detected at 0-6.4 MPN/L of water of in the Grand River Watershed, Ontario (Dorner et al., 2007). In Quebec, enteric viruses have been detected at up to 40% of 45 different water treatment sites near Saint Lawrence River (Payment and Locas, 2011). An outbreak of AGID can increase the public health risk if fecal contamination were to occur. This is because an infected individual sheds extremely high levels of virus; from 10^5 viral particles per gram of stool for some *Enteroviruses* up to 10^{10} viral particles per gram for rotavirus (Gerba, 2000). Enteric virus samples from sewage have been cultured at 10^4 plaque forming units per litre (PFU/L)(Carter, 2005).

Although enteric viruses cannot multiply in the environment, they are known to survive in various aquatic environments for prolonged periods of time and can pose a threat long after their release into the environment (Health Canada, 2014b). In general, enteric viruses are more stable at temperatures below 50 °C and pH levels over 3 (Bertrand et al., 2012; Carter, 2005), but viability is also influenced by water type and water quality. For example, it may take 671 days at 4 °C to inactivate 90% of the poliovirus particles in saltwater (Rajtar et al., 2008). Poliovirus can survive in different soil types for over a month, and can migrate in higher concentrations than originally applied due to accumulation over time when mobilized using simulated rainfall (Sobsey et al., 1980).Furthermore, several viruses are able to withstand low pH levels (3-5) for 3 hours or longer (Carter, 2005). Viruses are vulnerable to exposure to sunlight, which can inactivate 99.9% of poliovirus particles in just 24 hours (Rajtar et al., 2008). While enteric viruses are generally susceptible to high temperatures and chlorination (Carter, 2005), there are some exceptions. Hepatitis A requires temperatures over 90 °C to be completely inactivated (Croci et al., 1999) and one strain of coxsackievirus can survive levels of chlorination used in water treatment for over 100 minutes (Payment et al., 1985). Therefore, it is not unusual to detect enteric viruses in surface water.

Contamination of groundwater with enteric viruses is also possible. Although it was initially believed that enteric viruses are unlikely to enter aquifers due to low permeability aquitards and slow transport, resilience and small relative size of enteric viruses allow them to travel 60 meters and penetrate low permeability aquitards to reach groundwater (Borchardt et al., 2007). In a survey of 2210 groundwater sites across the US and Canada, 15% were found to contain enteric pathogens, although only a small fraction of studies tested for presence of enteric viruses (Hynds et al., 2014). The authors pointed out the need to test for enteric viruses in

groundwater samples because their size allows them to travel relatively unimpeded compared to other enteric pathogens (Hynds et al., 2014). Furthermore, adenovirus can remain infectious in ground water from four months to a year (Charles et al., 2009; Ogorzaly et al., 2010). As a result, outbreaks of AGID originating from groundwater sources have been documented (Fong et al., 2007; Parshionikar et al., 2003). Groundwater sources cannot be assumed to be free of enteric viruses and improved surveillance would not only help understand their occurrence in the environment but also reduce exposure to these pathogens.

1.2.3 Primary Concentration for Virus Detection

Due to their low environmental concentrations, the detection of enteric viruses requires sampling large volumes of water (hundreds to thousands of litres) followed by a primary concentration step (Borchardt et al., 2004; Locas et al., 2008; Lodder and de Roda Husman, 2005). This concentration step takes advantage of virus charge, which is based on the isoelectric point of the capsid determined by its amino acid composition. The isoelectric point of a protein is the pH at which it has a net zero charge (the number of positive charges and negative charges from amino acids are the same). The majority of viruses have an isoelectric point below neutral pH (2-7) (Michen and Graule, 2010). Therefore, viral capsids have a net negative charge in neutral or basic pH environments due to deprotonated state of the amino acid functional groups (Michen and Graule, 2010). Furthermore, under low pH conditions, the same functional groups would be protonated, giving the virions a net positive charge. Knowing isoelectric points and solution pH can be used to adsorb viruses to different matrices (Zerda et al., 1985). This is the basis of different kinds of filters which capture enteric viruses from environmental waters for detection.

Electronegative filters were the first kind of microfilters to be used for the concentration of enteric viruses. Membranes made of nitrocellulose (which, at neutral pH, has negative nitrate groups creating a net negative charge) have been used since the 1930s for microfiltration to separate bacteria and viruses from suspensions (Elford, 1931).Early studies found that viruses would adsorb to the membrane filters, despite being much smaller than the pore sizes (Cliver, 1965; Parkman et al., 1964). The addition of different concentrations of salt has also been found to increase the adsorption of viruses to Millipore filters (Lukasik et al., 2000). One mechanism suggested for this is the formation of salt bridges between negatively charged molecules (Farrah et al., 1981; Lukasik et al., 2000). Pre-treating negatively charged membrane filters with protein solutions was found to reduce virus adsorption to the filters, due to the proteins occupying any potential binding sites for viruses (Ver et al., 1968; Wallis and Melnick, 1967). These two mechanisms formed the basis for an "adsorption-elution" method of concentrating viruses from large volumes (10s to 1000s of litres) of solutions (Ikner et al., 2012).

Different "adsorption-elution" methods using negative filters have been developed, using a variety of filters, salts, and protein-rich eluents. Two commonly compared negative filter media include nitrocellulose and epoxy-fiberglass membrane filters (Farrah and Bitton, 1979; Jakubowski et al., 1975). Similar experiments with these filters would often yield variable recoveries (see Table 1 for examples).One study had>90% recovery of poliovirus spiked into tap water from both filters using beef extract at pH 9 (Farrah and Bitton, 1979), while another had <50% recoveries from both types of filters using a glycine eluent at pH of 11.5 (Jakubowski et al., 1975), and a third study had less than 5% recovery of polio viruses seeded into 378 L of tap water and eluted with glycine at pH of 9.5-11.5 (Sobsey and Jones, 1979).

In addition to beef extract and glycine, other proteinaceous eluents include tryptose-phosphate broth (Farrah et al., 1978) and concentrated nutrient broth (Hill et al., 1974). The ability of monovalent and divalent salts to increase adsorption of viruses to negatively charged filters have been evaluated since the start of the adsorption-elution method (Wallis and Melnick, 1967). The addition of divalent (Fields and Metcalf, 1975; Sobsey et al., 1985) or trivalent salts (Hill et al., 1974) to enhance absorption became common practice. As well, water samples were acidified to pH 3.5 prior to filtration(Payment and Trudel, 1979; Sobsey et al., 1985). This pH is below the isoelectric point of most enteric viruses (Michen and Graule, 2010), and is thought to enhance the attractive forces between the viruses and negatively charged filters.

The early methods of virus concentration had a number of limitations. pH adjustment of large volumes (e.g. >100 L) of water prior to filtration is difficult, requiring large volumes of acid and special equipment (Sobsey and Jones, 1979). Additionally, filtering large volumes of water is difficult due to low-flow rates (e.g. initial flow rates of 12 L/min filtering tap water through membrane filters slows to 4 L/min) and clogging (Farrah et al., 1976; Payment and Trudel, 1979). For large volumes of unfinished waters, pre-treatment before concentration is required to prevent filters from clogging. This pretreatment could be in the form of clarifying agents (Hill et al., 1974) or filtration using a matrix with larger pore sizes (Hsu et al., 2007; Wallis et al., 1972). Overall, although electronegative filters can have high recoveries (<5% up to 100%, see Table 1.1), these methods require a lot of optimization, are labour-intensive, and may not be reproducible (Farrah and Bitton, 1979; Jakubowski et al., 1975; Sobsey and Jones, 1979).

Table 1.1 Selected studies of enteric virus recovery from water using electronegative filters (from tap water unless indicated otherwise).

Citation	Filter type	Elution condition notes	Virus	Recovery and notes
(Hsu et al., 2007)	Nitrocellulose	Sulfuric acid/sodium hydroxide	Enterovirus	100% (surface)
	Nitrocellulose	Glycine/beef extract at pH 9.5	Enterovirus	7-21%
	Electronegative membrane	Sulfuric acid/sodium hydroxide	Enterovirus	24-83%
	Electronegative membrane	Glycine/beef extract at pH 9.5	Enterovirus	8-64%
(Farrah et al., 1976)	Nitrocellulose	Glycine at pH 10.5	Poliovirus	40-50%
(Fields and Metcalf, 1975)	Nitrocellulose	Glycine at pH 9.5	Adenovirus	<10% (sea)
		Beef extract at pH 9		100% (sea)
(Jakubowski et al., 1975)	Nitrocellulose	Glycine pH 11.5	Poliovirus	31%
	Epoxy-fiberglass			20%
	Borosilicate glass			45%
	Yarn-wound fiberglass + expoxy-fiberglass			29%
(Sobsey and Jones, 1979)	Nitrocellulose	Glycine pH 9.5-11.5	Poliovirus	<5%

To overcome some of the difficulties with electronegative filters, electropositive filters were developed. These filters work based on the attractive charge between the positive filters and the negative charges of most viruses that are in water, which means that pH adjustment of the water samples is not required. Two of the early electropositive filters tested were the Virosorb 1MDS and the Zeta Plus S-series. One of the first studies to compare the efficacy of these traditional epoxy fibreglass filters found that between pH 3.5 to 7.5, both electropositive filters had greater adsorption of poliovirus seeded into tap water than did the electronegative filter from water adjusted to pH 3.5 (Sobsey and Jones, 1979). Overall virus recoveries from the Virosorb filter were comparable to those of the electronegative filters at 30% (Sobsey and Jones, 1979). Another study testing the concentration of Hepatitis A virus from tap water found that recoveries from electropositive and electronegative filters were not significantly different when both were used under optimized conditions (Sobsey et al., 1985). Over the years these electropositive filters have been tested with different types of viruses and water types (see **Table 1.2** for examples).

Although electropositive filters do not require the water sample to be pH adjusted, pre-treatment of environmental samples, such as pre-filtration to prevent clogging of the filters, may be required before the concentration step (Pang et al., 2012). In addition to still being labour-intensive, the Virosorb filters are too expensive for routine use since their cost is $150-180 USD per filter, especially when compared to the much cheaper electronegative nitrocellulose filters (Cashdollar and Dahling, 2006; Li et al., 2010). This led to the development of cheaper electropositive filters incorporating nanoalumina fibres, notably the NanoCeram cartridge filter (Ikner et al., 2012).There were no significant differences in adenovirus recovery from 1L of seawater using either an electronegative membrane filter or a nanoalumina disk filter, though it is worth noting that the nanoalumina filter can process larger volumes of water without clogging

(Li et al., 2010). In addition, the NanoCeram filter performs as well or better than the 1MDS filter in the recovery of several enteric viruses from tap and river waters, though it clogs faster (Karim et al., 2009). Currently, the US Environmental Protection Agency (EPA) approves of the use of both the NanoCeram and the Virosorb 1MDS filters for detecting enteric viruses from water samples (Cashdollar et al., 2013). The most critical factor influencing virus recovery is the virus type according to a meta-analysis comparing both electronegative and positive filters; in the study, water matrix, sample volume, and filter type did not have any significant influence (Cashdollar and Wymer, 2013). Recovery may vary between viruses due to differing isoelectric points, sizes, and structures (Cashdollar and Wymer, 2013). Therefore, different methods of primary concentration can be optimized for the detection of one type of virus, but no single method is able to capture multiple types of viruses equally well. Given the difficulties of working with both types of charged filters and the highly variable recoveries, alternative methods of virus concentration need to be developed.

Table 1.2 Selected studies of enteric virus recovery from water using electropositive filters (from tap water unless indicated otherwise)

Citation	Filter type	Elution Used	Virus	Recovery and notes
(Sobsey and Jones, 1979)	Zeta Plus	Glycine pH 9.5-11.5	Poliovirus	22-64%
(Hsu et al., 2007)	Electropositive membrane	Sulfuric acid/sodium hydroxide	Enterovirus	0-6% (surface)
	Electropositive membrane	Glycine/beef extract at pH 9.5	Enterovirus	0-28%
(Cashdollar and Dahling, 2006)	Zetaspor 1-MDS	Beef extract at pH 9.5	Poliovirus	32-38%; 68-100% (river)
(Karim et al., 2009)	NanoCeram	Beef extract at pH 9-9.5	Poliovirus	54%
				38% (river)
			Coxsakievirus	27%
			Echovirus	32% (river)
(Lambertini et al., 2008)	Glass wool	Glycine/beef extract at pH 9.5	Poliovirus	70%
			Coxsackievirus	14%
			Echovirus	19%
			Adenovirus	21%
			Norovirus	29%
(Ikner et al., 2011)	NanoCeram	Salt-based or beef extract at pH 7.5-9.3	Poliovirus	66%
			Echovirus	83%
			Coxsakievirus	77%

			Adenovirus	14%
			MS2	56%
(Li et al., 2010)	Nanoalumina filter	Beef extract at pH 6	Adenovirus	82% (seawater) 86% (secondary sewage) 91% (reverse osmosis)
(Pang et al., 2012)	NanoCeram	Beef extract at pH 9.75	Norovirus	42% (DI) 29% 18% (river)
			Rotavirus	47% (DI) 42% 78% (river)
			Adenovirus	19% (DI) 21% 19% (river)

1.3 Chemical Contaminants

Chemical contamination of source waters is a growing concern in North America. There are many types of chemicals which can pollute source waters, including industrial by-products, personal care products, pharmaceuticals, disinfection by-products, and pesticides. While there are technologies that can remove chemical contaminants from water, such as Reverse Osmosis (RO) or activated carbon filters, use of these treatments requires testing and maintenance to prevent biological fouling or reduced effectiveness caused by saturation of the contaminants. Due to the ubiquity of chemical contaminants in source waters, the primary intervention to preventing their consumption by humans is to monitor their levels in drinking water treatment plants. Health Canada and the US EPA require routine monitoring for levels of chemical contaminants that have been widely established as harmful to human health based on toxicological studies. Health Canada currently has MACs for more than 80 different chemicals (Health Canada, 2014a).

Detection of chemical contaminants is based on analytical techniques that rely on the physical and chemical properties of the contaminant of interest. Many chemicals are present in source waters at trace levels (concentrations in the ng-µg/L range). Therefore, accurate detection of trace chemical contaminants requires a concentration step prior to analysis using highly sensitive analytic techniques. Both analytical techniques and concentration methods are dependent on the physical and chemical properties of the contaminant of interest. The wide variety of chemical contaminants found in source waters can be classified into two groups: inorganic compounds and organic compounds. Inorganic chemicals include metals and anions; common organic contaminants include phenols, pesticides, polycyclic aromatic hydrocarbons, and surfactants. The physical and chemical properties differ greatly not only between these

groups but also within them, which can make detection a challenge. Volatility, polarity, hydrophobicity, and size of the target compound influence the type of concentration step and analytical method used for detection. The large number of diverse chemical compounds means that routine detection requires the use of many different methods, which can be expensive and time-consuming. Pesticides, in particular, are widely used across Canada and have been regularly found in both ground and surface waters as trace contaminants, necessitating a concentration step for accurate and reliable detection (Bruzzoniti et al., 2000; Environment Canada, 2011).

1.4 Pesticides in Source Waters

Pesticides are a large, diverse group of chemicals that are commonly found as trace contaminants in source waters (Environment Canada, 2011). Pesticides include organic and inorganic compounds which are used to reduce or eliminate organisms that are considered pests. Such pests include insects, fungi, rodents, bacteria, and unwanted plants. It is well known that pesticides can contaminate source waters from both point and non-point sources through several types of pesticide transport (Reichenberger et al., 2007). These include surface runoff, ground water recharge and discharge, and atmospheric volatilization (Environment Canada, 2011). Pesticide use in Canada largely falls into two groups: agriculture and landscaping. A study of 15 rural communities in the Canadian prairies found 27 different herbicides in their drinking water reservoirs, with seven herbicides found consistently across all sources with mean total concentrations ranging from 125 to 1062 ng/L (Donald et al., 2007). As well, pesticides used for landscaping also contribute to environmental contamination; acidic herbicides, such as glyphosate, have been detected in many urban rivers and streams across Canada (Glozier et al., 2012). Though there are more than 500 active ingredients registered as pesticides in Canada (Brimble et al., 2005), Health Canada's Guidelines for Canadian Drinking Water Quality only

require routine monitoring for 23 of these compounds. The lack of routine surveillance can be attributed, in part, due to their low environmental concentrations, which requires the sampling and processing of large volumes (>10 L) of water.

1.4.1 Impacts on Human Health

The potential for pesticides to have negative impacts on human health through environmental exposure gained widespread attention since the banning of dichlorodiphenyltrichloroethane (DDT) by the US EPA in 1972 (Grier, 1982). In Canada, pesticide use is regulated by Health Canada's Pest Management Regulatory Agency. While Health Canada determines acceptable use of individual pesticides based on toxicological data, the fate of pesticides and their degradation products after application remains an ongoing area of concern. Negative health impacts from exposure to some types of pesticides and their byproducts through contaminated water sources have been documented. The triazine herbicides, such as atrazine and simazine, are frequently detected in surface waters at levels ranging from 10 to 3,000 ng/L (Byer et al., 2011; Struger et al., 2004). Pesticides have also been detected in ground waters at lower concentrations than in surface water (Barbash et al., 2001). The six highest-use herbicides were detected below 100 ng/L 98% of the time in a three-year national study by the US Geological survey (Barbash et al., 2001). Increased incidences of Intrauterine Growth Retardation and pre-term birth have been correlated with exposure levels of triazine herbicides greater than the Health Canada's MACs in drinking water (Munger et al., 1997; Rinsky et al., 2012). Therefore, routine detection of these pesticides is necessary to ensure they remain below the MACs in drinking water in order to protect public health.

Other pesticides, such as organophosphates which inhibit the neurotransmitter acetyl cholinesterase, pose potential health hazards if acute exposure were to occur, as their mode of

action is not specific to their target organisms (Costa, 2006). One organophosphate pesticide which has come under increasing scrutiny, chlorpyrifos, and which is known to have negative impacts on childhood development due to neurotoxicity (Eaton, 2008), has been frequently found in surface waters across Canada (e.g. 77% of samples from 10 isolated lakes across Ontario; 43% of samples from sites across British Columbia), though at levels two or more orders of magnitude below the MAC (Environment Canada, 2011; Kurt-Karakus et al., 2011). While neurotoxicity from acute exposure is well-documented, studies have found pre-natal exposure to levels of chlorpyrifos below those causing acute toxicity can also impair cognitive development (Rauh et al., 2012). All of the acidic herbicides that Health Canada has established MACs for are associated with health impacts due to acute exposure, and impacts from chronic exposure continue to be studied (see **Table 1.3**). In addition to the routine monitoring of pesticides which have MACs, it is also important to have accurate risk assessments of exposure to all pesticides from environmental water sources to facilitate understanding of their environmental fate.

Table 1.3 Acidic Herbicides with MACs established by Health Canada and their potential health impacts.

Acidic Herbicide	MAC (mg/L)	IARC Group Classification (cancer associations)[1]	WHO Classification 2009 (measure of hazard)[2]	Primary health concern as per Health Canada Guidelines	Notes
Atrazine	0.005	3	III	Reduced body weight	Potential endocrine disruptor via testosterone suppression (Friedmann, 2002) Testicular morphology changes and oxidative stress (Abarikwu et al., 2010; Victor-Costa et al., 2010)
Bromoxynil	0.005	2B	II	Reduced liver to body ratio	Potential carcinogen (Environmental Protection Agency, 2014)
Dicamba	0.12	NA	II	Liver damage	Unlikely to be a carcinogen, though positively associated with colon and lung cancer through occupational exposure (Weichenthal et al., 2010)
2,4-D	0.1	2B	II	Kidney damage	Known to cause kidney damage in rats (Tayeb, 2012) Possibly carcinogenic, induces oxidative stress and immunosuppression (Loomis et al., 2015)

[1] IARC Group classifications are measure of how much evidence there is for a compound being carcinogenic. Group 1 is carcinogenic to humans, 2A probably carcinogenic to humans, 2B possibly carcinogenic to humans, 3 not classifiable as to its carcinogenicity to humans, 4 probably not carcinogenic to humans (World Health Organization, 2015)

[2] WHO classification is based on acute oral and dermal toxicity measures in rats. Class Ia is extremely hazardous, Ib highly hazardous, II moderately hazardous, III slightly hazardous, and U is unlikely to present acute hazard (World Health Organization, 2010)

[2] WHO classification is based on acute oral and dermal toxicity measures in rats. Class Ia is extremely hazardous, Ib highly hazardous, II moderately hazardous, III slightly hazardous, and U is unlikely to present acute hazard (World Health Organization, 2010)

Diclofop-methyl	0.009	NA	II	Liver damage	Increased liver weights and enzyme levels in rodents (Palut et al., 2001)
Diquat	0.07	NA	II	Cataract formation	Cataract formation from acute exposure (Clark and Hurst, 1970) Kidney and liver damage has been observed in rodents (Burk, 1980)
Glyphosate	0.28	2A	III	Body weight	Probable human carcinogen (Loomis et al., 2015) Association with B cell lymphoma has been found (Schinasi and Leon, 2014)
MCPA	0.1	NA	II	Kidney	High-dose exposure causes liver damage in dogs (Sadlonová et al., 2006) No evidence of genotoxicity in rodents (Elliott, 2005)
Metolachlor	0.05	NA	III	Liver	Increased lung and liver cancer risk with occupational exposure (Alavanja et al., 2004; Silver et al., 2015)
Picloram	0.19	3	NA	Body and liver weight	Increase in liver and kidney weights, as well as liver enzyme activity (Gorzinski et al., 1987)
Simazine	0.01	3	NA	Body weight and thyroid	Acute exposure can cause dermatitis (World Health Organization, 2003)

1.4.2 Occurrence in Canadian Source Waters

Pesticide use is prevalent throughout Canada (see **Table 1.4**), although pesticide concentrations rarely exceed recommended guidelines in Canada for both surface and groundwater based on data from monitoring sites (Parris, 2011). Pesticides can enter water sources through many routes: surface runoff and erosion, drainage of soils, leaching, cycling of groundwater through recharge and discharge, spray drift, atmospheric volatilization, and accidental point source pollution (for example a spill or leakage) (Environment Canada, 2011; Reichenberger et al., 2007). Though surface run-off and erosion remain the most studied modes of pesticide transport to water sources (Reichenberger et al., 2007), pesticides can also be dispersed over long distances by evaporation and subsequent atmospheric deposition. For example, clopyralid, MCPA, and glyphosate were detectable in wetlands of Canadian Prairie Pothole region, which was too far for runoff to explain the presence of these chemicals (Messing et al., 2011). Another study of pesticide concentration in Ontario lakes attributed the presence of three pesticides (alachlor, ametryn, and disulfoton) to atmospheric deposition (Kurt-Karakus et al., 2011).

As pesticides are widely used across Canada, their contamination of source waters has been readily documented. In British Columbia, 51 different pesticides were detectable at sub-nanogram levels in surface waters (Woudneh et al., 2009). The levels of many pesticides peaked in the spring compared to fall period, and a major portion (up to 40%) of pesticides were stably detectable in 100% of the samples collected as part of the study (Woudneh et al., 2009). Other studies have found similar peaks in pesticide concentrations in the spring (Byer et al., 2011; Kurt-Karakus et al., 2011). Carbamazepine and atrazine were detected in 31% and 56% of sampling sites across Canada, though the concentrations detected across the sites did not exceed

1 ng/L, with the highest level detected in two samples from Montreal at 1.0 ± 0.1 ng/L (Segura et al., 2011). A national study surveying the occurrence of 141 pesticides across Canadian provinces (excluding Newfoundland) found that while no samples exceeded Health Canada MACs (Environment Canada, 2011), every province had surface waters containing pesticides at levels above Canadian Environmental Quality Guidelines or equivalent benchmarks (these are conservative estimates of pesticides levels below which there is no evidence of harm to aquatic ecosystems) (Environment Canada, 2011).

Although the majority of pesticide use in Canada is for agriculture, urban use of pesticides is more concentrated (Brimble et al., 2005). This intensive use of pesticides over smaller areas of land (as compared to agriculture) is reflected in pesticide levels. For example, 2,4- dichlorophenoxyaceticacid (2,4-D), mecoprop, dicamba, glyphosate, and AMPA were linked to urban use and were detected at higher concentrations in Ontario compared to other geographic regions (Pacific, Prairies, Quebec and Atlantic) (Glozier et al., 2012). Southern Ontario lakes demonstrated higher detection frequency of current use pesticides at 45% compared to central (40%) and northern (36%) lakes, with higher (>1 ng/L) concentrations in the three southern lakes (Kurt-Karakus et al., 2011). In Ontario, atrazine and metolachlor levels also peaked during spring periods and exceeded recommended concentrations in southwestern parts of the province, where the levels of the two pesticides have been historically higher (Byer et al., 2011).

Table 1.4 Pesticide use in Canada based on data gathered by Environment Canada (Environment Canada, 2011).

Province/Region	**Total pesticides used (year)**	**Primary user industry**	**Most used class of pesticide**
British Columbia	4,666,709 kg (2003)	Forestry	Anti-microbial
Alberta	9,300,508 kg (1998)	Agriculture	Herbicide
Ontario	4,218,238 kg (2003)	Agriculture	Herbicide
Quebec	3,276,257 (2001)	Agriculture	Herbicide
Prince Edward Island	814,103 kg (2002)	Agriculture	Fungicide
Nova Scotia	441,601 kg (2003)	Agriculture	Herbicide
New Brunswick	781,923 kg (2003)	Agriculture	Fungicide

1.4.3 Primary Concentration of Pesticides

Pesticides are often present in the environment at nanogram per liter concentrations and require sensitive analytical methods for detection and quantification (Öllers et al., 2001). Prior to injection of a small volume (microliter range) into an analytical apparatus, such as HPLC, the analyte of interest is first concentrated in a primary step using a relatively large volume (liter range) of water (see for example 6410b from Standard Methods for the Examination Water and Wastewater). The electrochemical properties of pesticides are used to extract and concentrate these chemicals by exploiting solubility and ionic charge properties of the molecules (Öllers et al., 2001; Stoob et al., 2005).

The first method of extraction takes advantage of the charge of the molecule depending on the pH of the solution (Yang et al., 2004). The pKa of acidic pesticides is lower compared to the near-neutral pH of the environmental waters and therefore such pesticides exist in the de-protonated or anionic form in the environment (Öllers et al., 2001). Therefore, acidic pesticides, such as 2,4-D, usually have a negative charge in the environment and can be reversibly adsorbed to positively charged media (Aksu and Kabasakal, 2004). The second method of extraction and concentration relies on the difference in solubility of the target pesticide in water and an organic solvent. For example, 2,4-D, which has been adjusted to a pH below its pKa, can be extracted from environmental samples using small volumes of organic solvents because it is more soluble in organic solvents than water (Walters, 1999).

Liquid-liquid extraction (LLE) is mainly used to concentrate non-polar volatile or semi-volatile organic compounds prior to their analysis by chromatographic methods and/or mass spectrometry and is the oldest standard method used by EPA (Othmer et al., 1941). Most organic compounds are more soluble in organic solvents than in water. The simplicity and extraction

efficiency of the technique has made this particular method widely used for hydrophobic organic compounds. Different classes of pesticides can be recovered from samples with extraction efficiencies of 83-96% (Ahnoff and Josefsson, 1974). Some of the common chemical contaminants that are pre-concentrated by this method include polycyclic aromatic hydrocarbons, haloacetic acids, and chlorinated acids.

The main advantages of liquid-liquid extraction method are that it is simple, easy, quick, and relatively cheap. The primary disadvantage is LLE requires working with large volumes of solvents ranging from 1-10 L that may be harmful to humans (Ridal et al., 1997). Working with volatile compounds presents a number of challenges. Volatile compounds in particular require larger volumes due to their low concentrations in water due to atmospheric volatilization (Dewulf and Van Langenhove, 1999). Since the injection volume of solvent required for downstream analysis is relatively small (typically around 1ml or less), laborious evaporation steps are sometimes needed (Barrionuevo and Lanças, 2002). However, for highly volatile compounds, an evaporation step would also allow for loss of the target compound, which can result in low recoveries for LLE methods (Dewulf and Van Langenhove, 1999).

Solid phase extraction (SPE) is a separation process in which an aqueous mobile phase, containing the analytes of interest, passes through a solid stationary phase. Analytes are retained on the stationary phase based on their chemical properties (i.e. the more affinity for the stationary phase, the longer the retention time). Usually the stationary phase is made up of silica with functional groups that allow adsorption of depend on the chemical and physical properties of the analyte of interest. SPE has replaced many traditional LLE procedures (Hennion, 1999). SPE can be done independently of or in-line with chromatographic analysis. On-line sample concentration and extraction is advantageous because it allows for automation, which limits

contamination, increases reproducibility, and allows for analysis of the entire extracted analyte, as opposed to aliquot from an off-line extraction (Hennion, 1999). As SPE results in the extraction of analytes in an aqueous form, it is easily compatible with liquid chromatography, though there are ways to couple it on-line with gas chromatography as well.

The analytes of interest that are being pre-concentrated by SPE are dependent on the sorbent material used for the procedure (see **Table 1.5** for examples). During normal-phase SPE polar analytes are able to bind to polar stationary phase bound to the surface of silica with short carbon chains. The solid phase is subsequently washed with non-polar solvent to remove impurities (Bruzzoniti et al., 2000). After this wash step, the analyte of interest is eluted from the solid phase with a polar solvent. Reverse-phase SPE uses a stationary phase composed of hydrophobic compounds bound to the surface of silica (Kolpin et al., 2002). Analytes in the mobile phase to be retained are either non-polar or have low-polarity, and require a non-polar solution to be eluted from the column. Ion exchange SPE is based on electrostatic interactions between analytes and charged groups on the surface of the stationary phase (Carson, 2000). An anion stationary phase will retain negatively charged anions (acids). Strong anion exchange sorbents contain ammonium groups that remain positively charged in aqueous solutions (Hennion, 1999). Weak anion exchange sorbents have a negative charge at pH values higher than the pKa of the functional group on the sorbent. The stationary phase is washed with a neutralizing solution that disrupts the charged interactions, thus eluting the analyte of interest. Strong cation exchange sorbents contain functional groups that always remain negatively charged in an aqueous solution, which will bind any strong basic analytes. Weak cation exchange sorbents have a positive charge at pH values below the pKa of the functional groups. The

stationary phase is washed with a neutralizing solution that disrupts the charge interaction between the analyte of interest and the solute phase thus eluting the analyte.

Due to the large variety of sorbent materials, different types of SPE resins can be made to capture a wide array of analytes. C18 silica is a reverse phase sorbent that is bound to silica that is efficient at capturing non-polar and moderately polar analytes (Ternes et al., 2002). Alternately, when modified with alkyl chains and residual silanols are bound to the solid phase, polar analytes can be captured (Rodriguez-Mozaz et al., 2007). Although this SPE resin offers a broad capture range, it can also capture unwanted compounds, such as humic acids, which will interfere with downstream analysis (Alpendurada, 2000). Furthermore, silanol groups are less pH stable and therefore pH adjustment may be required with some samples (for example, acidic herbicides must be adjusted to pH 2 or 3) (Hennion, 1999; Wells and Yu, 2000). Poly(styrene-divinyl benzene) (PS-DVB) is also a reverse phase sorbent used for the capturing of non-polar analytes, although hydrophilic groups can be added to create a broad-range sorbent that captures a wider range of analytes.PS-DVB offers an alternative to C18 silica for pre-concentration of polar analytes because of greater pH stability at low and high pH ranges (Hennion, 2000). PS-DVB can also be used for extraction of highly ionic compounds because of interactions with the organic part of the compound (e.g. acidic herbicides, which can be extracted in an ionic form when water is pH 7-8) (Hennion, 2000). A pH range of 7-8 prevents the retention and co-extraction of fulvic and humic acids, which interfere with downstream analysis by chromatographic methods (Weigel et al., 2004).

Graphitized carbon blacks (GCB) are created by heating carbon at temperatures between 2500-3000 °C (Hennion, 2000). The process creates non-porous material with a hydrophobic surface that is efficient at adsorbing non-polar analytes (Hennion, 2000). The adsorption relies

on dispersion forces for the surface interactions. GCBs are more efficient than C18 silica or PS-DVB for trapping non-polar analytes (Crescenzi et al., 1996; Di Corcia et al., 1993). However, anions can also bind due to electrostatic interactions with positively charged oxygen complexes when the GCB is heated (Hennion, 2000). Desorption is achieved by addition of a solvent or by heat. The analytes of interest may be hard to desorb from GCB, which is the main disadvantage of this material (Hennion, 2000).

While SPE has largely replaced LLE, there are still some difficulties associated with this methodology. SPE is limited to sampling from the liquid phase and is not specific towards a single analyte, which may hinder the downstream analytical methods. The efficiency of SPE can be decreased due to interferences from other organic contaminants in the sampled water (Hennion, 1999). Sample volumes are highly variable, ranging from µL up to 100 L quantities (Kurt-Karakus et al., 2010). Recoveries are also highly variable, from 9 to over 100% (Hennion, 1999), depending on the type of analyte, sorbent used, other co-contaminants, and type of water being sampled. Pre-treatment of samples to adjust the pH adds additional time and labour (Rodriguez-Mozaz et al., 2007). Downstream analysis by chromatographic techniques, which are the most common methods of analysis, can be hindered by co-extraction of fulvic and humic acids (Wells and Yu, 2000). Developing better tools for concentrating pesticides, such as 2,4-D, would improve detection of these contaminants in environmental source waters.

1.5 Capacitive Deionization

Enteric viruses and pesticides are both diverse classes of water contaminants for which no single concentration method is currently used. Both contaminant classes pose similar challenges

Table 1.5 Examples of Solid Phase Extraction Sorbents

Solid phase	Target analyte properties	Notes	Advantages/ Disadvantages	References
C18 silica	Preferred for use with non-polar to moderately polar analytes	Alkyl chain modification allow capturing polar compounds	Offers broad capturing range May capture humic acids Requires sample adjustment	(Ternes et al., 2002) (Rodriguez-Mozaz et al., 2007) (Alpendurada, 2000) (Hennion, 1999; Wells and Yu, 2000)
Poly(styrene-divinyl benzene) or PS-DVB	Primarily used for non-polar analytes	Better pH stability than C18 silica at low or high pH	Can extract highly ionic compounds Does not retain humic or fulvic acids	(Hennion, 2000) (Weigel et al., 2004)
Graphitized carbon blacks	Primarily used for non-polar analytes	Binds anions to positively charged oxygen complexes when heated	More efficient at extracting non-polar compounds than C18 or PS-DVB Analytes may be hard to desorb	(Hennion, 2000) (Crescenzi et al., 1996) (Di Corcia et al., 1993)

for routine detection due to their low environmental concentrations. The current concentration methods are specialized to individual contaminants and can be expensive, time-consuming, and labour-intensive. Enteric viruses and acidic herbicides carry negative charges at neutral pH levels typical of most surface and ground waters. Therefore, a single electrochemical method of contaminant concentration and removal could potentially be used to take advantage of the negative charges of both acidic herbicides and enteric viruses. The development of a technology that can concentrate multiple contaminants in a single-step with high recovery and reproducibility could reduce the cost and time associated with detecting these classes of contaminants. This would improve contaminant detection and risk assessments of source waters. In this thesis, capacitive deionization (CDI) was explored as a possible technology to concentrate small molecules that occur in low concentrations in source waters (such as enteric viruses and acidic pesticides).

1.5.1 Technology

CDI is an electrochemical technology originally developed in the 1960s to desalinate brackish waters (Porada et al., 2013). Application of a potential difference across two oppositely charged electrodes (called plates) is the principal process upon which CDI is based. The potential difference between the electrodes for CDI is low, around 1V, in order to minimize water splitting on the face of the electrodes via electrolysis (Porada et al., 2013). The electrodes are made of a carbon-based material that is highly porous (micropore diameter is less than 50nm (Oren, 2008)) to maximize surface area. These plates are separated by an ionic solution, which transmits an electrical current. Ions suspended in the solution are attracted to the electrodes of opposite charge, and are adsorbed into the micropores of the electrodes, while the solution elutes from the cell with a lower ionic concentration. The adsorption of ions to the electrodes follows the

Electrical Double Layer (EDL) theory. An EDL appears when fluid is exposed to the surface of a solid. Two parallel layers exist at the interface of the solid and liquid (Oren, 2008). The surface layer (also called the Helmholtz Outer Layer) is closest to the solid and is made up of counter-ions interacting with the surface via chemical bonds. Outside the surface layer is the diffuse layer, which is made up of ions attracted to the surface by electrostatic charge. The electrical capacity of the electrodes, which is their ability to store energy as adsorbed ions, depends on the capacity of both components of the EDL (Oren, 2008; Porada et al., 2013). Capacity increases as the surface area increases and the distance between the plates decreases (the distance between plates is on the scale of mm to cm (Mayes et al., 2010; Wimalasiri, 2013; Xu et al., 2008))(Oren, 2008). As the electrode surface areas become saturated with ions, the electrodes become less efficient at removing ions from the solution. The electrodes can be regenerated by reversing the charges, which repels the ions from the surface of the electrode to the interstitial space, and are subsequently "purged" from the cell as a highly concentrated solution. Following regeneration, the electrodes can then begin a new cycle of ionic adsorption. The concentrated effluent is typically discarded, but it can also be used for the recovery or sampling of components which may be very dilute in the influent water.

Variations of the technology have evolved as CDI has progressed. The passage of water through the cell can occur through two different mechanisms: flow-through mode or flow-by mode. In flow-through mode, water passes through the pores of the electrode whereas in flow-by mode, water passes through the gap or flow channel between the electrodes (Porada et al., 2013). CDI units can be operated using either constant current or constant voltage, the latter being more commonly used. Constant current can be used when a specified effluent concentration is required (Porada et al., 2013), though this requires the addition of an ion-exchange membrane. The

addition of membranes to the cell is thought to improve ion adsorption by preventing co-ions (those of the same charge as the electrode) from being expelled from the electrode and into the water eluting from the machine (Porada et al., 2013). Instead, the co-ions are expelled out of the micropores, but are retained on the electrode by the membrane, which attracts more counter-ions on to the surface of the electrode (Porada et al., 2013). This is advantageous because more ions can be absorbed on to the electrode than using standard CDI, which will reduce energy consumption. Additionally, the use of ion-exchange membranes prevents the ions adsorbed during the purification cycle from adsorbing to the electrode of opposite charge during the reversal of charge which occurs during the purge step. Different high surface area carbon-based electrodes have been used for CDI, including activated carbon, carbon aerogels, carbon nanotubes, and graphene (Porada et al., 2013).

1.5.2 Current Applications

CDI has been increasingly tested as a competitor for current desalination technologies, such as Reverse Osmosis (RO) and electrodialysis (ED). It is an attractive alternative because CDI does not require the use of membranes under high pressure, as RO does. As well, CDI has electrodes with a regenerative capacity, which lowers energy costs (Anderson et al., 2010). Additionally, RO and ED have issues with membrane fouling and maintenance, which the regenerative capacity of the electrodes reduces (Porada et al., 2013). The electrode regeneration is also able to recover the energy stored during the adsorption step, though the efficiency of recovery is variable and is in need of further optimization (Anderson et al., 1999). One model suggests that even with an energy efficiency as low as 70%, CDI is more energy efficient than ED for brackish waters below 5,000 mg/L of salt (Anderson et al., 2010). CDI is not competitive with RO for the desalination of waters over 1000 ppm of salt due to the decrease in charge

efficiency (Oren, 2008), which is in agreement with another model comparing RO and CDI energy consumption across different feed solution concentrations (Porada et al., 2013).

Currently, CDI is used for the removal of dissolved ions from different water sources. A mobile CDI unit, the ESD, was used to recover compounds from an industrially contaminated groundwater system. The contaminated water contained 137 mg/L of nitrate, which after undergoing CDI, was reduced to 42.5 mg/L of nitrate (personal correspondence, ENPAR, 2016). The ESD was able to reduce the concentration of TDS by 92% from a surface drinking water source in Sudbury, Ontario (Oren, 2008). A test of a benchtop CDI unit was able to remove an average of 80.3% of ions (by weight) from greenhouse leach water, with 83.9% water recovery; the energy usage was 2.517 kWh/m^3 of treated water (for comparison, RO energy usage is 2.9–3.7 kWh/m^3 of water treated (Anderson et al., 2010)) (Enpar Technologies Inc, 2015). CDI has also been used to recover water from RO brines produced during purification of wastewater through the NEWater initiative in Singapore. CDI was able to reduce the RO brine from a conductivity of 2,060 µS/cm to 277.4µS/cm, and the amount of TDS from 1,275 mg/L to 176.5 mg/L, while operating with 85% water recovery (Tao et al., 2011). The potential for CDI to remove TDS from wastewater produced during natural gas extractions has also been identified, as these waters usually have ion concentrations of less than 6,000 ppm. Bench-scale tests using these waters have shown TDS removals of 75-90% (Christen, 2006).

1.5.3 Potential for Contaminant Concentration

Currently, studies of CDI have focused primarily on the removal of simple (single-atom) ions from different types of solutions. The removal of charged compounds, such as nitrates and phosphate, has also been demonstrated (see **Table 1.6**). Kim et al. (2010) examined the effect of octanol on the removal of salt from brackish waters. They observed that the compound partly

adsorbed and desorbed to the ion-exchange membrane overlaying the electrode (Kim, 2010). Zhou et al. (2015) examined the removal of salt from both simulated and real wastewaters, as well as the removal of organic compounds, using electroadsorption by measuring the chemical oxygen demand. Under optimized conditions, 76% removal of organic compounds was observed from refined cotton wastewater (Zhou et al., 2015).

CDI has been used primarily for removal of small charged chemical compounds from different types of waters. It has not been extensively tested for the removal of biological substances. Microorganisms, such as bacteria and viruses, carry net negative charges in water, at neutral pH, imparted on them by nucleic acids and proteins present on the cell membrane (Michen and Graule, 2010). The ability of positively charged porous carbon electrodes to remove *E. coli* from liquid suspension has been known for several decades (Oren et al., 1983). Some of the early tests using graphite mesh were able to remove 85-95% of bacteria, depending on the strain of *E. coli* used (Golub et al., 1987). It is important to note that researchers were able to demonstrate successful desorption of *E. coli* from electrodes. This feature is particularly valuable for the application of a CDI system used for removal and concentration of microorganisms from drinking water. In addition, preliminary bench-scale experiments in the Habash laboratory at the University of Guelph have found that a benchtop flow-by CDI unit can remove over 99% (2-log) of the bacteria *E. faecalis* from phosphate buffered saline (see Appendix 2). The potential for CDI to remove and concentrate viruses from water has not been tested.

Table 1.6 Applications of CDI technology for removal of various water contaminants

Author	CDI type	Electrode and membrane details	Target Ions	Results
(Kim and Choi, 2012)	Flow by; constant voltage; no membrane	Nitrate-selective composite carbon electrodes	NaCl and NaNO3	36% higher total adsorption and 2.3 times higher nitrate specific adsorption compared to membrane CDI
(Jeon, 2013)	Flow by; constant voltage; ion exchange membrane	Flow electrode of activated carbon in suspension	NaCl	95% removal of NaCl from seawater; more scalable than conventional CDI and does not require discharge step
(Kim et al., 2015)	Flow by; constant voltage; ion-exchange membrane	Carbon-graphite electrode treated with Poly(vinyl alcohol) and polysulfone-based polymer membrane	NaCl, CaSO4, MgCl2	Individual efficiencies: NaCl> CaSO4 > MgCl2 ; the combined efficiency was 88% for all three ions at 300 ppm
(Huang, 2016)	Flow by; constant voltage; no membrane	Activated carbon cloth (Submerged electrode assembly in solution)	Heavy metals: Cd, Pb and Cr	81%, 78%, and 42% removal of Pb2+, Cr3+, and Cd2h+ respectively at simulated contamination concentrations (≈0.05 mM)
(Huang et al., 2013)	Flow by; constant voltage; no membrane	Activated carbon	Phosphate	77% to 86% removal for solutions of 50 to 300 mg Phosphate per Litre

(Mishra and Ramaprabhu, 2012)	Flow by; constant voltage; no membrane	Iron oxide-graphene composite supercapacitor	Inorganic arsenic	Compared to other materials iron oxide-graphene demonstrated 3-30 and 3-60 fold higher adsorption capacity for arsenate and arsenite

1.6 Research Objectives

Prior work with a bench top CDI system evaluated the ability of the system to remove and recover bacteria from different water types (see Appendix 2). The objective of this proof-of-concept research was to expand on the initial bacterial work by evaluating CDI as a new concentration method for biological and chemical contaminants from large volumes of water for their subsequent detection and enumeration. For this research project enteric viruses and pesticides were the focus. The bacteriophage MS2 was chosen to act as a surrogate for enteric viruses and the pesticide 2,4-D was selected as a model acidic herbicide. The first objective was to determine if, and to what extent, CDI could remove virus and herbicide from different types of water. The second objective was to determine if, and to what extent, the removed virus and herbicide could be recovered in the purged effluent.

CDI was expected to achieve better than 2-log removal of both 2,4-D and MS2 based on the literature review and previous work in the Habash laboratory (see Appendix 2). Furthermore, the removal of both contaminants was expected to produce concentrated effluent from which the contaminants of interest could be recovered, detected and quantified. The removal and recovery of the MS2 was evaluated using both culture-based and molecular analytical methods in order to determine the amounts of infective and total virus in the effluents, in order to determine if CDI had an impact on virus infectivity.

The effects of different water matrices on contaminant removal and recovery via CDI were also examined. The effects of mineral concentration, dissolved organic matter, and conductivity on removal and recovery were compared between ground, surface, dechlorinated tap, and MilliQ waters, as well as varying concentrations of phosphate buffered saline. The performance of CDI was hypothesized to be negatively affected by higher mineral content and high concentrations of

organic material that may be found in ground and surface water respectively. The effect of conductivity on CDI was explored by using two different concentrations of PBS, which bridged the range of conductivities of the environmental samples. Furthermore, tap water was used as a representative polished water sample.

2. Methods and Materials

2.1 CDI Experimental Procedure

2.1.1 ESD Unit Description and Set-up

Capacitive deionization was performed using a proprietary, patented bench top ESD system (ENPAR Technologies INC, Guelph, ON) for all experiments. The unit utilized porous carbon electrodes with a total surface area of 0.7 m^2 and a potential difference of 1.3 V. The unit was operated at a flow rate of 300 mL/min for all experiments. A built-in conductivity meter monitored the ion concentration of the solution as it flowed past the electrodes. The conductivity probe cell constant, k, was equal to 1, measuring a range of values between 0-2000 µS/cm. Set points were programmed to control the minimum and maximum conductivity values for eluting purified and concentrated effluents. For conductivities outside the defined range of elution for each valve, the solution was recycled through a separate valve back into the influent. As well, hysteresis values (**Table 2.1**) were set for each valve to prevent valve switching during small fluctuations in conductivity. The set points for the valve operation were as follows for all experiments (P1 controlled pure effluent, P2 controlled purge effluent, and P3 programs the cell constant). For the conductivity meter within the ESD unit, (set point P3), the value of the cell constant, was 1, allowing for conductivity readings between 0-2000µS/cm, with resolution of 1 µS/cm. The program for each complete cycle consisted of three steps (**Table 2.2**). Each CDI experiment consisted of multiple cycles in order replicate how the unit would be used in the field.

Prior to conducting each experimental run, the ESD unit was disinfected with 3L of 0.1M hydrochloric acid (HCl). Residual HCl was cleared from the ESD unit using a 10mM PBS solution followed by deionized water. The pH of the effluents was monitored to ensure the target

range of 6.5 to 7.5 was obtained. The ESD unit was then conditioned with 5 L of the solution or water type to be tested (without virus or herbicide spike), with 1 L running through each valve (pure, purge, and return to feed) and the remaining volume (approximately 2 L) used for two regeneration cycles to purge the electrodes of any adsorbed ions.

Table 2.1 ESD Valve Set-point Values

Set Point	**Purpose**	**Threshold direction**	**Threshold Conductivity (µS/cm)**	**Hysteresis Value (µS/cm)**
P1	Defines pure effluent	Low	200	20
P2	Defines purge effluent	High	1800	200

Table 2.2 Steps of one CDI cycle

Step	**Electrode Status**	**Length of Time**
Purification	Adsorption of ions to electrodes, removal from influent water	420s
Regeneration	Reverse potential difference between electrodes	90s
Purge	Desorption of ions, elute in purge effluent	40s*

*Legend: *Purge step for 0.1 mM phosphate-buffered saline was shortened to 25s due to low conductivity.*

2.1.2 Sample Processing

I. Preparation of Water Samples

Influent solution (10L) was prepared for each water or solution to be tested. All phosphate-buffer, MilliQ, and surface water solutions were sterilized at 121°C for 60 minutes at 15 psi via autoclaving prior to the addition of the analyte of interest, MS2 or 2,4-D (Sigma Aldrich, St. Louis, MO). At each sampling event 40 L of each water type was collected on multiple occasions through the spring and summer of 2015. Surface water samples were collected from the Grand River where it passes through Inverhaugh Flats, with approximate co-ordinates of +43.625305, -80.448175 (Elora, Ontario). Raw, untreated groundwater samples were collected from a groundwater well in the Middleton Well field, in the Grand River watershed (Cambridge, Ontario). Surface and ground water were filtered with a polyethylene filter bag with pore size of 1 µm to remove sediment. Groundwater samples were not autoclaved as this caused precipitation of minerals. Tap water was collected at the University of Guelph and agitated by mixing for 24 hours to dechlorinate. In addition, 1 mL of 3% sodium thiosulphate was added for every 10 L of tap water to further reduce the free chlorine content in the water samples. This concentration is below that recommended in the Standard Methods (APHA et al., 2012), however, this was necessary to prevent precipitation in the ESD unit (previously tested). After preparation of the water samples, the analyte of interest was added and stirred constantly to achieve a homogeneous mixture (30 minutes for both MS2 and 2,4-D). Analyte preparation is described below.

II. Preparation of MS2 Spike

The bacteriophage MS2 (ATCC ® 15597-B1) was propagated in liquid culture, based on APHA Method 9224 B (APHA et al., 2012). An overnight culture of *E. coli* strain C-3000

(ATCC Number 15597) was prepared, from a freezer stock, in 10 mL Luria Bertani (LB) broth at 37 °C and 120 rpm. A volume of 200 µL from the overnight culture was used to inoculate 100 mL LB broth that was incubated at 37 °C and 120 rpm for 2 hours. One mL frozen MS2 stock (est. 10^9 PFU/mL) was added to the *E. coli* culture and incubated for four hours at 37 °C at 120 rpm. The *E. coli*-MS2 suspension was first filtered through 0.45 µm membrane filters (47 mm diameter, EMD Millipore, Darmstadt, Germany) to remove large debris, followed by 0.22 µm filters (47 mm diameter, EMD Millipore) to remove bacteria. All filters were first conditioned with autoclaved 1.5% Beef Extract solution, pH 7.0-7.5.

After filtration virus stocks were concentrated using a precipitation method (Thurston-Enriquez et al., 2003). For every 100 mL of phage suspension, 9 g of polyethylene glycol (PEG) 8000 and 5.8 g of NaCl were added for flocculation. The suspension was incubated overnight at 4 °C, shaking at 120 rpm. The following day, the suspension was centrifuged at 8,000 x G for 90 minutes at 4 °C. After centrifugation the supernatant was carefully decanted and discarded. The remaining pellet was re-suspended in 20 mL of sterile LB broth. This stock was enumerated via plaque assay (described below) and stored at 4 °C for short-term usage (one week or less) or aliquoted 1:1 with 50% glycerol solution (total volume 1.5 mL, final glycerol concentration 25%) for long-term storage at -80°C.

On the day of CDI experiments, previously enumerated MS2 stocks were diluted in LB broth to a final concentration of 10^8 PFU/mL. One mL of this stock was added to 999 mL of the water type being tested and mixed. This 1 L of spiked water was added to 9L of the water type being tested and stirred on a stir plate for 30 minutes at room temperature.

III. Preparation of 2,4-D Spike

2,4-D (Sigma Aldrich, St. Louis, MO) was weighed out (250 mg) and dissolved into 10 L of influent water by stirring for 30 minutes at room temperature. This solution was processed by CDI using the common method described above.

2.1.3 Sample Collection

Following conditioning of the CDI unit for a particular experiment, approximately 8L of spiked influent solution was processed by the CDI unit using the cycling conditions indicated previously (**Table 2.1** and **Table 2.2**). After each experiment, the total volume was measured for each of the three steps. An initial 500 mL of spiked "influent" sample was removed prior to the start of the experiment for enumerating the initial concentration of spiked analyte. The concentrated effluent (purge) was collected in a sterile flask and the purified effluent was collected in a sterile 10 L carboy (Figure 2.1). Using the above protocol, at least three independent replicates were performed for each experiment. Collected samples were processed differently as described below for each of the analytes of interest.

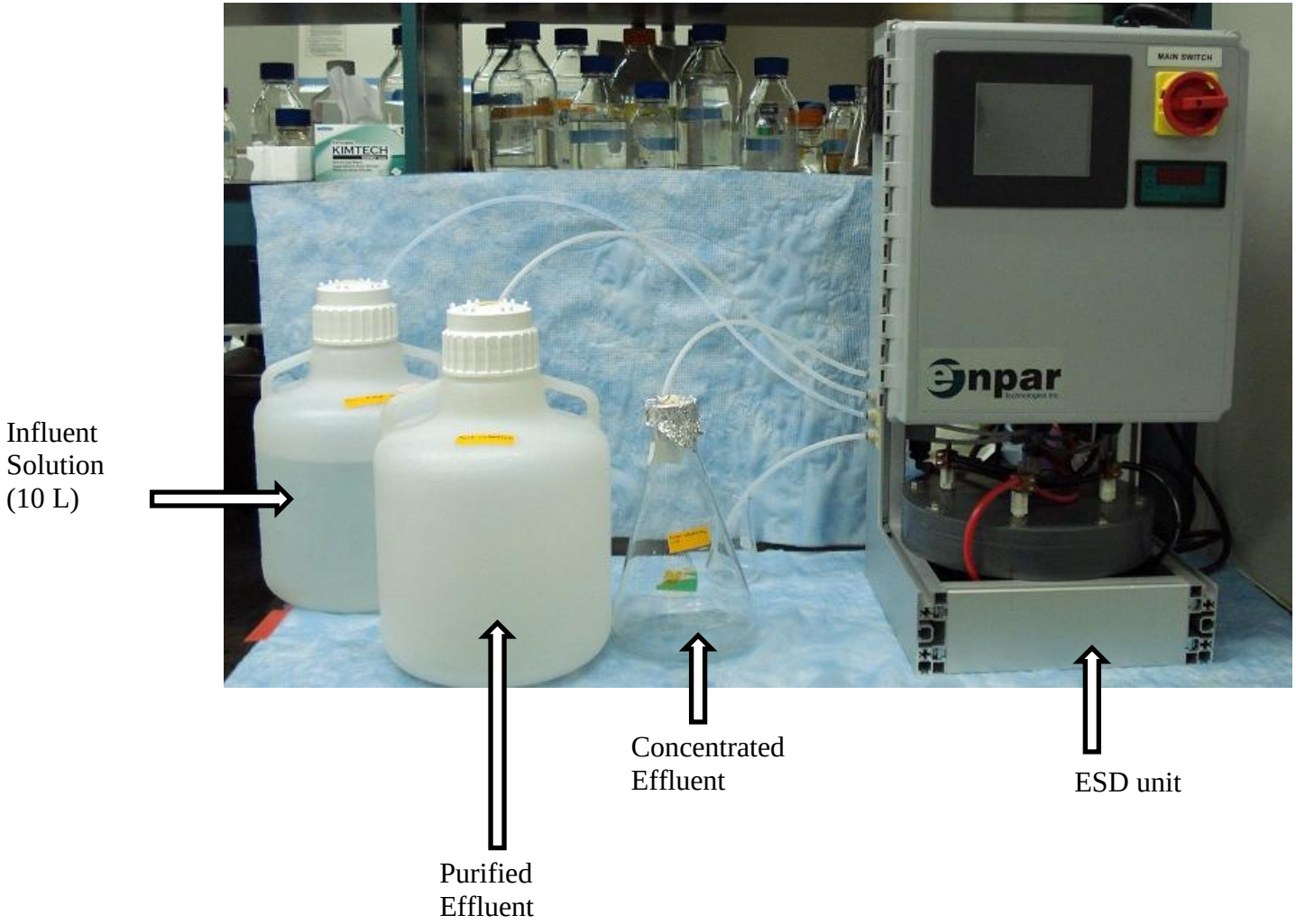


Figure 2.1 Benchtop ESD unit and sample collection set-up.

2.2 MS2 Experiments

2.2.1 MS2 Sample Concentration

For each CDI experiment, 500 mL of influent (collected prior to starting the experiment), 500 mL of purge (or the entire volume, if less than 500 mL was produced), and 1 L of pure went through a secondary concentration step, based on a method developed by Health Canada (Brassard et al., 2005). This secondary concentration step was necessary to increase the amount of genetic material for RT-qPCR analysis, as well as allow for a more reproducible analysis of the pure effluent via plaque assay. Each sample was passed through a 47 mm Zeta Plus 60S filter (3M, distributed by John Brooks Company Limited, Toronto, ON) as a secondary concentration step. Filters were conditioned with 10 mL sterile phosphate buffer solution (10mM phosphate, 137mM sodium chloride, and 2.7 mM potassium chloride), pH 7.0-7.4, before the sample was filtered. Following filtration, phage were eluted by placing the filter face-down in a sterile petri dish in 8 mL of 2.9% tryptose phosphate, 6% glycine broth (pH 9.5) and agitated gently at 85 rpm for 15 minutes at room temperature. The final 6mL sample removed from the petri dish was adjusted to pH 7.0-7.4 using 1M HCl and used as follows: 1 mL for the plaque assay, 280 µL for RNA extraction, and the remaining sample was split into three 1.5 mL aliquots and stored in -80 °C.

2.2.2 Enumeration via Plaque Assay

All MS2 stock enumerations, as well as CDI experiment samples of MS2 (influent, purge, and pure), were enumerated using an adapted plaque assay based on Standard Method 9224 B (APHA et al., 2012). 100 µL of thawed host, *E. coli* C-3000, was added to 100 mL of LB broth and incubated overnight (12-16 hours) at 37 °C and 120 rpm. 7 mL tryptone agar aliquots

(tryptone media + 0.28% agar) used as the top layer agar, were prepared in advance. The top layer agar was melted before use and cooled to 60 °C before pouring. The bottom agar consisted of petri plates containing 10 mL of 0.7% agar (tryptone media + 0.7% agar) prepared in advance and warmed to room temperature before use.

All samples were diluted in LB broth, 10-fold serial dilutions up to 10^{-8} were prepared in order to achieve an expected concentration of 0-10 PFU/mL per plate. 300 µL of overnight *E. coli* culture and 1 mL of MS2 diluted stock were added to each plate. One 7 mL aliquot of melted top agar was poured over top, and the plate was swirled in a figure 8 pattern for even distribution. This was repeated in triplicate for each dilution to be enumerated. Plates were incubated at 37 °C for 24 hours. Plated dilutions with counts between 10-100 PFU/mL, considered to be most accurate, were used for calculating stock and sample MS2 concentrations.

2.2.3 RNA Extraction

The MS2 RNA from each of the samples was extracted using the QIAamp Viral RNA extraction kit (Qiagen, Hilden, Germany). The extractions were carried out as per the manufacturer's instructions with the following modifications. The initial sample volume was increased from 140 µL to 280 µL to increase the amount of RNA to be extracted. Carrier RNA was not added as part of the binding step, as this interferes with fluorescence reading in reverse transcriptase (RT)-qPCR because a secondary product is created. During the final elution step, MS2 RNA was eluted in RNase-free H_20 instead of Buffer AVL. Buffer AVL contains sodium azide, which can interfere with Nanodrop readings as it is absorbed at wavelengths between 220 and 280 nm. The final volume of the RNA extract was 100 µL and was stored at -20°C until analysis.

2.2.4 cDNA Synthesis

iScript cDNA Synthesis Kit (Bio-rad, Mississauga, ON) was used as directed. A set volume of RNA was added to each reaction as the amount of RNA from each sample, even after a secondary concentration step, was too low for enumeration via Nanodrop (which has a detection limit of 2 ng/µL (Thermo Fisher Scientific, 2009)). Ten µL of sample RNA extract was added to each reaction, in addition to 4 µL 5x iScript Reaction Mix (containing random hexamer primers), 1 µL of iScript Reverse Transcriptase, and 5 µL of sterile water, for a total reaction volume of 20 µL. The thermocycler protocol followed manufacturer's directions.

2.2.5 qPCR

The qPCR assay used primers designed to target a 77 basepair region of the MS2 assembly protein based on Assay 1 (O'Connell and Bucher, 2006). This region was cloned into the pGEM Easy T Vector system (Promega, Fitchburg, WI), to create a standard plasmid to be used in the qPCR assay. This construct was used to create standard curves of serially diluted plasmid ($5x10^8$ to $5x10^2$ copies per reaction), which were prepared in triplicate for each run. qPCR assays were performed on an iQ5 PCR Thermal Cycler (Bio-rad, Mississauga, ON, Canada). SSoFast EvaGreen Supermix (Bio-rad, Mississauga, ON, Canada) was used as directed. Each reaction used 6 µL of template cDNA, 2 µL of each Forward and Reverse primer (final concentration 400 nM each), and 10 µL EvaGreen Supermix, for a total reaction volume of 20 µL. Samples were run in technical duplicates and every qPCR plate was run with no template, no enzyme, and no reverse transcriptase controls. The cycling protocol was based on Bio-rad's SSoFast EvaGreen Supermix recommendations, with the exception of the annealing temperature, which was based on Assay 1 (O'Connell and Bucher, 2006). This protocol consisted of an initial activation at 95 °C for three minutes, a melt step at 95 °C for 10s followed by annealing and extension at 58 °C

for 15s (repeated 40 times), and a melt curve from 65 °C to 95 °C, with readings taken at 0.5 °C intervals. For data analysis, the baseline threshold was determined by the Bio-rad iQ5 software. Only standard curves with efficiencies between 90-105% and R^2 values between 0.950 and 0.999 were used, as indicators of consistent product amplification between plates.

2.2.6 RT-qPCR Inhibition Tests

The concentration of large volumes of water can result in concentrating inhibitors, as well as target analytes for molecular analysis (Gibson et al., 2012). Inhibition of the RT-qPCR analysis by the purge samples was tested, due to an expected increased concentration of ionic species from CDI processing. To test for inhibition due increased ion concentration in the RNA extracts for each purge sample of each CDI experiment, an extra cDNA synthesis reaction was prepared alongside the original sample for analysis. This extra reaction contained a 5 µL spike of stock MS2 RNA (est. 10^9 PFU/mL), instead of the 5 µL of water used in the original sample reaction. A cDNA synthesis reaction of the 5 µL stock MS2 RNA with pure water was also prepared to act as a baseline control.

Inhibition of the RT-qPCR analysis would result in samples containing the purge matrix with stock MS2 RNA spike showing delayed amplification (e.g. every ten-fold decrease in copy number corresponds to a C_t value that is 3.33 units lower) when compared to the MS2 stock RNA in a pure water matrix. A no-template control and no-reverse transcriptase control were also prepared with each batch of cDNA synthesis reactions.

2.2.7 Recovery of MS2 from 60S Zeta Plus Filter

To test the recovery of MS2 from the 60S Zeta Plus filters, MS2 was spiked into 500 mL of 10 mM PBS, groundwater, surface water, and dechlorinated tap water to a final concentration of 10^4 PFU/mL, in triplicate experiments. Spiked samples were filtered and enumerated via plaque assay, as described above, in order to determine the percent recovery of spiked MS2 from each type of water filtered through the Zeta Plus 60S filters.

2.2.8 MS2 Viability During Sample Processing

To determine if there was a reduction in viability of MS2 during the length of sample processing (approximately 5 hours from influent being spiked until elution from secondary concentration step), each type of water (1 mM and 0.1 mM PBS, groundwater, surface water, and dechlorinated tap water) was spiked with 10^4 PFU/mL of MS2 and incubated at room temperature (22-23 ^oC) for 5 hours. Each sample was enumerated at time zero and again at five hours via plaque assay; plaque assays were conducted as described previously. Experiments were conducted in triplicate.

2.3 Herbicide Experiments

2.3.1 Solid-phase Extraction of Pure Effluent Samples

After all samples were collected, 100 mL of the pure effluent was concentrated using solid-phase extraction. Prior to extraction, the pure effluent sample pH was adjusted to 1.5-2.0 using 0.5 M H_2SO_4. The sample was then processed using the Fisher HyperSep C-18 Cartridge, (10 mL volume, 500 mg bedweight). An initial column wash with 20 mL methanol followed by 10 mL H_2O was passed through the column, after which the column was not allowed to dry. The sample was loaded at a rate of 5 mL/min and washed with 10 mL H_2O. The cartridge was then

dried for 20 minutes using a vacuum (10 mmHg). The analyte was eluted from the cartridge with 10 mL of acetonitrile. The sample was stored at 4°C and away from light until analysis by reverse phase high pressure liquid chromatography (HPLC), which was conducted within 24 hours of the initial experiment.

2.3.2 Reverse Phase-High Pressure Liquid Chromatography

Sample analysis of the influent, purge, and concentrated pure samples was conducted using a Zorbax ODS 4.6x12.5 mm 5µm guard column (Agilent, Santa Clara, CA) followed on-line by a Zorbax SB-C18 rapid resolution 4.6X75 mm 3.5 µm column (Agilent, Santa Clara, CA) for analysis. A gradient method adapted from EPA Method 555 was used to determine the amount of 2,4-D in each sample (Environmental Protection Agency, 1992). A mobile phase of 10% acetonitrile and 90% 0.025 M H_2PO_4 was established at a flow rate of 0.6 mL/min at 35 °C. This gradient was programmed to change at a constant rate to 90% acetonitrile and 10% H_2PO_4 over 30 minutes. The analyte concentration was determined using the 230 nm reading from the UV detector. A standard curve of 2,4-D serially diluted in acetonitrile was run with each set of samples, from 1 ng/µl to 1 µg/µL, in technical duplicates, as were a no-analyte control of acetonitrile and a blank of the environmental water type tested.

2.4 Statistical Analysis

Each treatment for each analyte (MS2 and 2,4-D) was performed in triplicate to calculate a mean and standard deviation. All statistical analysis was conducted using R version 3.2.3. Barlett's test of homogeneity of variance was performed for all MS2 and 2,4-D results prior to further analysis, with no significant differences found ($p>0.05$). For the comparisons of multiple treatments, a one-way ANOVA was used. If the F-statistic was significant ($p<0.05$), post-hoc

analysis was conducted using Tukey's HSD test, to determine which means were significantly different from each other ($p<0.05$). Tukey's HSD test was used because it allows for a comparison of all values to each other in order to determine significant differences, while minimizing the experiment-wise error rate. For comparisons of two mean values within a treatment, a student's t-test was used ($P<0.05$).

3. Results

3.1 MS2 Results

3.1.1 Optimization of MS2 Enumeration Method

The removal and recovery of MS2 via CDI were measured directly using a plaque assay and RT-qPCR enumeration without a secondary concentration step in the initial experiments. The initial concentration of MS2 (10^4 PFU/mL) was chosen to allow for enumeration of up to 4 log removal. Based on the input amount of MS2 (10^4 PFU/mL), the pure effluent was expected to produce 10^2 PFU/mL given 99% removal, as was hypothesized. However, the RT-qPCR method used was not sufficiently sensitive to enumerate the amount of MS2 present in the purge and pure samples below 500 genome copies per qPCR reaction (this RT-qPCR assay has previously been used to detect down to 2,000 genome copies per reaction (O'Connell and Bucher, 2006)). Furthermore, small volumes of 1 mL and 280 µl of pure or concentrated effluents used for the plaque assay and molecular enumeration methods respectively did not produce representative results (there was high variability between replicates).

3.1.2 Percent Removal of MS2 from different types of water

The adsorption of microorganisms to the porous carbon electrodes has been demonstrated previously (Golub et al., 1987). However, the adsorption and the subsequent removal of microorganisms, and specifically viruses, by the capacitive deionization technology have not been tested to date. In this study, MS2-spiked water samples were used to test the ability of CDI to remove viral particles from various water types (**Table 3.1**). The viral particles were enumerated using both a plaque assay (the standard method) and RT-qPCR methods. The culture-based method (plaque assay) only enumerates infectious MS2 while the molecular

Table 3.1 Water sample types used for all experiments**.**

Water type	**Abbreviation**	**Conductivity (µS/cm)**
0.1 mM PBS	PBS low	146.7 ± 11.5
1 mM PBS	PBS high	1758.7 ± 52.2
Ground water	GW	866.7 ± 57.7
Surface water	SW	566.7 ± 57.7
Dechlorinated tap water	TW	700.0 ± 20.0

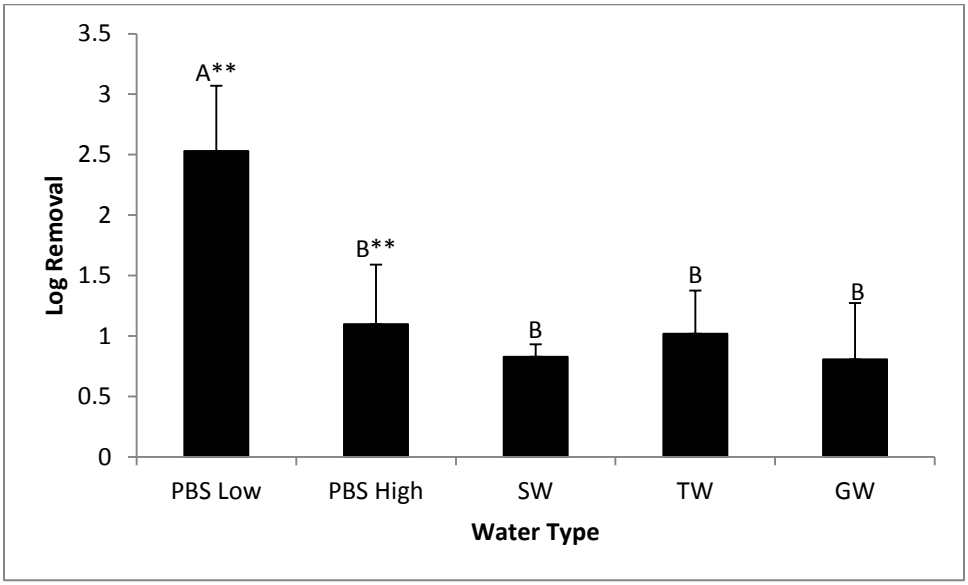
Legend: All values are mean ± SD; n=3

method (RT-qPCR) enumerates genome copies from both intact and inactivated virions and does not distinguish between infectious and non-infectious viral particles.

While the spiking of MS2 was based on enumeration via plaque assay, it was assumed that there would be at least as many gene copies of MS2 as there were infectious particles (i.e. if the influent spike was 10^4 PFU/mL, there would be at least 10^4gene copies per mL in the influent). Comparing the MS2 sample data from Appendix 4, all samples had more gene copies than infective viral particles. Based on the RT-qPCR enumeration results, the removal of MS2 from PBS-low was 2.5 ± 0.54 log and was different from all other sample types (**Figure 3.1**). The removal of the virus from PBS-high, surface, tap and ground water was not significantly different and ranged between 0.81 – 1.1 log (**Figure 3.1**). Of note, the molecular enumeration method found 1.76x and 2.56x less removal of the virus than plaque assay enumeration for PBS-low ($p<0.01$) and PBS-high ($p<0.005$) samples respectively. Furthermore, the two different methods of MS2 enumeration did not yield statistically different results for the amount of removal for the three types of environmental samples. Therefore, conductivity is not the only factor influencing degree of removal.

The removal of MS2 (initial concentration 10^4 PFU/mL) varied with different solution types based on the plaque assay results (**Figure 3.1**). MS2 removal was higher in PBS-low solution at 4.5 ± 0.48 log compared to PBS-high at 2.8 ± 0.15 log ($p<0.01$) (**Figure 3.1**). The removal of MS2 from environmental samples was lower ($p<0.05$) than either of the PBS solutions and varied based on the sample type. Surface water had the least amount of removal at 0.55 ± 0.32 log, and was statistically different from tap water at 1.8 ± 0.57 log ($p<0.02$) (**Figure 3.1**).

A. RT-qPCR



B. Plaque Assay

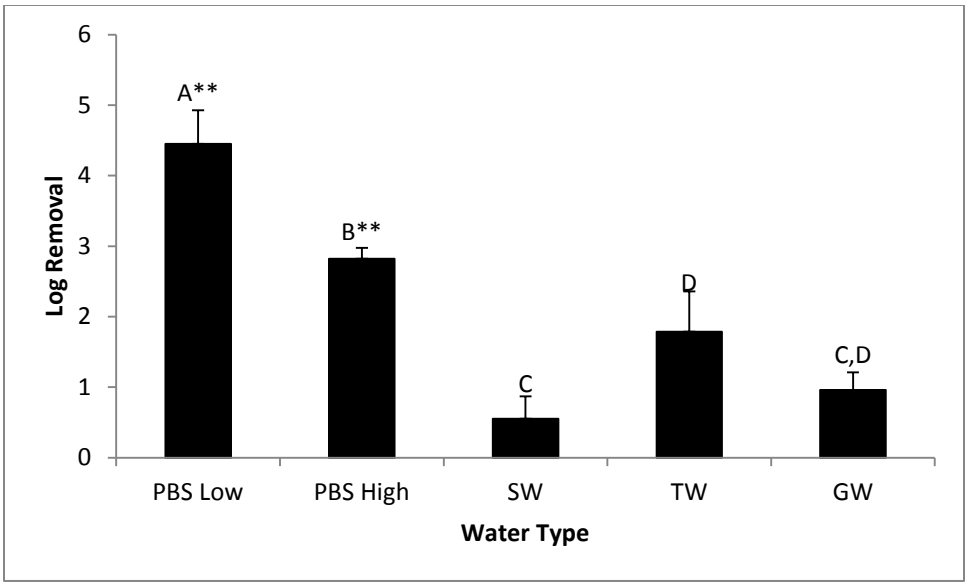


Figure 3.1 Log removal of MS2 bacteriophage spiked into 0.1 mM PBS (PBS low), 1 mM PBS (PBS high), surface water (SW), dechlorinated tap water (TW) and ground water (GW), and enumerated using RT-qPCR (A) and plaque assay (B) methods. ANOVA followed by Tukey's HSD were used to determine significant differences (A,B,C,D) within a given method for different water types, and the Student's t-test was used to determine significant differences (*) between the two methods for a given water type (mean, +SD, n=3; **$p<0.01$).

The removal of MS2 from ground water was 1.0 ± 0.25 log, and was not statistically different from either surface or tap water. In order to account for inactivation of the virus during the experiment, the enumeration was repeated using a molecular method (RT-qPCR), which enumerates viable and non-viable MS2. In summary, the removal of MS2 varied depending on the water type and, in some cases, the enumeration method used.

3.1.3 Purge recovery

The current methods of virus concentration are known to be time consuming, can be expensive and have variable recoveries thus necessitating research of novel methods of virus concentration (Cashdollar and Wymer, 2013). Given the ability of CDI to remove MS2 (0.55 to 4.45 log removal based on plaque assay and water type (see **Figure 3.1**)), it was hypothesized that the removed particles could be recovered from the concentrated effluent (purge) for detection and enumeration. The ability of the CDI technology to concentrate microorganisms was tested by enumerating the viral particles present in the purge using plaque assay and RT-qPCR-based methods. Although the purge was expected to consistently contain the majority of the removed particles, the virus recovery varied between 5.74 ± 5.0% and 118.8 ± 86.1% for plaque assay, and 59.0 ± 62.9% and 1038.3 ± 1585.0% for RT-qPCR (**Table 3.2**). Therefore, the purge recovery results were highly variable within a water type between analytical methods, and between water types using the same method (**Table 3.2**). The data were insufficient to evaluate the effects of conductivity or water type on the ability of CDI to concentrate MS2. This is due to the high variability within experimental replicates for each water type.

Table 3.2 Recovery of MS2 (%) from purge (concentrated effluent)

Sample type (conductivity)	**Plaque assay mean% Recovery**	**RT-qPCR Mean% Recovery**
PBS low (147 µS/cm)	6.76 ± 4.83	58.95 ± 62.87
PBS high (1759 µS/cm)	118.76 ± 86.13	1038.32 ± 1585.03
SW (567 µS/cm)	90.18 ± 84.72	329.14 ± 216.58
TW (700 µS/cm)	5.74 ± 5.03	743.27 ± 735.85
GW (867 µS/cm)	15.55 ± 6.61	107.88 ± 52.88

Legend: 0.1 mM PBS (PBS low), 1 mM PBS (PBS high), surface water (SW), dechlorinated tap water (TW) and ground water (GW). All values are mean % ± SD; n=3

3.1.4 Virus recovery off of Zeta Plus 60S filters varied with water type

To investigate the possible source of the variable recovery of MS2 from the influent, purge and pure samples, the recovery of MS2 from the Zeta Plus 60S filters was investigated. Virus recovery from positively charged filters is known to be variable, and is influenced by the filter elution method, water and virus types (Cashdollar and Wymer, 2013). Other studies have found MS2 recoveries of 45-56% from tap water concentrated using NanoCeram filters (Ikner et al., 2011), 0.04-1.7% from river water concentrated using Zeta Plus 60S filters (Jones et al., 2014), and 0.26-17.9% from groundwater using Zetapor membranes (De Keuckelaere et al., 2013). For this project, a preliminary check for virus recovery was performed using Zeta Plus 60S filters and 10 mM PBS. The mean recovery of MS2 was determined to be 14.1 ± 8.2% (**Figure 3.2**). In addition, the recovery of the virus from the three environmental water types was also tested. The recoveries from ground, surface and tap water samples were 3.9 ± 4.0%, 0.30 ± 0.11% and 1.8 ± 0.14%, respectively (**Figure 3.2**). Recovery of MS2 from surface water ($p<0.03$) and tap water ($p<0.05$) were significantly lower from that of PBS (**Figure 3.2**). Therefore, there was some variance in virus recovery from the Zeta Plus 60S filters based on water sample type. These results suggest two potential limitations. First, the comparison of results between some water types may be biased due to inconsistent recoveries between water types. Mean recovery from PBS was >7x and >40x greater than mean recovery from surface and tap water samples respectively (**Figure 3.2**). Second, reproducibility of results from a given water type may be difficult due to variable recoveries from the filter. The coefficient of variation for PBS and ground water recovery were 58% and 103%, respectively (**Figure 3.2**).

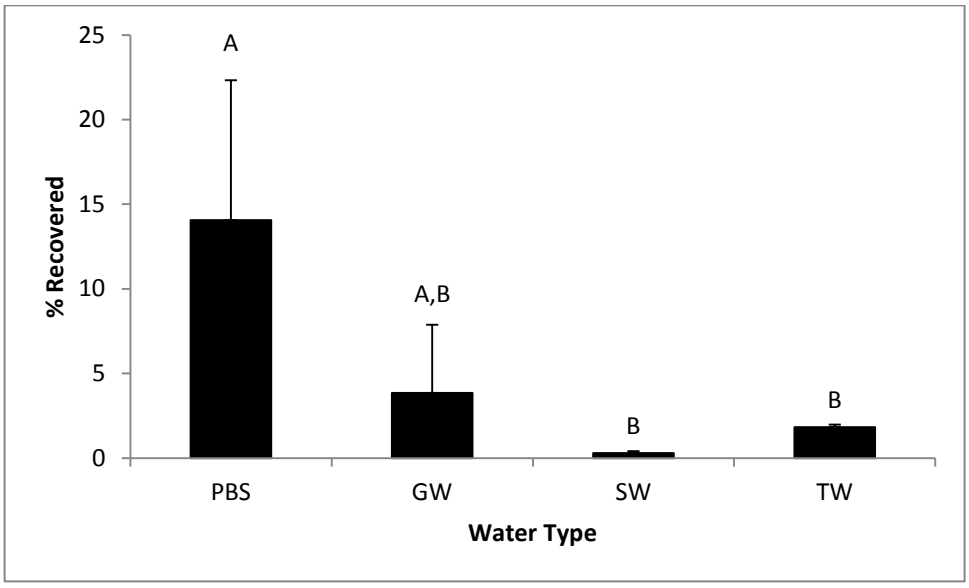


Figure 3.2 Percent of MS2 recovered from the Zeta 60S filters post-spike of 10^4 PFU/ml into 10 mM PBS, ground (GW), surface (SW) and dechlorinated tap water (TW). (mean; SD; n=3)

3.1.5 Influence of water sample type on the viability of MS2

Inactivation of MS2 due to varying water types was examined as a potentially confounding variable in the context of CDI when evaluating removal and recovery of the virus by plaque assay. The viability of MS2 has been previously shown to be variable (decay rate of 3200 to >60000 PFU/h) when suspended in different types of water (Olson et al., 2004). To investigate the effect of virus viability on removal and recovery of MS2 by CDI, the viability of MS2 was compared in surface, ground and dechlorinated tap water, as well as two concentrations of PBS, over five hours (average time from start of the experiment until end of the secondary filtration step). The percent of infective MS2 remaining after five hours was 98.1 ± 18.7% for 0.1 mM PBS, 81.3 ± 6.7% for 1 mM PBS, 80.9 ± 6.4% for surface water, 84.9 ± 9.5% for ground water, and 105 ± 11.9% for dechlorinated tap water (**Figure 3.3**). Although there are no differences in MS2 viability between water sample types (based on one-way ANOVA), viability of MS2 was less than 100% for 1 mM PBS and surface water samples (based on a one-way one-sample t-test, $p<0.05$) (**Figure 3.3**). Therefore, the amount of MS2 lost due to decreased viability in 1 mM PBS and surface water samples is negligible relative to the removal and the variable recoveries from the secondary concentration step. Sample type did not influence viability of MS2 over a 5-hour period.

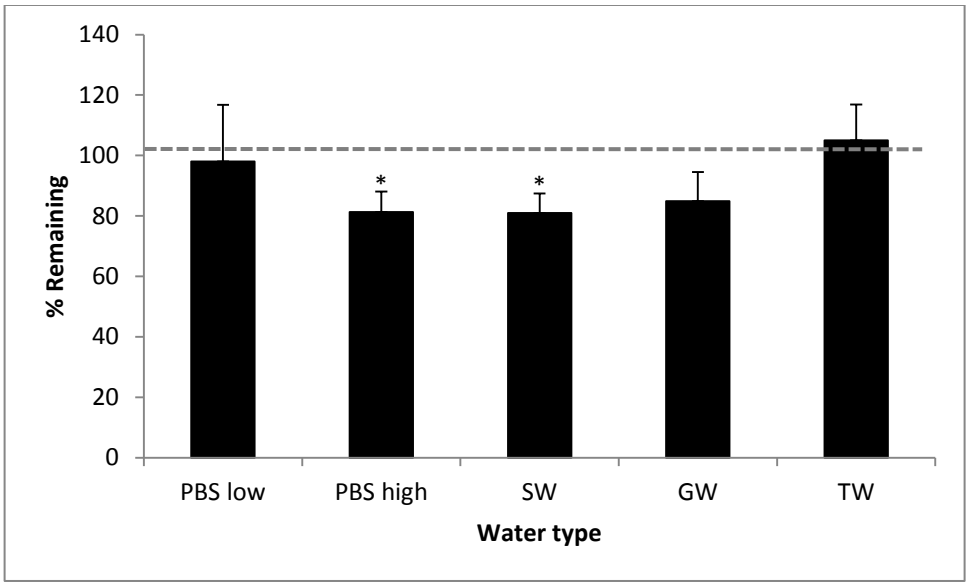


Figure 3.3 Percent of MS2 remaining in each water type 5 hours post-spike of 10^4 PFU/ml into 0.1 mM PBS (PBS low), 1 mM PBS (PBS high), ground (GW), surface (SW) and dechlorinated tap water (TW) (mean; SD; n=3). Values significantly less than 100% viability are indicated with * ($p<0.05$). Dotted line represents 100% MS2 remaining.

3.1.6 Inhibition of RT-qPCR reaction due to water contaminants

Water impurities, such as the presence of humic acids and heavy metals, are known to have inhibitory effects on RT-qPCR (Fong and Lipp, 2005), which can be further exacerbated when concentrated during filtration of large volumes of environmental water samples (Gibson et al., 2012). Since CDI is expected to collect ionic species in the concentrated effluent, it is important to investigate the effects of the concentrated effluent on enumeration by RT-qPCR. Therefore, inhibition of both reverse transcriptase and qPCR steps were examined for both influent and purge samples, since one or both steps can be inhibited. Typically the nucleic acid extracts are diluted to evaluate if inhibition can be alleviated without loss of the target signal. As well, the diluted extracts can be spiked with a known concentration of target RNA or DNA to evaluate inhibition. Since the purge samples were expected to contain higher concentrations of ions and potentially inhibitory compounds from the different water types, RT-qPCR inhibition of purge samples was determined by spiking the purge matrix from the RNA extraction of each experimental replicate with RNA extracted from stock MS2, this was compared to the same spiked MS2 amount in pure water matrix. It was hypothesized that all samples should have the same C_t value if no inhibition was present due to the purge matrix. Within each treatment, the mean C_t values of the Purge matrix +spike and the Spike-only were compared using a student's t-test. The only significant difference found was for "PBS - high" ($p<0.05$) (**Table 3.3**), indicating that this purge matrix caused some inhibition of RT-qPCR. However, it should be noted that the standard deviations for all samples in **Table 3.3**, with the exception of surface water, are larger than 0.3 (the acceptable standard deviation for technical replicates in qPCR).

Table 3.3 Purge matrix inhibition of RT-qPCR reactions

Experiment	Purge matrix + spike C_t (mean ± SD; n=3)	Spike-only C_t (mean ± SD; n=3)	t-test p-value
PBS – low	11.58 ± 0.42		>0.05
PBS - high	14.58 ± 0.37		<0.05
TW	11.86 ± 0.37	11.9 ± 1.32	>0.05
SW	11.31 ± 0.27		>0.05
GW	11.59 ± 0.38		>0.05

Legend: 0.1 mM PBS (PBS low), 1 mM PBS (PBS high), surface water (SW), dechlorinated tap water (TW) and ground water (GW)

The high variability may be due to the fact that these samples amplified outside the linear portion of the assay, with inconsistent replication occurring. Therefore the samples obtained following concentration via CDI and the secondary concentration step using Zeta plus 60S filters did not affect cDNA synthesis or target amplification during the RT-qPCR analysis, with the exception of the "PBS - high" sample.

3.2 2,4-Dichlorophenoxyacetic Acid Results

3.2.1 Optimization of solid phase extraction and HPLC methods for the quantification of 2,4-D

Solid phase extraction is a common technique used for concentrating 2,4-D from solution, and HPLC is commonly used to quantify the amount of analyte in the solution. In this study, the performance of the SPE method was tested using a recovery test. The performance of the HPLC method was validated by determining the linear range, as well as limits of detection and quantification as described in EPA Method 555 (Environmental Protection Agency, 1992). The recovery of 2,4-D from the SPE column was tested using a known amount of 2,4-D. A mean recovery of 94% was achieved for the laboratory-developed SPE method used in this study. Limits of detection and quantification for the HPLC method were determined to be 1.5 ng/µl and 4.6 ng/µl. The HPLC standard curve had a linear range from 1 ng/µl to 1 µg/µL. The limits of detection and quantification of the method used in present study were higher than the original EPA Method 555 (which had a method detection limit of 0.34 pg/uL). However, the difference in the equipment used makes the direct comparison inappropriate (Environmental Protection Agency, 1992).

3.2.2 Removal of 2,4-D by CDI

The adsorption of pesticides to carbon aerogel electrodes via CDI has not been previously tested. Since 2,4-D is an acidic pesticide (pKa = 2.73), it was hypothesized that it adsorbs to the carbon aerogel electrodes used in CDI technology due to negative charge in a typical water sample (pH 6-8). The ability of CDI to adsorb and remove 2,4-D from various water samples was tested by spiking the water samples with known amounts of the pesticide (250 µg/mL) and quantifying the concentrations using HPLC. The removal of 2,4-D varied with water type (**Figure 3.4**). The highest removal of 1.3 ± 0.03 log was achieved for MilliQ water compared to all other water types ($p<0.001$), while the lowest removal was detected for surface and ground water samples at 0.47 ± 0.04 log and 0.53 ± 0.01 log respectively (**Figure 3.4**). The removal of 2,4-D from tap water at 0.7 ± 0.02 log was lower compared to the MilliQ ($p<0.001$) and higher compared to the surface ($p<0.001$) and ground ($p<0.001$) water samples (**Figure 3.4**). The finished water (MilliQ and tap water samples) had higher removal of the pesticide than the unfinished water (surface and ground water samples), with tap water having approximately 0.2 log more removal than the unfinished waters, and the MilliQ water having more than double the removal. Additionally, pH measurements taken of the spiked influent and purified effluent were significantly different for all treatments (**Table 3.4**) ($p <0.05$). The drop in the pH level, which on its own was not expected to affect the removal of 2,4-D as it was still well above the pKa value of 2.73, was attributed to hydrolysis of water (personal communication, ENPAR 2015).

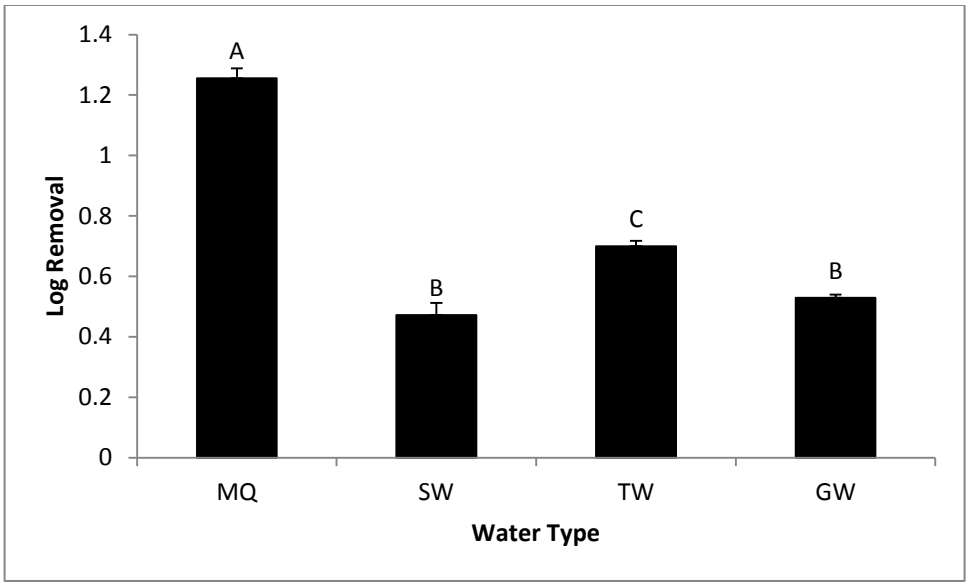


Figure 3.4 Log removal of 2,4-D as determined by HPLC for MilliQ (MQ), surface water (SW), dechlorinated tap water (TW) and ground water (GW). ANOVA followed by Tukey's HSD were used to determine significant differences (A,B,C) in removal between different water types (mean, +SD, n=3).

Table 3.4 Comparison of pH values from Influent, Pure, and Purge Samples.

Sample type (conductivity)	**Influent pH Mean ± SD**	**Purge pH Mean ± SD**	**Pure pH Mean + SD**	**p-values***
SW (550 µS/cm)	7.71 ± 0.41	7.34 ± 0.34	6.2 ± 0.77	<0.05
TW (700 µS/cm)	7.3 ± 0.15	7.46 ± 0.2	6 ± 0.2	<0.01
GW (900 µS/cm)	7.67 ± 0.18	7.93 ± 0.26	5.89 ± 0.18	<0.01

Legend: surface water (SW), dechlorinated tap water (TW) and ground water (GW); p-values were calculated using students t-test comparing Influent and Pure pH; All values are n=3

3.2.3 Recovery of 2,4-D in Purge (concentrated effluent)

Given the ability of CDI to remove 2,4-D (0.47 to 1.26 log removal depending on water type (see **Figure 3.4**)), it was hypothesized that the removed molecules could be recovered in the concentrated effluent (purge).The ability of the CDI technology to concentrate pesticides, such as 2,4-D, was tested by quantifying the amount of analyte present in the purge using HPLC. The recovery of 2,4-D varied with water type. The lowest recovery of 1.3 ± 0.68% was observed for MilliQ water samples compared to the three environmental sample types ($p<0.001$) (**Figure 3.5**). The highest recovery was achieved for ground and tap water at 19.3 ± 0.75% and 17.3 ± 1.0%, respectively, compared to MilliQ ($p<0.001$) and surface water samples ($p<0.001$) (**Figure 3.5**). Therefore, the recovery was influenced by water type, with lower conductivity waters having significantly lower recoveries of the pesticide.

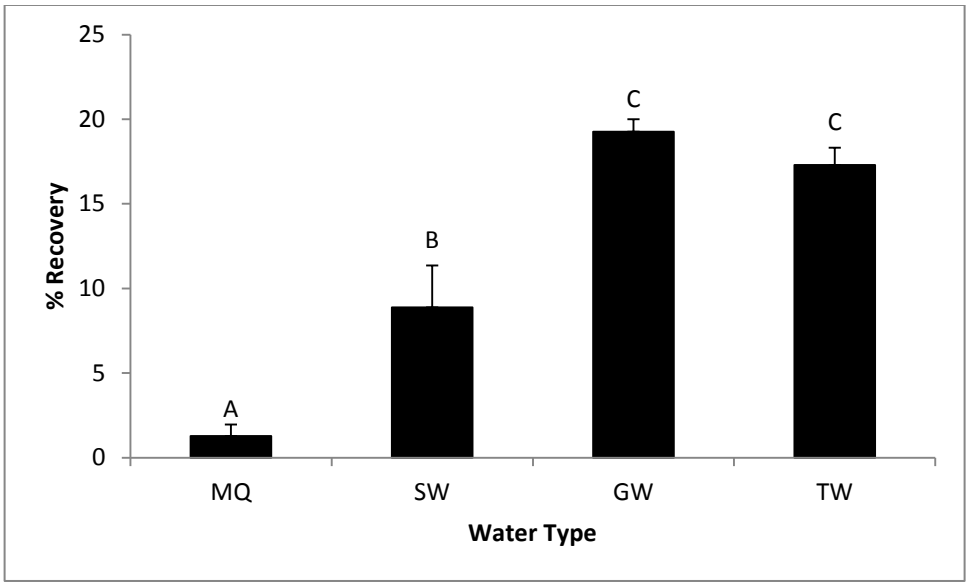


Figure 3.5 Percent recovery of 2,4-D as determined by HPLC for MilliQ (MQ), surface water (SW), dechlorinated tap water (TW) and ground water (GW). ANOVA followed by Tukey's HSD were used to determine significant differences (A,B,C) in recovery between different water types (mean, +SD, n=3).

4. Discussion

The aim of this project was to demonstrate the ability of CDI to remove and recover MS2 and 2,4-D from different types of water for the purposes of establishing CDI an alternative technology for the concentration of environmental contaminants from water. Current technologies for the concentration, such as charge-based filtration to capture viruses and solid-phase extraction of pesticides, of contaminants are time consuming, labour intensive and produce highly variable results; there are no universal concentration methods that capture a large range of each contaminant class equally well. Thus, the ease of use, cost, and energy efficiency make CDI an attractive alternative option. CDI was hypothesized to take advantage of the negative charge of both viruses and acidic herbicides at neutral pH by attracting them to the positively charged electrode. Thus, CDI could potentially remove these contaminants from different water types and subsequently recover them in the concentrated effluent for detection and quantification. In the present study CDI achieved up to 4-log removal (depending on the water type) of MS2, though the recovery of MS2 from the purge was highly variable. CDI also achieved greater than 1-log removal of 2,4-D, depending on the water type. Recovery of the pesticide from the purge was less than 25% for all the water types.

4.1 MS2:

4.1.1 Removal of MS2 by CDI is influenced by water type

CDI has been primarily used for removal of total dissolved solids from waste waters and desalination of brackish waters (Oren, 2008; Porada et al., 2013). However, the removal of microorganisms from water using CDI technology has not been examined in depth. While capacitive electroadsorption has been previously shown to remove bacteria (Golub et al., 1987;

Oren et al., 1983), the removal of viruses using this technology has not yet been demonstrated. In the present study, the ability of CDI to remove and recover MS2 from various water types, due to the net negative charge of the virus at neutral pH, was tested (Michen and Graule, 2010). Furthermore, the results obtained using two different methods for enumeration of MS2, culture-based and molecular, were compared.

CDI was shown to remove MS2 from a variety of solutions (**Figure 3.1**). The removal of MS2 from different water types using CDI ranged between 0.81 – 2.5 and 0.55 – 4.5 log as determined by RT-qPCR and plaque assay, respectively (**Figure 3.1**). Conventional water treatment methods, including coagulation, flocculation, clarification and rapid sand filtration, as outlined by Health Canada, is given credit for an overall 2 log removal of enteric viruses (Health Canada, 2012). The removal of MS2 from the phosphate buffered solutions is comparable to conventional methods, since removal from lower concentration PBS was greater than 2 log, as determined by both plaque assay and RT-qPCR. Similar results were obtained with the bacterium *E. faecalis*, in which the same conductivity PBS had approximately 2 log and 4 log removal of the bacteria based on culture and molecular data, respectively (Habash Lab, unpublished data, see Appendix 2). The removal of MS2 from higher concentration PBS was significantly lower than from the low concentration PBS, with removal of 1 and 2.8 log, as determined by RT-qPCR and plaque assay, respectively. Similar results were obtained using *E. faecalis*, where 0.7 and 3 log removal was observed from the higher conductivity PBS (culture and molecular data, respectively). Therefore, conductivity does have an effect on the ability of CDI to remove microorganisms, which is in agreement with previous work examining the impact on conductivity on the removal of nitrates (Broséus et al., 2009).

The differences in removal of MS2 from different water types were potentially due to increased competition for the electrode surfaces from a large number of ions. Increased levels of organic materials have been shown to lower electrode adsorption capacity for varying ionic species due to fouling of the electrode (Gabelich et al., 2002). The findings in the present study show a similar trend, with the PBS-low (147 µS/cm) having higher removal than PBS-high (1759 µS/cm), and both control solutions having higher removal than the environmental water samples (567-867 µS/cm) (**Figure 3.1**). Furthermore, the removal of MS2 from tap water (700 µS/cm) was higher than from surface water (567 µS/cm), when analyzed by plaque assay, despite surface water having a lower conductivity. Spiking experiments showed no difference in viability of MS2 when suspended in the different matrices over the course of sample processing time (5 hours) (**Figure 3.3**). Thus, the different matrices alone did not contribute to a decrease in MS2 viability for any of the experiments. These results suggest that both conductivity and matrix composition have an effect on the removal of MS2 by CDI.

Removal of the MS2 from various water samples varied depending on which enumeration method was used: RT-qPCR or plaque assay (**Figure 3.1**). RT-qPCR analysis found removal of MS2 from the environmental waters was approximately 1 log, although with non-statistical difference from the high-concentration of PBS, even though the conductivities of the environmental water were more than 800 µS/cm lower. However, plaque assay analysis found MS2 removal from the environmental water types was more variable, ranging from 0.5 (surface water) to 1.8-log (tap water). Ground water had approximately 1 log removal of MS2, and was not significantly different from surface or tap water, though it was significantly lower than both PBS treatments. This is in contrast to the *E. faecalis* experiments (see Appendix 2), which found that via molecular analysis, tap water did not have significantly different removal than either

PBS treatment. Culture-based enumeration did find significantly less *E. faecalis* removal from tap water than from PBS-low, but not from PBS-high. Therefore, CDI is able to take advantage of the negative charge of MS2 in water, similar to bacteria. Our findings expand on the potential applications of CDI with respect to removal of microorganisms from water, though further optimization is required to obtain levels of removal comparable to conventional water treatment for environmental waters.

4.1.2 Molecular and culture-based enumeration indicate degradation of MS2 by CDI

Two analytical methods were used to enumerate MS2 in the water samples: plaque assay (enumerates intact infective MS2) and RT-qPCR (enumerates genomic copies from virions independent of viability). These two methods are both commonly used for the enumeration of viruses from water as each provides information on the presence of the virus. The differences between plaque assay and RT-qPCR for the purposes of enumerating microorganisms in environmental samples are well known (Anderson et al., 2011; He and Jiang, 2005). Although qPCR is a more sensitive method, the inability to distinguish between infectious and non-infectious particles (Anderson et al., 2011; He and Jiang, 2005) may result in overestimating the number of viable microorganisms present in an environmental sample (He and Jiang, 2005). On the other hand, the plaque assay detects only viable virions and cannot distinguish between removal and inactivation. The use of both methods in the present study allowed for the analysis of removal and recovery while determining if CDI had an effect on the infectivity of the virus.

In the present study, there were no significant differences found by the two analytical methods for the levels of removal from the three environmental water types, although the concentrations of influent were consistently higher when enumerated using RT-qPCR compared to plaque assay (10-594 fold higher depending on the water type (see Appendix 4 for details))

(**Figure 3.1**). The plaque assay found significantly higher levels of removal than RT-qPCR from both PBS control solutions (**Figure 3.1**). One possible reason the plaque assay may have shown higher levels of removal is that this method is culture-based, and thus depends on the MS2 remaining infectious for enumeration. Any inactivated MS2 particles, even if they were not removed from the pure stream, would not be able to infect the host *E. coli* to produce plaques; this would prevent them from being enumerated in the pure via plaque assay. Therefore, the removal as determined by plaque assay would be overestimated due to the presence of inactivated MS2 virions in the pure solution. The RT-qPCR, which enumerates by detecting a target gene, does not depend on the virus remaining infectious. Thus, if there is inactivated MS2 remaining in the pure, as long as the genome is intact, it would be detected, and the removal would not be as high as a culture-based method of enumeration. The presence of inactivated MS2 in both the purge and pure samples, as well as the proportion of virions that were not recovered, suggest there may be some inactivation of the viral particles. In previous experiments with *E. faecalis* (Appendix 2), low recovery of the bacteria (around 25%) was determined by both methods of analysis. Taken together, our data indicate potential degradation of microorganisms and their genetic material by CDI.

4.1.3 Potential inactivation of MS2 by CDI via electrochemical disinfection

The removal of MS2 was hypothesized to be due to adsorption of the virus to the electrodes, which would allow for recovery in the purge. However, the recovery of MS2 in the purge was highly variable, with some experiments having very little of the total virus recovered (e.g. the low concentration PBS and tap water had less than 10% recovery as determined by the plaque assay enumeration) (**Table 3.2**); the high levels of removal and variable purge recoveries suggest that there may be some inactivation of the MS2 occurring during CDI. Electrochemical

disinfection of microorganisms via electrolysis is a well-described phenomenon (Kraft, 2008). Electrolysis is a process in which otherwise non-spontaneous chemical reactions may occur in due to the presence of an electric field. In the context of CDI, the flow of electric current through the solution may result in electrolytic reactions at the surface of the electrodes. For the PBS experiments, several possible known disinfecting agents may have been produced based on the content of the ionic species, in particular chlorine-containing salts. Hypochlorite, hypochlorous acid, hypochlorate, and perchlorate may form as the result of electrolytic oxidation of chloride ions from the PBS at the anode (Kraft, 2008). Hypochlorite and hypochlorous acid are both used as sources of free chlorine for microbial disinfection, including viruses, in water treatment plants (Health Canada, 2012). Free chlorine acts as a strong oxidizing agent, which can induce conformational changes in proteins on viral capsids, rendering them unable to replicate (Page et al., 2010; Sano et al., 2015). However, the exact mechanism by which replication is inhibited is still being studied (Li et al., 2011; Wigginton et al., 2012). The amount of viral inactivation due to free chlorine depends on pH, temperature, chlorine concentration, and exposure time (Environmental Protection Agency, 1999). Inactivation of MS2 via free chlorine cannot be ruled out in the present study due to the presence of chloride ions in the PBS (Section 2.2.1). Future studies are needed to examine the effects of chloride ion content on MS2 removal by testing different buffer compositions at varying concentrations. Additionally, the free chlorine and total chlorine of the influent and effluent solutions can be compared using a colourimetric N,N-diethyl-p-phenylenediamine test, such as Standard Method 4500-ClG (APHA et al., 2012). Additionally, if electrolytic reactions are occurring within the CDI cell, purified and concentrated effluent should be tested for the presence of harmful disinfection by-products, such as chlorite, chlorate and haloacetic acids (Health Canada, 2014a).

Electrolytic hydrolysis on the anode can also result in the oxidation of water to produce oxygen gas. The reduction of oxygen gas may occur on the cathode to form hydrogen peroxide in the presence of excess oxygen in the water (Kraft, 2008). Inactivation of viruses, including MS2, by hydrogen peroxide has been previously demonstrated (Pedahzur et al., 2000). The exact mechanism of inactivation of microorganisms is currently unclear, though it is commonly accepted that large biomolecules get oxidized and degraded by the by-products of H_2O_2 reactions and decomposition (Linley et al., 2012). These by-products may include superoxide and hydroxyl radicals (Linley et al., 2012). In particular, hydroxyl radicals are produced through a series of metal catalyzed reactions known as the Fenton process (Nieto-Juarez et al., 2010). MS2 inactivation from 10^7 PFU to $<10^3$ in under 15 minutes has been demonstrated to occur in the presence of a metal ion and H_2O_2, suggesting hydroxyl radicals as the cause of inactivation (Nieto-Juarez et al., 2010). Complete inactivation of viral particles has been observed using 1% solution of H_2O_2 exposed for a two hour period. However, solutions as low as 0.27% have also been commercially used for disinfection of viruses (Dembinski, 2014; Linley et al., 2012). Although hydrogen peroxide concentrations were not measured in the present study, there are numerous analytical methods available for the determination of hydrogen peroxide content in water. A colourimetric test based on the reduction of copper by hydrogen peroxide in the presence of 2,9-dimethyl-1,10-phenanthroline (DMP) allows for the detection of as little as 0.80 µM in a 1 mL cuvette (Kosaka et al., 1998). Monitoring the production of hydrogen peroxide in future experiments could serve to validate or disprove this mechanism of disinfection.

The Enpar ESD unit uses a 1.2V potential difference between the electrodes in order to minimize the electrolysis of water. However, an earlier study demonstrated the electroadsorption

of *E. coli* to carbon electrodes and evidence for chlorine disinfection at potential differences over 1.1V when using buffers containing chlorine (Oren et al., 1983). Lowering the potential difference to 0.8V did not impact the viability of the bacteria (Oren et al., 1983). In a different study which examined bacterial absorption from a saline solution with graphite electrodes at 1.5V potential difference, no water splitting occurred and *E. coli* was recovered after desorption (Golub et al., 1987). However, it was noted that bacteria attached directly to the electrode surface were more likely to be damaged than those adsorbed in the diffuse layer of the EDL (Golub et al., 1987). Another group later suggested that the inactivation was due to the direct oxidation of intracellular co-enzyme A from *E. coli* on the surface of the carbon cloth electrodes rather than the formation of disinfectants like hypochlorite and hydrogen peroxide (Matsunaga et al., 1992). Notably, the experiments were carried out at 0.6V and 0.7V as compared to a reference Saturated Calomel Electrode (Matsunaga et al., 1992). Future experiments with CDI could be conducted using a range of potential differences between the electrodes to determine what effect this has on the removal of microorganisms.

A more recent study examining the removal of poliovirus from modified tap water using a combination of a carbon anode and a titanium cathode coated in platinum iridium oxide (Kondo et al., 2004). Poliovirus was completely removed from solution when potential differences of 8.5V and 2.5V were applied, as determined by both immunofluorescence and a TCID-50 assay (Kondo et al., 2004). Additionally, RT-qPCR analysis of the samples revealed that genomic degradation increased as exposure time to the electric current increased (Kondo et al., 2004). The formation of hypochlorite, which remained below 0.02 mg/L, was monitored in order to account for possible chlorine disinfection (Kondo et al., 2004). Interestingly, both virus

and genetic material were shown to be degraded on the surface of an electrode even in the absence of chlorine disinfection (Kondo et al., 2004).

In addition to monitoring the effluents for known disinfectants, there is the possibility of virus adsorption without desorption from the electrodes. Although early experiments with porous carbon electrodes and bacteria demonstrated desorption (Golub et al., 1987; Oren et al., 1983), desorption of MS2 from the electrode surface in the ESD unit was not specifically tested. Imaging of the electrode surfaces at different points in the purification cycle may reveal the presence of MS2 on the electrode surface. Although Scanning Electron Microscopy (SEM) may be used to visualize the surface of an electrode, the current CDI set-up would make this difficult. A different set-up, which allows easy access to the electrodes, would enable the visualization of any accumulation of MS2 on the electrode surface due to poor desorption. Additionally, it may also be possible to visualize any structural changes or damage of MS2 capsid resulting from electroadsorption when compared to controls.

4.1.4 Influence of secondary concentration step on highly variable recovery of infective MS2

The amount of MS2 recovered in the concentrated effluent from all experiments was highly variable both within and between experiments and between analytical methods (**Table 3.2**). Nevertheless, viable MS2 particles were consistently detected for all treatments in the present study. The variability of the amount of MS2 in the concentrated effluents may be due to, or at least influenced by, several factors. The preferential adsorption and desorption of chemical ions or virions may vary depending on the ionic species present in the water matrices (Huang, 2016; Xu et al., 2008), which may account for some of the variability within and between the different water treatments. Additionally, the water matrix can influence the recovery of MS2 virus from the Zeta Plus 60S filters used during the secondary concentration step (**Figure 3.2**).

This has also been observed for other positively charged filters used to concentrate viruses (see **Table 1.2**). The recovery of MS2 from PBS was the highest, with a mean recovery of 14%, which was almost four times greater than the next highest recovery (ground water, with 3.8% recovery). Surface water and tap water both had recoveries of less than 2% and were not statistically different. Although the control PBS solution had higher conductivity than the environmental water types, it did not contain the mineral and organic materials present in the environmental water samples that may have reduced recovery of MS2 from the filters.

The variability of MS2 recovery from the filters among water types was evident in the MS2 sample data (Appendix 4). Since the influent solutions were all spiked to have 10^4 PFU/mL from MS2 freezer stocks with known concentrations and viability data did not indicate inactivation due to water type alone (**Figure 3.3**), the variability was attributed to the secondary concentration step. While the 0.1mM PBS samples had influent concentrations of infective MS2 at the expected level (10^4 PFU/mL), all other water types had lower concentrations. The 1mM PBS, tap and ground water samples all had concentrations on the order of 10^3 PFU/mL, while surface water had 10^2 PFU/mL in the influent. If a sample had a lower influent concentration due to poor recovery off of the filter, this would bias the results towards a lower level of removal. Furthermore, the reproducibility of the results from a given water type may be poor due to variable recoveries off of the filter. The coefficients of variation for PBS and ground water recoveries were 58% and 103%, respectively (**Figure 3.2**). In summary, the secondary concentration step introduced additional variability to the results.

4.1.5 Improving reproducibility of MS2 recovery from concentrated effluent

There are several ways that future studies of CDI could improve the reproducibility of the purge samples: ensure homogenous sampling, the use of a different secondary concentration

step, and the minimization of the purge volume to eliminate the need for a secondary concentration step. Virions have been shown to be non-homogenously distributed in water matrices (Floyd and Sharp, 1977). Aggregation of hundreds of virions from homogenously distributed virus stocks may be caused by a change in viral particle concentration (i.e. dilution) or ionic concentration, or a decrease in pH (Floyd and Sharp, 1977). Viral aggregation in the concentrated effluent may have resulted in non-representative samples being used for both the plaque assay and RT-qPCR. This could contribute to the variability of the mean recoveries both among different water types with the same analytical method, as well as within a water type between analytical methods. For example, the standard deviations for the mean recovery values for all the treatments, except ground water, were almost the same as the mean value. A non-homogenous distribution of viral particles could decrease reproducibility within experimental replicates. An additional sample processing step could be added to disrupt viral aggregates in all samples before plaque assay and RNA extraction. Common disaggregation techniques include sonication (Muller, 1976; Sharp et al., 1975) or vortexing the sample with an equal volume of chloroform (Thurston-Enriquez et al., 2003).

Although the long-term goal is to eliminate the need for a secondary concentration step, for future bench-scale studies, an alternative secondary concentration step with less variability could be used, such as filtration by size exclusion. For example, MS2 has a molecular weight of 3.5MDa (Kuzmanovic et al., 2003), which is large enough to be retained by the centrifugal ultrafiltration devices with a molecular weight cut off above 100,000 Da. Size exclusion filtration may also be less affected by water matrix composition as compared to charge-based filtration, reducing variability between treatments. Additionally, centrifugal ultrafiltration

devices allow for a much smaller final sample volume (i.e. on the scale of µL instead of mL), which would allow downstream analysis to be more representative of the sample.

Optimization of the ESD settings, such as the thresholds for the pure and purge streams, as well as the lengths of time for each step of the cycle, for each water type before conducting spike experiments may be used to accurately gauge the recovery of MS2. Direct sampling for the plaque assay could be employed, if the volume of the concentrated effluent can be minimized. For each experiment, the amount of water purified before the regeneration step could be increased. This would be possible by either increasing the flow rate of the water and/or increasing the length of time of the purification step until the electrodes are fully saturated, which would be determined by monitoring conductivity of the pure effluent. However, increasing the flow rate of the purification may decrease the time the ions have to adsorb to the electrodes and thus may reduce removal. Additionally, the concentrated effluent volume released during each purification cycle could be reduced by raising the set point for the low conductivity threshold, so that only highly concentrated effluent is released. Below this threshold, the rest of the concentrated effluent is cycled through the ESD unit again via the return-to-feed-valve. However, a continually increasing conductivity of the influent solution may result in reduced removal of MS2 due to increased competition for electrode surface area. Additionally, as the concentrated effluent volume is reduced and the concentration of ions increases, which may require dilution of the samples to eliminate any inhibitory effects for both plaque assay and RT-qPCR. However this may not be an issue when the technology is scaled up for water treatment. If there is always a new influent stream of water being added to the recirculating effluent from the return to feed valve, the influent conductivity would not continually increase. The use of one or more of the suggested additional steps in conjunction with the methodology described in the

present study may increase the reproducibility of the enumeration of the purge samples and allow for an accurate determination of the recovery of MS2 in the purge samples in the future.

4.2 2,4-D

4.2.1 Removal of 2,4-D by CDI is influenced by water type

While CDI has been previously used to remove and recover small molecules, such as salts and TDS, the removal of larger chemical compounds has not been studied. The level of removal of small molecules, from single-atom ions to molecules like nitrate and phosphate by CDI ranges from 42% to over 90% (**Table 1.6**), while the removal of 2,4-D in the present study ranged from 66 % to 95% (**Figure 3.4**) depending on the water type. The high removal observed in the MilliQ water treatment is only 5% lower than the levels of 2,4-D degradation achieved by other electrochemical technologies, such as electrolysis and photodegradation, which can achieve 100% removal of the pesticide in optimized experiments (Chaudhary and Grimes, 2001; Oturan, 2000; Zourab et al., 2009). Therefore, this study found that the acidic herbicide 2,4-D can be removed from different types of water samples, although the potential contribution of electrochemical degradation was not specifically examined (**Figure 3.4**). Furthermore, the removal of 2,4-D was comparable to the removal of other smaller contaminants.

The extent of removal varied between water types. As discussed previously, the performance of CDI can be influenced by the conductivity and the matrix composition (Appendix 2) (Gabelich et al., 2002). The highest degree of removal was achieved for MilliQ water, possibly due to a lack of competition for electrode surface area from any other ionic species. Tap water had the second highest removal, even though it had a higher conductivity than the surface water. This may be due to the fouling of the electrodes by organic materials

commonly present in the surface water (Gabelich et al., 2002). Preferential adsorption of ionic species in the surface water may have further reduced the removal of 2,4-D. Groundwater had the highest conductivity but showed lower 2,4-D removal than tap water, though not significantly different from surface water. The removal of 2,4-D from tap water was significantly higher than from the other environmental water types. This differs from the MS2 experiments, in which tap water was not significantly different from surface water (via both analytical methods) and ground water (according to RT-qPCR data only). Conductivity is therefore not the only factor influencing the degree of removal of 2,4-D. Experiments involving the spiking of 2,4-D into different simulated waters with the same conductivities could help determine which chemical compounds reduce the removal of 2,4-D while controlling for the influence of conductivity.

4.2.2 Low recovery of 2,4-Dfrom the concentrated effluent suggesting degradation

For the applications of desalination and removal of TDS, the recovery of ions from CDI is close to 100% (personal correspondence, ENPAR). In contrast, CDI recovery of 2,4-D was below 20% for all types of water samples in the present study (**Figure 3.5**). The MilliQ water had the highest removal rate of 2,4-D, but showed the lowest recovery rate. Since there was a minimum of other ions present to compete for electrode surface area compared to other sample types, 2,4-D may have participated in electrolytic reactions which occurred on the surface of the electrodes. In the environmental waters, the competition from other ionic species for electrode surface may have reduced the amount of 2,4-D on the electrodes, as increased conductivity has been shown to decrease removal of target analytes (Broséus et al., 2009). If there were less 2,4-D on the surface of the electrodes, degradation due to electrolytic reactions occurring on the electrode surface would be decreased, allowing for a higher recovery of the herbicide.

One possible reason for the low recovery of 2,4-D from all of the experiments may be that it was degraded during removal by CDI. Electrolysis of water on various electrode materials leading to the oxidative degradation of 2,4-D has been previously demonstrated (Zourab et al., 2009). Experiments examining the potential of electrolysis to degrade organic contaminants from water are often conducted with the addition of ferrous iron as a catalyst to produce hydroxyl free radicals (Chaudhary and Grimes, 2001; Li and Goel, 2010; Oturan, 2000). While the MilliQ experiments did not contain any added materials other than 2,4-D it is possible that impurities in the solution or ESD unit could have been oxidized to produce hydroxyl free radicals. Therefore, the mineralization of 2,4-D, leaving only carbon dioxide, hydrochloric acid, and water, cannot be ruled out (Oturan, 2000). Notably, the products of mineralization would not be detectable via HPLC analysis. As well, mineralization would potentially produce a more acidic purified effluent than influent, which was observed (**Table 3.4**). However, the acidic pure effluent may also be a result of water splitting alone (ENPAR, personal correspondence, 2015)

Lastly, it is possible that the HPLC method used in the present study did not detect polar degradation products; there were no additional peaks in the HPLC chromatograms of the concentrated and purified effluents when compared to the influent chromatograms (Appendix 3). The HPLC method would also not produce a signal for non-polar by-products (such as benzene) present in quantities below the LOD. Using a method of analysis that does not rely as heavily on the structure of 2,4-D as does HPLC may better determine if 2,4-D is being oxidized. One option is to take measurements of Total Organic Carbon (TOC) from the influent and effluent solutions. If the TOC declines from the influent to the purified effluent and is still present at low levels in the concentrated effluent, it suggests that 2,4-D is being degraded within the ESD unit. Another method to determine the fate of the 2,4-D during CDI would be to use radioisotopic-labeled2,4-

D. The fate of 2,4-D metabolism in plants as well as in bacteria in soil has been studied by substituting ^{14}C into the carbon ring of 2,4-D (Fulthorpe et al., 1996; Girardi et al., 2013; Schultz and Burnside, 1980). The [^{14}C]2,4-D content of the influent, pure, and purge samples would be analyzed using a method that can detect the beta emissions of the ^{14}C, such as Liquid Scintillation Counting (Girardi et al., 2013), in addition to HPLC. If the amount of ^{14}Crecovered is significantly less than was input, this suggests the loss of the carbon as CO_2, which would indicate the mineralization of the 2,4-D. As well, the fractions of the samples eluting off of the HPLC column at different times could be collected separately to correspond to different peaks and then analyzed for the presence of ^{14}C in order to determine if there are different degradation products present. If 2,4-D is being degraded via oxidation due to electrolysis in the ESD unit, the operating conditions of CDI would need to be optimized to minimize the electrolysis in order to increase recovery of the herbicide for purposes of concentration.

4.3 Potential Applications and Future Directions

The efficient removal of MS2 and 2,4-D (as well as *E. faecalis* from previous work, Appendix 2) combined with the low levels of recovery from the 2,4-D and the bacterial experiments suggest that CDI may have applications as an electrochemical method of disinfection of microorganisms and the degradation of organic contaminants. Short-term future work should focus on determining how these contaminants are being removed and if they are being degraded. As well, a variety of factors that could influence removal of contaminants should be studied (presence of different ions, conductivity, voltage, and pH). Once the mechanisms of removal and/or inactivation are better understood, CDI technology could be optimized for two potential purposes: as a tool for recovery, and as a tool for removal of various contaminants. CDI may be used as a concentration step for the detection of various contaminants

by decreasing inactivation/degradation of large molecules and microorganisms. Conversely, CDI may be used as a water treatment technology by increasing the inactivation of the contaminants.

This work originally set-out to study the potential for CDI to be used as a concentration step for the recovery of water contaminants which occur at low concentrations in the environment. The recoveries were consistently low for 2,4-D and *E. faecalis* within a given water type (**Figure 3.5** and Appendix 2). As well, infective MS2 was recovered for each water type, though the recovery of MS2 was too variable to be statistically analyzed (**Table 3.2**). If a consistent recovery of MS2 can be demonstrated through the use of a different secondary concentration step or through minimizing the purge volume, CDI may still have potential as a concentration technology, especially for enteric viruses. The current standard methods for concentrating enteric viruses from water utilize charged filters that have variable results, are labour-intensive, and have trouble with reproducibility even when the same protocols are used (see **Table 1.1** and **Table 1.2**). Given the ease of use for processing large volumes of water, CDI maybe established as a superior option even if the recovery levels are not 100%. Future work should address development and optimization of CDI settings to maximize recovery and reproducibility. Although our results suggest the ability of CDI to degrade large molecules, such as 2,4-D, better understanding of this phenomenon can be applied to future uses of this technology as a concentration tool. If the degradation of large molecules is accounted for, CDI can be further developed for concentration and subsequent recovery of a model pesticide, such as 2,4-D. Since CDI is user-independent, quick at processing large volumes of water, and does not require organic solvents, the technology remains an attractive alternative to the current methodologies.

The demonstration that CDI can simultaneously remove both biological and chemical contaminants suggests that studies should continue to focus on examining removal of a wide range of contaminants. The ability to remove and/or degrade different classes of contaminants in a single step would put CDI in a position to compete with other single-step technologies, such as RO and ED. CDI has the advantage of not using membranes under high pressure, as well as having electrodes with a regenerative capacity, which can reduce operating costs due to lower energy input requirements as well as reduce fouling of the unit. Additional experiments demonstrating the potential of CDI for effective removal should include examining the simultaneous removal of different types of bacteria, viruses, and chemical contaminants. Once the efficacy of CDI for removal of these classes of contaminants is established, different types of contaminants can be combined at increasing concentrations for developing CDI as an on-line, large-scale platform for water treatment. Finally, if the method of contaminant degradation is better understood, the operating parameters of the ESD unit can be optimized to achieve higher levels of contaminant removal that could improve current water treatment.

5. Conclusion

CDI has the potential to be used as a single technology to remove multiple classes of contaminants from different types of water. This study found that CDI is capable of more than 1-log removal of both MS2 and 2,4-D, depending on the conditions. The recovery of MS2 was highly variable across water types; the recovery of the 2,4-D was consistently below 25% for all water types examined. Short-term future work should focus on understanding the mechanisms of removal and chemical factors that influence the degree of removal of different contaminants. Furthermore, the efficacy of removing diverse contaminants in isolation as well as

simultaneously in different water types should be studied to better model its application to water treatment.

6. References

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7. Appendices

Appendix 1: Calculations for CDI Metrics

1. Log Removal – concentration of influent minus concentration of the pure divided by the concentration of the influent converted into %

% Removal= ([Influent]-[Pure])/[Influent]*100%

% Removal Log Transformed = log10 ([Influent]/[Pure])

2. % Recovered in Purge (observed vs. expected) – observed (volume of purge multiplied by concentration of purge) divided by expected amount removed from the influent (volume of pure multiplied by concentration of the influent times the % removal)

= Vpurge*[Purge] / (Vpure*[Influent]* % Removal)

Appendix 2: CDI *E. faecalis* Data

Methods

From an overnight culture of *E. faecalis* (ATCC 19433) in Tryptic Soy Broth, a fresh culture was grown for approximately three hours. The absorbance of the culture was tested at an optical density (OD) of 600 nm until an absorbance of 0.4 ± 0.1, indicating exponential phase growth. The cells were pelleted via centrifugation and the supernatant discarded. Cells were resuspended with 100 mL of 1 or 0.1 mM PBS or dechlorinated tap water and the absorbance at OD_{600} was determined. The concentrated bacterial culture was diluted in 1 or 0.1 mM PBS or dechlorinated (depending on experiment) to give an absorbance (OD_{600}) of approximately 0.008 in a volume of 100 mL.

The diluted culture was added to 9.9 L of influent to give a final volume of 10 L and 10^4 CFU/mL of the specific bacteria. Before running the experiment, samples were taken from the 10 L influent: 10 mL for qPCR and 15mL for drop counts. Cleaning of the ESD and experiment protocol unit followed previously described methods (see Section 2.1). After the experiment, 4 L of pure sample was retained for qPCR analysis, and 15 mL sampled for drop counts. The purge samples were 10 mL and 15 mL for qPCR analyses and drop counts, respectively.

For culture-based enumeration via drop counts, 10-fold dilution series in 10 mM PBS were prepared from each influent and purge sample. Each dilution was plated as a 20 µL drop, in triplicate, on Tryptic Soy Agar plates. For the pure sample, triplicate spread plates were created from volumes of 1, 0.1 and 0.01 mL. All plates were incubated at 37˚C for 24 hours, after which they were enumerated.

For molecular enumeration, the retained 10 mL influent and the purge samples were centrifuged at 4150 RPM for 20 minutes at 4 ˚C. The supernatant removed and the remaining

cell pellet was stored at -20 ˚C until the DNA extraction was performed using the DNeasy® Blood and Tissue kit (Qiagen, Hilden, Germany), following manufacturer's directions. The DNA was eluted with 100 µL nuclease-free water. The extracted DNA was stored at -20 ˚C until analysis via qPCR.

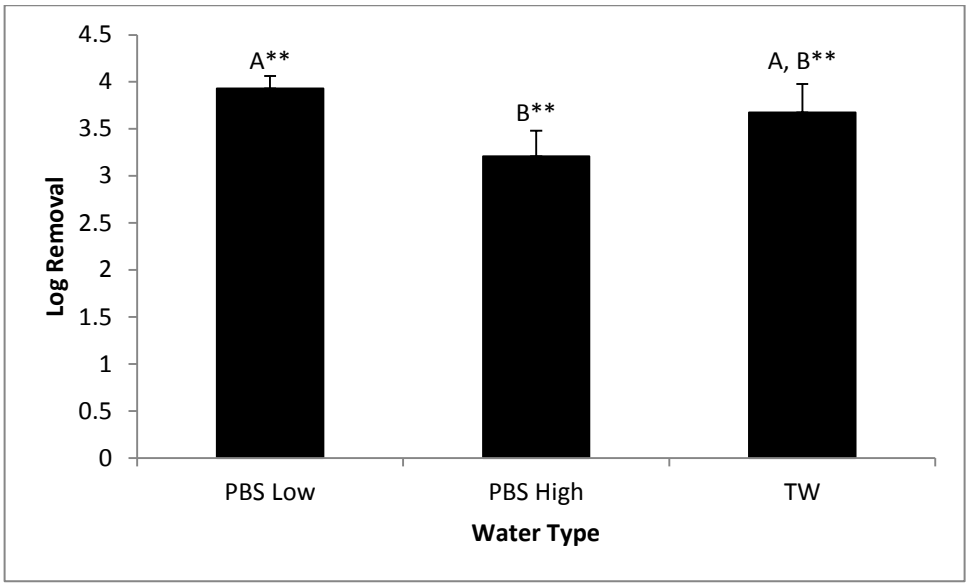
Prior to DNA extraction of the pure sample, a secondary concentration step for the 4 L of retained pure sample was used, based on "Standard operating procedure for DNA extraction from raw water. Version 4.0 MICSOP.021" (Weir, S., and Abbey, A.I. 2011). Briefly, 4 L of pure was passed through membrane filtration to capture *E. faecalis*. The filter was placed in a sterile centrifuge tube and 2 mL of 5 M guanidine isothiocyanate buffer was added to lyse the cells, after which DNA extraction was performed, using the DNeasy® Blood and Tissue kit (Qiagen, Hilden, Germany), following manufacturer's directions. The DNA was eluted with 100 µL nuclease-free water. The extracted DNA was stored at -20˚C until analysis via qPCR.

For qPCR, primers targeted the 23SrRNA gene of *E. faecalis* (Haugland et al., 2005). This region was cloned into the pGEM Easy T Vector system (Promega, Fitchburg, WI). This construct was used to create standard curves of serially diluted plasmid ($5x10^7$ to $5x10^1$ copies per reaction), which were prepared in duplicate for each run. An iQ5 PCR Thermal Cycler was used (Bio-rad, Mississauga, ON, Canada). SSoFast EvaGreen Supermix (Bio-rad, Mississauga, ON, Canada) was used as directed, with primers at a final concentration of 300 nM. The cycling protocol consisted of an initial activation step of 98 ˚C for three minutes; 98 ˚C for 5 seconds and then 60 ˚C for 10 seconds, repeated for 40 cycles; a melt curve from 60 ˚C to 95 ˚C, increasing by 0.5 ˚C intervals. For data analysis, the Bio-rad iQ5 software automatically determined the baseline threshold. Only standard curves with efficiencies between 90-105% and R^2 values

between 0.950 and 0.999 were used, in order to sure consistent product amplification between plates.

Results

A) qPCR



B) Culture-based Enumeration

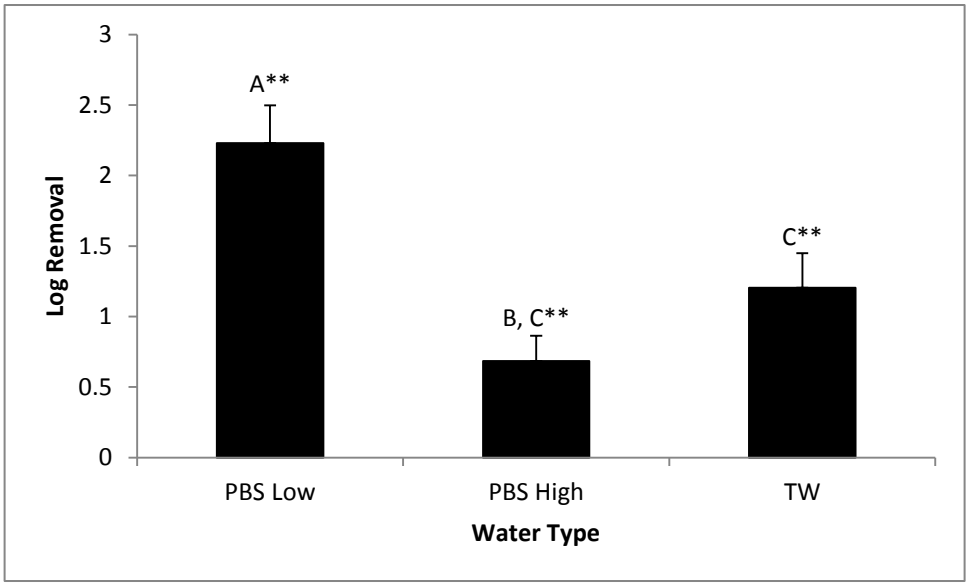
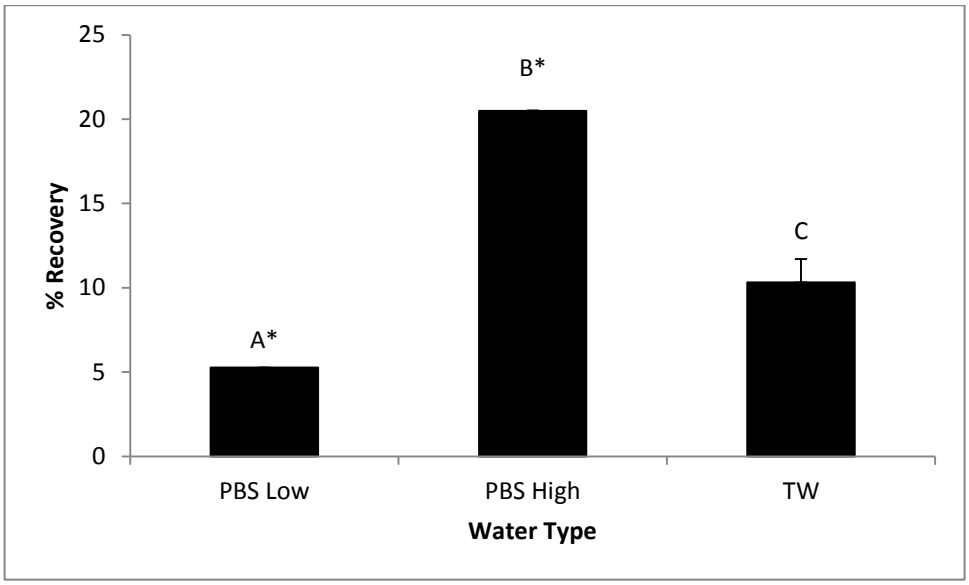


Figure 7.1 Log removal of *E. faecalis* by CDI enumerated using A) qPCR and B) culture-based methods. ANOVA followed by Tukey's HSD were used to determine significant differences (A,B,C) within a given method for different water types, and the Student's t-test was used to determine significant differences (*) between the two methods for a given water type (mean, +SD, n=3; **$p<0.01$).

A) qPCR



B) Culture-based Enumeration

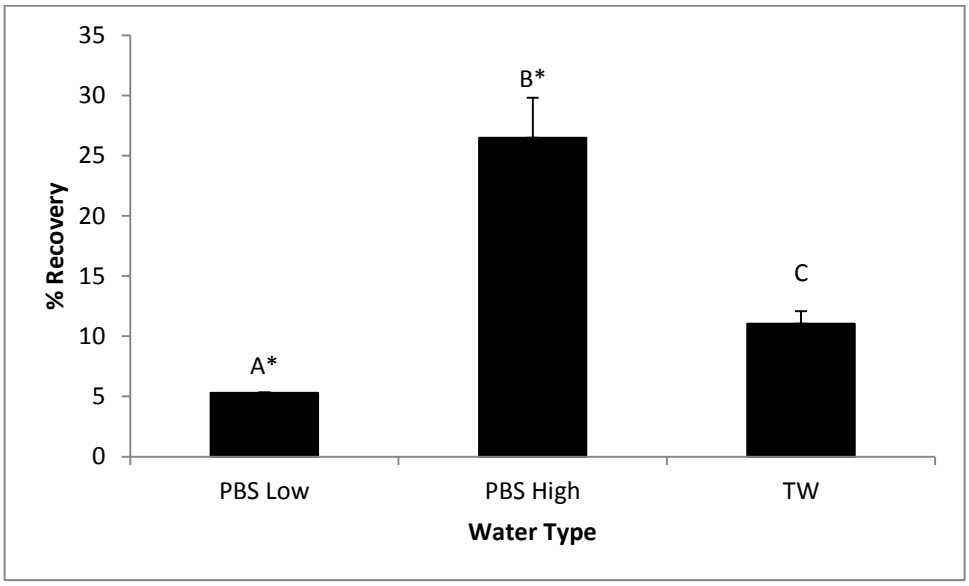
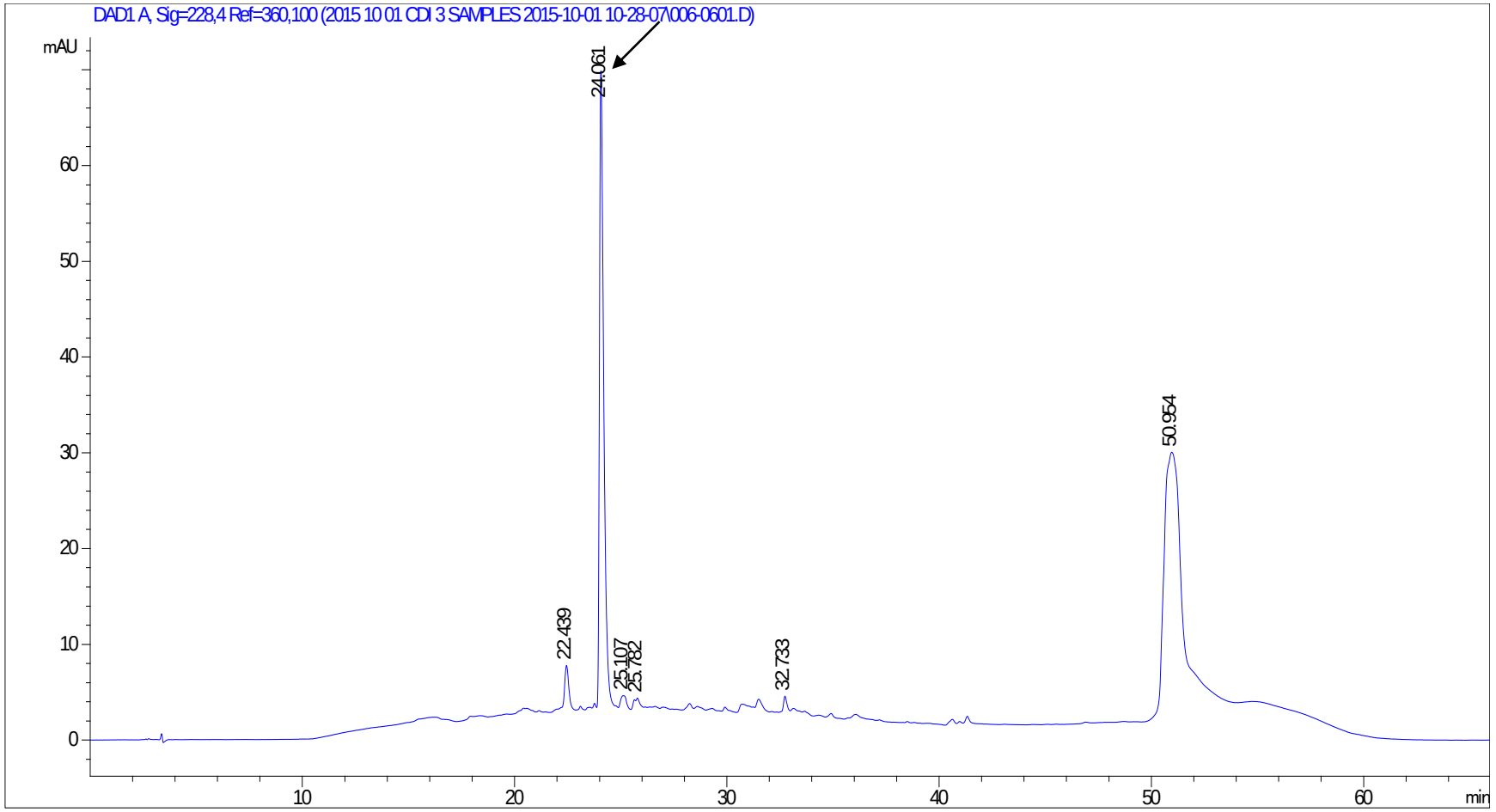


Figure 7.2 % Recovery of *E. faecalis* in purge samples enumerated using A) qPCR and B) culture-based methods. ANOVA followed by Tukey's HSD were used to determine significant differences (A,B,C) within a given method for different water types, and the Student's t-test was used to determine significant differences (*) between the two methods for a given water type (mean, +SD, n=3; *$p<0.05$).

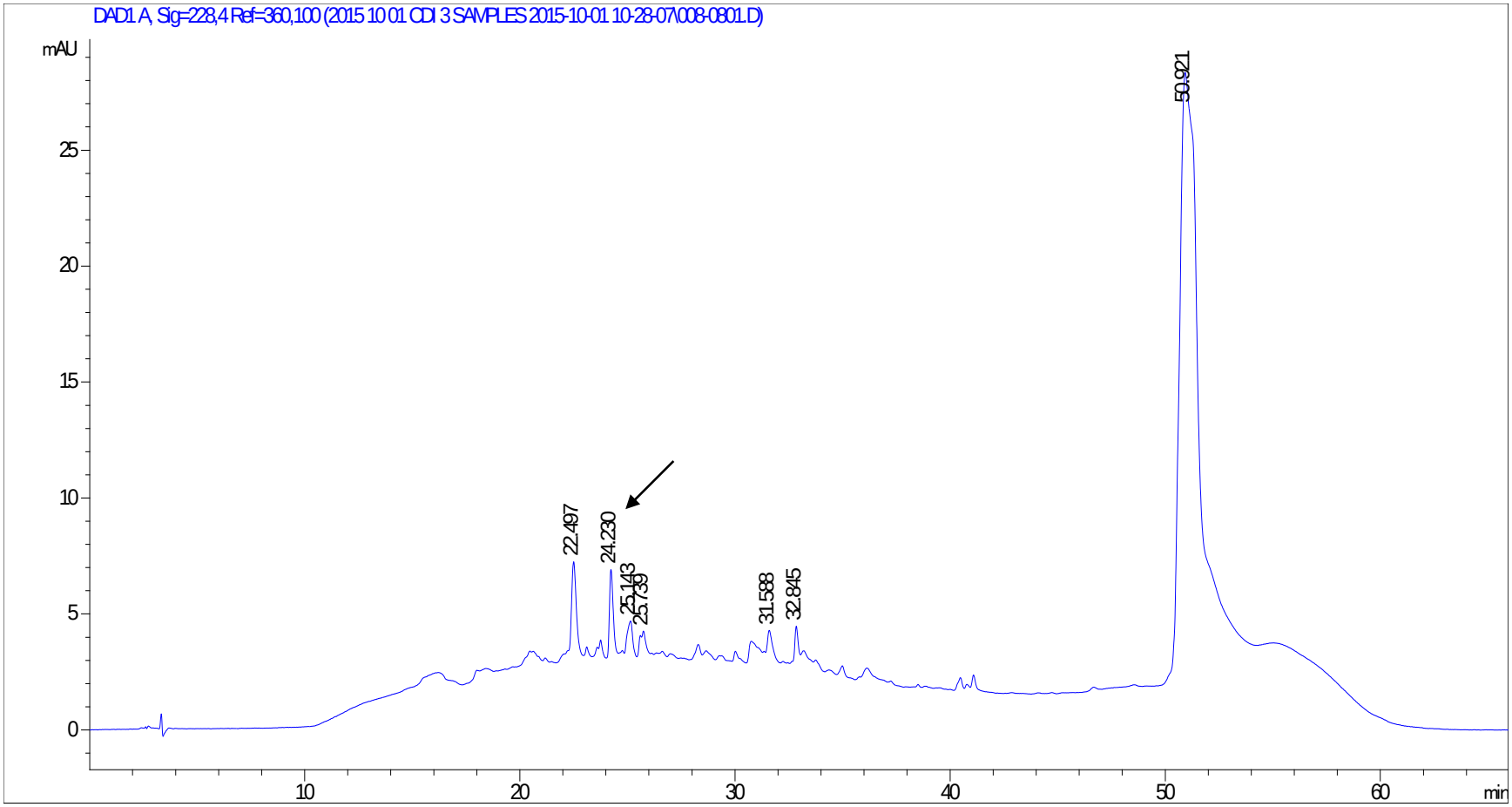
Appendix 3: Representative HPLC Chromatograms

Representative chromatograms of influent (A), pure (B), post-SPE pure (C) and purge (D) samples collected during a single CDI experiment with MilliQ water spiked with 2,4-D. 2,4-D elution peak is highlited with an arrow at ≈24 min.

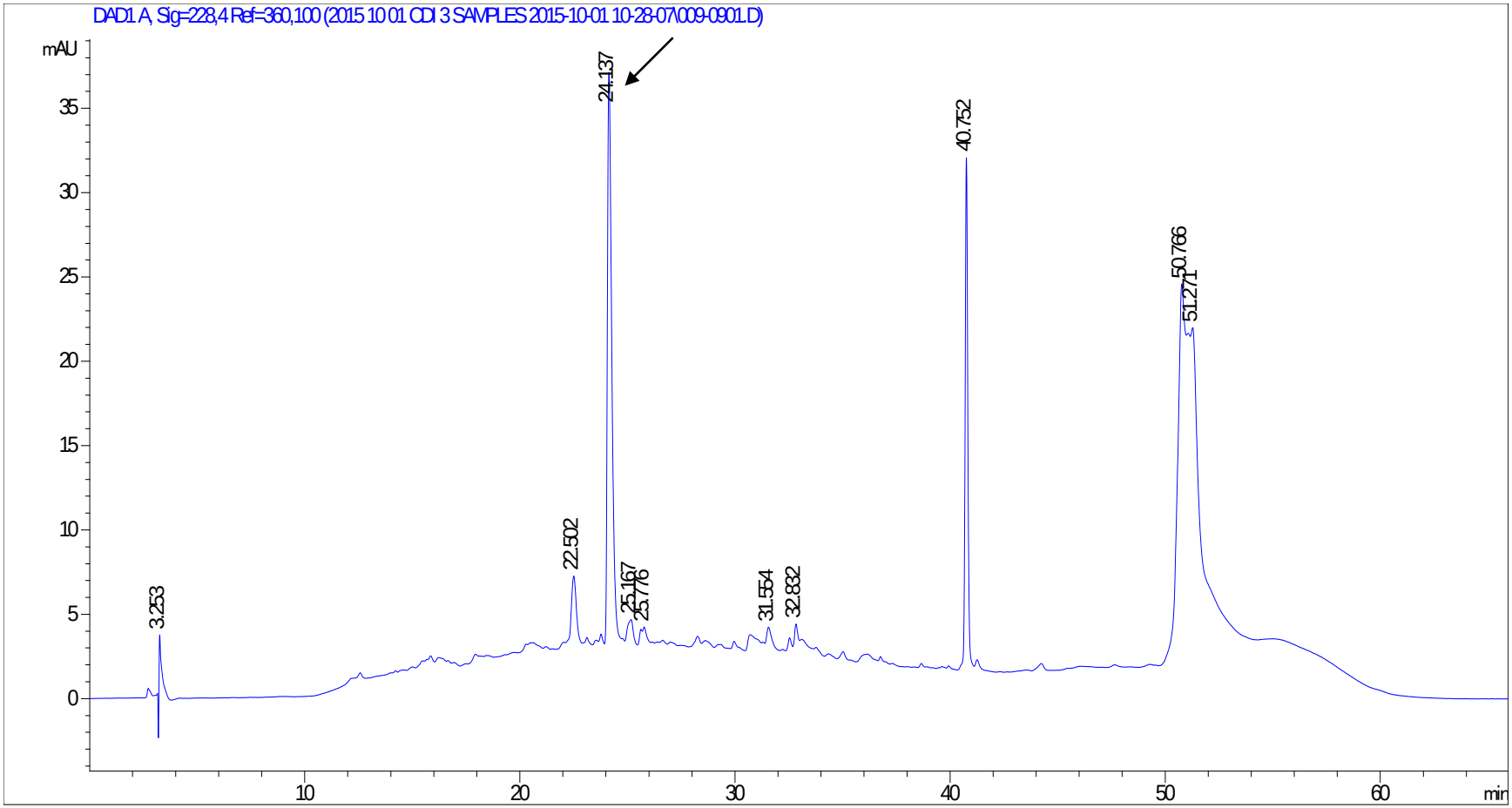
A. Influent



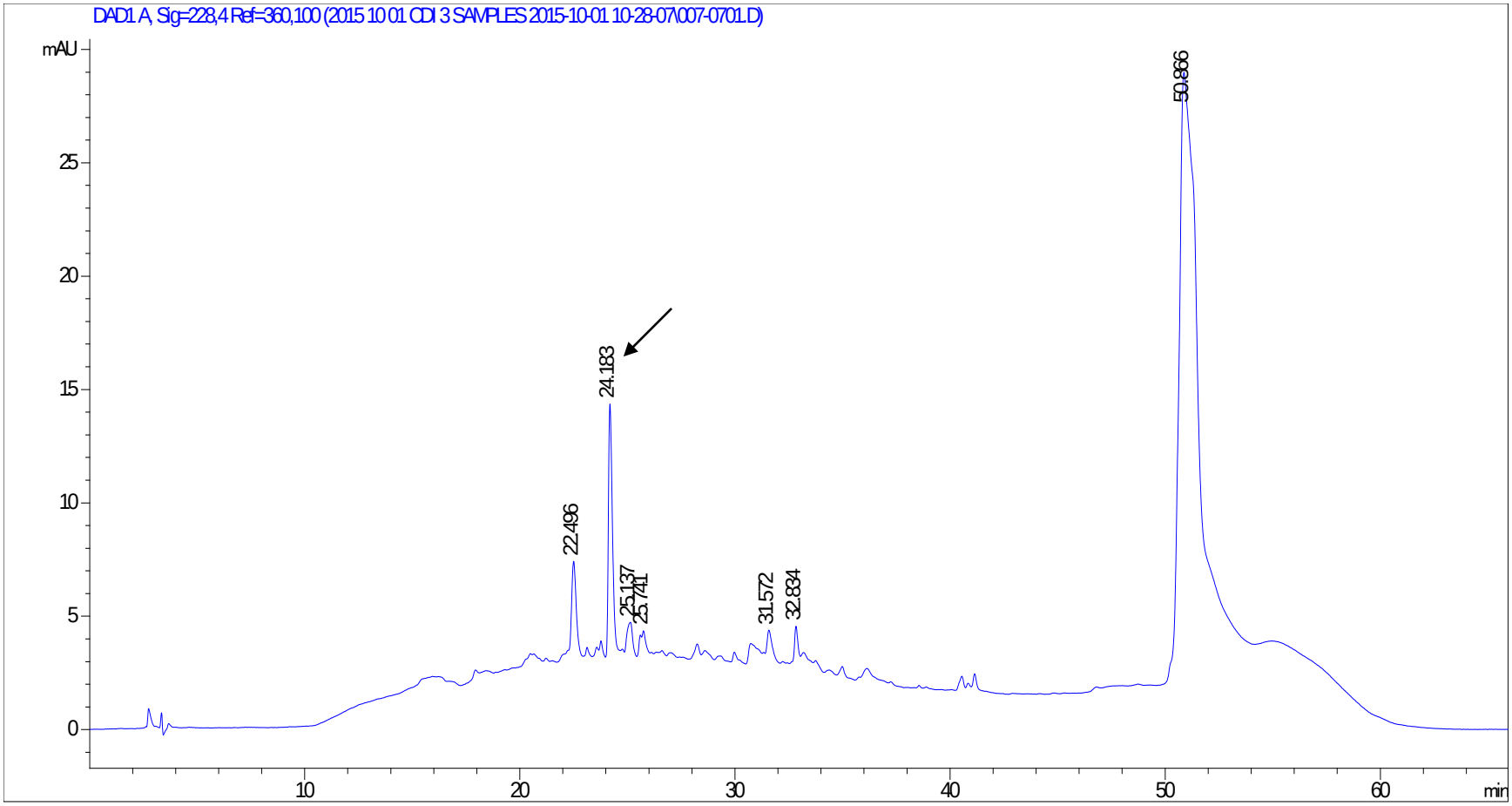
B. Pure


DAD1 A, Sig=228,4 Ref=360,100 (2015 10 01 CDI 3 SAMPLES 2015-10-01 10-28-07\008-0801.D)
mAU
25
20
15
10
5
0
22.497
24.230
25.143
25.739
31.588
32.845
50.921
10
20
30
40
50
60
min

C. Pure post SPE



D. Purge


DAD1 A, Sig=228,4 Ref=360,100 (2015 10 01 CDI 3 SAMPLES 2015-10-01 10-28-07\007-0701.D)
mAU
25
20
15
10
5
0
22.496
24.183
25.137
25.741
31.572
32.834
50.866
10
20
30
40
50
60
min

Appendix 4: MS2 Sample Data

Water type	Experimental replicate	Plaque Assay (PFU/mL)			RT-qPCR (genome copies/mL)		
		Influent	Purge	Pure	Influent	Purge	Pure
0.1 mM PBS	Rep 1	2.24E+02	5.74E+03	1.90E+00	1.67E+07	4.98E+07	1.01E+05
0.1 mM PBS	Rep 2	2.90E+04	4.88E+04	1.90E+00	6.88E+06	3.35E+07	4.83E+03
0.1 mM PBS	Rep 3	4.72E+04	9.20E+03	0.00E+00	9.89E+05	2.13E+07	5.94E+03
1 mM PBS	Rep 1	2.00E+03	9.58E+03	3.70E+00	1.78E+05	4.89E+05	5.39E+03
1 mM PBS	Rep 2	2.18E+03	1.55E+04	4.00E+00	3.87E+04	2.79E+06	1.08E+04
1 mM PBS	Rep 3	3.00E+03	4.13E+03	0.00E+00	2.01E+05	1.44E+06	1.22E+04
Surface	Rep 1	4.42E+02	2.20E+04	1.35E+02	1.87E+05	2.17E+07	2.76E+04
Surface	Rep 2	1.43E+02	3.71E+03	8.00E+01	1.50E+05	1.57E+07	2.82E+04
Surface	Rep 3	6.95E+02	1.38E+03	9.00E+01	2.15E+05	4.88E+06	2.54E+04
Tap	Rep 1	1.87E+03	1.91E+03	1.40E+02	2.49E+04	6.38E+06	1.36E+03
Tap	Rep 2	1.87E+03	1.51E+02	1.50E+01	2.40E+04	2.21E+05	5.92E+03
Tap	Rep 3	3.87E+03	6.06E+03	2.83E+01	1.27E+04	1.03E+06	8.28E+02
Ground	Rep 1	4.78E+03	1.58E+04	4.53E+02	5.00E+04	1.01E+06	1.48E+04
Ground	Rep 2	2.76E+03	7.10E+03	5.65E+02	2.29E+05	1.95E+06	1.04E+04
Ground	Rep 3	4.59E+03	4.94E+03	3.10E+02	2.20E+05	2.19E+06	6.27E+04