

EVALUATION OF A HOUSEHOLD CONTACT DISINFECTION DEVICE FOR
INACTIVATION OF BACTERIOPHAGE MS2 AND MURINE NOROVIRUS

By

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ABSTRACT

EVALUATION OF A HOUSEHOLD CONTACT DISINFECTION DEVICE FOR INACTIVATION OF BACTERIOPHAGE MS2 AND MURINE NOROVIRUS

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Obtaining safe drinking water is problematic in many developing nations around the world. The HaloPure disinfection canister designed by HaloSource was created to provide household water treatment for middle-class families in India. The Waterbird device was tested for its effectiveness in reducing viruses. The main objective of this study was to determine the effectiveness of the Waterbird device in removing or inactivating bacteriophage MS2 and murine norovirus as surrogates for human pathogens. Secondary objectives were to determine the potential impacts, if any, that added organic contaminants (in the form of raw sewage) and pH adjustment have on the effectiveness of the device. The Waterbird device was tested by adding MS2 and murine norovirus stock to five liters of well water (with or without sewage); the pH of the water was adjusted to 7.5 or 9 before treatment. The murine norovirus samples were all reduced to the detection limit, achieving a minimum of 2.0 to 4.0 log₁₀ reductions after treatment, but the performance of the device could not be accurately examined using these results. For the MS2, the Waterbird device inactivated or removed an average of 5.4 log₁₀. The MS2 log₁₀ reduction was affected by pH ($p=0.006$) and sampling times ($p<0.001$). Overall, the Waterbird device met the U.S. EPA's guidelines for water purifiers (which requires at least 4 log₁₀ removal of viruses).

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CHAPTER 1

INTRODUCTION

1.1 SAFE WATER FOR THE DEVELOPING WORLD

Obtaining clean, safe drinking water is something most people in developed countries take for granted, but in many parts of the world it is not so easy to come by. In 2011, the WHO estimated that approximately 11% of the world's population was without adequate access to safe drinking water (World Health Organization, 2011b). Roughly 2.5 million people die every year from diarrheal diseases, with 88% of those cases due to the combination of unsafe drinking water, inadequate sanitation, and poor hygiene (Kosek et al., 2003; World Health Organization, 2011b; Schwarzenbach et al., 2010). About two million of those deaths are children under the age of five, for which unsafe water is the number one killer (Boschi-Pinto et al., 2008; Elimelech, 2006). Diarrheal diseases caused by unsafe water and inadequate sanitation are responsible for 6.1% of all health-related deaths worldwide (Schwarzenbach et al., 2010). Unsafe water alone is thought to be responsible for 15-30% of all gastrointestinal illnesses (Schwarzenbach et al., 2010; Sobsey et al., 2003).

There has been an emergence of waterborne pathogens in recent years, due to an increase in the number of people sensitive to the pathogens, an increase in the importation of food from developing countries (where the water quality is poor), and the natural evolution of increased virulence in microbial pathogens (Reynolds et al., 2008). Environmental change, including climate change, has even been shown to play a role in the increase of infectious

diseases (Eisenberg et al., 2007; Lloyd et al., 2007). Environmental changes can also alter transmission cycles of infectious pathogens and modify human exposure to contaminated sources such as water or infected animals or other humans (Eisenberg et al., 2007). Drought has been shown to increase diarrheal disease, as people may be forced to use lower quality water sources; low rainfall has been shown to be a determinant of the prevalence of childhood diarrheal disease in developing countries (Lloyd et al., 2007). About 1.7 billion people currently live in a state of water scarcity; that number is expected to increase to 5 billion people by the year 2025 (Lloyd et al., 2007).

There are many bacterial, viral, and protozoan pathogens that can be spread through water. Common pathogenic bacteria include *E. coli* 0157:H7, *Salmonella spp.*, *Klebsiella spp.*, *Staphylococcus aureus*, *Vibrio spp.*, and *Campylobacter jejuni*; protozoa include *Cryptosporidium spp.*, *Naegleria fowleri*, *Toxoplasma gondii*, and *Giardia spp.* and viruses include adenovirus, enteroviruses, Hepatitis A and E, norovirus, and rotavirus (World Health Organization, 2011a; World Health Organization, 2011c). Bacteria have been shown to be the easiest to remove by chemical disinfection, and protozoa are larger and easier to remove by physical methods. However, the extremely small size and unique outer coating (capsid) of viruses makes them harder to physically remove or chemically disinfect. Table 1 lists some of the common viruses that are often found in water and that cause disease in humans.

Table 1. Viruses common in water that cause disease in humans.

Virus Name	Genome	Diseases	Year Discovered	Reference
Adenoviruses	DS ¹ DNA	Respiratory distress, gastroenteritis	1950	Goncalves and de Vries, 2006
Poliovirus	+ ² RNA	Gastroenteritis, paralysis, respiratory distress	1909	Skern, 2010
Hepatitis A	+ RNA	Liver inflammation	1973	WHO, 2000
Rotavirus	DS RNA	Gastroenteritis	1973	Flewett and Woode, 1978
Norovirus	+ RNA	Gastroenteritis	1972	Patel et al., 2009

Noroviruses in particular are becoming a worldwide concern. Human noroviruses are one of the major causes of non-bacterial gastroenteritis; they are responsible for more than 50% of general gastroenteritis and more than 90% of viral gastroenteritis worldwide (Wobus et al., 2006; Seitz et al., 2011; Patel et al., 2009; World Health Organization, 2011a, Seo et al., 2011). It is estimated that there are 218,000 deaths and 1.1 million hospitalizations among children in developing countries caused by norovirus infections each year (Seitz et al., 2011). Norovirus is responsible for about 23% of reported waterborne diseases;

¹ Double-stranded DNA.

² Positive single-stranded RNA. This genome enables the virus to proceed directly to translation following infection, without the need for genome replication or conversion.

there are approximately 23 million cases of norovirus each year in the United States alone (Keswick et al., 1985; Mead et al., 1999).

1.2 NOROVIRUS

Noroviruses (previously known as Norwalk-like viruses) are single-stranded RNA viruses that are part of the Caliciviridae family (World Health Organization, 2011a, Seo et al., 2011). They have a non-enveloped icosahedral capsid and are usually 35-40nm in diameter (World Health Organization, 2011a). Human noroviruses are small (27nm) enteric viruses (Bae and Schwab, 2008). There are currently seven genotypes of norovirus, five of which are found in humans (Lysén et al., 2009; Shirasaki et al., 2010). The noroviruses as a group have a genome that contains 7,500 nucleotides organized into three open reading frames (ORFs); ORF 1 encodes the RNA-dependent RNA polymerase and other proteins, ORF 2 encodes the major capsid protein (VP1), and ORF 3 encodes the small capsid protein (VP2) (Lysén et al., 2009). ORFs 1 and 2 are used for PCR and genotyping (Lysén et al., 2009). The virus was discovered in 1972 (Patel et al., 2009).

Gastroenteritis caused by human norovirus has a very rapid onset and resolution (about two days), but the virus can be shed by an infected individual for up to three weeks after infection, which leads to efficient transmission (Wobus et al., 2006; Lopman et al., 2004). Symptoms include severe vomiting and non-bloody diarrhea (Lopman et al., 2004; Pang et al., 2000). Children and the elderly usually have more severe symptoms, and individuals with compromised immune systems can experience long-term infections (Centers for Disease

Control and Prevention, 2012; Wobus et al., 2006). Norovirus is extremely infectious; a single infectious virus particle has a 49% chance of causing an infection (Teunis et al., 2008). Because of this, environmental contamination can prolong outbreaks (Cannon et al., 2006). As with many enteric viruses, there is no vaccine or drug available for prevention or treatment (Wobus et al., 2006).

Norovirus is mostly spread by the fecal-oral route, usually through consumption of contaminated water and food but also through exposure to vomitus (Seitz et al., 2011; Bae and Schwab, 2008; Cannon et al., 2006; Borchardt et al., 2010; Kim et al., 2005). It affects people of all ages and typically occurs in crowded areas; transmission through infectious vomit (from contact with contaminated surfaces or by inhaling aerosolized particles) accounts for its rapid spread in close quarters (Bae and Schwab, 2008; Wobus et al., 2006; Patel et al., 2009). Norovirus is a common cause of many foodborne epidemics; it could be responsible for 50% of all foodborne outbreaks in the US (Cannon et al., 2006). Norovirus is also frequently associated with outbreaks of gastroenteritis caused by contaminated drinking water (World Health Organization, 2011a; Chan et al., 2006; Kim et al., 2005). Lysén et al. (2009) estimated that 18% of norovirus outbreaks in Sweden were due to contaminated water. Norovirus has even been found in fecally-polluted drinking water that had been terminally disinfected; however these studies used PCR to detect the norovirus particles so infectivity cannot be determined (Keller et al., 2010; Maunula et al., 2005; Gallay et al., 2006; O'Reilly et al., 2007). Waterborne outbreaks of norovirus often occur after heavy rains, due to wastewater entering the drinking water system (Lysén et

al., 2009; Lloyd et al., 2007; Halonen et al., 2012) and during hot weather when people are more frequently visiting recreational water sites (Lysén et al., 2009). Viruses are the primary concern in groundwater contamination, due to their small size and related ease of transport (Reynolds et al., 2008).

Human noroviruses may be resistant to environmental degradation and chemical inactivation (Bae and Schwab, 2008; Seitz et al., 2011; Wu et al., 2005; D'Souza et al., 2006; Reynolds et al., 2008). A study by Seitz et al. (2011) used RT-PCR for detection of norovirus and human volunteers to determine infectivity; norovirus samples were spiked into groundwater and allowed to sit for varying amounts of time before being ingested by the volunteers or measured with RT-PCR. The authors found that norovirus can remain detectable in groundwater for over three years and remain infectious for at least 61 days (Seitz et al., 2011). Borchardt et al. (2010) found that in a conventional septic system (in Wisconsin), it would take 200 days to achieve the recommended four log₁₀ reduction in norovirus. Keller et al. (2010) found that murine norovirus did not degrade in raw river water at 4 or 25°C over 24 hours. Resistance to degradation may be due to aggregation of the virus, which is how the virus would be found in natural (fecal) contamination scenarios (Keswick et al., 1985).

Human noroviruses currently cannot be grown in cell culture or in an animal model, so different forms of polymerase chain reaction (PCR) are used to detect and quantify norovirus in samples. For studies where infectivity of the virus needs to be measured, surrogate viruses are used in place of human noroviruses. Feline calicivirus was historically the most common human

norovirus surrogate, but recent studies are turning to murine norovirus as a more appropriate surrogate. Murine (mouse) norovirus (MNV-1) is currently the only norovirus that replicates in cell culture and in a small animal model (Wobus et al., 2006; Duizer et al., 2004). There are many biochemical and genetic similarities between human noroviruses and murine norovirus, including pH stability (both are stable at high pH) (Wobus et al., 2006; Bae and Schwab, 2008; Cannon et al., 2006). Murine norovirus is also an enteric virus, its size is similar to human norovirus (27-35 nm), and its genome has the three open reading frames (ORFs) that are characteristic of noroviruses (Wobus et al., 2004; Shirasaki et al., 2010). Murine norovirus also seems to have a similar persistence against free chlorine disinfection (when comparing viral RNA reduction rates) (Kitajima et al., 2010).

MS2 bacteriophage is a common indicator and model for human enteric RNA viruses (Charles et al., 2009). Lim et al. (2010) found that murine norovirus and MS2 were inactivated similarly by chlorine and chlorine dioxide. Table 2 shows how murine norovirus and MS2 bacteriophage respond to different forms of chlorine disinfection. Both murine norovirus and MS2 have been shown to be sensitive to many forms of chlorine disinfection; murine norovirus may be slightly less sensitive than MS2. Free chlorine and chlorine dioxide appear to be more effective disinfectants than monochloramine, and low temperature may have a protective effect on the viruses.

Table 2. Disinfection of murine norovirus and MS2 with different forms of chlorine.

Virus	Disinfectant	Disinfectant Dose (mg/L)	Log₁₀ Reduction	Temperature	Reference
Murine norovirus	Free chlorine	0.1	4.00	20-25 °C	Kitajima et al., 2010
Murine norovirus	Monochloramine	1.89	<1	4 °C	Keller et al., 2010
			2.5	25 °C	
	Free chlorine	0.5	>4	4 °C	
			>4	25 °C	
Murine norovirus	Chlorine	0.184-0.193	3	5 °C & 25 °C	Lim et al., 2010
MS2		0.172-0.174	4	5 °C & 25 °C	
Murine norovirus	Chlorine dioxide	0.255-0.288	3	5 °C & 25 °C	
MS2		0.174-0.288	4	5 °C & 25 °C	

Past studies suggested that human noroviruses were resistant to chemical inactivation, but this may have been due to the methods used. Keswick et al. (1985) used human volunteers to determine the infectivity of norovirus after treatment with chlorine; they found that norovirus was more resistant to chlorine disinfection than poliovirus, rotavirus, or f2 bacteriophage. However, more recent research supports a different conclusion. Shin and Sobsey (2008) found that norovirus might not be very resistant to free chlorine disinfection. Their experiment, which used RT-PCR, found that norovirus is disinfected similar to MS2 bacteriophage, and faster than poliovirus (Shin and Sobsey, 2008). A study

by Cromeans et al. (2010) could achieve at least three $\log_{10}$ reduction of murine norovirus within five seconds using 0.2 mg/L of free chlorine. The experiments were run in flasks using buffered reagent-grade water of pH 7 and 8 at 5 °C, and infectivity was determined using plaque assay (Cromeans et al., 2010). Kitajima et al. (2010) found that free chlorine disinfection could result in approximately four $\log_{10}$ reduction of murine norovirus. Aggregation of virions and the presence of culture media have been found to hinder disinfection (Shin and Sobsey, 2008; Floyd et al., 1976).

Many characteristics of the water being used for testing can influence the disinfection capacity, especially temperature and pH. Both murine norovirus and MS2 bacteriophage were found to be more sensitive to inactivation at temperatures over 60 °C (Seo et al., 2011). Murine norovirus also appears to be somewhat resistant to strong acids (pH 2) but tolerant of slightly acidic or neutral conditions (pH 4 or 7) (Seo et al., 2011). Water temperature can influence the disinfection capacity of monochloramine on murine norovirus; murine norovirus appears to be resistant to disinfection at cooler temperatures (4 °C) (Keller et al., 2010). Lim et al. (2010) found that less chlorine is needed at higher temperatures to achieve the same amount of disinfection of murine norovirus and MS2 bacteriophage but it was unclear if this was synergistic or just additive effect of the heat and the disinfectant.

1.3 WATER TREATMENT & DISINFECTION

There is need for improving the methods to effectively disinfect drinking water, especially in developing countries. There are many reasons why water may be unsafe to drink: there may not be a public water supply available, the water supply may be unreliable and people are forced to frequently use unsafe sources of water, or the public water supply may actually distribute unsafe water (Wegelin et al., 1994; Mintz et al., 1995). Most of the world's population consumes untreated drinking water that they collect in small volumes and store in their home; most of this water is untreated and unprotected from further contamination (Sobsey et al., 2003). It is estimated that over 90% of diarrheal cases could be prevented through changes to the environment, which includes interventions to provide more clean water (McGuigan et al., 2012). Treating drinking water at the point of use or household level has been shown to be about twice as effective in reducing the incidence of diarrheal disease, compared to distributed water systems that treat the water and provide points of distribution (eg. community taps) (Clasen et al., 2006; McGuigan et al., 2012). Possible reasons for this include the potential for recontamination in the distribution system (due to faulty pipes, connections, etc.) and the potential for recontamination in storage containers in the home (Wegelin et al., 1994, Mintz et al., 1995). Treating drinking water at the point of use (in the household) has been shown to reduce diarrheal disease by up to 30-40%, even in the absence of improved sanitation or hygiene (Sobsey et al., 2008; Sobsey, 2002). Quick et al. (1999) found that the combination of a chlorine disinfectant and a safe water

storage container reduced the incidence of diarrhea by 44% within five months in Bolivia. A similar intervention studied by Sobsey et al. (2003) found a 20.8% reduction in diarrhea during an eight-month study in Bangladesh and a 43% reduction during a six-month intervention in Bolivia. It is also the most cost-effective method of preventing diarrheal disease (World Health Organization, 2002).

Household water treatment devices or point of use devices are now being developed for use in areas without consistent access to clean drinking water. Household water treatment devices that are designed to be used in developing countries must be inexpensive and easy to use to be successful (Sobsey et al., 2008; Clasen et al., 2006; McGuigan et al., 2012). Educational efforts also need to be considered when implementing a point of use water treatment device in a particular community, as the understanding that treating one's water will prevent diarrhea has been shown to contribute to the success of devices (Elimelech, 2006). Point of use devices are even being considered for people living in the United States that get their drinking water from shallow groundwater sources, or for small or rural communities (Abbaszadegan et al., 1997).

Devices utilize technology such as liquid halogen solution (chlorine, bromine, or iodine), ultraviolet (UV) light (in the form of lamps or sunlight (SODIS)), or filtration (through materials such as sand, gravel, ceramic, etc.). Liquid halogen solutions are simply added to a volume of water (at a specific concentration) and allowed to treat the water for a specific amount of time. Liquid chlorine is normally used to disinfect drinking water on both a community

and household scale, while bromine has historically only been used to treat water on navy ships (Dunk, 2007). Solar water disinfection (SODIS) uses clear plastic bottles that are filled with water and then set in the sun for several hours (more than six) to disinfect (Wegelin et al., 1994; McGuigan et al., 2012). It aims to treat two liters of water per person per day, or 10-15 liters for a family (Wegelin et al., 1994). Mäusezahl et al. (2009) found no significant effect of SODIS treatment of drinking water on the prevalence of diarrheal disease in rural Bolivia. Slow sand filters consist of 1-1.25m of medium sand over a layer of gravel through which the water flows (Sobsey, 2002). Particulate and microbial removal occurs in the slime layer that forms in the top few centimeters of the sand (Sobsey, 2002).

Table 3 shows some of the most common methods of disinfecting drinking water, as well as the advantages, disadvantages, and costs of each. SODIS and free chlorine or bromine treatment are by far the least expensive methods available, and along with ceramic filters are the ones most recommended for household use.

Table 3. Advantages and disadvantages of various methods of drinking water disinfection.

Disinfection Method	Advantages	Disadvantages	Recommended for Household Use	Reference
Boiling	Easy to use, effective against most pathogens, no disinfection by-products	Large energy requirement, no residual	No	Wegelin et al., 1994; Sobsey, 2002
Free chlorine/bromine	Safe, easy to distribute and use, effective against most pathogens, provides residual	Bad taste and smell (chlorine), often not used correctly, inhibited by turbidity, can form disinfection by-products	Yes	Wegelin et al., 1994; World Health Organization, 2011a; McGuigan et al., 2012; Mintz et al., 1995; Sobsey, 2002
Ceramic filter	Fairly sustainable, no disinfection by-products, easy to use, can be effective against most pathogens	Expensive, clog easily, often crack, less effective against viruses, no residual, requires regular cleaning	Yes	Wegelin et al., 1994; Clasen et al., 2006; McGuigan et al., 2012; Sobsey et al., 2008; Sobsey, 2002
SODIS (solar)	Low cost, sustainable, easy to use, mostly effective against pathogens, no disinfection by-products	Geographic variation; no residual, only treats small volumes, high interference (turbidity), long treatment time	Yes	Wegelin et al., 1994; McGuigan et al., 2012; Sobsey, 2002
Floculation/disinfection	Effective against most pathogens	Not sustainable, harder to use, expensive	No	McGuigan et al., 2012; Sobsey, 2002

Table 3 (cont'd).

Biosand filters	Very sustainable, no disinfection by-products, fairly inexpensive, can be effective against most pathogens	No residual, harder to maintain properly	No	Sobsey et al., 2008; Sobsey, 2002
UV irradiation	Easy to use, effective against most pathogens, no disinfection by-products	High cost, high interference (turbidity), requires electricity	No	Sobsey, 2002

Table 4 shows various forms of drinking water disinfectants and their efficacy on different viruses and bacteriophages. All methods of drinking water disinfection shown here appear to have good efficacy on various human and model viruses. Model viruses may actually be less sensitive to disinfection than the pathogenic viruses. Bromine also appears to be a more effective disinfectant than chlorine.

Table 4. Different methods of drinking water disinfection and their efficacies on viruses.

Disinfectant	Type of Method	Disinfectant Dose	Virus(es) Tested	Log ₁₀ Reduction	Reference
Ultraviolet light	Point of use (POU)	128 mWs/cm ²	Hepatitis A, Simian rotavirus, Poliovirus, MS2	4	Abbaszadegan et al., 1997
Bromine	Continuous flow apparatus, liquid bromine (lab)	0.13 mg/L	Poliovirus	3.5	Floyd et al., 1976
	Beakers, liquid bromine (lab)	0.07 mg/L	Reovirus	4	Sharp et al., 1975
	POU	Unknown	MS2	4	Enger et al., Draft Manuscript
			Human adenovirus	5	
	POU	Unknown	MS2	5	Coulliette et al., 2010
	POU	Unknown	Coliphage	1.8	McLennan et al., 2009
Chlorine	Beakers, liquid chlorine (lab)	3.75 mg/L	Norwalk virus (norovirus)	<1	Keswick et al., 1985
			Poliovirus	4	
			Simian rotavirus	4	
			Human rotavirus	4	
			F2 bacteriophage	2	
	Glass test tubes, liquid chlorine (lab)	1 mg/L	Norovirus	3	Shin & Sobsey, 2008
			Poliovirus & MS2	4	
	Flasks, liquid chlorine (lab)	0.5 mg/L	Norovirus	3.5	Kitajima et al., 2010
			Poliovirus	4	
	POU	Unknown	MS2	3	Coulliette et al., 2010
	POU	Unknown	Coliphage	1	McLennan et al., 2009
	POU	6 mg/L	MS2	7	Clasen et al., 2006
Ceramic filter	POU	n/a	MS2	1-2	Brown & Sobsey, 2010

Table 4 (cont'd).

SODIS	Glass tubes (lab & field)	199,800 mWs/cm ²	f2, encephalomyocarditis virus, bovine rotavirus	3	Wegelin et al., 1994
	POU	1,209,600 mWs/cm ²	Somatic phage, f2, rotavirus	3	McGuigan et al., 2012
	Simulation (lab)	1,836,000 mWs/cm ²	Poliovirus	4.4	
Coagulation-rapid sand filtration	Glass tubes (lab)	n/a	MS2	>3	Shirasaki et al., 2010
			rNV-VLPs	~3	
Ultrafiltration (LifeStraw)	POU	n/a	MS2	4.7	Clasen et al., 2009

Another consideration for safe water is the need for safe storage containers. Drinking water can be easily (re)contaminated when hands or utensils come in contact with water (Mintz et al., 1995). Mintz et al. (1995) found that mean coliform levels in water containers were significantly higher than in the water sources.

One common method of testing the efficacy of a point of use device is to challenge the device with bacteria, viruses, and protists, and compare the reduction in biological organisms to the US EPA's Guide Standard and Protocol for Testing Microbiological Water Purifiers. The Guide Standard states that a microbiological water purifier must be capable of reducing bacteria, virus, and protozoan pathogens by 99.9999% (six $\log_{10}$ reduction), 99.99% (four $\log_{10}$), and 99.9% (three $\log_{10}$), respectively (United States Environmental Protection Agency, 1987). Testing is done with general and worst-case water conditions over the life of the device (United States Environmental Protection Agency, 1987). The World Health Organization also has its own requirements for treatment capacity of household water treatment devices. The WHO recommends that a device be capable of four, five, and four $\log_{10}$ reductions of bacteria, viruses, and protozoa, respectively, to be labeled as highly protective; only two, three, and two $\log_{10}$ reductions of bacteria, viruses, and protozoa, respectively, are required for the device to be labeled as protective (World Health Organization, 2011c).

Contact disinfectants are relatively new and have been incorporated into household water treatment devices. One of these devices, the HaloSource Waterbird, was chosen to be tested as part of this research. The device uses physical removal and chemical disinfection to treat water to produce drinking water, through the use of a ceramic filter and brominated N-halamine polystyrene beads. N-halamines contain one or more nitrogen-halogen covalent bonds (Chen & Sun, 2006; Timofeeva & Klescheva, 2011). The polystyrene beads used in the device are 0.5mm diameter spherical polymer beads, with a large surface area to bind to halogen ions (Enger et al., Draft Manuscript). The N-halamine media has advantages over other methods of microbial disinfection: better overall performance, pH stability, rechargeability, less toxicity, and a lower price (Chen et al., 2003; Kenawy et al., 2007; Padmanabhuni et al., 2012). The halogenated polystyrene beads do not leach decomposition products (Chen et al., 2003). The suspected mechanism of action is that when a cell or virion makes contact with a halogenated bead, the halogen ion is transferred to the biological particle where it oxidizes proteins on or in the biological particle (Chen et al., 2003; Ahmed et al., 2008; Chen & Sun, 2006; Timofeeva & Klescheva, 2011). The N-halogen bond does not break to form free halogen in the water (Chen et al., 2003).

The device contains both a reservoir for untreated water (at the top), and a reservoir for treated water (at the bottom), so it can act as a safe water storage device as well as a system that disinfects the water. As a disinfectant, the device meets many of the features of a sustainable household water treatment

device specified by Sobsey et al. (2008): it can consistently produce enough safe water for an entire household for one day (9L), it has been shown to be effective against many groups of microorganisms (Dr. Jeff Williams, Personal Communication), it should effectively treat many different types and qualities of water, it requires very little user time to treat the water, and it is relatively inexpensive. As a safe water storage container, it also meets many of the criteria proposed by the Centers for Disease Control & Prevention (CDC) and the Pan American Health Organization (PAHO): it is made of translucent plastic that is durable, lightweight, nonoxidizing, and easy to clean, it has a stable base, it holds all the water that the device can disinfect at a single time, and it has a durable and nonrusting spigot to remove water (Mintz et al., 1995).

Previous experiments with an older model of the HaloSource device (same brominated polystyrene beads, but without the ceramic pre-filter) achieved four to six $\log_{10}$ reductions of *Salmonella* and four to seven $\log_{10}$ reductions of *Vibrio cholerae* (Enger et al., Draft Manuscript; Coulliette et al., 2012). There were four $\log_{10}$ reductions of MS2 bacteriophage, and six $\log_{10}$ reduction of human adenovirus type two (Enger et al., Draft Manuscript). Later time points had higher inactivation, accompanied by slower flow rate and higher total bromine residual (Enger et al., Draft Manuscript; Coulliette et al., 2010). The flow rate was found to decrease over time, but there was no significant impact on the halogen residual (Coulliette et al., 2010).

Traditionally, chlorine has been the halogen of choice for use in drinking water disinfection. Bromine has been used as a water disinfectant in hot tubs and swimming pools, but has really only been used to treat drinking water on navy ships (Dunk, 2007). However, recent studies suggest that certain forms of bromine may be a good option for drinking water disinfection. Studies comparing the chlorine and bromine forms of HaloPure N-halamine media have shown that the bromine disinfectant is actually more effective than its chlorine counterpart. Coulliette et al. (2010) found that the chlorine version of the media could remove an average of $2.98 \pm 0.26 \log_{10}$ of MS2, and that the bromine version could remove an average of $5.02 \pm 0.19 \log_{10}$ of MS2.

1.4 OBJECTIVES

The main objective of this study was to determine the effectiveness of the Waterbird device in removing or inactivating MS2 bacteriophage and murine norovirus with sub-objectives to determine if pH or added organic materials impact the device's effectiveness. The hypotheses are that the device will be able to reduce both MS2 bacteriophage and murine norovirus to levels required by the EPA standard (four $\log_{10}$ reduction), and that the addition of organic materials will not impact the efficiency of the device. I hypothesize that an increase in pH will decrease the effectiveness of the device.

CHAPTER 2

MATERIALS & METHODS

2.1 EXPERIMENTAL DESIGN

The main objective for this study was to determine the effectiveness of the Waterbird device in removing or inactivating MS2 bacteriophage and murine norovirus; sub-objectives were to determine if pH or added organic materials impact effectiveness of the device. To test these objectives, the study was designed to test three replicates of the device with both well water and well water seeded with approximately five percent raw sewage. The water was adjusted to either pH 7.5 or 9. Pure culture stock of MS2 and murine norovirus were added to each mixture of water before treatment. Each device was challenged in triplicate for each combination of pH (7.5 or 9) and sample types (well water only or well water with five percent raw sewage), giving a total of 36 experiments. For pH 7.5, 18 influent samples were collected and analyzed for MS2 and murine norovirus. Seventy-two effluent samples were collected at four time points for each experiment for a total of 90 samples for MS2 and murine norovirus. For pH 9, nine influent samples from the sewage treatment and six influent samples from the well water treatment were collected and analyzed for MS2; three influent samples from each treatment were collected and analyzed for murine norovirus. Effluent samples were collected at the same four time points (first flush, 15 minutes, 45 minutes, and 120 minutes) for each experiment, giving a total of 45 samples for the sewage treatment and 30 samples for the well water treatment for MS2, and 15 samples for each treatment for murine norovirus.

Figure 1 shows the device tested, where the upper reservoir was the chamber seeded with the viruses representing the influent and the clear chamber contains the effluent or treated water. Samples were collected from the tap. Figure 2 shows a schematic of the chlorinated system that is similar to the model of the device tested previously (McLennan et al., 2009).



Figure 1. The HaloSource Waterbird device. The white part at the top is where water is poured in; it flows through the ceramic pre-filter and the bromine cartridge before being deposited in the bottom (clear) reservoir. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

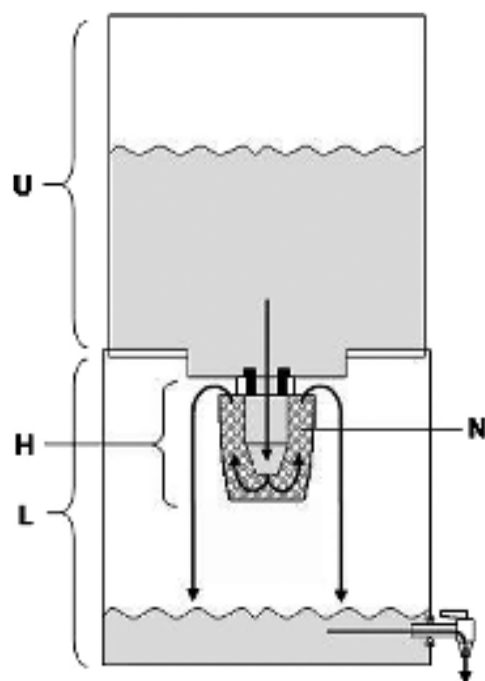


Figure 2. Diagram of a device similar to the HaloSource Waterbird. Arrows show the direction of water flow; water flows from the upper reservoir (U), through the bromine cartridge (H) containing packed N-bromine beads (N), to the lower reservoir (L), and finally is deposited in the lower reservoir (L) where it can flow out of the tap (McLennan et al., 2009).

To test the effectiveness of the Waterbird device, diluted mixtures of MS2 bacteriophage and murine norovirus were treated through the device and the concentrations of virus before and after treatment were measured and compared. A control of well water (from Williamston, MI) was used, as well as well water with a low volume of raw sewage added (from the East Lansing wastewater treatment plant). Two pH values were used (pH 7.5 and 9). Three Waterbird devices were analyzed in triplicate, giving nine replicates for each treatment and pH combination (36 total samples).

The Waterbird devices were pre-flushed with five liters of NanoPure water the night before each set of experiments. The influent mixture used to test the devices was made with 5L of well water. For the runs with raw sewage,

approximately 2-4% raw sewage was added. The exact amount of raw sewage added was adjusted based on the COD measured each day (the COD was adjusted to 8.1 mg/L (final concentration)). The influent was adjusted for pH (to either 7.5 or 9) using hydrochloric acid or sodium hydroxide, and temperature was measured using a digital thermometer. MS2 suspension in TSB (2mL) and purified murine norovirus in PBS (3mL) were added to the influent, and the mixture was stirred for 15 minutes. The mixed influent was poured into the top reservoir of the device (with the bromine cartridge and ceramic pre-filter already in place), and allowed to flow through the device by keeping the tap in the bottom reservoir open and flowing. Samples were taken of the untreated influent and of the treated water at first flush (0 minutes), 15 minutes, 45 minutes, and 2 hours. Flow rate (mL per minute) was also measured at each time point. The time from the start of the experiment to the first flow at the tap was recorded.

Bromine residual was measured using the Hach colorimetric DPD method was used, with an adjustment for bromine (total chlorine * 2.25) (Coulliette et al., 2010) at each time point.

2.2 BROMINE DEMAND OF THE ADDED SEWAGE

To determine the optimum concentration of raw sewage to use in the challenges, a bromine consumption experiment was run. Three concentrations (1%, 5%, and 10%) of raw sewage (from the East Lansing wastewater treatment plant) were analyzed to see how quickly they depleted the available bromine. An ideal concentration would likely show about 80% reduction in bromine residual over two hours (Eaton et al., 1992). The colorimetric DPD method was used and

adjusted for bromine (total chlorine * 2.25). A solution of hypobromous acid was prepared from 1.725 mL hydrogen bromide, 10.25 mL sodium hypochlorite, and 448.3 mL NanoPure water. Half a milliliter of the solution was added to 200mL of diluted raw sewage (diluted with NanoPure water), and allowed to react while continuously stirred for five hours. The 1% solution showed 54% reduction in bromine, the 5% solution showed 87% reduction, and the 10% solution showed 89% reduction; 5% raw sewage was determined to be the optimum sewage concentration for the challenge experiments.

2.3 CHEMICAL OXYGEN DEMAND OF THE ADDED SEWAGE

Since raw sewage varies by day in concentration of organic matter, chemical oxygen demand (COD) was chosen to standardize each experiment where raw sewage was added to the well water. The Hach COD kit was used (having a range of 0-1500 mg/L). Initial studies were undertaken to set up the experimental design based on COD measurements and then used to set up each experiment. Raw sewage samples were taken in the morning from the East Lansing wastewater treatment plant and immediately analyzed. Samples were added to the pre-made reagent tubes, and then digested on a heating block for two hours. Samples were then analyzed with a spectrophotometer to determine the COD. The absorbance reading from the spectrophotometer was converted to parts per million (PPM). The volume added each day was determined by dividing the day's COD PPM value by the initial COD value (162.5 PPM) to get a dilution factor; the initial volume (250 mL) was divided by the dilution factor to get the day's required volume of raw sewage (Table 5). Table 5 shows the chemical

oxygen demand values and percent raw sewage added for each raw sewage experiment. The overall COD concentration for the 5L influent was adjusted to be 8.1 mg/L.

Table 5. Chemical oxygen demand values (in mg/L) and percent raw sewage added for each raw sewage experiment.

Experiments	COD (mg/L) of Raw Sewage	Percent Raw Sewage (in 5L)
3,4,5	233	3.5
9,10,11	401	2.0
14,15,16	353	2.3
17,18,19	348	2.1
32,33,34	263	3.1
38,39,40	208	3.9

2.4 VIRUS PROPAGATION

Murine norovirus was propagated in RAW 264.7 host cells (ATCC# TIB-71); these cells are mouse macrophage cells. Frozen RAW 264.7 cells were thawed and started in 25cm² flasks with 8 mL growth medium (high-glucose DMEM (Hyclone, Logan, UT, #SH30243.02) with 10% low-endotoxin fetal bovine serum (FBS) (Hyclone, Logan, UT, #SH30070.03), 1% HEPES free acid (Amaresco, Solon, OH, #J848) 1% MEM NEAA (Lonza, Walkersville, MD, #13-114E), 1% penicillin/streptomycin (Hyclone, Logan, UT, SV30010), and 1% L-glutamine (Hyclone, Logan, UT, SH30034.02)) and incubated at 37 °C and 5% CO₂. Once the cells showed >80% confluence in the flasks they were removed by scraping and split at a 1:8 ratio to create new flasks. Subsequent passages of

the cells were grown in maintenance media (high-glucose DMEM with 2% low-endotoxin fetal bovine serum (FBS), 1% HEPES free acid, 1% MEM NEAA, 1% penicillin/streptomycin, and 1% L-glutamine).

Murine norovirus stock was obtained from Dr. Kellogg Schwab at Johns Hopkins University. To propagate the murine norovirus, >80% confluent RAW 264.7 cells in 150 cm² flasks were infected with 6 ml of diluted virus stock (diluted in maintenance media); the virus stock was diluted so that there are not more virus particles than cells in the flasks. Infected flasks were incubated for one hour at 37 °C and 5% CO₂, and were rocked every 15 minutes. After one hour of incubation, the inoculum was removed from the flasks and complete DMEM without FBS was added. Flasks were incubated until they showed cytopathic effect (CPE) for two to seven days. The flasks were then frozen at -80 °C overnight and subsequently thawed at room temperature; this was repeated three times. The resulting cell/virus mixture was filtered through a 0.45 and 0.22 micron filter (Millipore) and then frozen at -80 °C. Enger et al. (Draft Manuscript) found that 1X Eagle's MEM exerted a bromine demand, as observed by significantly lower bromine residuals, and protected MS2 from inactivation, so the filtered virus stock was ultrapurified to remove all media. Frozen virus stock was thawed and ultrapurified using Amicon ultrafilters (Millipore, Billerica, MA, #UFC910024) to remove the MEM and concentrate the virus. Resulting stock (at a concentration of approximately 1 x 10⁷ PFU/mL as determined by plaque assay) was frozen at -80 °C. Throughout the course of the study, approximately

200 flasks were prepared and 120 ml of a concentrated purified virus stock was prepared.

MS2 (ATCC# 15597-B1) was propagated in *E. coli* Famp host bacterial cells (ATCC# 700891). Frozen bacterial cells (frozen at -80 °C) were thawed and grown in tryptic soy broth (TSB) overnight at 37 °C. Cells were transferred to fresh TSB and incubated for four to six hours. Two milliliters of diluted MS2 stock (diluted in phosphate buffered water (PBW)) were added to 0.5 mL of the *E. coli* host cells and 2.5 mL of melted 1.5% trypticase soy agar (TSA). The melted TSA was boiled and then equilibrated to 50 °C before use. The resulting mixture was poured onto a thin layer of solidified TSA in a 75 cm² flask, thus described as the double agar overlay method. Plates were incubated for 16-24 hours at 37 °C, after which plaques or lysis in the bacterial monolayer could be visualized and counted. If sufficient concentrations of MS2 bacteriophage were present in the flasks (the flasks should have a lacey pattern, where the plaques are growing into one another), 30 ml of sterile TSB was added to each flask and the flasks were rocked at 4 °C for an hour to elute the viruses from the bacterial monolayer. The resulting cell/bacteriophage suspension was pipetted from the flask and filtered through 0.45 and 0.22 filters. The purified suspension was transferred to a sterile centrifuge tube, covered in aluminum foil, and stored at 4 °C.

2.5 VIRUS QUANTIFICATION

For the quantification of murine norovirus, the stock solution, influent and treated samples were all assayed using the plaque assay protocol. The treated water samples contained sodium thiosulfate (final concentration of 1% w/v) to neutralize the disinfectant. One-half milliliter of a 2% sterile sodium thiosulfate solution was added to the 15 ml tubes. Samples were frozen at -80 °C. Richards et al. (2012) have shown that norovirus can be frozen and thawed up to 14 times without decreasing infectivity. When ready to be processed, frozen samples were thawed and diluted with maintenance media. Samples were analyzed on RAW 264.7 host cells using the plaque assay method described below.

Confluent flasks of RAW 264.7 host cells were split to make 6-well 9.6 cm² cell culture plates (Corning, Corning, NY, #3516); approximately 3×10^6 cells were added to each well. The well plates were incubated for 24 hours in a 37 °C 5% CO₂ incubator. After 24 hours, the growth media was removed from each well and wells were infected with 1:10 dilutions of the samples (diluted in maintenance media) (0.5 mL inoculum was used per well). Duplicates were processed of each sample, as well as positive and negative controls. Well plates were inoculated and then incubated and rocked for one hour at room temperature. Overlay media was prepared using one half 1.5% agarose (1.5 g agarose (Cambrex, Rockland, ME, #50111) dissolved in 50 mL NanoPure water, autoclaved for 15 minutes, and brought to 48 °C) and one half 2xMEM (2xMEM (Sigma, St. Louis, MO, #M3024) with 10% low-endotoxin FBS, 2% L-glutamine,

2% penicillin/streptomycin, and 1% HEPES) brought to 37°C. Immediately before use the agarose and 2xMEM were combined and thoroughly mixed. Two milliliters of the overlay solution were added to each well, and the plates were left to solidify at room temperature for 30 minutes. After solidifying, the plates were incubated at 37°C and 5% CO₂ for 24 hours. After 24 hours, two milliliters of fresh overlay solution (with 2% neutral red (Sigma, St. Louis, MO, #N2889)) were added on top of the other overlay in each well to stain the living cells and visualize the plaques. The plates were allowed to solidify at room temperature and then incubated for 24 hours at 37°C and 5% CO₂. After 24 and 48 hours, plaques were counted to obtain a measurement of PFU per milliliter of sample.

For quantification of MS2 samples, a double agar overlay method was used. Sodium thiosulfate (final concentration of 1% w/v) was added to the treated water samples as described above. A 1/10 dilution series were prepared with PBS and then plated with *E. coli* Famp using the double agar overlay method as described above. Plates were incubated at 37°C for 16-24 hours, and then read to determine the plaque forming units (PFU) per milliliter sample.

2.6 STATISTICAL ANALYSIS

For the MS2 and murine norovirus results, the concentrations of virus before and after treatment were measured and compared to determine a log₁₀ reduction for each time point (the log₁₀ of the effluent was subtracted from the log₁₀ of the influent for each sample). The data were also converted to ratios

(the effluent virus concentration divided by the influent virus concentration) and then arcsine transformed. For the MS2 results, a repeated measures ANOVA was used to determine whether or not the water type (sewage or pH) or other variables (sampling time, cartridge, or ceramic) had a significant effect on the efficacy of the device in removing the virus. A Tukey's post-hoc test was used to distinguish which groups were significantly different. For the murine norovirus results, an ANOVA was used to look for significant effects. A Tukey's post-hoc test was used to distinguish which groups were significantly different. Linear regression analysis was used to look for correlations between the $\log_{10}$ reductions of the two viruses and the physical parameters (flow rate and bromine concentration). P-values equal to or less than 0.05 were considered significant.

CHAPTER 3

RESULTS

3.1 INTRODUCTION

This study set out to determine the effectiveness of the bromine-based disinfectant HaloSource Waterbird device in reducing or inactivating MS2 bacteriophage and murine norovirus as model enteric viruses. Sub-objectives were to determine the effect (if any) that the addition of raw sewage or the manipulation of pH had on the overall effectiveness of the device.

To test these objectives, the study was designed to test three replicates of the device with both well water and well water seeded with approximately five percent raw sewage. The water was adjusted to either pH 7.5 or 9. Each device was challenged in triplicate for each combination of pH (7.5 or 9) and sample types (well water only or well water with five percent raw sewage), giving a total of 36 experiments. For pH 7.5, 18 influent samples (nine for well water and nine for sewage) were collected and analyzed for MS2 and murine norovirus.

Seventy-one effluent samples were collected at four time points (first flush, 15 minutes, 45 minutes, and 120 minutes). For each experiment a total of 89 samples for MS2 and murine norovirus were analyzed. For pH 9, 18 influent samples (nine for well water and nine for sewage) were collected and analyzed for MS2; three influent samples from each treatment were collected and analyzed for murine norovirus. Effluent samples were collected at the same four time points for each experiment, however if the first flush was beyond 15 minutes this was considered first flush sample with no 15 minute sample collected, giving a

total of 44 samples for the sewage treatment and 40 samples for the well water treatment for MS2, and 14 samples for the sewage treatment and 13 samples for the well water treatment were analyzed for murine norovirus.

There were several issues with testing this device as a whole, mostly concerning the variability inherent in the device itself and in running samples across a long time period. The variability in the device comes from differences between cartridges and ceramic pre-filters, and variability within the cartridge itself (this shows in the variability in the bromine that is released or the flow rate through the cartridge). Variability due to the experimental design comes from the raw sewage addition. Variation due to the long time period during which experiments were run comes from differences in batches of virus stock produced and variation in sewage used (which could impact the MS2 concentration if additional natural bacteriophage is introduced). The study was designed to attempt to control the variation from these factors, but it is never possible to control all variation in an experiment. The variability due to the device itself could not be controlled. The same three ceramic pre-filters were used for all of the experiments, but the cartridges had to be replaced partway through the study due to inefficient performance and apparent exhaustion of the bromine. The raw sewage was measured for the chemical oxygen demand (COD) and adjusted so that the same amount of organic material was added to each experiment, however while this controlled for demand, this did not control for a variation in naturally-occurring coliphage that may be observed with the host used. The

same procedures were used to propagate and process virus stock, in attempt to reduce variability.

3.2 MS2 VIRUS

3.2.1 INFLUENT CHARACTERIZATION

Table 6 shows the average influent concentrations for the experimental challenges run using MS2. The goal was to keep the influent concentrations for each virus as consistent as possible between replicates. The MS2 stock used gave an average influent concentration of 6.9, 6.7, 7.3, and 7.7 log₁₀ for the experiments at pH 7.5 for well water and sewage and pH 9 for well water and sewage, respectively. The pH 9 experiments had slightly higher influent concentrations of MS2. The same MS2 stock was used for all experiments, so the variation might have been due to clumping of the virus particles (and not enough mixing). If the virus particles were clumped, the concentration would be underestimated in the assay. Differences between well water and sewage samples could also be due to the occasional higher level of naturally occurring coliphage added from the sewage.

Table 6. Influent concentrations for MS2 for the pH, sewage and well water variables. Shown are the average values for virus influent concentrations for each combination of pH and water treatment variables.

	pH 7.5		pH 9	
	Well Water	Sewage	Well Water	Sewage
MS2 Influent Concentration (log ₁₀ PFU/mL)	6.9 (n=9)	6.7 (n=9)	7.3 (n=9)	7.7 (n=9)

Figure 3 shows the average MS2 influent concentrations for the pH 7.5 samples. There was an average MS2 influent concentration of 6.9 log₁₀ for the well water samples and 6.7 log₁₀ for the sewage samples (Figure 3).

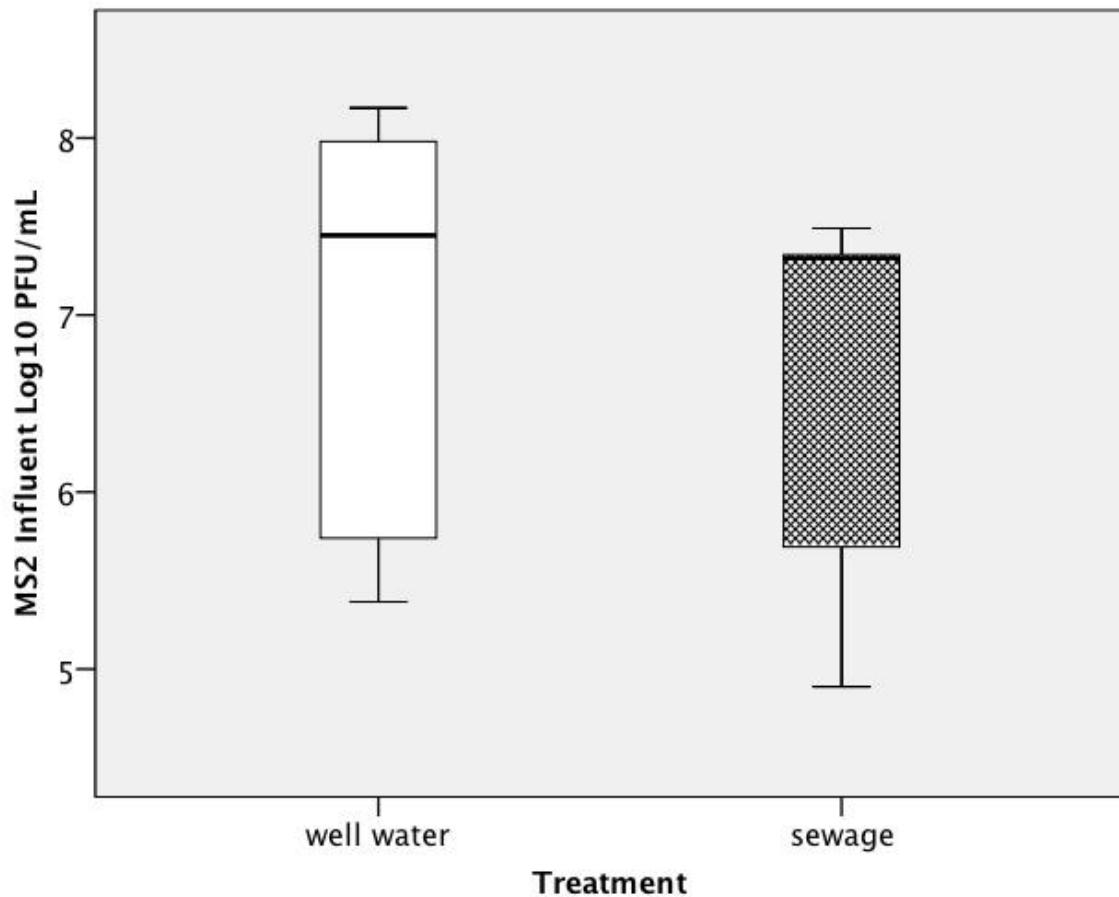


Figure 3. Average MS2 influent concentrations (in log₁₀) for the pH 7.5 samples. N=9 for well water and sewage (N=18 total).

Figure 4 shows the average MS2 influent concentrations for the pH 9 samples. There was an average MS2 influent concentration of 7.3 log₁₀ for the well water samples and 7.7 log₁₀ for the sewage samples (Figure 4).

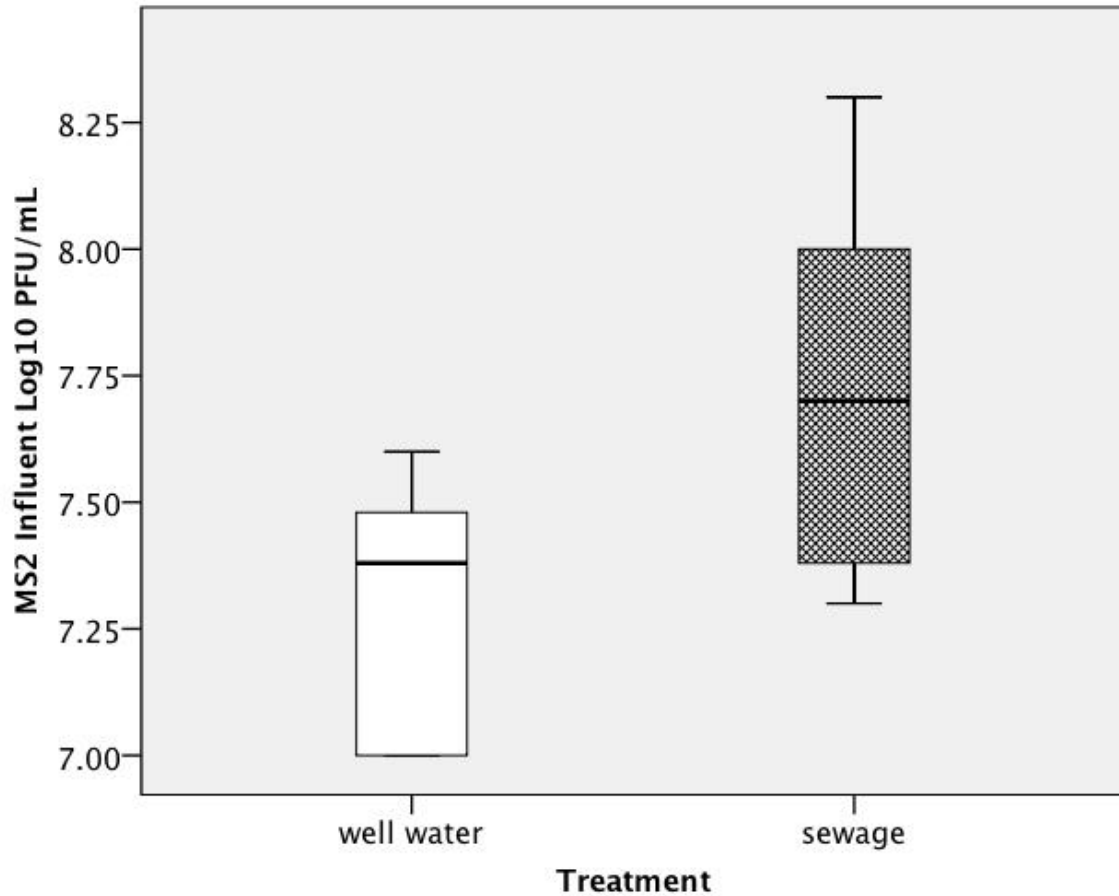


Figure 4. Average MS2 influent concentrations (in $\log_{10}$) for the pH 9 samples. N=9 for well water and sewage (N=18 total).

Overall, there was an average of 7.2 $\log_{10}$ of MS2 for all experiments when measuring the untreated influent samples. There was found to be no significant difference in the MS2 influent concentrations between the well water and sewage samples ($p=0.754$), but there was a significant difference between pH treatments ($p=0.011$) (Figure 5). Figure 5 shows the average MS2 concentrations between the pH 7.5 samples and the pH 9 samples. The pH 7.5 samples had a mean of 6.8 $\log_{10}$, while the pH 9 samples had a higher mean of 7.5 $\log_{10}$. Looking closer at the data, it appears that two sets of experiments

(one in the pH 7.5 sewage experiments and one in the pH 9 sewage experiments) may be responsible for the difference between pH variables. One set of pH 7.5 sewage experiments had an average MS2 influent concentration of $4.9 \log_{10}$, while the rest of those experiments had an average of $6.9 \log_{10}$; that set of experiments would have lowered the overall average. One set of pH 9 sewage experiments had an average MS2 influent concentration of $8.3 \log_{10}$, while the rest of those experiments had an average of $7.6 \log_{10}$; that set of experiments would have raised the overall average. Due to these two experimental results there was a statistically significant difference in the influent concentrations of the two pH experiments for MS2. When those two influent values are removed from the analysis, there is no significant effect of pH ($p=0.293$) on the influent concentrations. The role of the influent concentration in relationship to reduction of MS2 is discussed later.

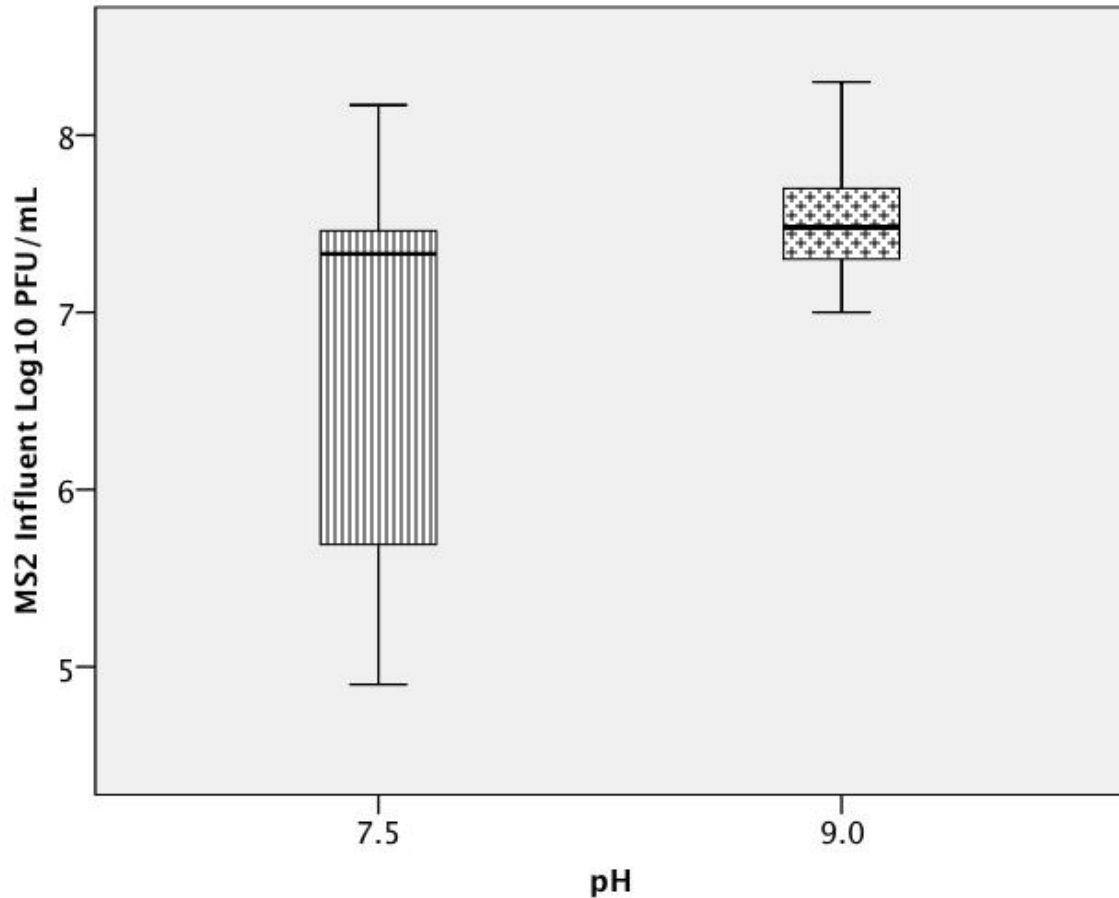


Figure 5. Average MS2 influent concentrations (in log₁₀) for all samples. N=18 for pH 7.5 and n=18 for pH 9 (N=36 total).

3.2.2 EFFLUENT CHARACTERIZATION

The time of the first sample (t_0 , or first flush) differed with each experiment. This is probably due to variation between cartridges and ceramic pre-filters used in each unit. For each combination of pH and water condition, three devices were tested in triplicate, giving a total of nine experiments. The results for bromine residuals, flows, device performance and effect of sewage and pH are presented below.

3.2.2.1 BROMINE RESIDUAL

Table 6 shows the average bromine residual values (+/- standard deviation) and which cartridges were used for each combination of influent experimental conditions (pH 7.5 or 9, well water or sewage). The bromine residual was not significantly different between cartridges ($p=0.127$) (Figure 6). Figure 6 shows the bromine residual for each cartridge used. The cartridges 4, 5 and 6 had bromine levels that were one third of the levels of the cartridges 1, 2 and 3. Only the values from the pH 9 sewage experiments were compared, since that was the only group of similar samples that used all six cartridges.

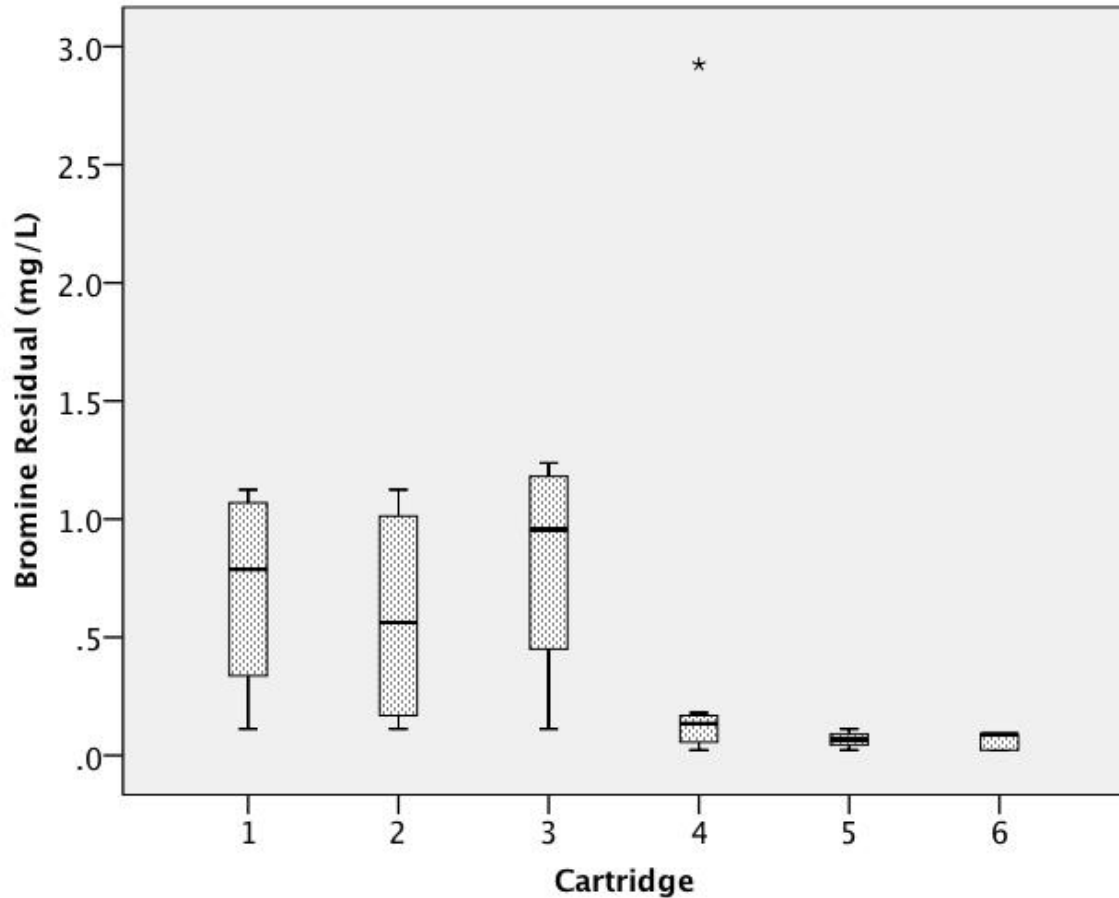


Figure 6. Bromine residual (mg/L) between cartridges used (for the pH 9 sewage samples). Values were averaged for all time points. The bromine residual was not significantly different between cartridges ($p=0.201$). $N=4$ for cartridge 1,2,3, $N=8$ for cartridge 4, $N=7$ for cartridge 5, $N=6$ for cartridge 6, $N=33$ total. The * symbol indicates an extreme outlier.

While the addition of sewage to the well water had no statistically significant effect on bromine residual ($p=0.409$) (Table 7), there was a slight increase in the bromine residuals when sewage was added.

Table 7. Average bromine residual (+/- standard deviation) and cartridges used for each type of influent (pH 7.5 or 9, well water or sewage).

	pH 7.5		pH 9	
	Well Water	Sewage	Well Water	Sewage
	N=35	N=36	N=31	N=34
Cartridges Used	1, 2, 3	1, 2, 3	4, 5, 6	1, 2, 3* 4, 5, 6** 1-6***
Bromine Residual (mg/L)	0.50 +/- 0.42	0.54 +/- 0.48	0.19 +/- 0.29	0.70 +/- 0.45* (N=12) ³ 0.22 +/- 0.62** (N=21) Avg. 0.39 +/- 0.61***

Figure 7 shows the bromine residual for each cartridge used in the pH 7.5 samples. There was no statistical difference between cartridges (Figure 7), however the lower levels could still have had an effect on disinfection. This will be addressed later.

³ N=12 is for cartridges 1,2,3 combined (*), and N=21 is for cartridges 4,5,6 combined (**). The average bromine residual shown is for all six cartridges combined (***).

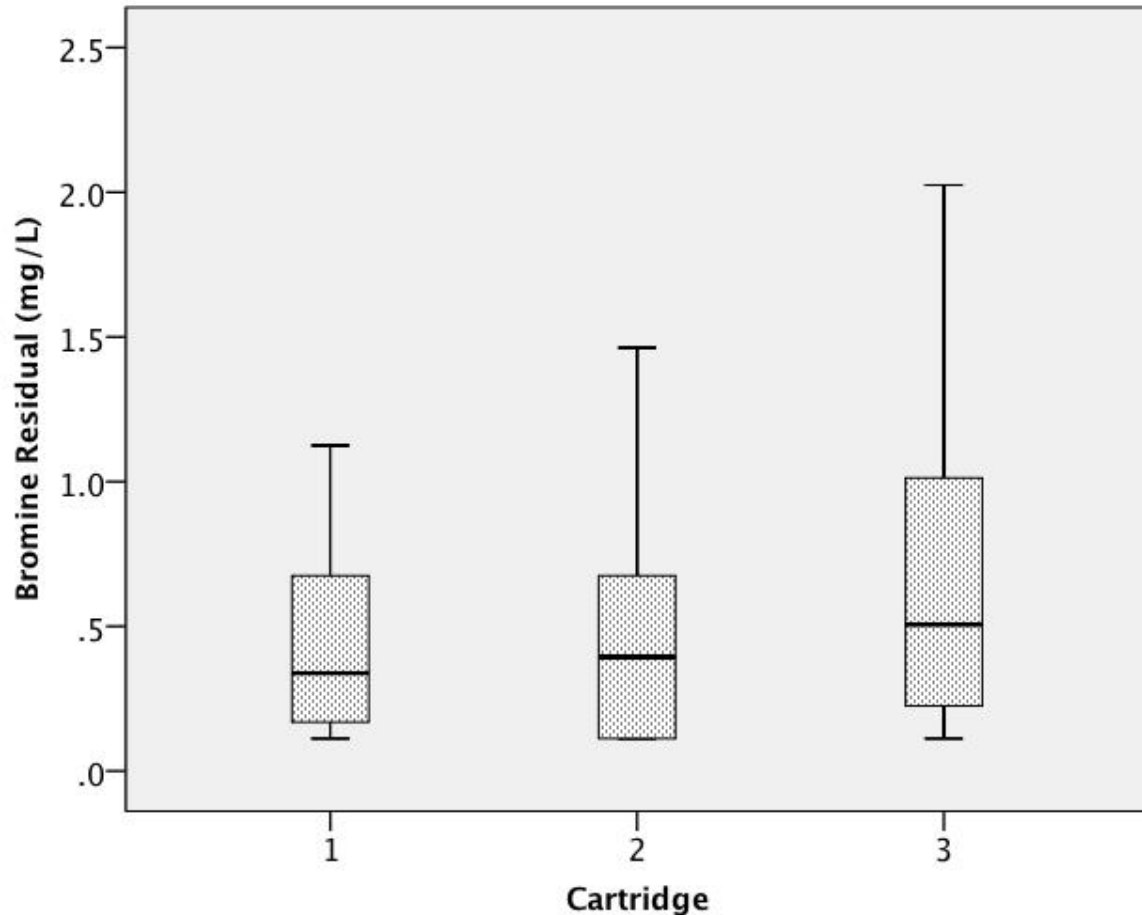


Figure 7. Bromine residual (mg/L) between cartridges used (for the pH 7.5 samples). N=71.

The bromine residual showed a statistically significant difference between timed samples ($p < 0.001$) with increasing residuals; the first flush sample differed from the 45 minute sample ($p = 0.001$) and the 120 minute sample ($p < 0.001$), and the 15 minute sample differed from both the 45 minute sample ($p = 0.044$) and the 120 minute sample ($p = 0.010$) (Figure 8). Figure 8 shows the bromine residual over sampling time, and compares the two pH variables. The first flush samples had an average bromine residual of 0.16 ± 0.31 mg/L, the 15 minute sample had an average of 0.27 ± 0.22 mg/L, the 45 minute sample had an average of

0.57 +/- 0.61 mg/L, and the 120 minute sample had an average of 0.62 +/- 0.48 mg/L.

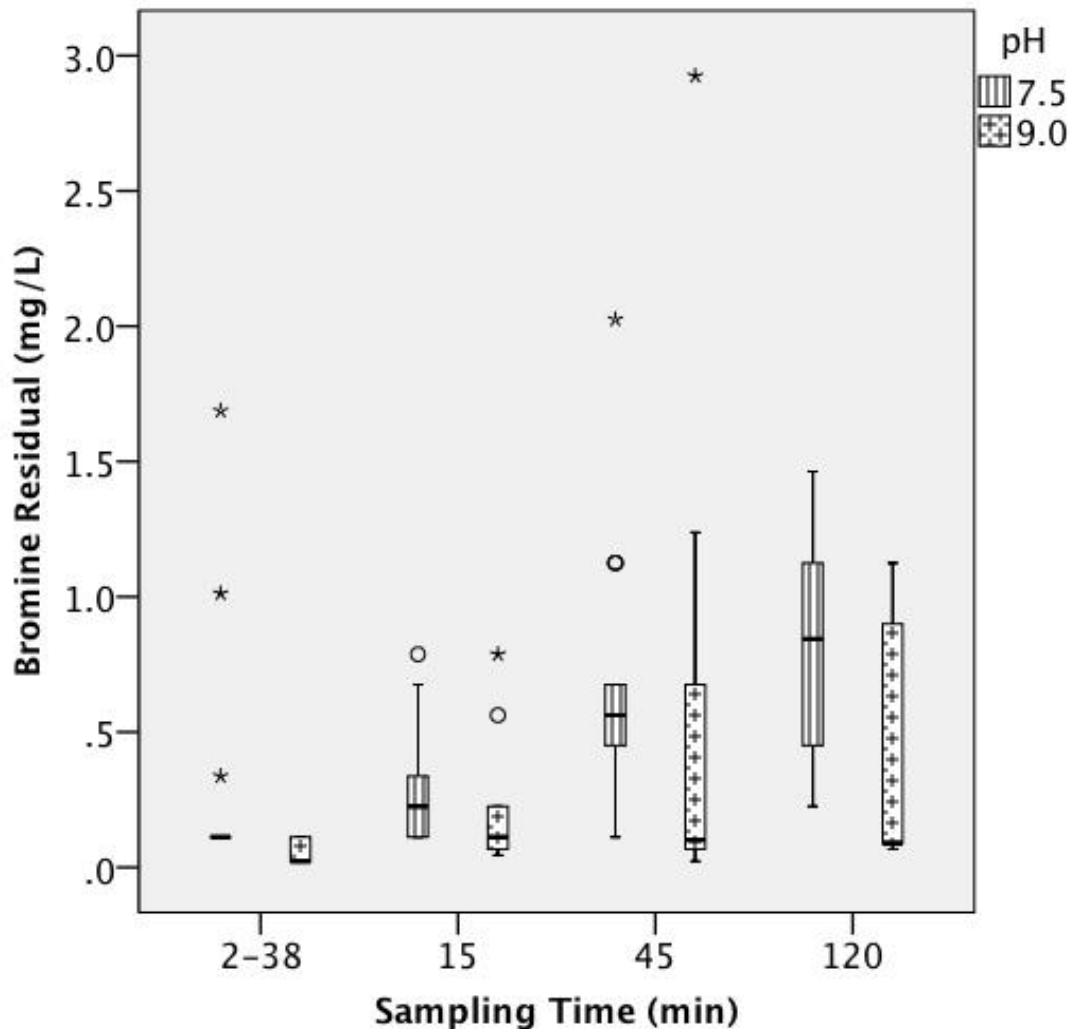


Figure 8. Average bromine residual (mg/L) for well water and sewage addition samples for pH 7.5 and 9 over sampling time. N=135. The $^{\circ}$ indicates an outlier, and the $*$ indicates an extreme outlier.

There was no significant effect of pH ($p=0.402$) on bromine residual (Figure 9). Figure 9 shows the bromine residual between the two pH variables. Only the experiments that used cartridges 1, 2, or 3 were considered (only the sewage samples could be analyzed). For this subset of data, the pH 7.5

samples had an average bromine residual of 0.54 +/- 0.48 mg/L; the pH 9 samples had an average bromine residual of 0.70 +/- 0.45 mg/L.

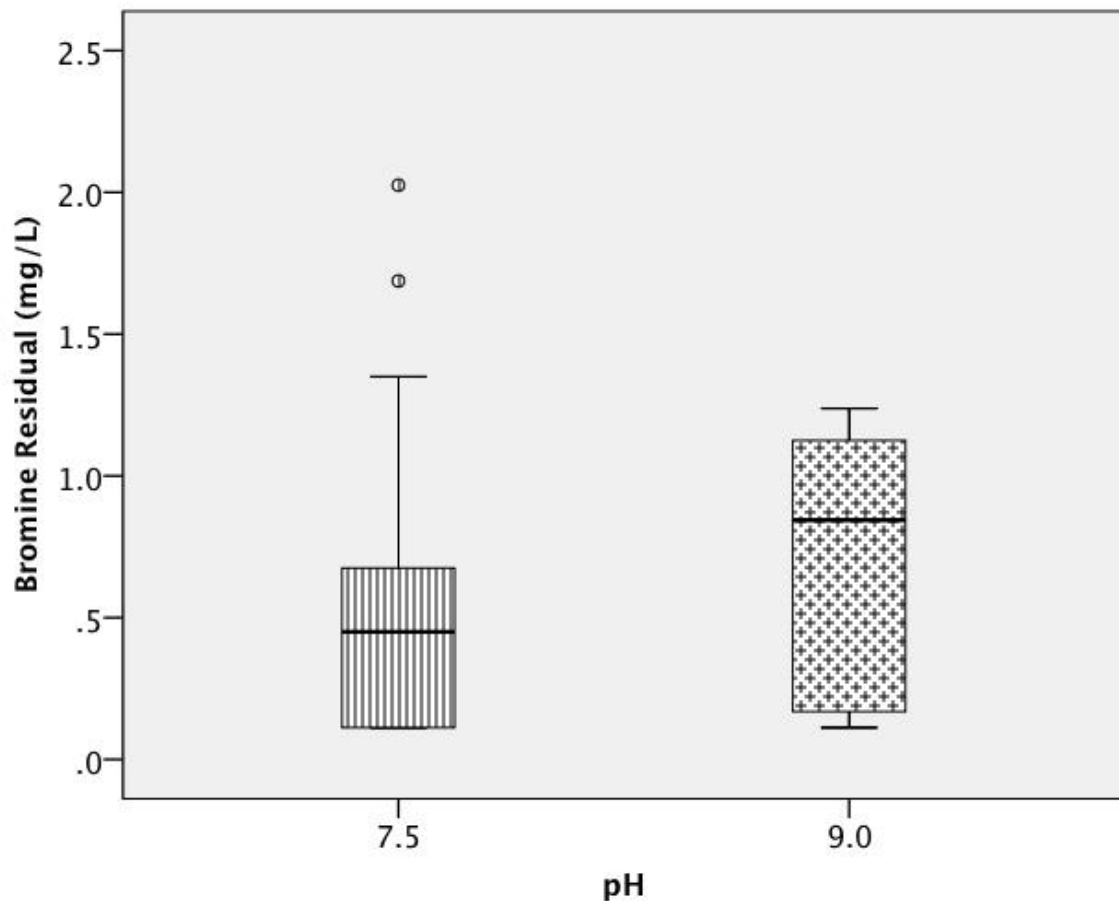


Figure 9. Bromine residual (mg/L) between the two pH variables. N=48 for cartridges 1, 2 and 3 only. The ^o indicates an outlier.

3.2.2.2 FLOW RATE

Overall, the flow rate did not differ significantly between treatments ($p=0.626$), pH ($p=0.869$), or cartridges ($p=0.433$) (Table 13). There was a significant difference in flow rates between timed samples ($p<0.001$) as expected with the decrease in the influent volume and head; the first flush sample was different than all other samples ($p<0.001$), the 15 minute sample was different than the 45 minute sample ($p=0.044$) and the 120 minute sample ($p<0.001$), and

the 45 minute sample was also significantly different from the 120 minute sample ($p=0.004$) (Figure 10). Figure 10 shows the flow rate between sampling times. The first flush samples had an average flow rate of 61 ± 25 mL/min, the 15 minute samples had an average of 38 ± 14 mL/min, the 45 minute samples had an average of 27 ± 14 mL/min, and the 120 minute samples had an average of 13 ± 5.3 mL/min.

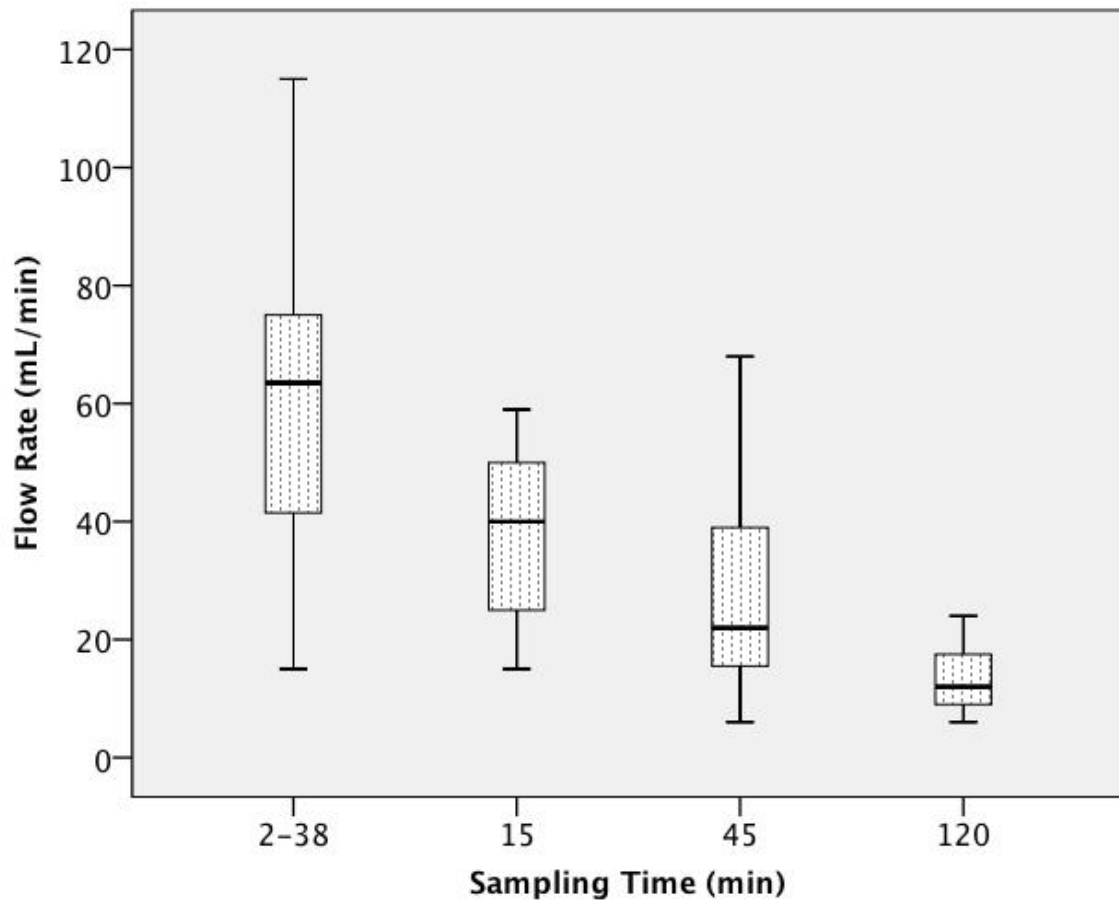


Figure 10. The flow rate (in mL/min) for all samples, separated by sampling time. There was a significant difference in flow rates between samples ($p<0.001$); the first flush sample was different than all other samples ($p<0.001$), the 15 minute sample was different than the 45 minute sample ($p=0.044$) and the 120 minute sample ($p<0.001$), and the 45 minute sample was also significantly different from the 120 minute sample ($p=0.004$). $N=135$.

3.2.2.3 DEVICE PERFORMANCE

For each of the influent and effluent samples, MS2 concentration was determined by plaque assay. The $\log_{10}$ reduction values were calculated by taking the $\log_{10}$ concentration of the influent minus the $\log_{10}$ concentration of the effluent. The same influent $\log_{10}$ concentration was used for four corresponding effluent $\log_{10}$ concentrations (in four separate calculations). Each effluent sample (from each of the four time points when effluent samples were taken) had a separate $\log_{10}$ reduction value calculated; thus, each experiment has one influent concentration and four effluent concentrations or $\log_{10}$ reduction values. For MS2, the detection limit was one PFU/mL; non-detect samples were considered to have one PFU/mL for statistical analysis.

The average influent concentration of MS2 was 7.16 $\log_{10}$ (n=36), and the average effluent concentration was 1.79 $\log_{10}$ (n=135 from all timed samples from all experiments). The overall reduction of MS2 was 5.4 $\log_{10}$. The device was capable of 4.7, 5.7, 5.3, and 5.9 $\log_{10}$ reduction of MS2 virus for the experiments at pH 7.5 for well water and sewage and pH 9 for well water and sewage, respectively (Table 8). Table 8 shows the average (+/- standard deviation) flow rate, bromine residual, MS2 influent and effluent concentrations, and $\log_{10}$ reduction. There was no correlation between influent concentration of MS2 and the $\log_{10}$ reduction ($R^2=0.000$).

Table 8. Physical characteristics (flow rate and bromine residual), and MS2 influent and effluent concentrations and log₁₀ reductions. The average values (+/- standard deviation) are given for each measurement for each combination of pH and water treatment variables.

	pH 7.5		pH 9		
	Well Water	Sewage	Well Water	Sewage	
	n=9	n=9	n=9	n=3	n=6
Cartridge	1, 2, 3	1, 2, 3	4, 5, 6	1, 2, 3	4, 5, 6
Flow Rate (mL/min)	37 +/- 23	32 +/- 20	32 +/- 30	42 +/- 20	33 +/- 28
Bromine Residual (mg/L)	0.50 +/- 0.42	0.54 +/- 0.48	0.19 +/- 0.29	0.70 +/- 0.45	0.22 +/- 0.62
MS2 Influent Concentration (log ₁₀)	6.9	6.7	7.3	7.6	7.7
MS2 Effluent Concentration (log ₁₀)	2.3	1.0	2.0	3.1	1.3
MS2 Log ₁₀ Reduction	4.7	5.7	5.3	4.6	6.7

For the pH 7.5 treatment, there was an average influent MS2 concentration of 6.8 log₁₀, an average effluent concentration of 1.6 log₁₀, and an average log₁₀ reduction of MS2 of 5.2 log₁₀ (Table 9 and 10). Table 9 shows the average MS2 concentrations and device characteristics (average flow rate and bromine residual) in the effluent post treatment for well water samples at pH 7.5. Table 10 shows the average MS2 concentrations and device characteristics

(average flow rate and bromine residual) in the effluent post treatment for sewage addition samples at pH 7.5.

Table 9. Average MS2 concentrations and device characteristics in the effluent post treatment for well water samples at pH 7.5.

Experiment #	Cartridge	Time (min) post addition of influent to upper reservoir	# of Samples	Average MS2 Concentration (log ₁₀)	Average Flow Rate (mL/min)	Average Bromine Residual (mg/L)
6, 13, 23	1	0 ⁴	3	4.1	0	0
		2;4;10	3	0.0	46	0.41
		15;15;17	3	3.1	41	0.19
		45	3	2.9	39	0.49
		120	3	1.7	15	0.83
2, 8, 12, 25	2	0	4	4.0	0	0
		3;3;5;24	4	0.3	53	0.11
		15;15;17	3	4.4	39	0.34
		45	4	3.5	27	0.62
		120	4	1.4	14	0.87
7, 24	3	0	2	3.8	0	0
		4;7	2	0.0	84	0.11
		15	2	4.7	48	0.28
		45	2	3.9	40	0.62
		120	2	2.7	18	1.18
			Total n=44		39	0.50

⁴ Time of 0 (zero) is the untreated influent sample.

Table 10. Average MS2 concentrations and device characteristics in the effluent post treatment for sewage addition samples at pH 7.5.

Experiment #	Cartridge	Time (min) post addition of influent to upper reservoir	# of Samples	Average MS2 Concentration ($\log_{10}$)	Average Flow Rate (mL/min)	Average Bromine Residual (mg/L)
5, 11, 15	1	0 ⁵	3	6.7	0	0
		3;11;13	3	0.0	43	0.19
		15	3	1.2	30	0.26
		45	3	1.3	28	0.64
		120	3	0.8	12	0.56
4, 10, 14	2	0	3	6.8	0	0
		2;3;5	3	0.0	36	0.11
		15	3	2.3	31	0.30
		45;45;46	3	0.6	28	0.68
		120	3	0.7	15	0.75
3, 9, 16	3	0	3	6.6	0	0
		3;3;9	3	0.8	64	0.64
		15	3	2.0	43	0.43
		45	3	1.4	31	0.99
		120	3	0.8	18	0.94
			Total n=45		32	0.54

For the pH 9 treatment, there was an average influent MS2 concentration of 7.5 $\log_{10}$, an average effluent concentration of 2.0 $\log_{10}$, and average $\log_{10}$ reduction of MS2 was 5.6 $\log_{10}$ (Table 11 and 12). Table 11 shows the average MS2 concentrations and device characteristics (average flow rate and bromine residual) in the effluent post treatment for well water samples at pH 9. Table 12

⁵ Time of 0 (zero) is the untreated influent sample.

shows the average MS2 concentrations and device characteristics (average flow rate and bromine residual) in the effluent post treatment for sewage addition samples at pH 9.

Table 11. Average MS2 concentrations and device characteristics in the effluent post treatment for well water samples at pH 9.

Experiment #	Cartridge	Time (min) post addition of influent to upper reservoir	# of Samples	Average MS2 Concentration (log ₁₀)	Average Flow Rate (mL/min)	Average Bromine Residual (mg/L)
29, 36, 44	4	0 ⁶	3	7.3	0	0
		9;10;13	3	1.7	62	0.05
		15	3	3.4	31	0.07
		45	3	3.3	19	0.17
		120	3	2.8	11	0.35
30, 37, 45	5	0	3	7.4	0	0
		20;22;37	3	0.9	61	0.06
		45	3	1.9	11	0.11
		120	3	0.0	8	0.35
31, 35, 46	6	0	3	7.1	0	0
		12;20;26	3	1.7	95	0.07
		14	1	5.1	26	0.11
		45	3	2.4	17	0.26
		120	3	1.2	8	0.44
			Total n=40		32	0.19

⁶ Time of 0 (zero) is the untreated influent sample.

Table 12. Average MS2 concentrations and device characteristics in the effluent post treatment for sewage addition samples at pH 9.

Experiment #	Cartridge	Time (min) post addition of influent to upper reservoir	# of Samples	Average MS2 Concentration (log ₁₀)	Average Flow Rate (mL/min)	Average Bromine Residual (mg/L)
18	1	0 ⁷	1	7.3	0	0
		2	1	0.0	24	0.11
		15	1	5.1	59	0.56
		45	1	5.0	40	1.13
		120	1	3.0	19	1.01
17	2	0	1	7.4	0	0
		4	1	0.0	64	0.11
		15	1	4.2	56	0.23
		45	1	4.6	46	0.90
		120	1	3.0	15	1.13
19	3	0	1	8.3	0	0
		3	1	0.0	80	0.11
		15	1	4.6	48	0.79
		45	1	3.9	38	1.24
		120	1	3.3	20	1.13
32, 40	4	0	2	7.7	0	0
		6	2	0.0	42	0.02
		15;24	2	3.7	31	0.10
		45	2	5.7	29	1.54
		120	2	4.0	11	0.17
33, 38	5	0	2	7.7	0	0
		10;18	2	0.0	83	0.02
		17	1	0.0	28	0.07
		45	2	0.0	19	0.09

⁷ Time of 0 (zero) is the untreated influent sample.

Table 12 (cont'd).

		120	2	0.0	10	0.09
34, 39	6	0	2	7.8	0	0
		25	2	0.0	84	0.02
		45	2	0.0	16	0.09
		120	2	0.0	10	0.09
			Total n=42		38	0.47

3.2.2.3.1 EFFECT OF pH AND SEWAGE ADDITION

For the pH 7.5 samples, the sewage samples showed higher $\log_{10}$ reduction of MS2 than the well water samples ($p=0.005$) (Figure 11). Figure 11 shows the $\log_{10}$ reduction of MS2 between well water and sewage addition samples, for pH 7.5. The well water samples had an average influent concentration of MS2 of 6.9 $\log_{10}$ and an effluent concentration of 2.3 $\log_{10}$, giving an average $\log_{10}$ reduction of 4.7. The sewage samples had an average influent concentration of MS2 of 6.7 $\log_{10}$ and an effluent concentration of 1.0 $\log_{10}$, giving an average $\log_{10}$ reduction of 5.7.

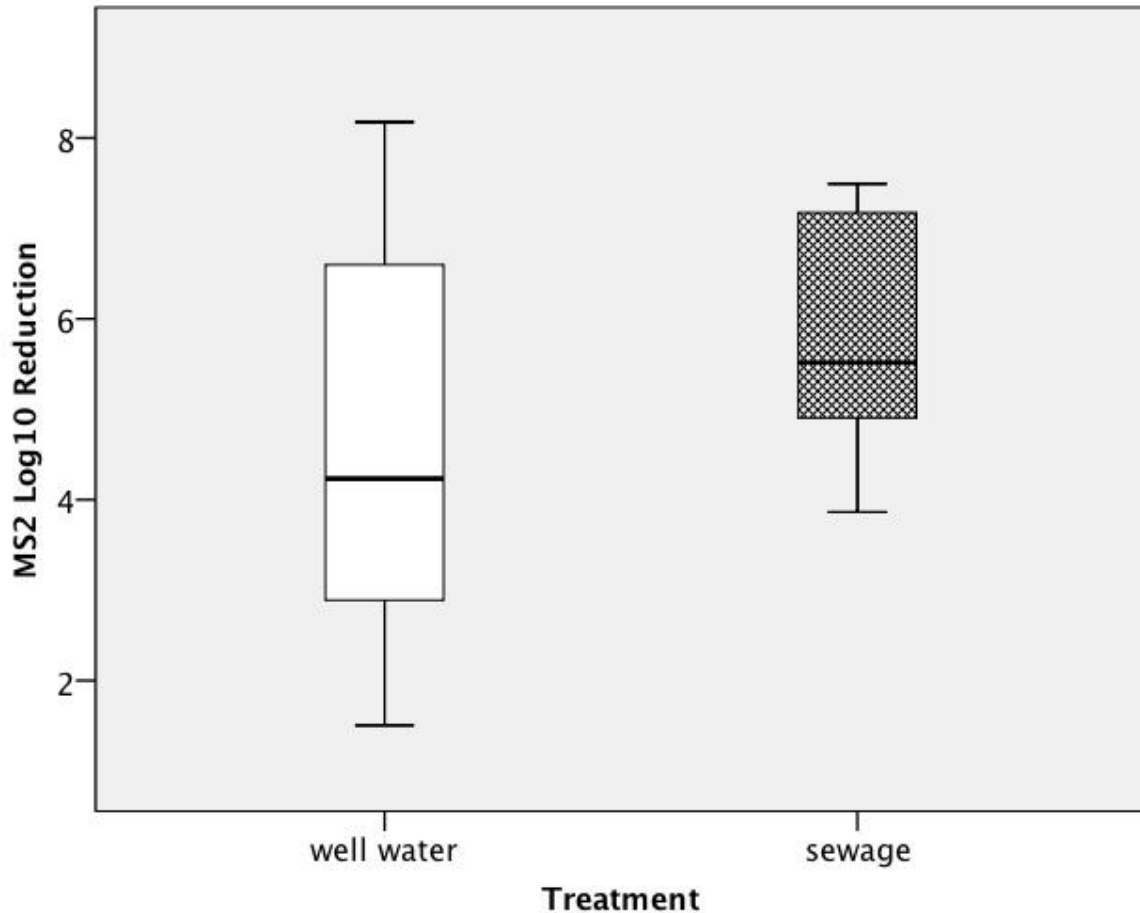


Figure 11. $\log_{10}$ reduction of MS2 between well water and sewage addition samples (pH 7.5). The sewage samples did have significantly different $\log_{10}$ reduction compared to the well water samples ($p=0.005$). $N=9$ for well water and 9 for sewage ($N=18$ total).

For the pH 9 samples, the well water samples had an average influent concentration of MS2 of 7.3 $\log_{10}$ and an effluent concentration of 2.0 $\log_{10}$, giving an average $\log_{10}$ reduction of 5.3. The sewage samples had an average influent concentration of MS2 of 7.7 $\log_{10}$ and an effluent concentration of 1.9 $\log_{10}$, giving an average $\log_{10}$ reduction of 5.9. There was no significant effect of sewage addition on the $\log_{10}$ reduction of MS2 ($p=0.389$), however the data did

show an increased removal overall with the addition of sewage. Only the samples that used cartridges 4, 5, and 6 were used for this comparison, which may have influenced the statistical significance.

Overall, the sewage samples showed an average $\log_{10}$ reduction of MS2 of 5.8 and the well water samples had an average $\log_{10}$ reduction of 5.0 (Figure 12). Figure 12 shows the $\log_{10}$ reduction of MS2 between well water and sewage addition samples.

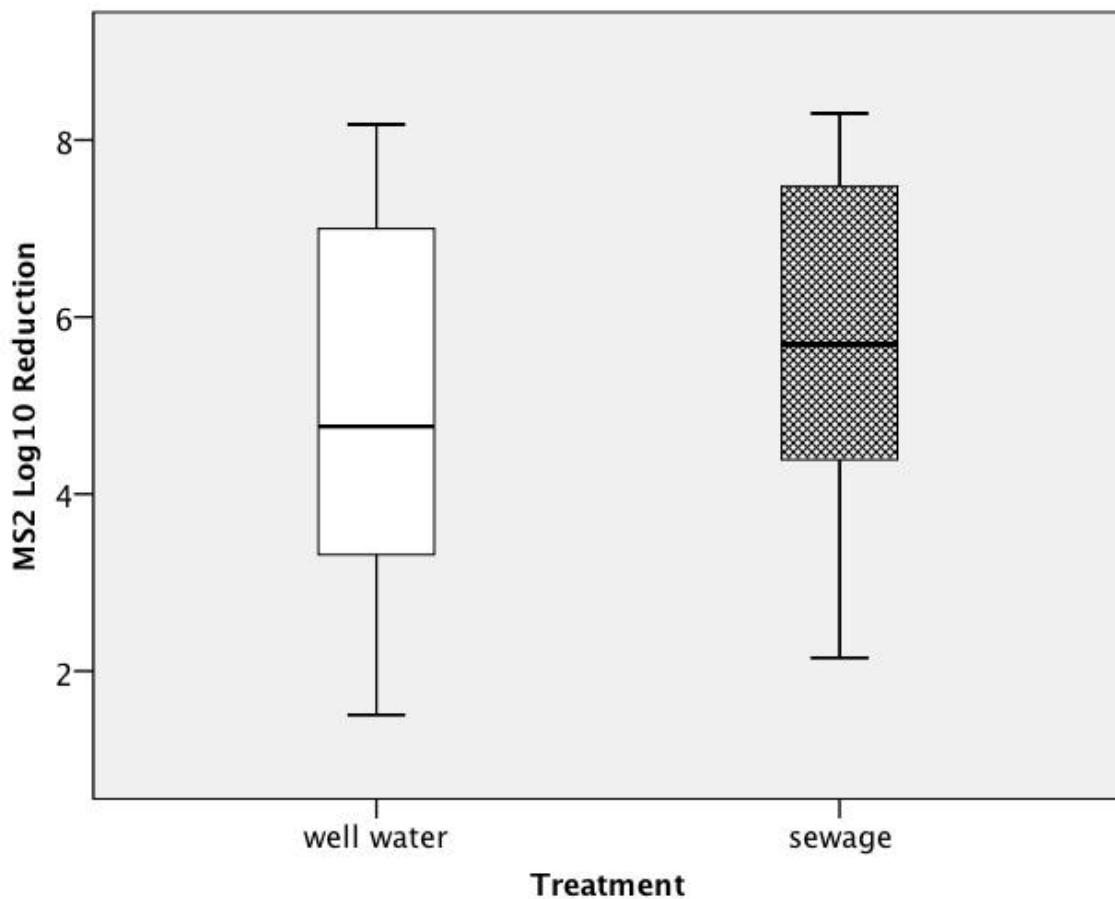


Figure 12. $\log_{10}$ reduction of MS2 between well water and sewage addition samples (pH 7.5 and 9 samples together). N= 18 for well water and 18 for sewage (N=36 total).

When pH effects were compared with the sewage only samples that used cartridges 1, 2, or 3 it was found that there was a statistically significant effect of pH on the $\log_{10}$ reduction of MS2 ($p=0.006$) (Figure 13). Figure 13 shows the $\log_{10}$ reduction of MS2 between pH 7.5 and pH 9 samples for this comparison. Otherwise pH could not be shown to impact reductions.

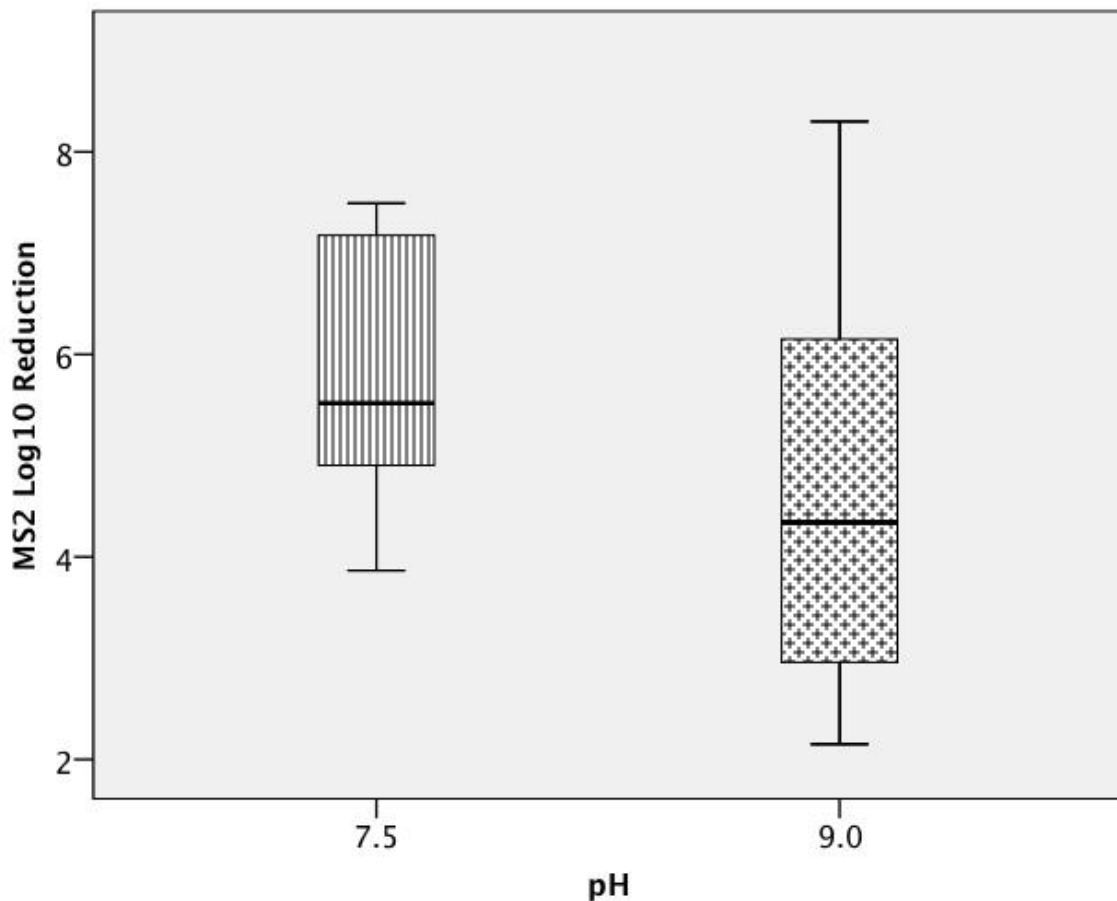


Figure 13. $\log_{10}$ reduction of MS2 between pH 7.5 and pH 9 sewage samples. There was a significant difference in the $\log_{10}$ reduction of MS2 between the samples ($p=0.006$). $N=9$ for pH 7.5 and 3 for pH 9 ($N=12$ total).

3.2.2.3.2 DEVICE VARIABILITY

For the pH 7.5 samples, the $\log_{10}$ reduction of MS2 starts high at the first flush sample (2-38 minutes) (about 7 $\log_{10}$), drops to around 4 $\log_{10}$ when the second sample was collected at 15 minutes, and then goes back up to about 5 $\log_{10}$ for the 45 and 120 minute time points. There was a significant effect of sampling time ($p < 0.001$); the first flush sample differed significantly from both the 15 minute sample ($p < 0.001$) and the 45 minute sample ($p < 0.001$). The 15 minute sample also differed significantly from the 120 minute sample ($p = 0.005$) (Figure 14). Figure 14 shows the $\log_{10}$ reduction of MS2 for the pH 7.5 samples. There was no significant effect of cartridge ($p = 0.979$) or ceramic ($p = 0.979$). Cartridge 1 had an average $\log_{10}$ reduction of MS2 of 5.4, cartridge 2 had an average of 5.3, and cartridge 3 had an average of 4.8. Ceramic 1 had an average $\log_{10}$ reduction of MS2 of 5.2, ceramic 2 had an average of 5.3, and ceramic 3 had an average of 5.1.

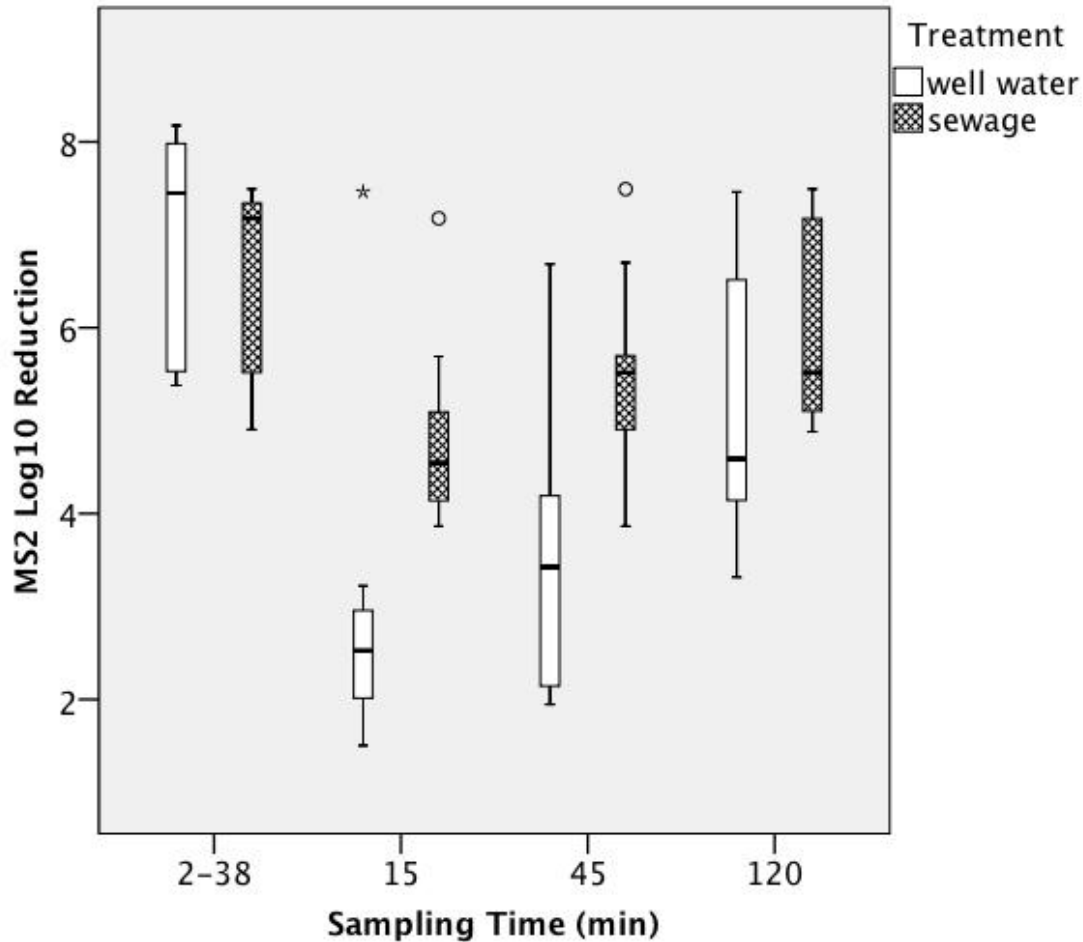


Figure 14. Log₁₀ reduction of MS2 for the pH 7.5 samples. There was also a significant difference between samples ($p < 0.001$); the first flush (2-38 minutes) sample differed significantly from both the 15 minute sample ($p < 0.001$) and the 45 minute sample ($p < 0.001$). The 15 minute sample also differed significantly from the 120 minute sample ($p = 0.005$). $N = 18$. The $^{\circ}$ indicates an outlier, and the * indicates an extreme outlier.

For the pH 9 samples, the log₁₀ reduction of MS2 started around 7 log₁₀ for the first flush sample, dropped to around 4 log₁₀ for the 15 minute sample, and then increased back to about 7 log₁₀ for the 45 and 120 minute samples. There was a significant effect of sample ($p = 0.002$), but only the first flush sample and 45 minute sample differed significantly ($p = 0.004$) (Figure 15). Figure 15

shows the $\log_{10}$ reduction of MS2 for the pH 9 samples. There was no significant effect of ceramic ($p=0.270$) or cartridge ($p=0.542$). Cartridge 4 had an average $\log_{10}$ reduction of MS2 of 4.7, cartridge 5 has an average of 7.1, and cartridge 6 has an average of 6.1. Ceramic 1 had an average $\log_{10}$ reduction of MS2 of 5.9, ceramic 2 had an average of 5.3, and ceramic 3 had an average of 5.6.

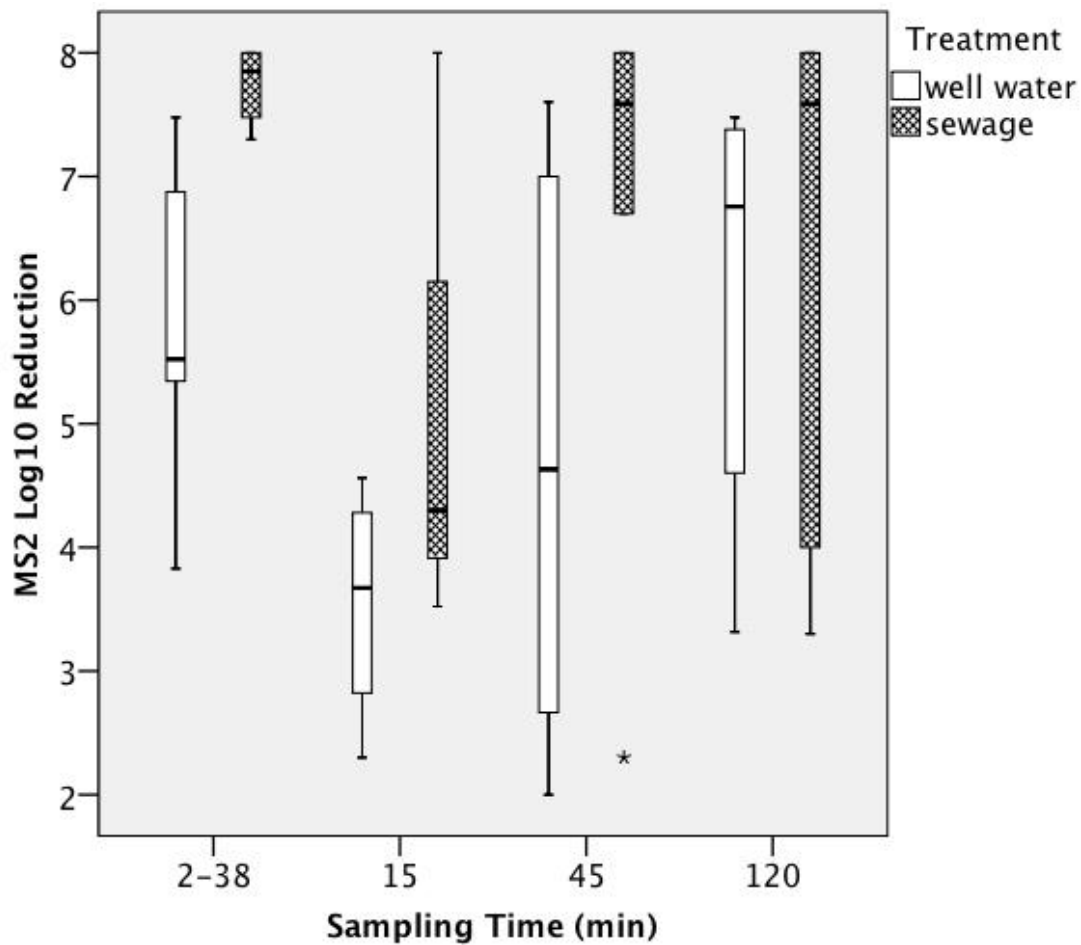


Figure 15. $\log_{10}$ reduction of MS2 for the pH 9 samples. There was a significant effect of sampling time ($p=0.002$), but only the first flush sample and 45 minute sample differed significantly ($p=0.004$). $N=18$. The * indicates an extreme outlier.

Overall, there was a significant effect of sampling time on the $\log_{10}$ reduction of MS2 ($p < 0.001$); the first flush and the 15 minute samples ($p < 0.001$), the first flush and 45 minute samples ($p = 0.001$), the 15 minute and 120 minute samples ($p = 0.001$), and the 45 minute and 120 minute samples ($p = 0.006$) were all significantly different (Figure 16). Figure 16 shows the MS2 $\log_{10}$ reduction at each sampling time. The first flush samples showed an average $\log_{10}$ reduction of MS2 of 6.7, the 15 minute samples had an average of 3.9, the 45 minute samples had an average of 4.8, and the 120 minute samples had an average of 5.7. There was no significant effect of ceramic ($p = 0.517$). No correlation was found between MS2 $\log_{10}$ reduction and flow rate ($R^2 = 0.005$, $p = 0.421$) or with bromine residual ($R^2 = 0.025$, $p = 0.069$). There was also no significant effect of cartridge ($p = 0.479$). Only the pH 9 sewage results were used for this analysis, since that was the only group of the same type of samples that used all six cartridges.

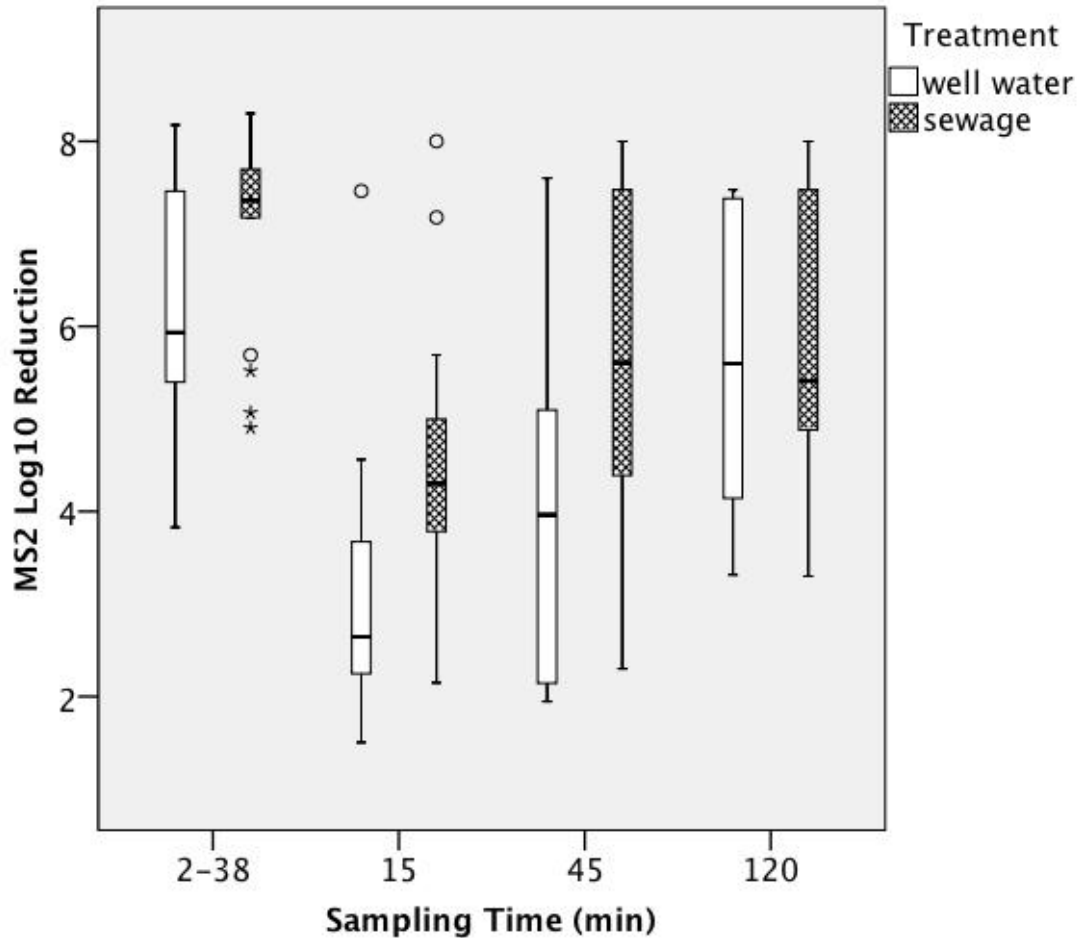


Figure 16. MS2 log₁₀ reduction at each sampling time. There was a significant effect of sample on the log₁₀ reduction of MS2 ($p < 0.001$); the first flush and the 15 minute samples ($p < 0.001$), the first flush and 45 minute samples ($p = 0.001$), the 15 minute and 120 minute samples ($p = 0.001$), and the 45 minute and 120 minute samples ($p = 0.006$) were all significantly different. $N = 18$ for well water and 18 for sewage ($N = 36$ total). The $^{\circ}$ indicates an outlier, and the * indicates an extreme outlier.

3.3 MURINE NOROVIRUS

3.3.1 INFLUENT CHARACTERIZATION

Table 13 shows the average influent concentrations for the experimental challenges using murine norovirus. The murine norovirus stock used gave an average influent concentration of 4.0, 4.3, 2.8, and 2.3 log₁₀ PFU/mL for the experiments at pH 7.5 for well water and sewage and pH 9 for well water and

sewage, respectively. The pH 7.5 experiments had higher influent concentrations of murine norovirus. All of the murine norovirus stock was prepared using the same procedure and materials; the differences seen in the influent concentrations cannot be explained at this time. Murine norovirus stocks were purified on 9 different dates (2-15-12, 2-29-12, 3-19-12, 3-21-12, 4-12-12, 5-10-12, 10-23-12, 11-5-12, and 12-14-12). The stocks were stored at -80°C until use. Due to issues with a working plaque assay, only the stock that was purified on 5-10-12 was assayed to determine the concentration; the pure stock had a concentration of 10^6 PFU/mL (assay run 10-15-12). The purified stocks produced 2-15-12 to 5-10-12 were used for the pH 7.5 experiments, and the purified stocks produced 5-10-12 to 12-14-12 was used for the pH 9 experiments. The purified stocks were a maximum of five months old when used, but most of the stocks were less than a month old when used. The differences seen in the influent concentrations of murine norovirus are probably due to slight differences in the starting concentrations of the unpurified norovirus stock. Even though the same conditions and protocols were used to grow up each batch of virus, slight differences in initial cell and virus concentrations could result in large differences in the amount of new viruses produced in each batch.

Table 13. Influent concentrations for murine norovirus for the pH, sewage and well water variables. Shown are the mean values (+/- standard deviation) for virus influent concentrations for each combination of pH and water treatment variables.

	pH 7.5		pH 9	
	Well Water	Sewage	Well Water	Sewage
Murine Norovirus Influent Concentration (log ₁₀)	4.0 (n=9)	4.3 (n=9)	2.8 (n=3)	2.3 (n=3)

Figure 17 shows the average murine norovirus influent concentrations for the pH 7.5 samples. There was an average murine norovirus influent concentration of 4.0 log₁₀ for the well water samples and 4.3 log₁₀ for the sewage samples (Figure 17).

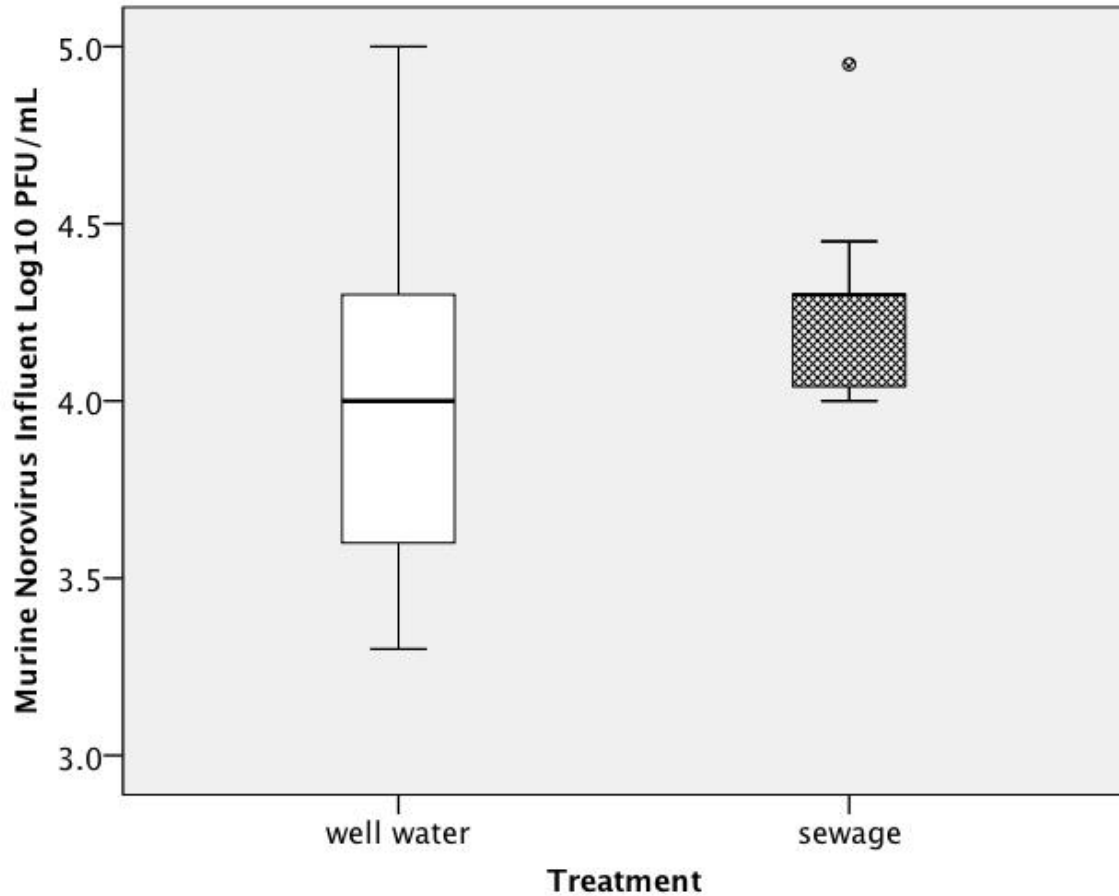


Figure 17. Average murine norovirus influent concentrations (in $\log_{10}$) for pH 7.5 samples. N=9 for well water and sewage (N=18 total). The $^{\circ}$ indicates an outlier.

Figure 18 shows the average murine norovirus influent concentrations for the pH 9 samples. There was an average murine norovirus influent concentration of $2.8 \log_{10}$ for the well water samples and $2.3 \log_{10}$ for the sewage samples (Figure 18).

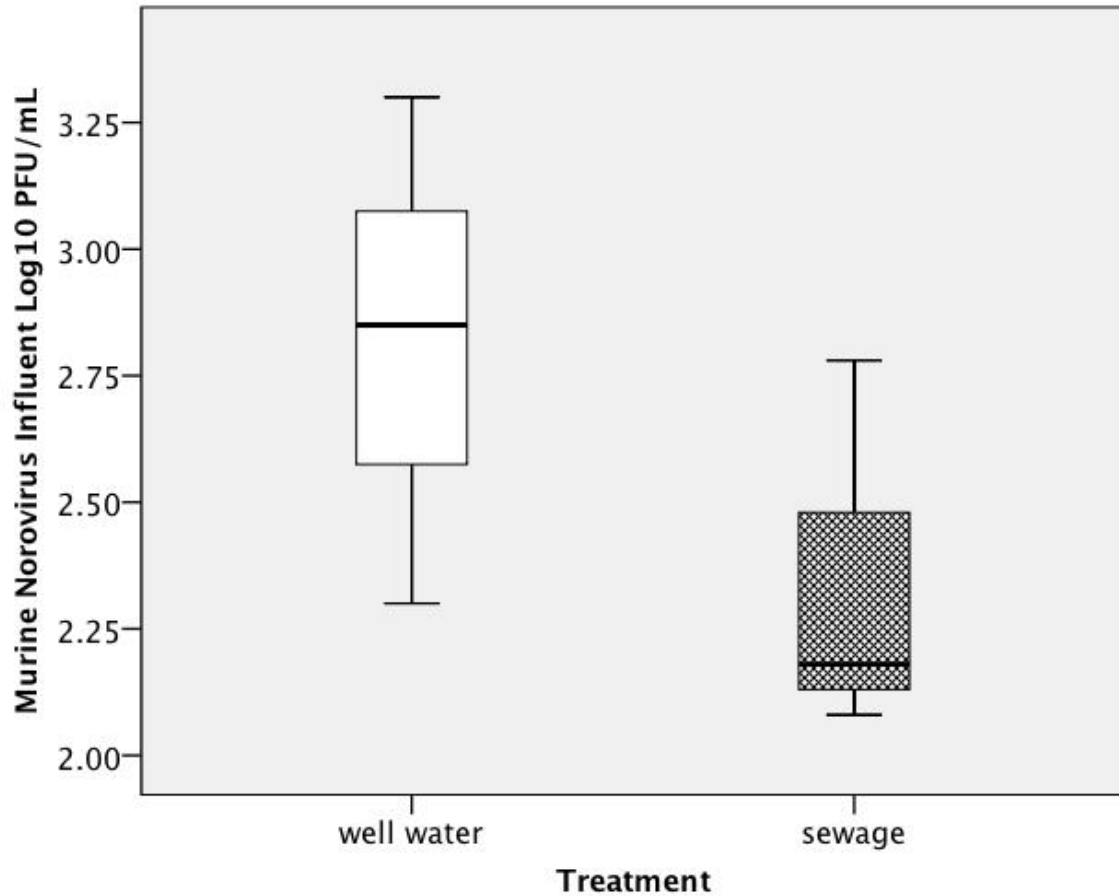


Figure 18. Average murine norovirus influent concentrations (in $\log_{10}$) for pH 9 samples. N=3 for well water and sewage (N=6 total).

For the murine norovirus samples, there was an average of 3.8 $\log_{10}$ in the untreated influent samples (Figure 19). Figure 19 shows the average murine norovirus influent concentrations for all samples. There was found to be no significant difference in the murine norovirus influent concentrations between the water treatments ($p=0.768$), but there was a significant difference between pH treatments ($p<0.001$). The pH 7.5 samples had a mean of 4.1 $\log_{10}$, while the pH 9 samples had a mean of 2.6 $\log_{10}$. All of the norovirus samples have not been analyzed due to time constraints.

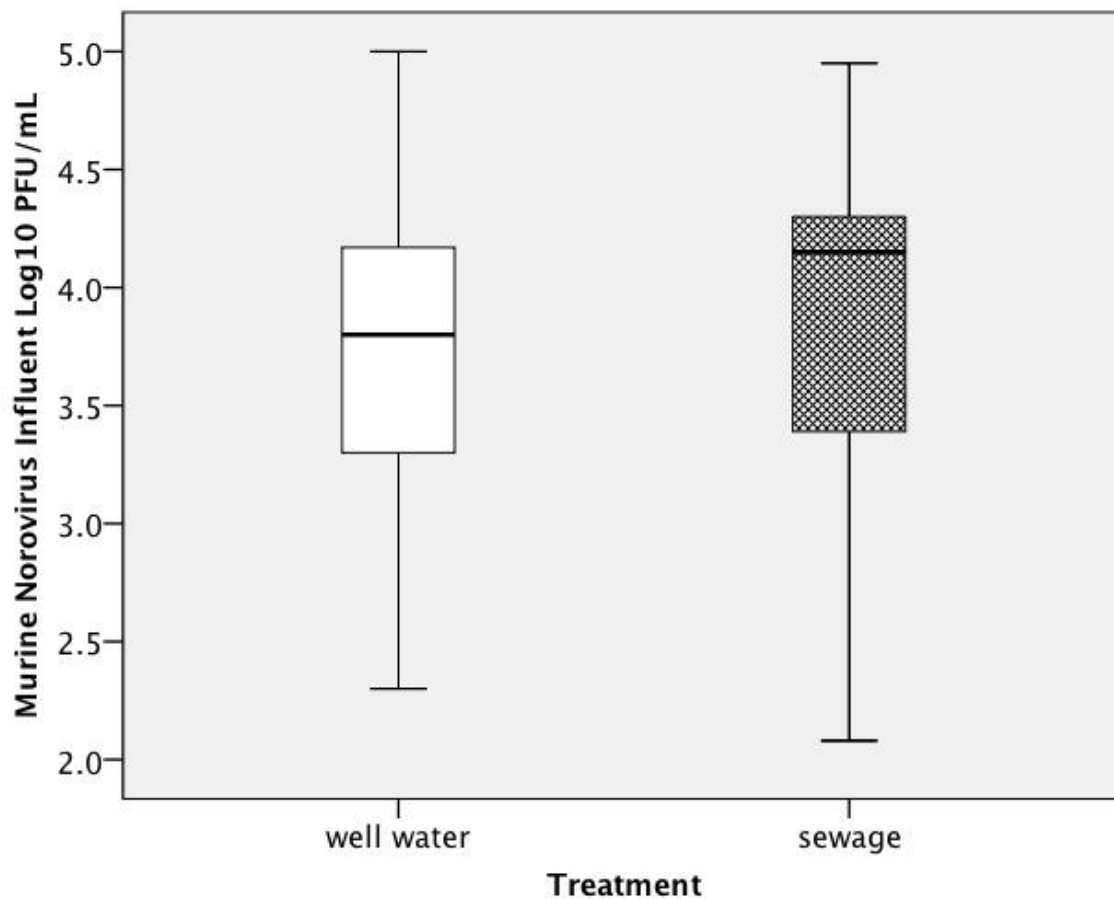


Figure 19. Average murine norovirus influent concentrations (in log₁₀) for all samples. N=12 for well water and sewage (N=24 total).

3.3.2 EFFLUENT CHARACTERIZATION

3.3.2.1 DEVICE PERFORMANCE

For the murine norovirus samples, all of the treated samples were reduced to the detection limit (2 PFU/mL), so no sample variation could be analyzed (Figure 20). Log₁₀ reductions are presented as “greater than or equal to” the values given.

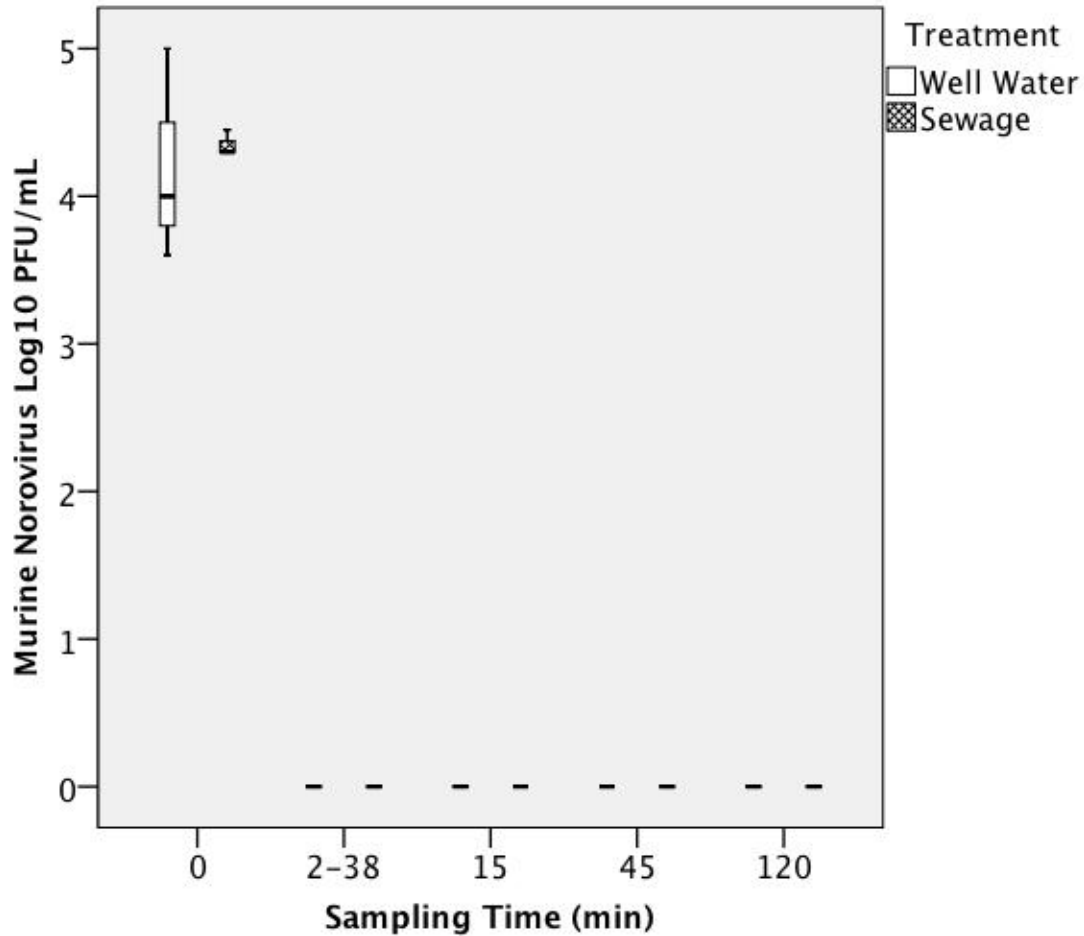


Figure 20. The $\log_{10}$ concentrations of murine norovirus in untreated influent (time=0, n= 24) and treated samples. The treated samples were all reduced to the detection limit (2 PFU/mL).

For each of the influent and effluent samples, murine norovirus concentration was determined by plaque assay. The $\log_{10}$ reduction values were calculated by taking the $\log_{10}$ concentration of the influent minus the $\log_{10}$ concentration of the effluent.

Overall, the average influent concentration of murine norovirus was 3.8 $\log_{10}$, the average effluent concentration was <0.3 $\log_{10}$, and the average $\log_{10}$ reduction was >3.8 $\log_{10}$ (Table 14). Table 14 shows the mean norovirus

influent and effluent concentrations, as well as the log₁₀ reductions for each combination of pH and water treatment variables. Although a true statistical comparison is not possible due to the effluent samples being reduced to the detection limit, it is important to note that the well water and sewage samples did perform similarly in regards to log₁₀ reduction of murine norovirus. All of the samples (both well water and sewage) were reduced to the detection limit, so it is possible to presume that the device is capable of the same performance even with contaminated water.

Table 14. Murine norovirus influent and effluent concentrations and log₁₀ reductions. The mean values (+/- standard deviation) are given for each measurement for each combination of pH and water treatment variables.

	pH 7.5		pH 9	
	Well Water	Sewage	Well Water	Sewage
	N=9	N=9	N=3	N=3
Cartridge	1, 2, 3	1, 2, 3	1, 2, 3	4, 5, 6
Murine Norovirus Influent Concentration (log ₁₀)	4.0	4.3	2.8	2.3
Murine Norovirus Effluent Concentration (log ₁₀)	<0.3	<0.3	<0.3	<0.3
Murine Norovirus Log Reduction (log ₁₀)	>3.7	>4.0	>2.5	>2.0

For the pH 7.5 samples, the average influent concentration of murine norovirus was $4.1 \log_{10}$, the average effluent concentration was $<0.3 \log_{10}$, and the average $\log_{10}$ reduction was $>3.9 \log_{10}$ (Table 15 and 16). Table 15 shows the average murine norovirus concentrations and device characteristics (average flow rate and bromine residual) in the effluent post treatment for well water samples at pH 7.5. Table 16 shows the average murine norovirus concentrations and device characteristics (average flow rate and bromine residual) in the effluent post treatment for sewage addition samples at pH 7.5.

Table 15. Average murine norovirus concentrations and device characteristics in the effluent post treatment for well water samples at pH 7.5.

Experiment #	Cartridge	Time (min) post addition of influent to upper reservoir	# of Samples	Average Murine Concentration (log ₁₀)	Average Flow Rate (mL/min)	Average Bromine Residual (mg/L)
6, 13, 23	1	0 ⁸	3	4.4	0	0
		2;4;10	3	0.3	46	0.41
		15;15;17	3	0.3	41	0.19
		45	3	0.3	39	0.49
		120	3	0.3	15	0.83
2, 8, 12, 25	2	0	4	3.9	0	0
		3;3;5;24	4	0.3	53	0.11
		15;15;17	3	0.3	39	0.34
		45	4	0.3	27	0.62
		120	4	0.3	14	0.87
7, 24	3	0	2	3.8	0	0
		4;7	2	0.3	84	0.11
		15	2	0.3	48	0.28
		45	2	0.3	40	0.62
		120	2	0.3	18	1.18
			Total n=44		39	0.50

⁸ Time of 0 (zero) is the untreated influent sample.

Table 16. Average murine norovirus concentrations and device characteristics in the effluent post treatment for sewage addition samples at pH 7.5.

Experiment #	Cartridge	Time (min) post addition of influent to upper reservoir	# of Samples	Average Murine Concentration (log ₁₀)	Average Flow Rate (mL/min)	Average Bromine Residual (mg/L)
5, 11, 15	1	0 ⁹	3	4.2	0	0
		3;11;13	3	0.3	43	0.19
		15	3	0.3	30	0.26
		45	3	0.3	28	0.64
		120	3	0.3	12	0.56
4, 10, 14	2	0	3	4.3	0	0
		2;3;5	3	0.3	36	0.11
		15	3	0.3	31	0.30
		45;45;46	3	0.3	28	0.68
		120	3	0.3	15	0.75
3, 9, 16	3	0	3	4.3	0	0
		3;3;9	3	0.3	64	0.64
		15	3	0.3	43	0.43
		45	3	0.3	31	0.99
		120	3	0.3	18	0.94
			Total n=45		32	0.54

For the pH 9 samples, the average influent concentration of murine norovirus was 2.6 log₁₀, the average effluent concentration was <0.3 log₁₀, and the average log₁₀ reduction was 2.2 log₁₀ (Table 17 and 18). Table 17 shows the average murine norovirus concentrations and device characteristics (average

⁹ Time of 0 (zero) is the untreated influent sample.

flow rate and bromine residual) in the effluent post treatment for well water samples at pH 9. Table 18 shows the average murine norovirus concentrations and device characteristics (average flow rate and bromine residual) in the effluent post treatment for sewage addition samples at pH 9.

Table 17. Average murine norovirus concentrations and device characteristics in the effluent post treatment for well water samples at pH 9.

Experiment #	Cartridge	Time (min) post addition of influent to upper reservoir	# of Samples	Average Murine Concentration (log ₁₀)	Average Flow Rate (mL/min)	Average Bromine Residual (mg/L)
29	4	0 ¹⁰	1	2.3	0	0
		10	1	0.3	55	0.02
		15	1	0.3	16	0.05
		45	1	0.3	15	0.05
		120	1	0.3	9	0.07
30	5	0	1	2.8	0	0
		20	1	0.3	56	0.05
		45	1	0.3	11	0.07
		120	1	0.3	7	0.09
31	6	0	1	3.3	0	0
		20	1	0.3	100	0.07
		45	1	0.3	16	0.09
		120	1	0.3	7	0.09
			Total n=14		30	0.06

¹⁰ Time of 0 (zero) is the untreated influent sample.

Table 18. Average murine norovirus concentrations and device characteristics in the effluent post treatment for sewage addition samples at pH 9.

Experiment #	Cartridge	Time (min) post addition of influent to upper reservoir	# of Samples	Average Murine Concentration (log ₁₀)	Average Flow Rate (mL/min)	Average Bromine Residual (mg/L)
32	4	0 ¹¹	1	2.1	0	0
		6	1	0.3	46	0.02
		24	1	0.3	28	0.11
		45	1	0.3	20	2.92
		120	1	0.3	10	0.18
33	5	0	1	2.2	0	0
		10	1	0.3	75	0.02
		17	1	0.3	28	0.07
		45	1	0.3	20	0.11
		120	1	0.3	12	0.09
34	6	0	1	2.8	0	0
		25	1	0.3	70	0.02
		45	1	0.3	18	0.09
		120	1	0.3	7	0.09
			Total n= 14		30	0.34

3.4 CONCLUSIONS

Overall, the device was shown to be capable of removing an average of 5.4 log₁₀ PFU/mL of MS2. Due to the low influent concentrations for the murine norovirus samples, the effectiveness of the device against that virus cannot be fully determined, but the device did remove an average of 3.5 log₁₀ PFU/mL of

¹¹ Time of 0 (zero) is the untreated influent sample.

murine norovirus. The addition of raw sewage did not have a significant effect on the performance of the device overall. However, for the pH 7.5 samples, the sewage addition samples did have significantly higher $\log_{10}$ reduction of MS2 compared to the well water samples. The increased pH did have a negative effect on the performance of the device but was only shown for those samples with sewage.

CHAPTER 4

DISCUSSION

The HaloSource Waterbird is a novel type of point of use device. Rather than using a liquid bromine solution, the bromine is attached to polystyrene beads (HaloPure media). As water rushes over the beads, bromine comes in contact with the biological particles and inactivates them. The unique delivery system for bromine makes the device difficult to evaluate as a disinfectant because the active dose of bromine cannot be measured, only the residual that is left after treatment. The exact contact time can also not be evaluated, and the mechanism is still not understood. The device utilizes both ceramic filtration and halogen disinfection (bromine) to remove and/or inactivate bacteria and viruses. The HaloPure media has been tested and approved by the EPA to be capable of six $\log_{10}$ reduction of bacteria and four $\log_{10}$ reduction of viruses (poliovirus and rotavirus) (Dr. Jeff Williams, Personal Communication). This study aimed to test the Waterbird device as a whole and particularly bromine media disinfection, similar to how a buyer would use it to treat water in their home. The main objective for the study was to determine the effectiveness of the Waterbird device for inactivating and or removing MS2 bacteriophage and murine norovirus. Sub-objectives were to determine if pH or added organic materials (in the form of raw sewage) had an impact on the device's performance.

Past studies suggest that MS2 is easier to inactivate with chlorine than murine norovirus; higher $\log_{10}$ reductions can be achieved for MS2 with the same contact time and chlorine concentration (Lim et al., 2010). The study by

Lim et al. (2010) used demand-free water at pH 7.2; experiments were run at 5°C and 20°C. The experiments were set up in batch reactors, with 30 mL volume per experiment. Four log₁₀ reduction of MS2 was possible after four minutes of contact time with 0.17 mg/L chlorine; only two minutes of contact time with 0.19 mg/L chlorine resulted in three log₁₀ reduction of murine norovirus. While the present study showed a lower titer of the murine norovirus in the influent than anticipated, it was possible to show at least two to four log₁₀ reductions, which seems to be somewhat comparable result.

The same disinfection delivery system (HaloPure bromine media) has been used previously in three studies (Enger et al., Draft Manuscript; Coulliette et al., 2010; McLennan et al., 2009). However, all of these studies simply tested the effectiveness of the HaloPure media (without the carbon pre-filter), while the present study also included the ceramic pre-filter during the device testing. For MS2 bacteriophage, Coulliette et al. (2010) found that the device was capable of an average log₁₀ reduction of 5.0, which was not different overall from the present study (5.4). Their system had an average flow rate of 82 mL/min, and an average bromine residual (for samples taken at 60 and 90 minutes) of 1.2 mg/L (Coulliette et al., 2010). The average flow rate for the present study was half this (34 mL/min), and the average bromine residual (for the samples taken at 45 and 120 minutes) was lower (0.60 mg/L). The slightly higher log₁₀ reduction seen in the present study could be due to the presence of the ceramic pre-filter, which

might be able to remove some viruses. The ceramic pre-filter also lowered the flow rate. The second study by Enger et al. (Draft Manuscript) showed an average of 4.7 log₁₀ reduction of MS2; the average flow rate was 160 mL/min, and the average bromine residual was 1.0 mg/L. As with the Coulliette et al. (2010) study, the absence of the ceramic pre-filter could explain the lower log₁₀ reduction values for MS2 and the higher flow rate (Enger et al., Draft Manuscript). The final study by McLennan et al. (2009) used natural coliphage from raw sewage (10%) instead of adding pure stock of MS2. They observed an average log₁₀ reduction of 1.8, but their starting influent concentration of coliphage was much lower than in the present study. The chlorine system produced residuals ranging from zero to 0.60 mg/L and bromine system had residuals of 0.68 to 1.8 mg/L. All of their coliphage samples were reduced to the detection limit for the bromine but only one log₁₀ reduction for the chlorine, so the bromine device could be capable of more than a 1.8 log₁₀ reduction of coliphage and was more effective than the chlorine.

The study by Enger et al. (Draft Manuscript) used human adenovirus (Adenovirus Type 2), where by a log₁₀ reduction of 4.9 was observed. As mentioned above, this study used a different type of cartridge (though the HaloPure media inside was about the same) and no ceramic pre-filter. Adenoviruses are double-stranded DNA viruses, so they may be more resistant to bromine. The Waterbird device has been tested with the EPA protocol, and

has been shown to be capable of reducing four log₁₀ of both poliovirus and rotavirus (Dr. Jeff Williams, Personal Communication).

Two other studies tested liquid bromine against human viruses (Floyd et al., 1976; Sharp et al., 1975). Floyd et al. (1976) found a 3.5 log₁₀ reduction of poliovirus, and Sharp et al. (1975) found a four log₁₀ reduction of reovirus. Floyd et al. (1976) used 0.13 mg/L bromine, and it took 12 seconds of contact time to get 3.5 log₁₀ reduction of poliovirus. The experiment was done at 10°C and pH 7. Sharp et al. (1975) used 0.07 mg/L bromine, and it took 1.5 minutes of contact time to get a four log₁₀ reduction of reovirus. The experiment was done at 2°C and pH 7. Reoviruses are double-stranded RNA viruses, and poliovirus is a positive-strand RNA virus like norovirus. These studies demonstrate that bromine residual similar to what is delivered with the HaloPure media can inactivate both DNA and RNA viruses similar to what was found for the murine norovirus. It is important to note that these two studies used a low temperature which also affects disinfection efficacy (lowering it) and buffered water (no raw sewage added) to do their testing (which should increase the efficacy) compared to the present study of the WaterBird device.

In the present study, for the MS2 samples, there was a significant effect of pH, as would be expected (but only the sewage experiments could be compared). Previous studies suggest that halogen disinfection works better at a lower pH. One study using a chlorine solution found that it took more chlorine and longer contact time to get the same level of poliovirus inactivation at high pH

(10.0) than at low pH (6.0) (Kott et al., 1975). Amiri et al. (2010) found that it was more difficult to inactivate *E. coli* at pH 8.0 than at pH 6.0 or 6.9 when using organic N-chloramines as a disinfectant. Another study with chlorine dioxide (and six different viruses) found that the disinfectant loses its effectiveness at pH 9.0 (Junli et al., 1997). In accordance with those results, this study found that the device works better at pH 7.5 than at pH 9. At pH 7.5, bromine is mostly in the form of hypobromous acid (HOBr); at pH 9 it is mostly OBr^- (Song et al., 1996). At a low pH, the amino acids in the virus capsid are likely to be neutral, while at high pH the amino acids are likely to be negatively charged. If both the bromine and virus capsid are neutral the two won't repel one another, but if they are both negative they will repel one another and disinfection will be less successful.

For the MS2 samples overall, there was no significant effect of sewage addition. Additionally, neither bromine residual nor flow rate differed between the well water and sewage addition samples. It might be expected that the increased organic materials in the raw sewage would exert an additional bromine demand on the device, resulting in a higher bromine residual, and that the increased turbidity in the raw sewage treatment could result in the ceramic pre-filter clogging more rapidly and lowering the flow rate. However, neither observation occurred often enough to be statistically significant. Enger et al. (Draft Manuscript) also found that the addition of raw sewage had no effect on the performance of the HaloPure bromine media. It is unusual that the addition of raw sewage did not negatively affect the performance of the device, as that is the common result with many other types of disinfection systems. It appears that the

HaloPure media cartridges are less susceptible to variation in the organic content of the water to be treated. In addition to increasing the disinfectant demand, raw sewage can physically block contact and light-based disinfectants (if the virus particles are inside the organic material) (McGuigan et al., 2012). For the Waterbird device, the ceramic pre-filter is instrumental in removing the solid organic particulates present in the raw sewage, thus decreasing the bromine demand and allowing the bromine contact disinfectant to work better.

When considering only the samples taken at the 15 minute time point, the trends for the effect of sewage addition and pH manipulation are the same as when all the data are considered; there was no significant effect of sewage addition or pH, but the pH 9 samples did have significantly lower $\log_{10}$ reduction of MS2 than the pH 7.5 samples (when only the sewage samples were analyzed). The average $\log_{10}$ reduction of MS2 was 4.7, 5.7, 5.3, and 5.9 for the pH 7.5 well water and sewage samples, and the pH 9 well water and sewage samples, respectively. The 15 minute samples had the lowest $\log_{10}$ reduction of MS2 compared to almost all of the samples overall. The average flow rate for the 15 minute samples was 38 mL/min, compared to an overall average of 34 mL/min. The average bromine residual was 0.27 mg/L, compared to an overall average of 0.41 mg/L. The low bromine residual may be responsible for the lower $\log_{10}$ reduction of MS2 seen in the 15 minute samples.

The rechargeability of the HaloPure beads may be responsible for the differences seen in MS2 $\log_{10}$ reduction between sampling times. In the first

flush samples, the bromine on the outside of the polystyrene beads is released to disinfect the water that flows through the cartridge early on; there is more bromine available, so there is more reduction of viruses. In the short amount of time between the first flush sample and the 15 minute sample, there may not be enough time for the outside of the polystyrene beads to become covered with bromine atoms again (atoms from the inside move to the outside of the bead); not enough bromine is available to treat the water, so there is less reduction of viruses. By the 45 and 120 minute samples, there has been ample time for the displaced bromine atoms from the outside of the beads to be replaced by new bromine atoms from the inside, so there is once again enough bromine to treat the water flowing through the cartridge. However, it is impossible to measure the actual disinfecting dose of bromine released from the cartridges, so it may not be possible to verify this theory.

There was also a significant difference in bromine residual between sampling times. The bromine residual typically would start very low and increase over time. This result might explain the variability seen in the $\log_{10}$ reductions of MS2 between sampling times. However, no statistical correlation was found between MS2 $\log_{10}$ reduction and bromine residual. The bromine residual was lowest for some of the samples when the MS2 $\log_{10}$ reduction was highest (the first flush samples). Flow rate also differed significantly between sampling times, changing the contact times (less contact at the beginning when flow rate was faster). For almost every device, the flow rate decreased with increased

sampling time. This is due to the decreasing volume in the top reservoir of the device; since the device is gravity fed, as the top volume decreases, there is less pressure to force water through the device, so the flow rate decreases.

One area of concern with the Waterbird device is the short lifespan that the HaloPure media cartridges seem to have, compared to the manufacturer's stated lifespan. The first three HaloPure bromine cartridges used for the study only treated approximately 100 L of water before losing effectiveness (reducing less than one $\log_{10}$ of MS2) when challenged with sewage contaminated well water. HaloSource claims that the cartridges have a lifespan of 1,500 L. The cartridges have a built-in end-of-life shut-off device that doesn't allow water to flow through the cartridge after approximately 1,500 L, but the three cartridges did not have the shut-off device engaged.

Bromine has typically been avoided for use in drinking water treatment systems because of the potential to form more hazardous disinfection by-products such as bromate, bromoform, and dibromoacetic acid (Guo and Chen, 2009; Song et al., 1996). Disinfection by-products have been shown to cause cancer and reproductive anomalies in lab animals (Krasner, 2009). Brominated organic disinfection by-products can actually be more dangerous than the chlorinated forms, and hypobromous acid has been shown to react better and faster with organic matter than hypochlorous acid (Singer, 1999; Guo and Chen, 2009). The HaloPure delivery system for bromine has been tested for the potential to form disinfection by-products (bromate and bromide), and has received the National Sanitation Foundation (NSF) certification (standard 042)

(National Sanitation Foundation, 2012). The NSF testing determined that up to 94 g of HaloPure bromine media could be placed in a cartridge and still release acceptable levels of bromate and bromide; a normal cartridge for the Waterbird device holds an average of 15 g of media (National Sanitation Foundation, 2012).

There is still more work that could be done with the Waterbird device to better understand how well it works. The virus challenges should be repeated, but rather than taking samples during the treatment process, all of the water should be allowed to pass through the device before taking samples. This would more closely represent how consumers use the device in their own homes. It would also be interesting to do an observation study on how the device actually is used in the field. No household water treatment device is going to be used with 100% compliance by consumers, so understanding how consumers actually use the water is always helpful.

Overall, I do feel that the Waterbird device is a worthy drinking water disinfection device. It has been shown to be capable of a 5.4 log₁₀ reduction of MS2, and at least a 3.5 log₁₀ reduction of murine norovirus. I would recommend that the device is used as the manufacturer suggests, adding water at night and letting it treat for at least 8 hours before consumption. That would provide the bromine residual in the treated water additional time to inactivate some of the remaining microorganisms that may still be present in the treated water.

The device is a bit expensive for use in third world countries, but it seems to be a good option for the intended market (middle-class India). The device reportedly has a good lifespan (1,500 L), but after that point a new HaloPure

cartridge must be purchased. Using the purchase price for the entire device, this technology costs \$0.02/L of water. The replacement cost for new cartridges would be less than the original purchase price, so the cost would go down over time. However, the Waterbird device still ends up being a more expensive way to disinfect drinking water compared to traditional chlorination (Sobsey, 2002).

APPENDIX

APPENDIX

Table A-1. Results of all challenge experiments.

Experiment	Date	Mat rix	Cartri dge	Cera mic	Tempe rature	pH	Sam ple	Flow Rate (mL/min)	Total Chlori ne (mg/L)	Bromi ne Resid ual (mg/L)	MS2 Concentr ation (PFU/mL)	Murine Norovirus Concentrat ion (PFU/mL)
2	22- Mar	ww	2	3	10.5	7.40	inf		0.00	0.00	2.80E+07	2.00E+03
2	22- Mar	ww	2	3	10.5	7.40	t0	45	0.05	0.11	1.00E+00	2.00E+00
2	22- Mar	ww	2	3	10.5	7.40	t1	25	0.10	0.23	7.00E+04	2.00E+00
2	22- Mar	ww	2	3	10.5	7.40	t2	22	0.25	0.56	1.80E+03	2.00E+00
2	22- Mar	ww	2	3	10.5	7.40	t3	13	0.30	0.68	1.00E+00	2.00E+00
3	10- Apr	se wa ge	3	3	14.6	7.44	inf		0.00	0.00	8.00E+04	1.00E+04
3	10- Apr	se wa ge	3	3	14.6	7.44	t0	35	0.75	1.69	1.00E+00	2.00E+00
3	10- Apr	se wa ge	3	3	14.6	7.44	t1	40	0.12	0.27	1.00E+00	2.00E+00
3	10- Apr	se wa ge	3	3	14.6	7.44	t2	30	0.27	0.61	1.00E+00	2.00E+00

Table A-1 (cont'd).

3	10-Apr	se wa ge	3	3	14.6	7.44	t3	22	0.45	1.01	1.00E+00	2.00E+00
4	10-Apr	se wa ge	2	2	14.5	7.65	inf		0.00	0.00	3.30E+05	2.00E+04
4	10-Apr	se wa ge	2	2	14.5	7.65	t0	27	0.05	0.11	1.00E+00	2.00E+00
4	10-Apr	se wa ge	2	2	14.5	7.65	t1	20	0.05	0.11	4.00E+01	2.00E+00
4	10-Apr	se wa ge	2	2	14.5	7.65	t2	18	0.20	0.45	1.00E+00	2.00E+00
4	10-Apr	se wa ge	2	2	14.5	7.65	t3	10	0.30	0.68	1.00E+00	2.00E+00
5	10-Apr	se wa ge	1	1	14.7	7.51	inf		0.00	0.00	4.90E+05	2.00E+04
5	10-Apr	se wa ge	1	1	14.7	7.51	t0	35	0.15	0.34	1.00E+00	2.00E+00
5	10-Apr	se wa ge	1	1	14.7	7.51	t1	15	0.05	0.11	1.00E+00	2.00E+00

Table A-1 (cont'd).

5	10-Apr	se wa ge	1	1	14.7	7.51	t2	15	0.20	0.45	1.00E+00	2.00E+00
5	10-Apr	se wa ge	1	1	14.7	7.51	t3	9	0.30	0.68	1.00E+00	2.00E+00
6	14-Apr	ww	1	2	11.3	7.54	inf		0.00	0.00	2.40E+05	1.10E+04
6	14-Apr	ww	1	2	11.3	7.54	t0	72	0.45	1.01	1.00E+00	2.00E+00
6	14-Apr	ww	1	2	11.3	7.54	t1	50	0.10	0.23	4.90E+02	2.00E+00
6	14-Apr	ww	1	2	11.3	7.54	t2	68	0.25	0.56	9.00E+01	2.00E+00
6	14-Apr	ww	1	2	11.3	7.54	t3	20	0.50	1.13	1.40E+01	2.00E+00
7	14-Apr	ww	3	1	11.4	7.48	inf		0.00	0.00	3.10E+05	2.00E+04
7	14-Apr	ww	3	1	11.4	7.48	t0	115	0.05	0.11	1.00E+00	2.00E+00
7	14-Apr	ww	3	1	11.4	7.48	t1	42	0.10	0.23	1.10E+03	2.00E+00
7	14-Apr	ww	3	1	11.4	7.48	t2	40	0.25	0.56	5.70E+01	2.00E+00
7	14-Apr	ww	3	1	11.4	7.48	t3	15	0.45	1.01	8.00E+00	2.00E+00
8	14-Apr	ww	2	3	11.6	7.45	inf		0.00	0.00	5.50E+05	1.00E+05

Table A-1 (cont'd).

8	14-Apr	ww	2	3	11.6	7.45	t0	60	0.05	0.11	1.00E+00	2.00E+00
8	14-Apr	ww	2	3	11.6	7.45	t1	46	0.15	0.34	3.30E+02	2.00E+00
8	14-Apr	ww	2	3	11.6	7.45	t2	38	0.30	0.68	4.50E+02	2.00E+00
8	14-Apr	ww	2	3	11.6	7.45	t3	21	0.50	1.13	7.00E+00	2.00E+00
9	17-Apr	se wa ge	3	1	16.6	7.6	inf		0.00	0.00	2.10E+07	1.10E+04
9	17-Apr	se wa ge	3	1	16.6	7.6	t0	75	0.05	0.11	1.80E+02	2.00E+00
9	17-Apr	se wa ge	3	1	16.6	7.6	t1	38	0.10	0.23	6.00E+02	2.00E+00
9	17-Apr	se wa ge	3	1	16.6	7.6	t2	18	0.15	0.34	4.20E+01	2.00E+00
9	17-Apr	se wa ge	3	1	16.6	7.6	t3	7	0.20	0.45	1.00E+00	2.00E+00
10	17-Apr	se wa ge	2	3	16.1	7.58	inf		0.00	0.00	3.10E+07	2.80E+04

Table A-1 (cont'd).

10	17-Apr	se wa ge	2	3	16.1	7.58	t0	15	0.05	0.11	1.00E+00	2.00E+00
10	17-Apr	se wa ge	2	3	16.1	7.58	t1	16	0.05	0.11	2.50E+02	2.00E+00
10	17-Apr	se wa ge	2	3	16.1	7.58	t2	22	0.20	0.45	1.00E+00	2.00E+00
10	17-Apr	se wa ge	2	3	16.1	7.58	t3	21	0.20	0.45	1.00E+00	2.00E+00
11	17-Apr	se wa ge	1	2	16.4	7.56	inf		0.00	0.00	1.50E+07	1.80E+04
11	17-Apr	se wa ge	1	2	16.4	7.56	t0	30	0.05	0.11	1.00E+00	2.00E+00
11	17-Apr	se wa ge	1	2	16.4	7.56	t1	24	0.05	0.11	1.00E+00	2.00E+00
11	17-Apr	se wa ge	1	2	16.4	7.56	t2	22	0.15	0.34	3.00E+00	2.00E+00
11	17-Apr	se wa ge	1	2	16.4	7.56	t3	10	0.15	0.34	1.00E+00	2.00E+00

Table A-1 (cont'd).

12	19-Apr	ww	2	1	11.6	7.57	inf		0.00	0.00	3.40E+06	4.00E+03
12	19-Apr	ww	2	1	11.6	7.57	t0	68	0.05	0.11	1.00E+01	2.00E+00
12	19-Apr	ww	2	1	11.6	7.57	t2	12	0.05	0.11	1.70E+02	2.00E+00
12	19-Apr	ww	2	1	11.6	7.57	t3	7	0.10	0.23	1.00E+00	2.00E+00
13	19-Apr	ww	1	2	13	7.53	inf		0.00	0.00	2.90E+07	2.60E+04
13	19-Apr	ww	1	2	13	7.53	t0	22	0.05	0.11	1.00E+00	2.00E+00
13	19-Apr	ww	1	2	13	7.53	t1	22	0.05	0.11	1.00E+00	2.00E+00
13	19-Apr	ww	1	2	13	7.53	t2	6	0.10	0.23	6.00E+00	2.00E+00
13	19-Apr	ww	1	2	13	7.53	t3	9	0.10	0.23	1.00E+00	2.00E+00
14	24-Apr	se wa ge	2	1	16.7	7.51	inf		0.00	0.00	2.20E+07	2.00E+04
14	24-Apr	se wa ge	2	1	16.7	7.51	t0	65	0.05	0.11	1.00E+00	2.00E+00
14	24-Apr	se wa ge	2	1	16.7	7.51	t1	57	0.30	0.68	1.06E+03	2.00E+00

Table A-1 (cont'd).

14	24-Apr	se wa ge	2	1	16.7	7.51	t2	44	0.50	1.13	7.00E+01	2.00E+00
14	24-Apr	se wa ge	2	1	16.7	7.51	t3	15	0.50	1.13	1.10E+02	2.00E+00
15	24-Apr	se wa ge	1	2	16.9	7.54	inf		0.00	0.00	2.20E+07	1.00E+04
15	24-Apr	se wa ge	1	2	16.9	7.54	t0	64	0.05	0.11	1.00E+00	2.00E+00
15	24-Apr	se wa ge	1	2	16.9	7.54	t1	50	0.25	0.56	3.00E+03	2.00E+00
15	24-Apr	se wa ge	1	2	16.9	7.54	t2	46	0.50	1.13	3.00E+03	2.00E+00
15	24-Apr	se wa ge	1	2	16.9	7.54	t3	18	0.30	0.68	2.90E+02	2.00E+00
16	24-Apr	se wa ge	3	3	18	7.51	inf		0.00	0.00	2.90E+07	9.00E+04
16	24-Apr	se wa ge	3	3	18	7.51	t0	82	0.05	0.11	1.00E+00	2.00E+00

Table A-1 (cont'd).

16	24-Apr	se wa ge	3	3	18	7.51	t1	52	0.35	0.79	2.10E+03	2.00E+00
16	24-Apr	se wa ge	3	3	18	7.51	t2	46	0.90	2.03	4.40E+02	2.00E+00
16	24-Apr	se wa ge	3	3	18	7.51	t3	24	0.60	1.35	2.30E+02	2.00E+00
17	27-Apr	se wa ge	2	2	9.7	8.97	inf		0.00	0.00	2.40E+07	
17	27-Apr	se wa ge	2	2	9.7	8.97	t0	64	0.05	0.11	1.00E+00	
17	27-Apr	se wa ge	2	2	9.7	8.97	t1	56	0.10	0.23	1.70E+04	
17	27-Apr	se wa ge	2	2	9.7	8.97	t2	46	0.40	0.90	4.00E+04	
17	27-Apr	se wa ge	2	2	9.7	8.97	t3	15	0.50	1.13	1.00E+03	
18	27-Apr	se wa ge	1	1	11	9.02	inf		0.00	0.00	2.00E+07	

Table A-1 (cont'd).

18	27-Apr	se wa ge	1	1	11	9.02	t0	24	0.05	0.11	1.00E+00	
18	27-Apr	se wa ge	1	1	11	9.02	t1	59	0.25	0.56	1.40E+05	
18	27-Apr	se wa ge	1	1	11	9.02	t2	40	0.50	1.13	1.00E+05	
18	27-Apr	se wa ge	1	1	11	9.02	t3	19	0.45	1.01	1.00E+03	
19	27-Apr	se wa ge	3	3	12.3	8.96	inf		0.00	0.00	2.00E+08	
19	27-Apr	se wa ge	3	3	12.3	8.96	t0	80	0.05	0.11	1.00E+00	
19	27-Apr	se wa ge	3	3	12.3	8.96	t1	48	0.35	0.79	4.00E+04	
19	27-Apr	se wa ge	3	3	12.3	8.96	t2	38	0.55	1.24	8.00E+03	
19	27-Apr	se wa ge	3	3	12.3	8.96	t3	20	0.50	1.13	2.00E+03	

Table A-1 (cont'd).

23	11-May	ww	1	2	9.1	7.48	inf		0.00	0.00	9.60E+07	1.00E+04
23	11-May	ww	1	2	9.1	7.48	t0	45	0.05	0.11	1.00E+00	2.00E+00
23	11-May	ww	1	2	9.1	7.48	t1	50	0.10	0.23	3.00E+06	2.00E+00
23	11-May	ww	1	2	9.1	7.48	t2	42	0.30	0.68	1.09E+06	2.00E+00
23	11-May	ww	1	2	9.1	7.48	t3	17	0.50	1.13	6.90E+03	2.00E+00
24	11-May	ww	3	1	10.1	7.53	inf		0.00	0.00	1.50E+08	2.00E+03
24	11-May	ww	3	1	10.1	7.53	t0	52	0.05	0.11	1.00E+00	2.00E+00
24	11-May	ww	3	1	10.1	7.53	t1	54	0.15	0.34	2.20E+06	2.00E+00
24	11-May	ww	3	1	10.1	7.53	t2	40	0.30	0.68	1.08E+06	2.00E+00
24	11-May	ww	3	1	10.1	7.53	t3	20	0.60	1.35	2.50E+04	2.00E+00
25	11-May	ww	2	3	14	7.58	inf		0.00	0.00	1.10E+08	1.00E+04
25	11-May	ww	2	3	14	7.58	t0	38	0.05	0.11	1.00E+00	2.00E+00
25	11-May	ww	2	3	14	7.58	t1	45	0.20	0.45	7.00E+05	2.00E+00
25	11-May	ww	2	3	14	7.58	t2	37	0.50	1.13	1.00E+06	2.00E+00

Table A-1 (cont'd).

25	11-May	ww	2	3	14	7.58	t3	16	0.65	1.46	5.30E+04	2.00E+00
29	27-Oct	ww	4	3	9	9.03	inf		0.00	0.00	4.00E+07	2.00E+02
29	27-Oct	ww	4	3	9	9.03	t0	55	0.01	0.02	3.00E+01	2.00E+00
29	27-Oct	ww	4	3	9	9.03	t1	16	0.02	0.05	1.10E+03	2.00E+00
29	27-Oct	ww	4	3	9	9.03	t2	15	0.02	0.05	1.00E+00	2.00E+00
29	27-Oct	ww	4	3	9	9.03	t3	9	0.03	0.07	7.00E+00	2.00E+00
30	27-Oct	ww	5	1	9.1	9.02	inf		0.00	0.00	3.00E+07	7.00E+02
30	27-Oct	ww	5	1	9.1	9.02	t0	56	0.02	0.05	4.00E+00	2.00E+00
30	27-Oct	ww	5	1	9.1	9.02	t2	11	0.03	0.07	7.00E+02	2.00E+00
30	27-Oct	ww	5	1	9.1	9.02	t3	7	0.04	0.09	1.00E+00	2.00E+00
31	27-Oct	ww	6	2	8.6	9.03	inf		0.00	0.00	1.00E+07	2.00E+03
31	27-Oct	ww	6	2	8.6	9.03	t0	104	0.03	0.07	3.00E+01	2.00E+00
31	27-Oct	ww	6	2	8.6	9.03	t2	16	0.04	0.09	8.00E+01	2.00E+00
31	27-Oct	ww	6	2	8.6	9.03	t3	7	0.04	0.09	5.00E+00	2.00E+00

Table A-1 (cont'd).

32	31-Oct	se wa ge	4	3	11.5	8.97	inf		0.00	0.00	1.00E+08	1.20E+02
32	31-Oct	se wa ge	4	3	11.5	8.97	t0	46	0.01	0.02	1.00E+00	2.00E+00
32	31-Oct	se wa ge	4	3	11.5	8.97	t1	28	0.05	0.11	3.00E+04	2.00E+00
32	31-Oct	se wa ge	4	3	11.5	8.97	t2	20	1.30	2.92	5.00E+05	2.00E+00
32	31-Oct	se wa ge	4	3	11.5	8.97	t3	10	0.08	0.18	1.00E+04	2.00E+00
33	31-Oct	se wa ge	5	2	11.1	9.00	inf		0.00	0.00	1.00E+08	1.50E+02
33	31-Oct	se wa ge	5	2	11.1	9.00	t0	75	0.01	0.02	1.00E+00	1.00E+00
33	31-Oct	se wa ge	5	2	11.1	9.00	t1	28	0.03	0.07	1.00E+00	1.00E+00
33	31-Oct	se wa ge	5	2	11.1	9.00	t2	20	0.05	0.11	1.00E+00	1.00E+00

Table A-1 (cont'd).

33	31-Oct	Se wa ge	5	2	11.1	9.00	t3	12	0.04	0.09	1.00E+00	1.00E+00
34	31-Oct	se wa ge	6	1	11.9	8.99	inf		0.00	0.00	1.00E+08	6.00E+02
34	31-Oct	se wa ge	6	1	11.9	8.99	t0	70	0.01	0.02	1.00E+00	1.00E+00
34	31-Oct	se wa ge	6	1	11.9	8.99	t2	18	0.04	0.09	1.00E+00	1.00E+00
34	31-Oct	se wa ge	6	1	11.9	8.99	t3	7	0.04	0.09	1.00E+00	1.00E+00
35	6-Nov	ww	6	1	13.4	9.00	inf		0.00	0.00	1.00E+07	
35	6-Nov	ww	6	1	13.4	9.00	t0	110	0.01	0.02	1.00E+00	
35	6-Nov	ww	6	1	13.4	9.00	t2	10	0.01	0.02	1.00E+00	
35	6-Nov	ww	6	1	13.4	9.00	t3	7	0.04	0.09	1.00E+00	
36	6-Nov	ww	4	2	13.7	8.95	inf		0.00	0.00	1.00E+07	
36	6-Nov	ww	4	2	13.7	8.95	t0	86	0.01	0.02	4.00E+01	

Table A-1 (cont'd).

36	6-Nov	ww	4	2	13.7	8.95	t1	25	0.02	0.05	1.00E+03	
36	6-Nov	ww	4	2	13.7	8.95	t2	23	0.01	0.02	1.00E+05	
36	6-Nov	ww	4	2	13.7	8.95	t3	13	0.04	0.09	3.00E+03	
37	6-Nov	ww	5	3	13.5	8.99	inf		0.00	0.00	3.00E+07	
37	6-Nov	ww	5	3	13.5	8.99	t0	64	0.01	0.02	1.00E+00	
37	6-Nov	ww	5	3	13.5	8.99	t2	7	0.01	0.02	1.00E+00	
37	6-Nov	ww	5	3	13.5	8.99	t3	6	0.03	0.07	1.00E+00	
38	9-Nov	se wa ge	5	3	10.7	8.99	inf		0.00	0.00	3.00E+07	
38	9-Nov	se wa ge	5	3	10.7	8.99	t0	90	0.01	0.02	1.00E+00	
38	9-Nov	se wa ge	5	3	10.7	8.99	t2	18	0.03	0.07	1.00E+00	
38	9-Nov	se wa ge	5	3	10.7	8.99	t3	8	0.04	0.09	1.00E+00	

Table A-1 (cont'd).

39	9-Nov	se wa ge	6	2	11.3	8.96	inf		0.00	0.00	5.00E+07	
39	9-Nov	se wa ge	6	2	11.3	8.96	t0	98	0.01	0.02	1.00E+00	
39	9-Nov	Se wa ge	6	2	11.3	8.96	t2	14	0.04	0.09	1.00E+00	
39	9-Nov	se wa ge	6	2	11.3	8.96	t3	12	0.04	0.09	1.00E+00	
40	9-Nov	se wa ge	4	1	12.5	8.97	inf		0.00	0.00	2.00E+07	
40	9-Nov	se wa ge	4	1	12.5	8.97	t0	38	0.01	0.02	1.00E+00	
40	9-Nov	se wa ge	4	1	12.5	8.97	t1	34	0.04	0.09	1.00E+03	
40	9-Nov	se wa ge	4	1	12.5	8.97	t2	38	0.07	0.16	4.00E+05	
40	9-Nov	se wa ge	4	1	12.5	8.97	t3	12	0.07	0.16	1.00E+04	

Table A-1 (cont'd).

44	20-Dec	ww	4	3	8.5	9.04	inf		0.00	0.00	3.10E+07	
44	20-Dec	ww	4	3	8.5	9.04	t0	46	0.05	0.11	1.40E+02	
44	20-Dec	ww	4	3	8.5	9.04	t1	52	0.05	0.11	1.40E+04	
44	20-Dec	ww	4	3	8.5	9.04	t2	18	0.20	0.45	6.70E+04	
44	20-Dec	ww	4	3	8.5	9.04	t3	10	0.40	0.90	1.50E+04	
45	20-Dec	ww	5	1	8.1	9.00	inf		0.00	0.00	2.40E+07	
45	20-Dec	ww	5	1	8.1	9.00	t0	63	0.05	0.11	1.30E+02	
45	20-Dec	ww	5	1	8.1	9.00	t2	14	0.10	0.23	6.60E+02	
45	20-Dec	ww	5	1	8.1	9.00	t3	10	0.40	0.90	1.00E+00	
46	20-Dec	ww	6	2	7.8	8.98	inf		0.00	0.00	2.40E+07	
46	20-Dec	ww	6	2	7.8	8.98	t0	72	0.05	0.11	3.60E+03	
46	20-Dec	ww	6	2	7.8	8.98	t1	26	0.05	0.11	1.20E+05	
46	20-Dec	ww	6	2	7.8	8.98	t2	24	0.30	0.68	2.10E+05	

Table A-1 (cont'd).

46	20- Dec	ww	6	2	7.8	8.98	t3	11	0.50	1.13	6.00E+02	
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Table A-2. Murine norovirus influent concentrations.

Experiment	Date	Date Stock Purified	Water Type	pH	Norovirus Influent Concentration
2	22-Mar	2/15/12	well water	7.5	2.00E+03
3	10-Apr	2/29/12	sewage	7.5	1.00E+04
4	10-Apr	2/29/12	sewage	7.5	2.00E+04
5	10-Apr	2/29/12	sewage	7.5	2.00E+04
6	14-Apr	3/19/12	well water	7.5	1.10E+04
7	14-Apr	3/19/12	well water	7.5	2.00E+04
8	14-Apr	3/19/12	well water	7.5	1.00E+05
9	17-Apr	3/19/12	sewage	7.5	1.10E+04
10	17-Apr	3/19/12	sewage	7.5	2.80E+04
11	17-Apr	3/19/12	sewage	7.5	1.80E+04
12	19-Apr	3/21/12	well water	7.5	4.00E+03
13	19-Apr	3/21/12	well water	7.5	2.60E+04
14	24-Apr	3/21/12	sewage	7.5	2.00E+04
15	24-Apr	3/21/12	sewage	7.5	1.00E+04
16	24-Apr	3/21/12	sewage	7.5	9.00E+04
23	11-May	5/10/12	well water	7.5	1.00E+04
24	11-May	5/10/12	well water	7.5	2.00E+03
25	11-May	5/10/12	well water	7.5	1.00E+04
29	27-Oct	5/10/12	well water	9.0	2.00E+02
30	27-Oct	5/10/12	well water	9.0	7.00E+02
31	27-Oct	5/10/12	well water	9.0	2.00E+03
32	31-Oct	10/23/12	sewage	9.0	1.20E+02
33	31-Oct	10/23/12	sewage	9.0	1.50E+02
34	31-Oct	10/23/12	sewage	9.0	6.00E+02

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