METAL NANOPATCH FORMATION IN CERAMIC POROUS MEDIA FOR POINT-OF-USE WATER FILTRATION

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APPROVAL SHEET

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ABSTRACT

Ceramic water filters, a common point-of-use technology used to treat water in the home, are produced around the world using local labor and materials. Current production methods frequently use silver nanoparticles as an antimicrobial agent to prevent fouling in the filter and release silver at safe concentrations into the effluent for residual disinfection. Silver nanoparticles are expensive and difficult to acquire in developing world countries, so the use of silver nitrate was investigated.

In the lab, filter disks made with silver nanoparticles (AgNP) and disks made with silver nitrate ($AgNO_3$) were evaluated using miscible displacement flow-through experiments with pulse and continuous-feed injections of *E. coli*. Experiments using pulse injections of *E. coli* showed similar performance in breakthrough curves between the two application methods. Long-term challenge tests performed with a continuous feed of *E. coli* and growth medium resulted in similar log removal rates, but the removal rate by AgNP filters decreased over time. $AgNO_3$ filters provided consistent removal with lower silver levels in the effluent and effective bacterial disinfection. Results from continued use with synthetic groundwater over 4 weeks, with a pulse injection of *E. coli* at 2 and 4 weeks support similar conclusions—AgNP filters perform better initially, but after 4 weeks of use, AgNP filters suffer larger decreases in performance.

To build on this, full-size filters were produced with AgNP, $AgNO_3$, and with copper nitrate ($Cu(NO_3)_2$) (applied with the same method as the silver nitrate) at PureMadi Filter Production Facilities in South Africa and evaluated for total coliform and *E. coli* removal, and metal concentrations in the effluent. Results of these experiments showed total coliform and *E. coli* removal was greater for $AgNO_3$ filters (log reductions of 4.06 and 4.11) relative to AgNP filters (log reductions of 3.85 and 3.92). $Cu(NO_3)_2$ filter had log total coliform and *E. coli* removals of 3.33 and 3.54, respectively. $AgNO_3$ filters produced with the same method and the same amount of silver as AgNP filters release smaller amounts of silver into the treated water (average 2 μg/L versus 12 μg/L).

MS2 bacteriophage is commonly used as a model virus for evaluating the antiviral abilities of point-of-use technologies. Both Ag and Cu were tested for disinfection ability against MS2. Ag and Cu, at the concentration of their drinking water standard, showed 0.46 and 1.87 log removal, respectively after 24 h of exposure. Additionally, ceramic filters were made with $AgNO_3$ and with $Cu(NO_3)_2$ and compared for MS2 removal in a miscible displacement experiment. When comparing performance in breakthrough curves between the two application methods, $Cu(NO_3)_2$ filters performed slightly better. Concerns exist as to the validity of MS2 as a model virus for disinfection performance, so disinfection experiments were performed with adenovirus. Silver and copper, at the concentration of their drinking water standard, showed 0.91 and 1.61 log removal, respectively after 8 h of exposure, with little added inactivation afterwards.

Results suggest that the silver nitrate method may effectively reduce costs, improve silver retention in the filter, increase effective lifespan and maintain effective pathogen removal while also eliminating the risk of exposure to inhalation of silver nanoparticles by workers in developing-world filter production facilities.

LIST OF FIGURES AND TABLES

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PUBLICATIONS AND PRESENTATIONS

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Nunnelley, K. G., Smith, J.A., Smith, M.Y. and Samie, A., 2016, A New Method for Nanosilver Application in Ceramic Water Filters: Proceedings of The World Environmental and Water Resources Congress, May 2016, West Palm Beach, FL.

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Jackson, K. N., Smith, J.A., and Edokpayi, J., (Submitted) Application of a New Method for the Deposition of Metallic Silver and Metallic Copper on Full- Size Porous Ceramic Water Filters, *Environmental Engineering Science*

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Chapter 1: Introduction

The work presented in this chapter resulted in a published book chapter.

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1.1 INTRODUCTION

Approximately 1 billion people worldwide do not have access an “improved water supply”, which is defined by the World Health Organization (WHO) as “a household connection or access to a public standpipe, a protected well or spring, or a source of rainwater collection”(Cosgrove & Rijsberman, 2000). This definition also requires that at least 20 liters per person per day are available within 1 km of a person’s home. Because this definition does not refer to water quality, reliability of service, or even cost, it is estimated that an additional 2-3 billion people also have unsafe water supplies, at least for part of the time (Hutton & Haller, 2004). Studies have also shown that numbers reported by the Joint Monitoring Programme of the proportion of drinking water coming from improved sources are likely substantial overestimations when water quality data is included (Bain et al., 2012). Access as well as quality is key.

The dangers of consuming unsafe water are substantial, particularly for children. The WHO estimates that consuming unsafe water causes the deaths of over 4 million people per year, with more than 1.5 million of these deaths being children under the age of 5 (Dillingham & Guerrant, 2004). Not included in this figure are the added health burdens on children who experience cognitive impairment and growth stunting as a result of gastrointestinal infections caused by consumption of water with pathogenic organisms like Shigella, pathogenic strains of *Escherichia coli*, *Vibrio cholerae*, and *Cryptosporidium parvum* (Dillingham & Guerrant, 2004). People living with AIDS are particularly susceptible to infections from waterborne pathogens because of their weakened immune systems (Dillingham et al., 2006). In many parts of sub-Saharan Africa, there is an unfortunate confluence of low-quality drinking water and high rates of HIV infection. Providing safe water to our global population has been identified as one of the Grand Challenges of Engineering by the National Academy of Engineering.

In most cities and suburban areas of the developed world, water is treated at centralized water-treatment plants and delivered directly to homes through distribution systems with chlorine residual, generally preventing gastrointestinal infections by waterborne pathogens. This service is usually not available in the developing world or even in some suburban and rural areas of the developed world. These regions cannot economically sustain centralized water treatment plants that meet the full demands of the population. The treatment plants plus the distribution system is not practical in developing rural areas due to the financial and structural requirements for safe treatment and delivery of water. This leads to using untreated natural water sources (rivers, lakes, groundwater or rain) (Peter-Varbanets, Zurbrugg, Swartz, & Pronk, 2009). The WHO has suggested that one possible solution is to take a more decentralized approach to water treatment, wherein people treat their water in their households immediately before consumption. This idea, often referred to as point-of-use (POU) water treatment, has the potential to significantly improve the microbial quality of household water and reduce the risk of diarrheal disease and death, particularly among children (Thomas Clasen, Nadakatti, & Menon, 2006).

Without centralized treatment and direct piping into the home, there are many steps before consumption including collection from the source, storage, contamination, and treatment (J. E. Mellor, Smith, Learmonth, Netshandama, & Dillingham, 2012). Common practices for point of use water treatment not utilizing filtration include boiling, chlorination, or solar disinfection. Membrane systems for use in the home have become increasingly popular, but still remain too expensive for the poorest of countries. Microbial pollution is the most threatening issue with water supplies, and fecal contamination of a water supply leads to water-borne diseases. Boiling of water is the most common form of treatment in developing countries, but is not as effective as hoped. Boiling also creates a high-energy demand and poses the additional problem of deforestation when wood fires are used to boil the water, while places like Bangladesh also lack access to the fuel necessary to boil water (A. Huq et al., 1996). Chlorine has been tested as an in-home option and has proven effective, but unpopular; household users complain about the taste and have reported a refusal to drink the water since the smell is similar to that of household bleach (Kirchhoff & McClelland, 1985). Solar disinfection has been proven as effective but requires sunlight and a large supply of plastic bottles while only treating a limited amount of water at one time (D. S. Lantagne, Quick, & Mintz, 2006). When there is limited water quantity, common solutions include groundwater wells and rainwater harvesting. However, groundwater wells are not always well planned and can become polluted, while temporal and spatial variations in rainfall can make rainwater harvesting unreliable in the absence of large-volume storage (Peter-Varbanets et al., 2009).

1.2 DISSERTATION AIMS

This dissertation discusses the development and testing of a new method for embedding nanoscale metallic silver or copper in ceramic porous media for use in a point-of-use water treatment technology. Chapter 2 presents a literature review of some point-of-use water treatment technologies currently used. Chapter 3 evaluates the new method of metallic nanosilver formation in ceramic filter disks in the lab. Filters made with current production practices and with the new method are compared for *E. coli* removal. Chapter 4 compares these two methods of silver application, and copper applied in the same method, for total coliform and *E. coli* removal by full-size filters produced and tested in South Africa. Metal concentrations in effluents are also compared. Viral disinfection efficacy of silver and copper and the quality of MS2 bacteriophage as a model virus is evaluated in Chapter 5.

1.3 REFERENCES

Clasen, T., Nadakatti, S., & Menon, S. (2006). Microbiological performance of a water treatment unit designed for household use in developing countries. *Tropical Medicine and International Health, 11*(9), 1399-1405.

Cosgrove, W. J., & Rijsberman, F. R. (2000). *World Water Vision: Making Water Everybody's Business*. London: Earthscan Publications.

Dillingham, R., & Guerrant, R. L. (2004). Childhood stunting: measuring and stemming the staggering costs of inadequate water and sanitation. *The Lancet, 363*, 94-95.

Huq, A., Xu, B., Chowdhury, M. A. R., Islam, M. S., Montilla, R., & Colwell, R. R. (1996). A Simple Filtration Method To Remove Plankton-Associated Vibrio cholerae in Raw Water Supplies in Developing Countries. *Applied and Environmental Microbiology, 62*(7), 2508-2512.

Hutton, G., & Haller, L. (2004). Evaluation of the costs and benefits of water and sanitation improvements at the global level. *World Health Organization, Geneva*.

Kirchhoff, L. V., & McClelland, K. E. (1985). Feasibility and efficacy of in-home water chlorination in rural North-eastern Brazil. *The Journal of Hygiene, 94*(2), 173-180.

Lantagne, D. S., Quick, R., & Mintz, E. D. (2006). Household Water Treatment and Safe Storage Options in Developing Coutries: A Review of Current Implementation Practices .

Mellor, J. E., Smith, J. A., Learmonth, G. P., Netshandama, V. O., & Dillingham, R. A. (2012). Modeling the Complexities of Water, Hygiene, and Health in Limpopo Province, South Africa. *Enviromental Science and Technology, 46*, 13512-13520.

Peter-Varbanets, M., Zurbrugg, C., Swartz, C., & Pronk, W. (2009). Decentralized systems for potable water and the potential of membrane technology. *Water Research, 43*, 245-265.

Chapter 2: Literature Review

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2.1 ABSTRACT

With significant infrastructure investments required for centralized water treatment, in home treatment technologies, known as point-of-use, have become a popular solution in the developing world. This review discusses current filtration-based point-of-use water treatment technologies in three major categories: ceramics, papers and textiles. Each of these categories has used silver for added antimicrobial effectiveness. Ceramics have had the most development and market infiltration, while filter papers are a new development. Textiles show promise for future research as a cheap, socially acceptable, and effective method.

2.2 BACKGROUND

High surface area to volume ratio leads to better bactericidal activity, making nanomaterials excellent adsorbents (Praveena & Aris, 2015). This chapter will focus on one particular nanomaterial, silver. Silver has been used for disinfection for millennia and in wound treatments since the 1960s (LeOuay & Stellacci, 2015). Silver has bactericidal activity in its oxidized state, Ag^+ (Karel, Koparal, & Kaynak, 2015). Silver nanoparticles are between 1 and 100 nm in size (T. A. Dankovich, 2012).

Growth studies of microbial cultures have been performed with both silver and copper nanoparticles. One study showed that silver and copper nanoparticles have great promise as antimicrobial agents against *E. coli, B. subtilis*, and *S. aureus (Ruparelia, Chatterjee, Duttagupta, & Mukherji, 2008). E. coli* is a common choice for testing the effectiveness of a technology since it is a gram-negative, rod-shaped bacterium, 1-2 μm long and 0.1-0.5 μm in diameter, and a common water microbe with strains that cause gastrointestinal infections (Brown, Chai, Wang, & Sobsey, 2012). Silver nanoparticles perform better as an antimicrobial agent against *E. coli* and *S. aureus* than copper. Copper nanoparticles form an oxide layer and demonstrate better antimicrobial activity towards *B. subtilis* than silver, possibly due to a greater affinity to the surface-active groups. Ruparelia et al. admits that the antimicrobial mechanism of the silver and copper nanoparticles is not yet fully established, and a combination of silver and copper nanoparticles may produce a more complete bactericidal effect against mixed bacterial populations that would be more common in actual use (Ruparelia et al., 2008).

Silver is usually in concentrations of approximately 0.1 mg/kg in aquatic systems at subnanomolar levels. Higher silver concentrations in aquatic environments often come from waste streams of commercial or industrial processes. Silver in nature can be monovalent (Ag(I)) or metallic (Ag(0)). Studies have shown that free or hydrated Ag(I) exhibits toxic effects on a variety of aquatic organisms ranging from zooplankton to rainbow trout (Herrin, Andren, & Armstrong, 2001). Several studies have demonstrated that silver ions are selectively toxic for prokaryotic microorganisms, with little effect on eukaryotic cells (Park & Jang, 2003). However, the exact reasons for the antimicrobial ability of the nanoparticles are still unknown. Among all antimicrobial nanomaterials, nanosilver is probably the most widely used (Li et al., 2008). Silver's antimicrobial property is related to the amount and the rate at which it is released. Metallic state silver is inert, but silver will react with moisture in the skin or fluid in a wound to become ionized. Ionized silver is highly reactive, binding to bacterial DNA and RNA by denaturing. The nanoparticles bind to the cell membrane, a vital point of protection, and accumulate to causing irreparable damage (Pradeep & Anshup, 2009). This inhibits a bacterial cell from replication. In binding to tissue proteins, the silver brings structural changes to the cell wall and nuclear membranes causing the cell to distort and die (Rai, Yadav, & Gade, 2009). It has also been found that if the antimicrobial and the bacterial cell (negative charge) have the same surface charge, there is repulsion and less contact (Pradeep & Anshup, 2009). Oxygen presence in a system with bulk silver provides complete destructive oxidation of microorganisms (Pradeep & Anshup, 2009).

Health concerns are the chief barrier to the implementation of new technologies that use silver nanoparticles. Silver release from a treatment material is affected by the water's pH, temperature and chemical composition. Just 60 milligrams can be toxic to humans, while 1.3 to 6.2 grams is lethal (D. S. Lantagne, 2001). The WHO sets a limit to ensure that these technologies do not cause harm, stating that up to .1 mg/L can be tolerated without risk. The United States Environmental Protection Agency sets a maximum silver concentration for drinking water at .1 mg/L (Quang et al., 2013). The retention rate of silver in humans is only 0-10 percent, and silver is mainly stored in the liver and skin. The WHO set a guideline of 10 grams of silver in a lifetime (D. S. Lantagne, 2001). Potential human risks of silver consumption at high levels include high blood pressure, kidney damage, gastrointestinal irritation, cancer and neurological damage, in addition to DNA damage (Praveena & Aris, 2015). The silver levels after treatment must be within a safe range for human consumption.

Risks to environment and human health are a rising concern. The technologies discussed in the chapter release silver at levels below the drinking water standard, meaning they are safe and may cause a substantial improvement in water quality and human health, particularly in resource-limited settings. Some of the ceramic technologies are made with silver nitrate instead of silver nanoparticles and thus release silver ions instead of nanoparticles. There is a strong understanding of the health effects of silver in ion form, but the effects of silver in the nano form are less understood.

2.3 CURRENT POINT-OF-USE WATER TREATMENT TECHNOLOGIES

This chapter discusses current point-of-use water treatment practices that use silver nanoparticles in porous media. The three main categories of treatment type that will be addressed are ceramic, paper and textile. These technologies use filtration, which benefit from removal of turbidity and are gravity fed, which saves on energy costs (Albert, Luoto, & Levine, 2010). Table 2.1 shows a summary of some point-of-use systems currently in use and their effectiveness against *Escherichia coli* (*E. coli*). In addition, we present new data on a novel “silver-nanopatch” fabrication technique as applied to ceramic filters.

Table 2.1 A summary of *E. coli* removal efficiency in some POU technologies that use silver nanoparticles (Praveena & Aris, 2015)

Type of material	Removal Efficiency (LRV)	Removal Efficiency (%)	Silver Concentration (mg/L)	References
Ceramic				
Ceramic materials (clay from Indonesia)	3		10	(Rayner et al., 2013)
Ceramic materials (clay from Tanzania)	2.8		10	(Praveena & Aris, 2015)
Ceramic materials (clay from Nicaragua)	2.5		11	(Praveena & Aris, 2015)
Ceramic materials (clay mixed with sawdust)		92	.02	(Kallman, Vinka A. Oyanedel-Craver, & and James A. Smith, 2011)
Ceramic filter (40%soil, 10%flour, 50%grog)		97.8-100	<.1	(V. A. Oyanedel-Craver & J. A. Smith, 2008)
Fiber				
Synthetic fiber		100	10	(S. Chen, Liu, & Zeng, 2005)
Natural fiber pure cellulose paper	8.7		.1	(T. A. Dankovich & Gray, 2011)

2.3.1 CERAMIC

Silver has been imbedded in clay and can be designed to control silver release kinetics (Karel et al., 2015). Silver imbedded clay can also be used with textiles by soaking a textile then oven drying (Kasuga et al., 2011). Although this method has not been used for water, it has been used for air purifiers, and the clay has been used in plates to treat drinking water (Kasuga et al., 2011). Fired clay makes a ceramic that can be used for ceramic filtration which has been shown to have high effectiveness against bacteria and protozoa (D. S. Lantagne et al., 2006). Potters for Peace is a US-based nongovernmental organization (NGO) that has been teaching communities to produce and manufacture ceramic pots that filter water (D. S. Lantagne, 2001). The pots are made from water, clay and a combustible material like sawdust and shaped like a pot. The kiln is typically fired at 900 ˚C for 8 hours, below the melting point of silver (Praveena & Aris, 2015; Ren & Smith, 2013).

During firing, the clay forms a ceramic and the sawdust combusts, leaving behind porosity for water flow. Some filter factories then perform a pressure test by submerging the filter to the rim in water for 10 seconds and to see if any wet spots appear on the interior of the filter pot. A wet spot would suggest a macropore that in turn may limit filter performance for pathogen removal (Rayner, 2009). Filters are then soaked and subjected to a flow test. If the filters pass a flow rate test they are then painted or dipped in colloidal silver solution that provides antimicrobial effects (Rayner, 2009). Water containing a colloidal silver has been filtered through as a way to embed the silver in the filters, but has been proven less efficient (D. S. Lantagne, 2001). Also, painting on silver nitrate is less efficient due to high washout, and silver nanoparticles are recommended (Rayner et al., 2013). Mixing in silver nanoparticles prior to firing has shown higher retention of silver in the ceramic and potentially better long term use when compared to the paint on or dipped application method which release silver at high levels quickly during early use (Ren & Smith, 2013). This has not been tested for bacterial disinfection nor has it been field tested, but it could be a promising alternative. The ceramic sits inside a 20-30 liter plastic bucket with a spigot. Figure 2.1 is a diagram of a completed ceramic water filter (Brown & Sobsey, 2010). These can be manufactured locally and pots are a common storage container for water already, so there is no break to the social norm (D. S. Lantagne et al., 2006). The filtering of water is gravity-fed and would save on energy cost. The clay-to-sawdust ratio by volume of 47% has proven best in optimizing flow rate without losing coliform removal (D. Lantagne et al., 2010). Also, the type of combustible used has been shown to make a difference even if the same size and amount is used, like sawdust versus coffee or rice husk (D. Lantagne et al., 2010).

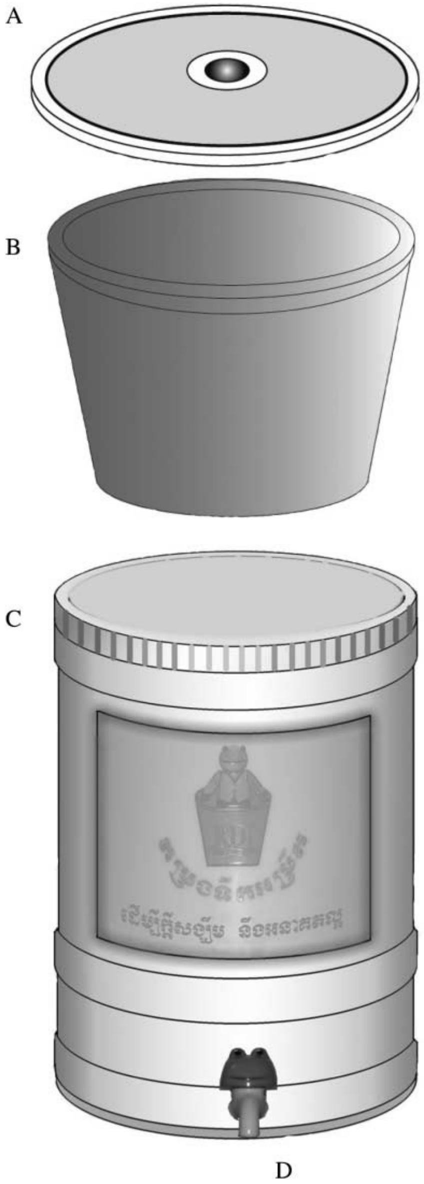

Figure 2.1: Schematic of the ceramic water filter produced by Resource Development International—Cambodia (courtesy of Mickey Sampson). The complete filter unit consists of a lid (A), ceramic filter element (B) in a plastic container (C) that gathers treated water and dispenses through a tap at the base (D). (Brown & Sobsey, 2010).

The silver-impregnated ceramic water filters have shown to be both sustainable and socially acceptable as well as effective with physical filtration and chemical treatment both contributing to pathogen removal (Abebe et al., 2014). The ceramic filter without silver has been shown to successfully remove *E.* coli but the addition of silver improves the water quality of the effluent (Kallman et al., 2011). Porosimetry of the filters has shown that the pores of the ceramic are too big to remove bacteria by only size exclusion-- there are several other mechanisms like depth straining, particle bridging inertial impaction, interception, diffusion and electrostatic attraction that help with capturing bacteria (Matthies et al., 2015). The filter has also been shown to be a preferred option by a study of 400 households in Kenya. Households were given the option of three different technologies, PUR (chlorine-based), Waterguard (chlorine-based) and a ceramic water filter. The households had a two-month trial with each, and then were asked their preference again at the conclusion of the trial. The ceramic water filter was preferred both before and after (Albert et al., 2010).

The method of application of silver does not appear to be a factor affecting disinfection efficiency (D. Lantagne et al., 2010; V. Oyanedel-Craver & J. A. Smith, 2008). Instead the mass of colloidal silver that remains after the saturation period determines effectiveness. The bacterial removal may be helped by the ceramic pores being filled by silver instead of the bacteria in transport. Testing has shown between 97.8 and 100% removal of *E.* coli (V. Oyanedel-Craver & J. A. Smith, 2008). It is also important that the silver release remains below the regulatory limit for silver in drinking water, which is 0.1 mg/L. This level was not exceeded for any of the filters tested after passing water through the filter for about 200 min (V. Oyanedel-Craver & J. A. Smith, 2008). The shape of the filter, flat bottom versus round bottom, also has no effect (D. Lantagne et al., 2010).

Ceramic candles are often used in a gravity-fed, ceramic water filter system. The set up involves two 20 L plastic buckets stacked on top each other with the silver impregnated ceramic candles vertical allowing their effluent to flow to the bottom bucket and be dispensed out a spigot. Each candle can treat 20000 L depending on water quality (Thomas Clasen, Parra, Boisson, & Collin, 2005). This technique has been associated with 70% lower diarrheal rates, and 92% of users report "feeling better" after using the filter (T. F. Clasen, Brown, Collin, Suntura, & Cairncross, 2004). Ceramic candles have shown that after 16 months, only 48.7% are still operating properly, and 54% of those produce coliform free water (T Clasen & Boisson, 2006). The main problem with this technology is breakage. The ceramic filters are popular with the end user, but with so many parts, any failure commonly leads to discontinued use because of difficulty to repair (T Clasen & Boisson, 2006). It has been found that the treatments are not always done correctly by the user (D. S. Lantagne & Clasen, 2012). This lowers the effectiveness of the technology and therefore does not provide recipients with the safe drinking water they should be receiving. Proper education on use and maintenance of the treatment system is vital (D. S. Lantagne, 2001). Proper maintenance of ceramic water filters can reduce early childhood diarrhea by an extra 45% (J. Mellor, Abebe, Ehdaie, Dillingham, & Smith, 2014). Often the new technology may be provided to a developing country, but with English directions. Keeping methods simple and similar to current practices could improve the utilization phase of treatment processes (D. S. Lantagne & Clasen, 2012).

The benefits of ceramic filtration include its proven effectiveness against bacteria and protozoa, its simplicity, its long-term use, and its low cost due to the ability to locally produce. One study of the ceramic filter reported a 90-99% removal of MS2, a viral pathogen (Brown & Sobsey, 2010), but conversely it has also been claimed that there is a minimal efficiency against viruses (Matthies et al., 2015). But with that, there is a risk of recontamination in the lower reservoir and need for education for optimal use (T. F. Clasen et al., 2004). Ceramic filters provide health improvements, cost-effectiveness and are environmentally friendly, making them sustainable and a better option than a centralized treatment system in the developing world (Ren, Colosi, & Smith, 2013). Unfortunately, ceramic filters have shown a 2% decrease in likelihood of use after each month of ownership with the most common reason being the filter breakage (Brown, Proum, & Sobsey, 2009), and 67% of the discontinued use is because of breakage (Sobsey, Stauber, Casanova, Brown, & Elliott, 2008).

As for human health effects, a study has shown that the use of silver-impregnated ceramic filters reduced diarrhea rates by 49% across age groups and sexes (Brown, Sobsey, & Loomis, 2008), and by 80% among HIV-positive users (Abebe et al., 2014). This is important since individuals who are immunocompromised can be particularly susceptible to gastrointestinal infections from waterborne pathogens

(Abebe et al., 2014). There has also been epidemiological research on ceramic filter use and reduction of the hepatitis A virus (T. F. Clasen et al., 2004). Ceramic water filters have been found to reduce early childhood diarrhea by 41% over two years (J. Mellor et al., 2014), but the effectiveness is reduced after 3 years (J. Mellor et al., 2014).

Other ceramic technologies have been developed that do not use filtration. Silver-coated ceramic beads can be used in water, and has been tested by pouring through columns rather than a batch method (Han, Lee, Lee, Uzawa, & Park, 2005; Lin et al., 2013). Recently, a novel method of metallic silver formation in a porous ceramic was reported by Ehdaie et al. (2014). These researchers combined clay, sawdust, water, and silver nitrate, pressed the mixture into the form of a ceramic tablet, and fired the tablet in a kiln at 900 ˚C. During firing, the clay formed a ceramic, the sawdust combusts, and the ionic silver is reduced to metallic silver, forming metallic silver "nanopatches" with an average diameter of 2.71 nm as seen in Figure 2.2. The ceramic tablet can be added to a 5-15-L household water storage container and it passively releases ionic silver for the inactivation of *E. coli* and other gram-negative waterborne pathogenic bacteria. A 3-log reduction (99.9% removal) was shown for *E.* coli in a 10 L water source after 8 hours. This technology shows promise as a successful stand-alone technology for household water treatment for very low cost, or in combination with other filter or boiling treatments, where recontamination is a common issue (Ehdaie, Krause, & Smith, 2014). Similarly, colloidal silver has been coated on a ceramic brick for suspension in large drinking water storage tanks

(Nover, McKenzie, Joshi, & Fleenor, 2013). This provides continuous, low-maintenance disinfection quickly (Nover et al., 2013), but the silver levels may become too high and require attention to residence time and size of the water container (Nover et al., 2013).

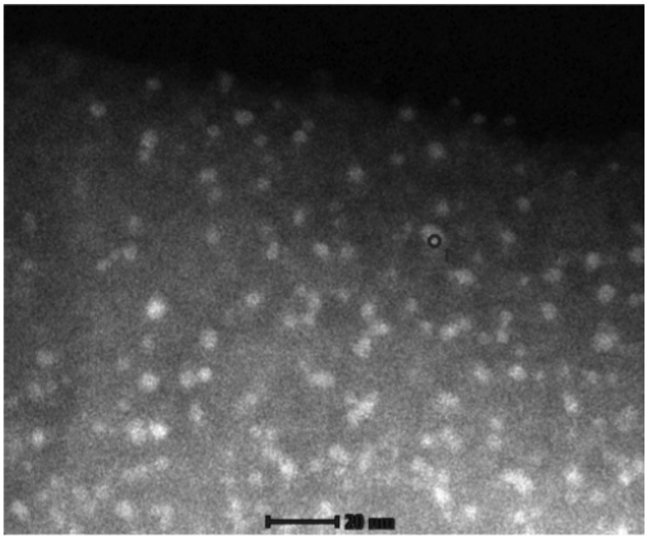

Figure 2.2. Transmission electron microscopy image of silver nanopatches in the center of a ceramic tablet using scanning electron microscopy mode. The scale bar represents 20 nm in length (Ehdaie et al., 2014).

2.3.2 PAPER

Paper is mostly made up of cellulose, the most abundant natural polymer. It is readily available, inexpensive, renewable, and biodegradable as well as having a great ability for metal ion sorption (Tang, Meng, Lu, & Zhu, 2009) (T. A. Dankovich, 2012). Paper is a cellulosic non-woven material like some fabrics (Ngo, Li, Simon, & Garnier, 2011). Silver nanoparticles have been formed on cellulose in bandages, clothing, food packaging, and much more. This proves a potential use of paper for water treatment (T. A. Dankovich, 2012). The most common way of synthesizing silver nanoparticles on cellulosic material is *in situ* chemical reduction of metal ions, with a reducing agent commonly being sodium borohydride (T. A. Dankovich, 2012;

Tang et al., 2009; Zeng et al., 2007). Dankovich (2012) has described a method of creating silver nanoparticle (AgNP) paper by immersing blotting paper sheets (6.5 cm by 6.5 cm) in 20 mL of silver nitrate solutions at concentrations from 0 to 100 mM for 30 minutes. The sheets are then rinsed with ethanol to remove excess silver nitrate that was not absorbed into the blotter. To form nanoparticles, the paper is placed in aqueous $NaBH_4$ solutions (ranging from 1:1 to 10:1 molar ratio, $NaBH_4/AgNO_3$) for 15 min. Lastly, the paper is soaked in water for 60 min, and dried in an oven at 60° C for 2 to 3 hours (T. A. Dankovich & Gray, 2011).

A microware irradiation method for *in situ* preparation of silver nanoparticles in paper has also been developed. Using a microwave, or a conventional oven, a silver nanoparticle covered paper can be produced in a "green" way. Blotting papers are submerged in aqueous solutions of silver nitrate and glucose (T. Dankovich, Clinch, Weinronk, Dillingham, & Smith, 2014). The papers are then heated in either a microwave or a conventional oven before being soaked in water to remove excess reagents (T. Dankovich et al., 2014). Glucose functions as the reducing agent with higher concentrations leading to smaller, more uniform nanoparticles and a faster reaction time. The average size of the resulting nanoparticles can be measured with TEM images to be 5.5 nm (T. A. Dankovich, 2012). Figure 2.4 shows scanning electron microscope (SEM) images of AgNP on paper (T. A. Dankovich, 2014). This way of producing the papers is more benign than the use of sodium borohydride as the reducing agent, while being just as effective (T. Dankovich et al., 2014).

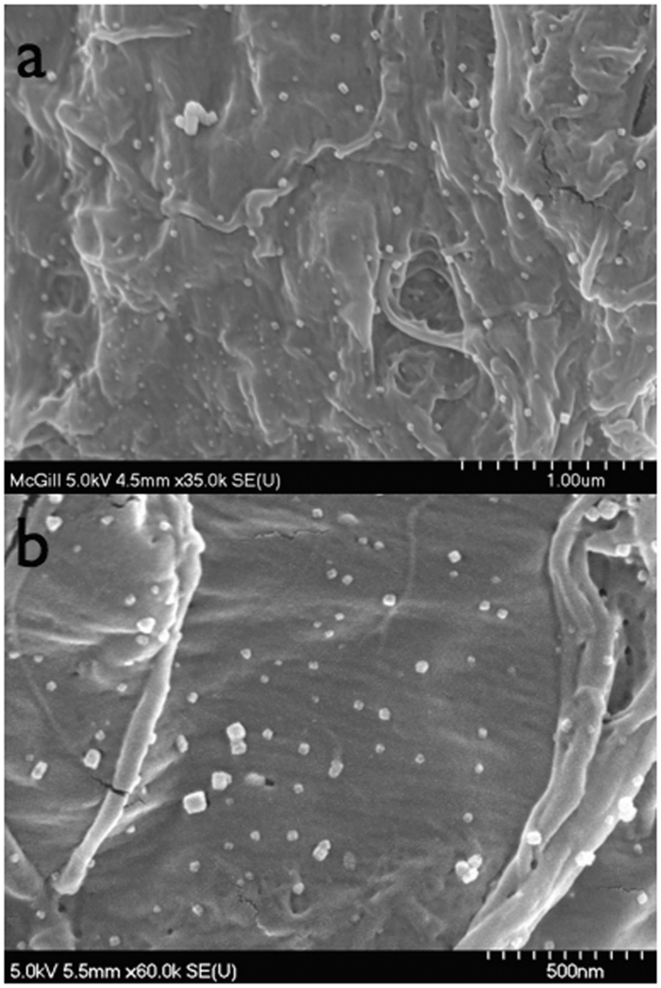

Figure 2.4: SEM image of AgNP paper with 2.5 mg Ag g−1 paper, (a) 35 000× and (b) 60 000× magnification (T. A. Dankovich, 2014).

Silver nanoparticles have the largest antibacterial effects with the smallest particle sizes, with average diameters under 10 nm being most effective. Results showed the AgNP paper was effective with *E. coli* bacteria suspensions poured through the sheets. After passage through the paper, effluent bacteria was isolated and used for plate counts, showing log 7.6 (±1.3) and log 3.4 (±0.9) reductions of viable *E. coli* and *E. faecalis* bacteria, respectively. Bacteria were reduced by log 0.95 (± 0.5), probably because some bacteria remained on the fiber surfaces in the blotting paper. Graphite furnace atomic absorption was used to measure the silver content in the effluent, which measured 0.0475 (±0.0177) ppm meeting the USEPA guideline for drinking water of less than 0.1 ppm (T. A. Dankovich & Gray, 2011).

Gottesman et al. (2010) describe a way of depositing silver nanoparticles onto paper by ultrasonication. The paper is held immersed in a solution of silver nitrate, ethanol, ethylene glycol and water which is then irradiated with a high intensity ultrasonic horn.. These papers have been shown to have minimal loss of silver from the coated surface, which is important for the longevity of the material. They were also shown to be effective against both gram-negative and gram-positive bacteria. This shows promise for water treatment and the avoidance of cross contamination (Gottesman et al., 2010).

Silver nanoparticles have been imbedded in paper by first immobilizing initiators on a paper's surface. Then a surface-initiated atom transfer radical polymerization technique has been developed to graft poly(tert-butyl acrylate) brushes to cellulose filters. Then the brushes are transferred into poly(acrylic acid) by hydrolyzation. Silver nitrate is then reduced to Ag^+ *in situ* by reducing agent sodium borohydride. These filters display good antibacterial ability against *E. coli* (Tang et al., 2009).

2.3.3 TEXTILE

Silver nanoparticles are commonly used in clothing, especially socks, as an antimicrobial agent (Benn & Westerhoff, 2008). Antimicrobial silver is also popular for improving dressing for burn wounds. An ethanol/water medium with silver nitrate uses cation exchange between silver and sodium from sodium carboxymethyl cotton gauze (Parikh et al., 2005). The use of textiles for filtration is not a new idea. Different types of sari material have been used in Bangladesh as an inexpensive and easily available option for point-of-use water filtration (Colwell et al., 2003; A. Huq et al., 1996). Shesh, a traditional textile of North Africa, and more modern textiles like denim or shower curtains have also been tested for water filtration performance (Saidani-Scott, Tierney, & Sánchez-Silva, 2009). Textiles are very effective at removing turbidity when used for filtration (Tammisetti, 2010). The waterborne pathogen *Vibrio cholerae* often attaches to something larger like zooplankton, particularly copepods (Colwell et al., 2003; A. Huq et al., 1996). This makes for easier removal by fabric filtration. The larger particles are more likely to get filtered out via fabric filtration, while smaller organisms could pass straight through the holes in the fabric. Folding of the fabric is encouraged to increase efficiency so more levels of fabric are used for filtration (Colwell et al., 2003). The simple filtration technique of pouring water through a fabric folded at least four times has been shown to effectively remove zooplankton, most phytoplankton and particulates bigger than 20 micrometers (Colwell et al., 2003). Most importantly, follow up studies have shown that the method is accepted and sustainable by villagers in Bangladesh and also reduced the incidence of cholera by 48% (Anwar Huq et al., 2010). Gram-negative bacterium *E. coli* and Gram-positive bacterium *Staphylococcus aureus* are commonly used for antimicrobial activity with fabric testing and fungus *C. albicans* is also used frequently (Ilic et al., 2010; Ilic et al., 2009; Maneerung, Tokura, & Rujiravanit, 2007; Mecha & Pillay, 2014). Textiles show this promise because without alteration they remove larger suspensions and reduce turbidity.

Radetić (2013) offers a comprehensive overview of nanoparticle-embedded textiles. The quick push for silver nanoparticles in textiles led to a growing industry with lack of insight into the possible environmental effects. Cotton seems to be the original textile of choice, especially pre-dyed or printed. The natural fiber allows for silver application to be commonly done without any prep work. Cotton is also a common fiber that is easily available and widely used. Like paper, sodium borohydride and glucose are common reducing agents used for the formation of silver nanoparticles (Maneerung et al., 2007; Reda M. El-Shishtawy, 2011). Silk has also be used with the reduction of silver nitrate with reducing agents like hydrazine and glucose (Gulrajani, Gupta, Periyasamy, & Muthu, 2008). Wool is also a common fiber, but not as well explored for AgNP applications. It has been shown to have antimicrobial abilities, and its main benefit is said to be the bright brilliant colors it produces (Radetic, 2013). Silver nanoparticle wool composites have been made by the reduction of silver ions in solution by trisodium citrate in solution with merino wool fibers or fabrics (Kelly & Johnston, 2011). This process creates a bind between the silver metal nanoparticles and the amino acids of the keratin protein in the wool fibers with the reducing agent functioning as the linker (Kelly & Johnston, 2011). Cotton fabrics also do not need pre-treatment to be able to load silver nanoparticles efficiently, even though 3-aminopropyltriethoxysilane has been used for surface modification with improved results (Guo et al., 2013; Ilic et al., 2009; Liu, Lee, Norsten, & Chong, 2013), but, the ability of cotton textiles to retain moisture leads to an excellent environment for growing microorganisms (C.-Y. Chen & Chiang, 2008). Because of surface charges, inorganic particles and polymeric materials, like manmade textiles, demonstrate repelling between silver and the fiber at the interface; this requires a modification be made to the surface, but this commonly washes away with laundering leading to leaching (Dastjerdi, Montazer, & Shahsavan, 2009). Chelating monomers, like glycidyl methacrylate-iminodiacetic acid, can be used to graft onto cotton fibers to increase the amount of Ag^+ sorbed. The Ag^+ can then be reduced by UV radiation to form silver nanoparticles on the surface (C.-Y. Chen & Chiang, 2008). Polyester and polyamide offer two synthetic options, and polypropylene fibers (PP) are widely used in the manufacturing of medical and hygienic textile materials where a high level of antimicrobial activity is required (surgical masks, diapers, hygienic bands, etc.) (Radetic, 2013). Figure 2.5 show untreated PP fiber as well as a PP fiber loaded with silver nanoparticles. Activated carbon fibers have high adsorption capacities and can be impregnated with silver. Because of the high adsorption capacity and large surface area, bacteria will breed on the activated carbon filter (Yoon, Byeon, Park, & Hwang, 2008). Silver-deposited activated carbon filters have been shown to be effective for air filters, but also have a low life expectancy with greatly decreasing performance quickly when used for water filtration (LePape et al., 2002, 2004; Yoon et al., 2008). When designing a method to incorporate silver nanoparticles with textiles, it is key to control the chemistry of the solution including the pH, ionic strength, and surface complexation (Yin, Walker, Chen, & Yang, 2014). A crosslinkable polysiloxane layer can be used to permanently modify the surface of polyester with a polymeric layer on the surface that is resistant to light, heat, chemical and microbial attacks making it safer during washing (Dastjerdi et al., 2009; Dastjerdi, Montazer, & Shahsavan, 2010).

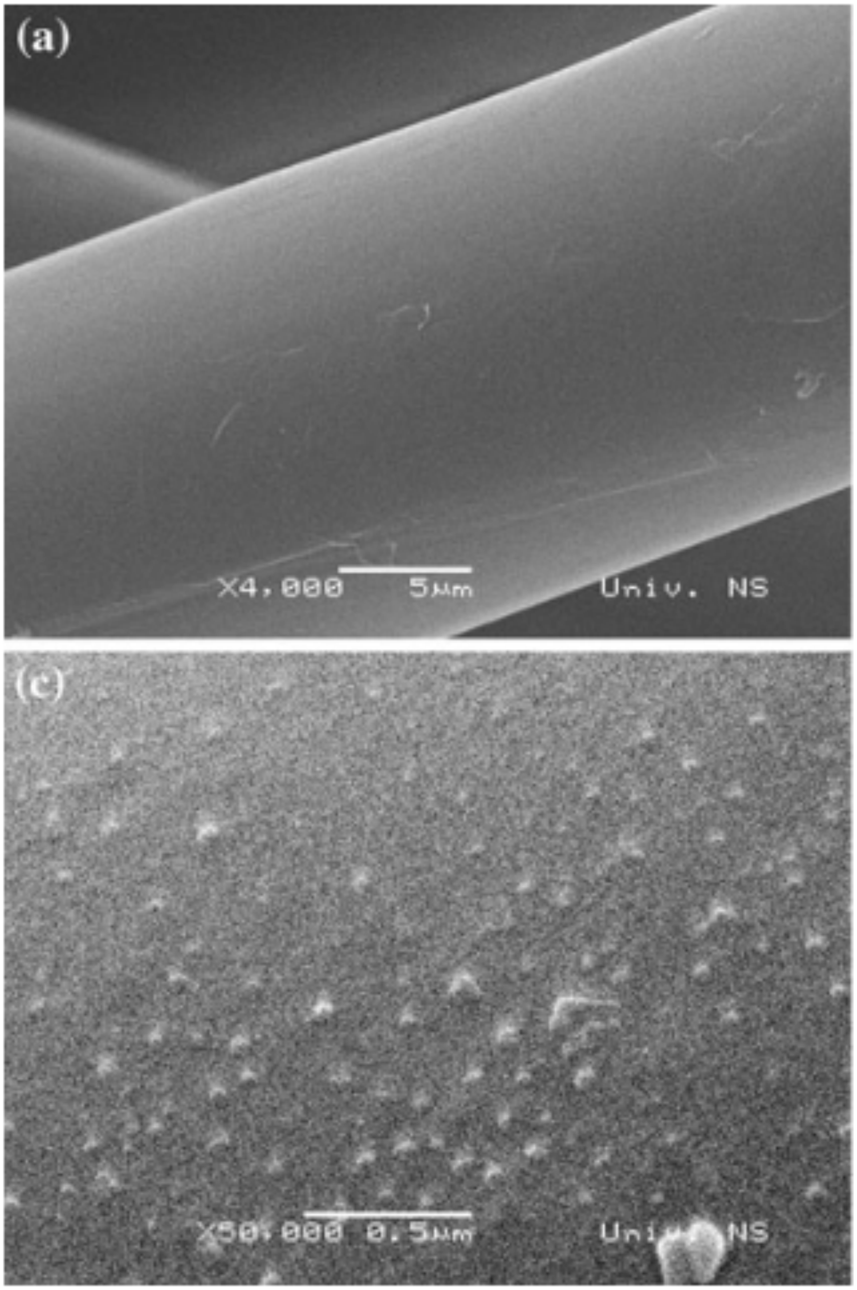

Figure 2.5: SEM image of untreated PP fiber and PP fiber loaded with AgNP (Radetic, 2013).

Another way of synthesizing silver nanoparticles in textiles involves using Tollen's reagent; silver nitrate is combined with NaOH to form Ag_2O powder. An ammonia solution is added and sonicated to produce an aqueous solution of Tollen's reagent, a complex ion, $[Ag(NH_3)_2]^+$. Cotton fabric is soaked in a boiling bath of Tollen's reagent solution then washed with deionized water and dried at room temperature forming silver nanoparticles (Montazer, Alimohammadi, Shamei, & Rahimi, 2012). Using electrostatic interactions, cellulose can be modified to be positively charged by using an epoxy substitution reaction on the abundant hydroxyl groups on cellulose to graft ammonium ions. This now positively charged cellulose fiber surface can be involved in adsorption of either negatively charged metal complex ions followed by reduction or with metal nanoparticles capped with negative citrate ions (Dong & Hinestroza, 2009).

In efforts to take a more "green approach", natural extracts from *Eucalyptus citriodora* and *Ficus bengalenis* are used in production of silver nanoparticles. Silver nanoparticles are produced on cotton fibers by an aqueous silver nitrate solution with fresh leaf extract. The leaf broth reduces Ag^+ to silver nanoparticles through the interactions of functional groups present in the leaf extracts (Ravindra, Mohan, Reddy, & Raju, 2010). This does not require any harsh chemicals or dangerous byproducts in the production process. *Curcuma longa* tuber powder and extract have also been used for their water-soluble organics to reduce silver ions from aqueous silver nitrate to nano-sized silver particles on cotton cloth. The extract works better than the powder since there is more of the reducing agent to form the nanoparticles (Sathishkumar, Sneha, & Yun, 2010).

Low-temperature radio frequency plasma has been used to activate the surface of fibers for improved binding ability of colloidal silver nanoparticles onto polyester fabrics. The plasma treatment reduces the number of coatings necessary for the same level of antibacterial ability in half. These fabrics have been tested for antimicrobial ability as well as laundering ability and have proven to be an improved method. The antibacterial ability was tested against the common Gram-negative bacterium *E. coli* and Gram-positive bacterium *S. aureus* (Ilic et al., 2010).

As with papers, the cellulose structure of fabric can be used to soak the fabric in silver nitrate solution followed by either physical (heat/UV) or chemical (sodium borohydride) reduction (Fernández et al., 2009). Microwaves are a way of delivering irradiation and heat after soaking with a reducing agent, like sodium citrate (Guo et al., 2013). Mecha and Pillay uses a modified version of the Dankovich method described in the previous section on woven fabric for microfiltration membranes (T. A. Dankovich, 2014). This is a point-of-use filter that is gravity fed and has coated membranes that are hydrophilic and have high water permeability ($p<0.05$). Filtration of turbid water (40–700 NTU) produced clear permeate (<1 NTU) and treatment of water spiked with bacteria (2500– 77,000 CFU/100 mL *E. coli*) showed the removal efficiency of coated membranes was 100% (Mecha & Pillay, 2014).

It has been reported that silver-nanoparticle-coated textile fabrics possess antibacterial activity against *S. aureus*. Silver nanocomposite fibers have been made containing silver nanoparticles incorporated inside the fabric but scanning electron microscopy has concluded that the silver nanoparticles incorporated in the sheath part of fabrics possessed significantly better antibacterial ability compared to the fabrics with silver nanoparticles in the core. The same results came from silver nanoparticles on polyester nonwovens (Rai et al., 2009). Aqueous silver nitrate has been electrospun with polymers to create a fiber that is then reduce with UV irradiation to create silver nanoparticle fibers (Lala et al., 2007). These fibers could then be woven into a textile for use in filtration. An interesting application method has also been developed for applying silver to TiO_2 thin film. An aqueous solution of 1 M silver nitrate ($AgNO_3$) is mixed with ethanol and a 0.6 mL aliquot of the solution is uniformly cast on the TiO_2 thin film. The film is then irradiated with UV light for 1 min, to deposit Ag nanoparticles in the porous film by photocatalytic reduction of Ag^+. The resulting $Ag–TiO_2$ film is thoroughly rinsed with DI water and dried by blowing air with an electric duster (Naoi et al., 2004). Schoen et al., presents an interesting textile technology that combines several mechanisms into one process. It is a "textile based multiscale device for the high speed electrical sterilization of water using silver nanowires, carbon nanotubes, and cotton" (Schoen et al., 2010). This gravity fed device can operate at 100,000 L/(h m^2) while inactivating >98% of bacteria with only seconds of total incubation time. An electrical mechanism rather than size exclusion, allows for the flow to be fast but effective with a very high surface area and large electric field with silver nanowire (Schoen et al., 2010).

2.4 CONCLUSIONS

Increasing the market penetration of point-of-use (POU) technologies will require continued development of inexpensive, easy to use, socially acceptable technologies that do not change the taste of water. These design efforts must be complimented by education and training. Current filtration technologies possess many of the requisite design criteria. Each of the technologies introduced in the chapter have their own strengths and weaknesses. Ceramics filters are the most widely used technology and have the most market penetration to date, but the fragile nature of ceramics makes brakeage a concern. Fabric filters can successfully remove copepods, reducing cholera, but their effectiveness against other pathogens is unknown. Adding silver to fabrics and evaluating performance and social acceptance would be a significant advancement. Paper filters are the least developed technology but may be effective once the proper way of incorporating the technology into household point-of-use is determined. The new method of silver incorporation by the reduction of silver nitrate in papers and ceramics also shows promise as a way to avoid harsh chemicals and the potential health risks of silver nanoparticles.

Moving forward, important work should also be done to understand the anti-bacterial activity of silver against all classes of bacteria (Pradeep & Anshup, 2009). New POU technologies, in addition to being effective, need to be culturally appropriate to encourage use and motivate the purchasing of the treatment system (D. S. Lantagne et al., 2006). An optimized POU technology should be easy to use, low maintenance, independent of energy and chemicals and have a low cost (Peter-Varbanets et al., 2009). Finding a technology that satisfies all these requirements is challenging. Other nanomaterials have proven effective against other waterborne pathogens and the potential for combination of silver with another metal could improve technologies (Ruparelia et al., 2008). Long term effectiveness is important, making the antifouling ability necessary (Praveena & Aris, 2015). Expanding production of, marketing and distributing any technology will also require research and knowledge of underlying economic factors (Sobsey et al., 2008). It has also been suggested that the introduction of multiple interventions to prevent the transmission of disease is the most effective way to improve the quality of all of the interventions (J. E. Mellor et al., 2012). Solving one point of exposure will not maximize effect. Multiple interventions to improve knowledge and treatments will have the most effect.

2.6 REFERENCES

Abebe, L. S., Smith, J. A., Narkiewicz, S., Oyanedel-Craver, V., Conaway, M., Singo, A., . . . Dillingham, R. (2014). Ceramic water filters impregnated with silver nanoparticles as a point-of-use water-treatment intervention for HIV-positive individuals in Limpopo Province, South Africa: a pilot study of technological performance and human health benefits. *Journal of Water and Health, 12*(2), 288-300. doi:10.2166/wh.2013.185

Albert, J., Luoto, J., & Levine, D. (2010). End-User Preferences for and Performance of Competing POU Water Treatment Technologies among the Rural Poor of Kenya. *Environmental Science & Technology, 44*(12), 4426-4432. doi:Doi 10.1021/Es1000566

Bain, R. E., Gundry, S. W., Wright, J. A., Yang, H., Pedley, S., & Bartram, J. K. (2012). Accounting for water quality in monitoring access to safe drinking-water as part of the Millennium Development Goals: lessons from five countries. *Bulletin of the World Health Organization*. doi:10.2471/BLT.11.094284

Benn, T. M., & Westerhoff, P. (2008). Nanoparticle Silver Released into Water from Commercially Available Sock Fabrics. *Enviromental Science and Technology, 42*(11), 41334139.

Brown, J., Chai, R., Wang, A., & Sobsey, M. D. (2012). Microbiological Effectiveness of Mineral Pot Filters in Cambodia. *Enviromental Science and Technology, 46*, 12055-12061.

Brown, J., Proum, S., & Sobsey, M. D. (2009). Sustained use of a household-scale water filtration device in rural Cambodia. *Journal of Water and Health, 7*(3), 404-412.

Brown, J., & Sobsey, M. D. (2010). Microbiological effectiveness of locally produced ceramic filters for drinking water treatment in Cambodia. *Journal of Water and Health, 8*(1), 1-11.

Brown, J., Sobsey, M. D., & Loomis, D. (2008). Local drinking water filters reduce diarrheal disease in Cambodia: A randomized, controlled trial of the ceramic water purifier. *American Journal of Tropical Medicine and Hygiene, 79*(3), 394-400.

Chen, C.-Y., & Chiang, C.-L. (2008). Preparation of cotton fibers with antibacterial silver nanoparticles. *Matterials Letters*(62), 3607-3609.

Chen, S., Liu, J., & Zeng, H. (2005). Structure and antibacterial activity of silver-supporting activated carbon fibers. *Journal of Materials Science, 40*, 6223-6231.

Clasen, T., & Boisson, S. (2006). Household-Based Ceramic Water Filters for the Treatment of Drinking Water in Disaster Response: An Assessment of a Pilot Programme in the Dominican Republic. *Water Practice & Technology, 1*(2). doi:10.2166/WPT.2006031

Clasen, T., McLaughlin, C., Nayaar, N., Boisson, S., Gupta, R., Desai, D., & Shah, N. (2008). Microbiological Effectiveness and Cost of Disinfecting Water by Boiling in Semi-urban India. *The American Society of Tropical Medicine and Hygiene, 79*(3), 407-413.

Clasen, T., Nadakatti, S., & Menon, S. (2006). Microbiological performance of a water treatment unit designed for household use in developing countries. *Tropical Medicine and International Health, 11*(9), 1399-1405.

Clasen, T., Parra, G. G., Boisson, S., & Collin, S. (2005). Household-Based Ceramic Water Filters for the Prevention of Diarrhea: A Randomized, Controlled Trial of a Pilot Program in Columbia. *The American Society of Tropical Medicine and Hygiene, 73*(4), 790-795.

Clasen, T. F., Brown, J., Collin, S., Suntura, O., & Cairncross, S. (2004). Reducing diarrhea through the use of household-based ceramic water filters: A randomized, controlled trial in rural Bolivia. *American Journal of Tropical Medicine and Hygiene, 70*(6), 651-657.

Colwell, R. R., Huq, A., Islam, M. S., Aziz, K. M. A., Yunus, M., Khan, N. H., . . . Russek-Cohen, E. (2003). Reduction of cholera in Bangladeshi villages by simple filtration. *Proceedings of the National Academy of Sciences, 100*(3), 1051-1055.

Cosgrove, W. J., & Rijsberman, F. R. (2000). *World Water Vision: Making Water Everybody's Business*. London: Earthscan Publications.

Dankovich, T., Clinch, C., Weinronk, H., Dillingham, R., & Smith, J. A. (2014). Inactivation of bacteria from contaminated streams in Limpopo, South Africa by silver- or copper-nanoparticle paper filters. *Water Research, Submitted*.

Dankovich, T. A. (2012). *Bactericidal Paper Containing Silver Nanoparticles for Water Treatment.* (Doctor of Philosophy Thesis), McGill University.

Dankovich, T. A. (2014). Microwave-assisted incorporation of silver nanoparticles in paper for point-of-use water purification. *Royal Society of Chemistry, 1*, 367-378.

Dankovich, T. A., & Gray, D. G. (2011). Bactericidal Paper Impregnated with Silver Nanoparticles for Point-of-Use Water Treatment. *Eniviromental Science and Technology, 45*(5), 1992-1998.

Dastjerdi, R., Montazer, M., & Shahsavan, S. (2009). A new method to stabilize nanoparticles on textile surfaces. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 345*, 202-210.

Dastjerdi, R., Montazer, M., & Shahsavan, S. (2010). A novel technique for producing durable multifunctional textiles using nanocomposite coating. *Colloids and Surfaces B: Biointerfaces, 81*, 32-41.

Dillingham, R., & Guerrant, R. L. (2004). Childhood stunting: measuring and stemming the staggering costs of inadequate water and sanitation. *The Lancet, 363*, 94-95.

Dillingham, R., Pinkerton, R., Leger, P., Severe, P., Pape, J., & Fitzgerald, D. (2006). *Mortality in Haitian patients treated with antiretroviral therapy (ART) in a community setting*. Paper presented at the 13th Conference on Retroviruses and Opportunistic Infections, Denver, CO.

Dong, H., & Hinestroza, J. P. (2009). Metal Nanoparticles on Natural Cellulose Fibers: Electrostatic Assembly and In Situ Synthesis. *Applied Materials & Interfaces, 1*(4), 797-803.

Ehdaie, B., Krause, C., & Smith, J. A. (2014). Porous Ceramic Tablet Embedded with Silver Nanopatches for Low- Cost Point-of-Use Water Purification. *Eniviromental Science and Technology, 48*, 13901-13908.

Fernández, A., Soriano, E., López-Carballo, G., Picouet, P., Lloret, E., Gavara, R., & Hernández-Muñoz, P. (2009). Preservation of aseptic conditions in absorbent pads by using silver nanotechnology. *Food Research International, 42*, 1105-1112.

Gottesman, R., Shukla, S., Perkas, N., Solovyov, L. A., Nitzan, Y., & Gedanken, A. (2010). Sonochemical Coating of Paper by Microbiocidal Silver Nanoparticles. *Langmuir, 27*(2), 720-726.

Gulrajani, M. L., Gupta, D., Periyasamy, S., & Muthu, S. G. (2008). Preparation and Application of Silver Nanoparticles on Silk for Imparting Antimicrobial Properties. *Journal of Applied Polymer Science, 108*, 614-623.

Guo, R., Li, Y., Lan, J., Jiang, S., Liu, T., & Yan, W. (2013). Microwave-Assisted Synthesis of Silver Nanoparticles on Cotton Fabric Modified with 3-Aminopropyltrimethoxysilane. *Journal of Applied Polymer Science*. doi:10.1002/app.39636

Han, D.-W., Lee, M. S., Lee, M. H., Uzawa, M., & Park, J.-C. (2005). The use of silver-coated ceramic beads for sterilization of Sphingomonas sp. in drinking mineral water. *World Journal of Microbiology & Biotechnology, 21*, 921-924.

Herrin, R. T., Andren, A. W., & Armstrong, D. E. (2001). Determination of Silver Speciation in Natural Waters. 1. Laboratory Tests of Chelex-100 Chelating

Resin as a Competing Ligand. *Environmental Science & Technology, 35*(10), 1953-1958.

Huq, A., Xu, B., Chowdhury, M. A. R., Islam, M. S., Montilla, R., & Colwell, R. R. (1996). A Simple Filtration Method To Remove Plankton-Associated Vibrio cholerae in Raw Water Supplies in Developing Countries. *Applied and Environmental Microbiology, 62*(7), 2508-2512.

Huq, A., Yunus, M., Sohel, S. S., Bhuiya, A., Emch, M., Luby, S. P., . . . Colwell, R. R. (2010). Simple Sari Cloth Filtration of Water Is Sustainable and Continues To Protect Villagers from Cholera in Matlab, Bangladesh. *mBio, 1*(1). doi:10.1128/mBio.00034-10

Hutton, G., & Haller, L. (2004). Evaluation of the costs and benefits of water and sanitation improvements at the global level. *World Health Organization, Geneva*.

Ilic, V.,´, Z. S. a., Vodnik, V.,´, S. L.,´, S. D.,´, P. J., . . .´, M. R. (2010). Bactericidal Efficiency of Silver Nanoparticles Deposited onto Radio Frequency Plasma Pretreated Polyester Fabrics. *Ind. Eng. Chem. Res., 49*(16), 7287-7293.

Ilic, V.,´, Z. S. a., Vodnik, V., Potkonjak, B.,´, P. J.,´, J. N., &´, M. R. (2009). The influence of silver content on antimicrobial activity and color of cotton fabrics functionalized with Ag nanoparticles. *Carbohydrate Polymers, 78*, 564-569.

Kallman, E. N., Vinka A. Oyanedel-Craver, A. M. A., & and James A. Smith, M. A. (2011). Ceramic Filters Impregnated with Silver Nanoparticles for Point-of-Use Water Treatment in Rural Guatemala. *Journal of Enviromental Engineering, 137*, 407-415. doi: 10.1061/(ASCE)EE.1943-7870 .0000330

Karel, F. B., Koparal, A. S., & Kaynak, E. (2015). Development of Silver Ion Doped Antibacterial Clays and Investigation of Their Antibacterial Activity. *Advances in Materials Science and Engineering, 2015*. doi:10.1155/2015/409078

Kasuga, E., Kawakami, Y., Matsumoto, T., Hidaka, E., Oana, K., Ogiwara, N., . . . Honda, T. (2011). Bactericidal activities of woven cotton

and nonwoven polypropylene fabrics coated with hydroxyapatite-binding silver/titanium dioxide ceramic nanocomposite "Earth-plus". *International Journal of Nanomedicine, 6*, 1937-1943.

Kelly, F. M., & Johnston, J. H. (2011). Colored and Functional Silver NanoparticleWool Fiber Composites. *Applied Materials & Interfaces, 3*, 1083-1092.

Kirchhoff, L. V., & McClelland, K. E. (1985). Feasibility and efficacy of in-home water chlorination in rural North-eastern Brazil. *The Journal of Hygiene, 94*(2), 173-180.

Lala, N. L., Ramaseshan, R., Bojun, L., Sundarrajan, S., Barhate, R. S., Ying-jun, L., & Ramakrishna, S. (2007). Fabrication of Nanofibers With Antimicrobial Functionality Used as Filters: Protection Against Bacterial Contaminants. *Biotechnology and Bioengineering, 97*(6), 1357-1365.

Lantagne, D., Klarman, M., Mayer, A., Preston, K., Napotnik, J., & Jellison, K. (2010). Effect of production variables on microbiological removal in locally-produced ceramic filters for household water treatment. *International Journal of Environmental Health Research, 20*(3), 171-187.

Lantagne, D. S. (2001). *Investigation of the Potters for Peace Colloidal Silver Impregnated Ceramic Filter*

Report 2: Field Investigations. Retrieved from

Lantagne, D. S., & Clasen, T. F. (2012). Use of Household Water Treatment and Safe Storage Methods in Acute Emergency Response: Case Study Results from Nepal, Indonesia, Kenya, and Haiti. *Eniviromental Science and Technology, 46*, 11352-11360.

Lantagne, D. S., Quick, R., & Mintz, E. D. (2006). Household Water Treatment and Safe Storage Options in Developing Coutries: A Review of Current Implementation Practices .

LeOuay, B., & Stellacci, F. (2015). Antibacterial activity of silver nanoparticles: A surface science insight. *Nano Today*. doi:10.1016/j.nantod.2015.04.002

LePape, H., Solano-Serena, F., Contini, P., Devillers, C., Maftah, A., & Leprat, P. (2002). Evaluation of the anti-microbial properties of an activated carbon fibre supporting silver using a dynamic method. *Carbon, 40*, 2947-2954.

LePape, H., Solano-Serena, F., Contini, P., Devillers, C., Maftah, A., & Leprat, P. (2004). Involvement of reactive oxygen species in the bactericidal activity of activated carbon fibre supporting silver: Bactericidal activity of ACF(Ag) mediated by ROS. *Journal of Inorganic Biochemistry, 98*, 1054-1060.

Li, Q., Mahendra, S., Lyon, D. Y., Brunet, L., Liga, M. V., Li, D., & Alvarez, P. J. J. (2008). Antimicrobial nanomaterials for water disinfection and microbial control: Potential applications and implications. *Water Research, 42*(18), 4591-4602.

Lin, S., Huang, R., Cheng, Y., Liu, J., Lau, B. L. T., & Wiesner, M. R. (2013). Silver nanoparticle-alginate composite beads for point-of-use drinking water disinfection. *Water Research, 47*, 3959-3965.

Liu, H., Lee, Y.-Y., Norsten, T. B., & Chong, K. (2013). In situ formation of anti-bacterial silver nanoparticles on cotton textiles. *Journal of Industrial Textiles*. doi:10.1177/1528083713481833

Maneerung, T., Tokura, S., & Rujiravanit, R. (2007). Impregnation of silver nanoparticles into bacterial cellulose for antimicrobial wound dressing. *Carbohydrate Polymers, in press*.

Matthies, K., Bitter, H., Deobald, N., Heinle, M., Diedel, R., Obstaand, U., & Brenner-Weissa, G. (2015). Morphology, composition and performance of a ceramic filter for household water treatment in Indonesia. *Water Practice & Technology, 10*(2), 361-370. doi:10.2166/wpt.2015.044

Mecha, C. A., & Pillay, V. L. (2014). Development and evaluation of woven fabric microfiltration membranes impregnated with silver nanoparticles for potable water treatment. *Journal of Membrane Science, 458*, 149-156.

Mellor, J., Abebe, L., Ehdaie, B., Dillingham, R., & Smith, J. (2014). Modeling the sustainability of a ceramic water filter intervention. *Water Research, 49*, 286-299.

Mellor, J. E., Smith, J. A., Learmonth, G. P., Netshandama, V. O., & Dillingham, R. A. (2012). Modeling the Complexities of Water, Hygiene, and Health in Limpopo Province, South Africa. *Enviromental Science and Technology, 46*, 13512-13520.

Montazer, M., Alimohammadi, F., Shamei, A., & Rahimi, M. K. (2012). In situ synthesis of nano silver on cotton using Tollens' reagent. *Carbohydrate Polymers, 87*, 1706-1712.

Ngo, Y. H., Li, D., Simon, G. P., & Garnier, G. (2011). Paper surfaces functionalized by nanoparticles. *Advances in Colloid and Interface Science, 163*, 23-38.

Nover, D. M., McKenzie, E. R., Joshi, G., & Fleenor, W. E. (2013). Assessment of colloidal silver impregnated ceramic bricks for small-scale drinking water treatment applications. *International Journal for Service Learning, 8*(1), 18-35.

Oyanedel-Craver, V., & Smith, J. A. (2008). Sustainable colloidal-silver-impregnated ceramic filter for point-of-use water treatment. *Environmental Science and Technology, 42*(3), 927-933.

Oyanedel-Craver, V. A., & Smith, J. A. (2008). Sustainable Colloidal-Silver-Impregnated Ceramic Filter for Point-of-Use Water Treatment. *Enviromental Science and Technology, 42*, 927-933.

Parikh, D. V., Fink, T., Rajasekharan, K., Sachinvala, N. D., Sawhney, A. P. S., Calamari, T. A., & Parikh, A. D. (2005). Antimicrobial Silver/Sodium Carboxymethyl Cotton Dressings for Burn Wounds. *Textile Research Journal, 75*, 134-138. doi:10.1177/004051750507500208

Park, S.-J., & Jang, Y.-S. (2003). Preparation and Characterization of Activated Carbon Fibers Supported with Silver Metal for Antibacterial Behavior. *Journal of Colloid and Interface Science, 261*(2), 238-243. doi:10.1016/S0021-9797(03)00083-3

Peter-Varbanets, M., Zurbrugg, C., Swartz, C., & Pronk, W. (2009). Decentralized systems for potable water and the potential of membrane technology. *Water Research, 43*, 245-265.

Pradeep, T., & Anshup. (2009). Noble metal nanoparticles for water purification: A critical review. *Thin Solid Films, 517*, 6441-6478.

Praveena, S. M., & Aris, A. Z. (2015). Application of Low-Cost Materials Coated with Silver Nanoparticle as Water Filter in Escherichia coli Removal. *Water Qual Expo Health*. doi:10.1007/s12403-015-0167-5

Quang, D. V., Sarawade, P. B., Jeon, S. J., Kim, S. H., Kim, J.-K., Chai, Y. G., & Kim, H. T. (2013). Effective water disinfection using silver nanoparticle containing silica beads. *Applied Surface Science, 266*, 280-287.

Radetic, M. (2013). Functionalization of textile materials with silver nanoparticles. *Journal of Material Science, 48*, 95-107. doi:10.1007/s10853-012-6677-7

Rai, M., Yadav, A., & Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances, 27*(1), 76-83.

Ravindra, S., Mohan, Y. M., Reddy, N. N., & Raju, K. M. (2010). Fabrication of antibacterial cotton fibres loaded with silver nanoparticles via "Green Approach". *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 367*, 31-40.

Rayner, J. (2009). *Current Practices in Manufacturing of Ceramic Pot Filters for Water Treatment.* (Master of Science), Loughborough University.

Rayner, J., Zhang, H., Schubert, J., Lennon, P., Lantagne, D., & Oyanedel-Craver, V. (2013). Laboratory Investigation into the Effect of Silver Application on the

Bacterial Removal Efficacy of Filter Material for Use on Locally Produced Ceramic Water Filters for Household Drinking Water Treatment. *ACS Sustainabe Chemical Engineering, 1*, 737-745. doi:10.1021/sc400068p

Reda M. El-Shishtawy, A. M. A., Nayera A. M. Abdelwahed, Maha M. Al-Otaibi. (2011). In situ production of silver nanoparticle on cotton fabric and its antimicrobial evaluation. *Cellulose, 18*, 75-82.

Ren, D., Colosi, L. M., & Smith, J. A. (2013). Evaluating the Sustainability of Ceramic Filters for Point-of-Use Drinking Water Treatment. *Enviromental Science and Technology, 47*, 11206-11213.

Ren, D., & Smith, J. A. (2013). Retention and Transport of Silver Nanoparticles in a Ceramic Porous Medium Used for Point-of-Use Water Treatment. *Eniviromental Science and Technology, 47*, 3825-3832.

Ruparelia, J., Chatterjee, A., Duttagupta, S., & Mukherji, S. (2008). Strain specificity in antimicrobial activity of silver and copper nanoparticles. . *Acta Biomaterialia, 4*(3), 707-716.

Saidani-Scott, H., Tierney, M., & Sánchez-Silva, F. (2009). Experimental Study of Water Filtering Using Textiles as in Traditional Methods. *Applied Mechanics and Materials, 15*, 15-20.

Sathishkumar, M., Sneha, K., & Yun, Y.-S. (2010). Immobilization of silver nanoparticles synthesized using Curcuma longa tuber powder and extract on cotton cloth for bactericidal activity. *Bioresource Technology, 101*, 7958-7965.

Schoen, D. T., Schoen, A. P., Hu, L., Kim, H. S., Heilshorn, S. C., & Cui, Y. (2010). High Speed Water Sterilization Using One-Dimensional Nanostructures. *Nano Letters, 10*, 3628-3632.

Sobsey, M. D., Stauber, C. E., Casanova, L. M., Brown, J. M., & Elliott, M. A. (2008). Point of Use Household Drinking Water Filtration: A Practical, Effective Solution for Providing Sustained Access to Safe Drinking Water in the Developing World. *Enviromental Science and Technology, 42*, 4261-4267.

Tammisetti, R. (2010). Research on the Effectiveness of Using Cloth as a Filter to Remove Turbidity from Water.

Tang, S., Meng, X., Lu, H., & Zhu, S. (2009). PVP-assisted sonoelectrochemical growth of silver nanostructures with various shapes. *Materials Chemistry and Physics, 116*, 464-468.

Yin, T., Walker, H. W., Chen, D., & Yang, Q. (2014). Influence of pH and ionic strength on the deposition of silver nanoparticles on microfiltration membranes. *Journal of Membrane Science, 449*, 9-14.

Yoon, K. Y., Byeon, J. H., Park, C. W., & Hwang, J. (2008). Antimicrobial Effect of Silver Particles on Bacterial Contamination of Activated Carbon Fibers. *Enviromental Science and Technology, 42*, 1251-1255.

Zeng, F., Hou, C., Wu, S., Liu, X., Tong, Z., & Yu, S. (2007). Silver nanoparticles directly formed on natural macroporous matrix and their anti-microbial activities. *Nanotechnology, 18*. doi:10.1088/0957-4484/18/5/055605

Chapter 3: A New Method for the Deposition of Metallic Silver on Porous Ceramic Water Filters

The work presented in this chapter resulted in two published papers: one proceedings paper and one journal article.

Nunnelley, K. G., Smith, J.A., Smith, M.Y. and Samie, A., 2016, A New Method for Nanosilver Application in Ceramic Water Filters: Proceedings of The World Environmental and Water Resources Congress, May 2016, West Palm Beach, FL.

Jackson, K. N. and Smith, J. A., 2018, A New Method for the Deposition of Metallic Silver on Porous Ceramic Water Filters, Journal of Nanotechnology, vol. 2018, Article ID 2573015, 9 pages. doi:10.1155/2018/2573015.

3.1 ABSTRACT

A new method of silver application to a porous ceramic water filter used for point-of-use water treatment is developed. We evaluated filter performance for filters manufactured by the conventional method of painting an aqueous suspension of silver nanoparticles onto the filter and filters manufactured with a new method that applies silver nitrate to the clay-water-sawdust mixture prior to pressing and firing the filter. Filters were evaluated using miscible displacement flow-through experiments with pulse and continuous-feed injections of *E. coli*. Flow characteristics were quantified by tracer experiments using $[^3H]H_2O$. Experiments using pulse injections of *E. coli* showed similar performance in breakthrough curves between the two application methods. Long-term challenge tests performed with a continuous feed of *E. coli* and growth medium resulted in similar log removal rates, but the removal rate by nanosilver filters decreased over time. Silver nitrate filters provided consistent removal with lower silver levels in the effluent and effective bacterial disinfection. Results from continued use with synthetic groundwater over 4 weeks, with a pulse injection of *E. coli* at 2 and 4 weeks support similar conclusions—nanosilver filters perform better initially, but after 4 weeks of use, nanosilver filters suffer larger decreases in performance. Results show that including silver nitrate in the mixing step may effectively reduce costs, improve silver retention in the filter, increase effective lifespan and maintain effective pathogen removal while also eliminating the risk of exposure to inhalation of silver nanoparticles by workers in developing-world filter production facilities.

3.2 INTRODUCTION

The World Health Organization ("WHO") estimates that over 4 million deaths per year, of which more than 1.5 million involve children under the age of 5, are attributable to unsafe drinking water [1]. Centralized water treatment facilities, like those found in cities and suburban areas in the developed world, are not feasible for many developing communities due to the large infrastructure investment. Alternatively, the WHO has suggested a decentralized approach of treatment in home immediately prior to consumption—commonly referred to as point-of-use ("POU") water treatment [2,3]. POU technologies have the potential to significantly improve microbial quality of drinking water and reduce the risk of diarrheal disease and death, particularly in children [4]. A POU technology must be effective with respect to removal and/or deactivation of waterborne pathogens under a wide range of water chemistries, and simple to use to ensure use, long-term effectiveness, and reduce risk of recontamination [3,5]. The technology much also be socially acceptable and affordable, commonly achieved by the use of local labor and materials [6-8]. Ceramic water filters, produced with local labor and materials, are an appealing POU water treatment technology, and over 50 production facilities exist worldwide [9]. Clay, sawdust and water are mixed and then molded into a pot shape. The filter is then fired in a kiln, causing the clay to sinter into a ceramic and sawdust to combust. This creates pore channels that allow water flow. After quality testing, the filter is painted with a silver nanoparticle solution, where the silver acts as an well studied antimicrobial agent without changing the taste, color or odor of treated water [10-14]. The ceramic filter is suspended inside a plastic bucket with a spigot on the bottom for personal use. Source water is poured into the ceramic filter, then the water percolates through to the lower reservoir and clean water is dispensed through the spigot. The relatively small pore size (mean around 10 μm) of the ceramic filter helps remove turbidity and larger particles [15]. The silver release rate has generally been reported to produce silver concentrations below the secondary drinking water standard of 0.1 mg/L set by the USEPA [16] and the World Health Organization [17]. The "nano" size of the silver particles results in a high surface area to volume ratio, leading to better bactericidal activity [18]. However, silver nanoparticles have poor retention in ceramic, shortening the effective lifespan of the filter and possibly causing silver levels in the effluent water above drinking-water standards [19]. Mixing silver nanoparticles into the clay mixture prior to firing has been shown to result in higher retention of silver in the ceramic and a potentially longer lifespan than filters made with the silver nanoparticle method—which release silver at high levels quickly during early use [19]. This method has not been tested for bacterial disinfection nor has it been field tested, but it could be a promising alternative, despite the fact that it still relies on silver nanoparticles as a raw material. The method of silver application does not appear to be a factor affecting disinfection efficiency [15,20]. Instead, the mass of colloidal silver in the ceramic determines effectiveness.

Ehdaie et al. [21] reported on the formation of silver nanopatches in a ceramic porous tablet. In this work, they mixed silver nitrate, a Redart clay, sawdust, and water together in different proportions. The mixture was pressed into the shape of a disk of varying thickness and diameter and fired in a kiln at a final temperature of 900 °C. Characterization of the resulting silver-ceramic tablet revealed patches of silver throughout the pore structure with mean diameters in the range of 2-3 nm.

This chapter evaluates an alternative silver application method to ceramic water filters that adds silver nitrate to the clay-water-sawdust mix, similar to the methodology described by Ehdaie et al (21) for ceramic tablets and building on results from Nunnelley et al. (22). During firing, we hypothesize the formation of silver nanopatches in the porous medium. Compared to silver nanoparticles, silver nitrate is less expensive, more accessible in developing regions of the world, and easier to apply to ceramic water filters. We hypothesize that our new application method will keep more silver in the filter compared to conventional methods. This may increase the effective performance lifespan of the filter and result in less ingestion of silver by the end users. We evaluate this method with a series of miscible flow experiments using $[^3H]H_2O$ as a conservative tracer and a non-pathogenic strain of *E. coli* as an indicator organism targeted for removal. Different formulations of the silver-nitrate method are compared against the conventional silver-nanoparticle application method.

3.3 MATERIALS AND METHODS

3.3.1 MATERIALS

For ceramic filters, 200-mesh Redart clay produced by Cedar Heights Clay Company and sawdust sieved to 20-mesh from a commercial lumberyard in Earlysville, Virginia (USA) were used. The clay has a particle-size distribution (PSD) of 30.7%, 54.9%, and 14.4% for particles <2, 2− 20, and >20 μ m, respectively [15]. For the silver nanoparticle method of production, silver proteinate (7-8% Ag by weight) from Argenol Laboratories was used.

For the silver nitrate method, 99.5% pure silver nitrate from Acros Organics was used for the ceramic filter disk fabrication. For bacterial tests, a nonpathogenic strain of *E. coli* was purchased from IDEXX Laboratories (cat. 982900700, Lot 042313) and cultured, used and stored in the same method outlined in Ehdaie et al. [21]. A 10 mM phosphate buffer solution (PB) composed of 11.2 g/L of dipotassium phosphate, 4.8 g/L of potassium phosphate monobasic, 0.02 g/L of netetraacetic acid and deionized, organic-free water was used to preserve viability of *E. coli* in solution while preventing growth. A 60 g/L solution of sodium thiosulfate, prepared by dissolving anhydrous sodium thiosulfate (Fisher Scientific) in deionized water, was used to treat samples at the collection time to inhibit continued disinfection during incubation. All materials and solutions used for microbial analyses were sterilized before use. 4.3 μCi [^{3}H]H_2O was used for conservative tracer tests.

3.3.2 CERAMIC FILTER SYNTHESIS.

Two types of filters were studied in this investigation and they will be referred to as follows throughout the manuscript: (i) silver nanoparticle filters and (ii) silver nitrate filters. Silver nanoparticle filters use a conventional synthesis method similar to that described by Oyandedel-Craver and Smith [15]. Because of the results in Oyandedel-Craver and Smith [15] showing silver nanoparticles improve performance of ceramic water filters and that current production methods utilize silver nanoparticles, only filters with nanosilver were compared. 168.75 g of Redart clay and 18.75 g of sawdust (total mass of 187.5 g) were mixed by hand. Then 57 mL of deionized, organic free water was added and thoroughly mixed by hand. This mix was then separated by hand into three portions of equal weight, placed in a 6.5-cm-diameter PVC mold and compressed at 1000 psi for 1 minute. The resulting filter was an approximately 1 cm thick disk, providing a one-dimensional simplified geometry for lab testing (Figure 3.1). After air-drying for 48 h, the ceramic filters were fired in a kiln with the following temperature program: increase temperature from 20˚C at 150 ˚C/h to 600 ˚C, then increase at 300 ˚C/h to 900 ˚C, then isothermal for 3 h.

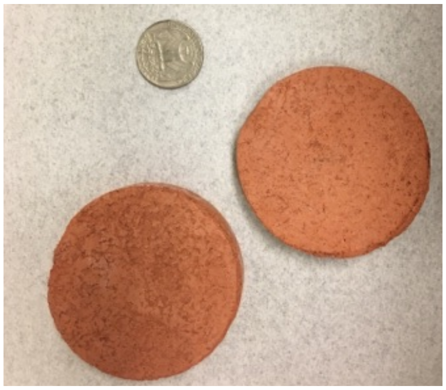

Figure 3.1. Ceramic filter disks used for laboratory testing

In order to produce the silver nanoparticle filter, a 496 mg/L silver nanoparticle suspension in deionized water was used. Silver amounts applied to the ceramic filter were chosen to be proportional to silver used in a full-size filter containing 0.3 g of silver. This ratio and silver amounts were chosen because of their current use at the PureMadi Mukondeni Production Facility in Mukondeni, Limpopo Province, South Africa. 10 mL of this solution was painted with a brush on both sides and the edges of the filter [23]. This impregnates 4.96 mg of Ag per filter.

For the silver nitrate filters, the same dry mix described above was combined with either 117 mg $AgNO_3$ ("5X filters") or 234 mg $AgNO_3$ ("10X filters") dissolved in 57 mL deionized water. These higher levels of silver were chosen since silver nitrate is much less expensive and the removal of the painting step saves labor costs, also, due to concern that some silver nitrate would end up in dead-end pore channels, having no contact with water as it filters through. The silver nitrate filters were then pressed into the shape of a cylinder, air dried, and fired as described above for the silver nanoparticle filters.

After firing, silver nitrate filters do not require any additional fabrication steps, as the silver forms metallic silver nanopatches like those seen in the TEM micrograph in Figure 2.2.

The porosity of each filter was measured gravimetrically by weighing a dry filter, saturating in deaired, deionized water for 24 h, then reweighing. The difference in mass equates to the volume of water inside the filter. The porosity is then calculated as the volume of water divided by the volume of the filter.

3.3.3 MISCIBLE DISPLACEMENT TRANSPORT EXPERIMENTS WITH E. COLI

The cylindrical ceramic filters were loaded into a flexible-wall permeameter, holding a 10-psi pressure on the cell to ensure flow through the filter, rather than around. A high-performance liquid chromatography (HPLC) pump (Acuflow series IV), a 1.0 mL syringe and the inflow valve of the permeameter chamber were connected with a three-way stopcock. The HPLC pump maintained a constant flow rate of 0.6 mL/min to mimic the average flow of pot filters (1.5 L/h). For initial bacterial pulse testing, 10 mM phosphate buffer solution was used as the inflow solution. The effluent valve of the permeameter chamber was open to the atmosphere for collection of effluent water samples. Filters were saturated by pumping inflow solution through the filter for 24 hours prior to the experiment. During the saturation period, effluent water samples were collected for silver analysis.

After the saturation period, a 1.0 mL syringe was used for a pulse injection of approximately 10^{10} MPN/100mL *E. coli.* Effluent samples were collected and analyzed over time to define the breakthrough of the *E. coli*. Viable *E. coli* were quantified in each sample using the Colilert Defined-Substrate Technology System, a method approved by the U.S. EPA and recommended by the WHO for microbiological testing [24-26]. Colilert media (cat. WP200I) was added to 100 mL of sample and mixed thoroughly, before being poured into IDEXX Quanti-trays (cat. WQT-2K) and incubated for 24 hours at 37 °C. A fluorescent UV lamp was used to count the number of fluorescing wells in the tray and correlated to *E. coli* concentrations using a most-probable-number table provided by the manufacturer. Samples were taken over time to measure nitrate in the effluent with Hach TNT835 Kit and the DR 3900 bench top spectrophotometer. Total silver was tested with a graphite furnace atomic adsorption spectrometer (PerkinElmer HGA900). Upon collection, bacteria samples were treated with a 60 g/L solution of sodium thiosulfate, prepared by dissolving anhydrous sodium thiosulfate (Fisher Scientific) in deionized water, to deactivate silver from continuing to disinfect during sample incubation. Silver methods were tested in triplicates.

3.3.4 LONG-TERM PERFORMANCE EVALUATION WITH CONSTANT EXPOSURE

To evaluate the performance of each filter type over an extended period of time, silver-ceramic filters were again loaded in to a flexible wall permeameter with a 10-psi cell pressure. The saturation period was performed as described above, with one HPLC pump providing a constant feed of 10 mM phosphate buffer solution for 24 h. After 24 h of saturation, hydraulic conductivity was measured using a falling head analysis. Then, as in Oyanedel-Craver et al. [15], a 1.0 mL syringe was used to inject a 0.6 mL pulse of 4.3 μCi $[^3H]H_2O$ into the ceramic disk. Effluent samples were collected overtime and measured by a liquid scintillation counter to define a conservative tracer breakthrough curve. Effluent tracer concentrations were simulated using a transient one-dimensional form of the advection-dispersion equation with first order decay:

$$R\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} - v\frac{\partial c}{\partial x} - \mu c$$

Subject to the following initial and boundary conditions:

$$c(x,0) = 0$$

$$c(0,t) = c_0 \; for \; t < t_0$$

$$c(0,t) = 0 \; for \; t > t_0$$

$$\frac{\partial c(L,t)}{\partial x} = 0$$

[15]

R is the retardation coefficient, c is the concentration of $[^3H]H_2O$ in counts per minute per mL, t is time in minutes, t_0 is the tracer injection time, D is the dispersion coefficient in cm^2 /min, x is distance in cm, v is the linear velocity in cm/min, μ is the first-order decay coefficient, and L is the thickness of the disk. CXTFIT [27] was used to provide the optimum fit of the model to experimental data. D and v were determined with R=1 and μ=0 from the $[^3H]H_2O$ transport experiment.

After the completion, two high-performance liquid chromatography (HPLC) pumps (Acuflow series IV) were connected and mixed at a three-way push to connect fitting immediately before the inflow valve of the permeameter chamber maintaining a constant flow rate of 0.6 mL/min to mimic the average flow of pot filters (1.5 L/h). For these experiments, one pump contained an inflow of EPA semi-hard synthetic groundwater solution plus acetate at a concentration of 6 mg/L (to allow bacterial growth similar to natural water) [28]. The other pump contained $\sim 10^6$ MPN/100 mL *E. coli* in synthetic groundwater without the added acetate. The growth medium was kept separate from the *E. coli* until immediately prior to entering the cell, which ensued a constant influent concentration. The effluent valve of the permeameter chamber was open to the atmosphere for collection of effluent water samples. Pumping and sampling of effluent for silver levels and *E. coli* concentration continued for 2 weeks. A final hydraulic conductivity was measured and another $[^3H]H_2O$ breakthrough experiment was performed to compare initial and final porous medium flow characteristics. For *E. coli* concentration, the same quantifications were used as described above. A graphite furnace atomic adsorption spectrometer (PerkinElmer HGA 900) was used for total silver quantification. Silver methods were tested in duplicate.

3.3.5 PERFORMANCE EVALUATION AFTER 2 AND 4 WEEKS OF FLOW

To evaluate the long-term performance of the ceramic filters under conditions mimicking real-world use, we conducted experiments using 2- and 4-week pulse injections of *E. coli* without an added growth substrate. Ceramic filters were again in to a flexible wall permeameter with a 10-psi cell pressure. Like above, a HPLC pump, a 1.0 mL syringe and the inflow valve of the permeameter chamber were connected with a three-way stopcock. The HPLC pump maintained a constant flow rate of 0.6 mL/min this time with an EPA semi-hard synthetic groundwater solution as the inflow solution [28]. The effluent was collected the same as before for silver and *E. coli* sampling. After two weeks, a 0.6 mL pulse of ~10^9 *E. coli* was injected through the filter via the syringe. Effluent samples were collected to trace the bacterial breakthrough. The pump was left to continually pump synthetic groundwater for another two weeks with effluent sampling for silver levels. After a total of four weeks of pumping, another 0.6 mL pulse of ~10^9 *E. coli* was injected through the filter via the syringe. Effluent samples were collected to trace the bacterial breakthrough. For *E. coli* concentration, Colilert media (cat. WP200I) was added to 100 mL of sample and mixed thoroughly, before being poured into the IDEXX Quanti-trays (cat. WQT-2K) and incubated for 24 h at 37 °C. A fluorescent UV lamp was be used to count the number of fluorescing wells in the tray and correlated to *E. coli* concentrations using a most-probable-number table provided by the manufacturer. A graphite furnace atomic adsorption spectrometer (PerkinElmer

HGA 900) was used for total silver quantification. Silver methods were tested in duplicate.

3.4 RESULTS AND DISCUSSION

3.4.1 MISCIBLE DISPLACEMENT TRANSPORT EXPERIMENTS WITH E. COLI

The results of the *E. coli* pulse injections into the ceramic filter disks are shown in Figures 3.2 and 3.3. Figure 3.2 shows the log removal of *E. coli* versus the pore volumes of flow. Both methods of silver application, silver nanoparticle and silver nitrate, performed similarly. Further, both levels of silver nitrate application (5X and 10X) performed similarly, showing the potential of the new method as a viable substitution. To calculate results, the effluent concentration (C) was divided by influent concentration (C_0), followed by taking the log of C/C_0.

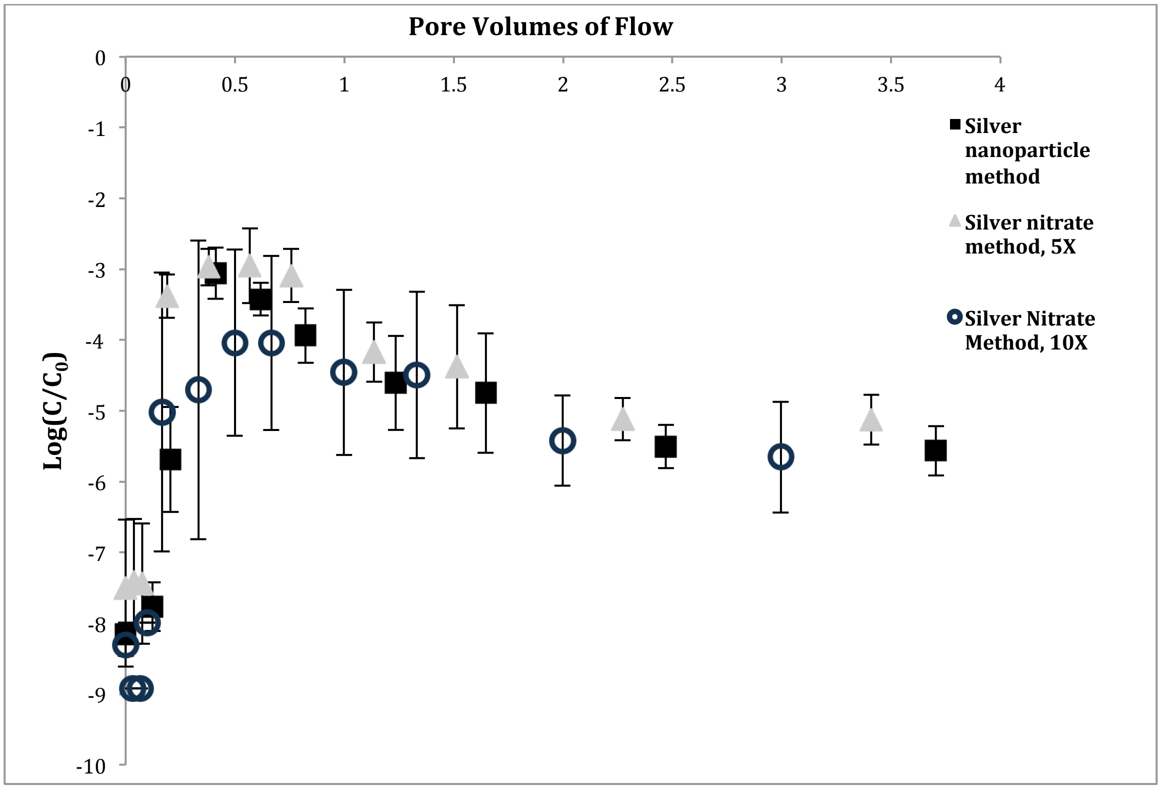

Figure 3.2. Average log change of *E. coli*, log(C/C0), versus pore volumes of flow after a one-min pulse injection of *E. coli* to ceramic filters manufactured using the silver nanoparticle method and the silver nitrate application method. Error bars show one standard error above and below the mean. For the silver nitrate application method, the mass of added silver was five times (5X) or ten times (10X) the mass of silver applied to the ceramic filter fabricated using the silver nanoparticle method. Samples were taken at the same time for each disk, and converted to pore volume. Since pore volumes varied minimally (3%) between disks, samples at the same time were averaged.

Figure 3.3 shows the total silver measured in the effluent from the ceramic filters over time. The effluent from filters made with the silver nanoparticle method has the highest silver levels—even above the drinking water standard for sampling times less than 5 h—while the new silver nitrate method results in lower levels. These lower silver levels, while still an effective antimicrobial agent, suggest a safer filter with a longer lifespan because silver is retained in the filter media rather than released into the treated water.

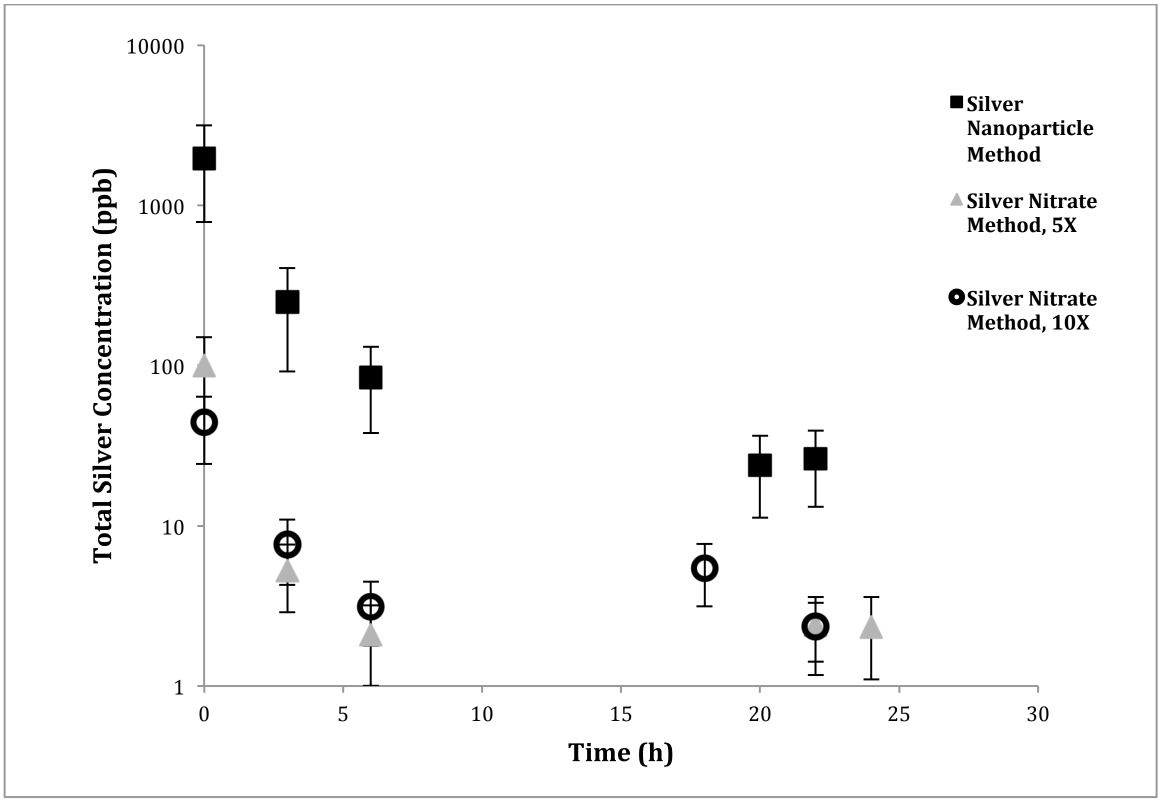

Figure 3.3. Total silver concentration as a function of time in effluent from silver-ceramic filter media. Data is for three filter types with varying silver application methods and amounts. Error bars show one standard error above and below the mean.

These graphic results suggest that at least during short-term experiments, the new silver nitrate method provides no benefit in regards to bacterial removal since the error bars overlap. However, in Figure 3.3, the error bars do not overlap between the silver nanoparticle and silver nitrate methods, showing a statistical difference. Water chemistry will effect the rate of silver release from the ceramic filter [29], however, under the same conditions, the silver nitrate application method results in lower silver effluent levels, potentially improving long-term silver retention in the filter and the overall useful filter lifetime. This fits well with literature that a significant fraction of silver nanoparticles is being washed off over time, decreasing the long-term effectiveness of ceramic water filters [19]. This is particularly notable since there is five and ten times as much silver in the silver nitrate filters as the silver nanoparticle filters. New filters commonly require the first few liters be discarded in case of high silver levels [30], and these silver levels are safely below the drinking water standard quickly after first use.

3.4.2 LONG-TERM PERFORMANCE EVALUATION WITH CONSTANT EXPOSURE

In response to the bacterial tracer testing results, a longer-term experiment was performed in order to evaluate the hypothesis that ceramic filters made with the silver nanoparticle method have longer lifespans. Figure 3.4 shows the results of exposing the ceramic filter disks to a constant flow of *E. coli* with synthetic groundwater and an added carbon source. This ensures maximal bacterial growth and can potentially cause biofilm formation and bacteria growth. Each point represents a single data point due to the nature of sampling over such a large time scale with slight variance in pore volume between replicates. Replicates of the same silver method had data pooled together to present the trends seen in Figure 3.4.

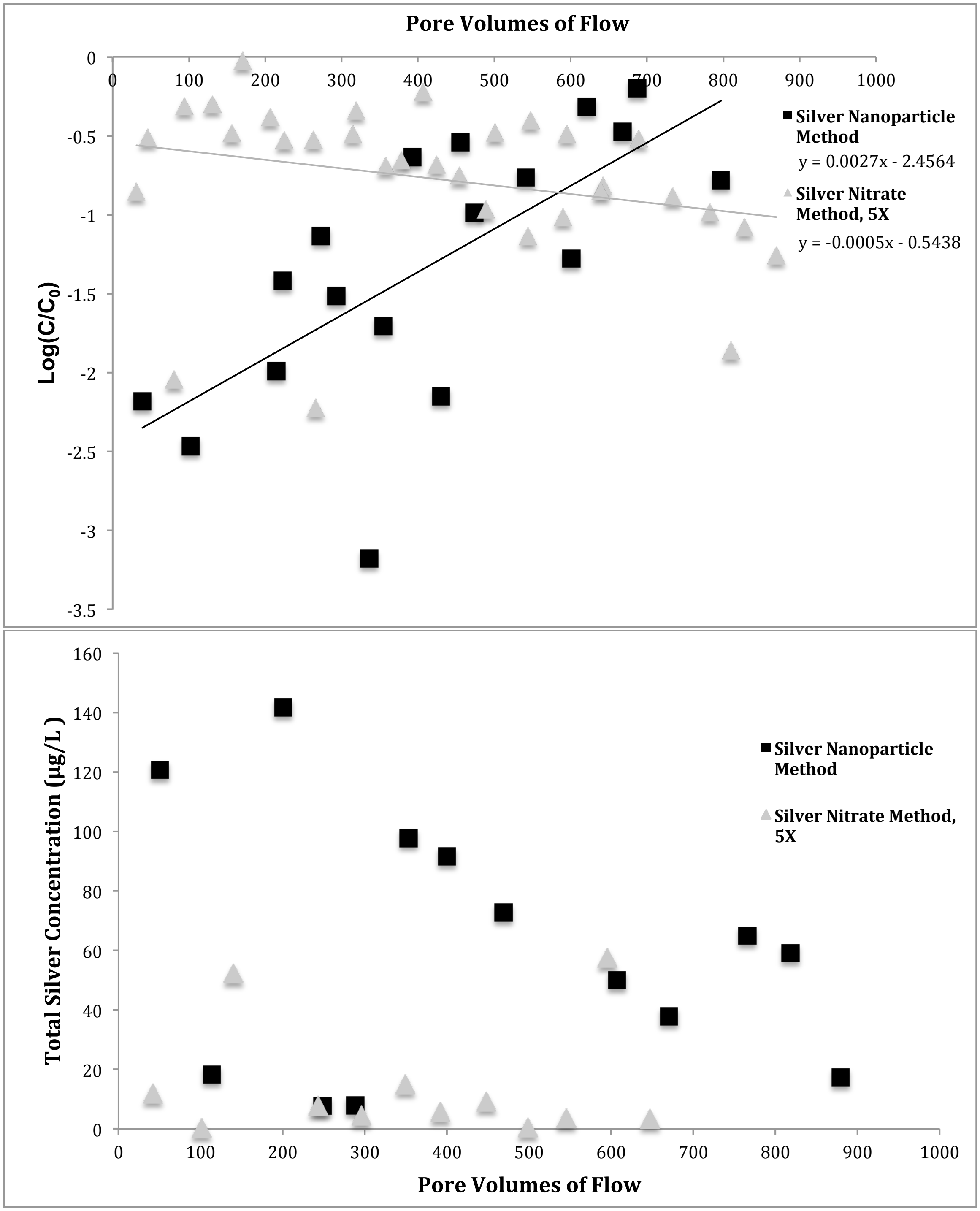

Figure 3.4. The top graph is average log change of *E. coli*, Log(C/C_0), versus pore volume of flow with a long-term constant exposure to *E. coli* by silver application method. Trend lines show the change in performance of *E. coli* removal over time. The slope of the trend line for the silver nanoparticle filters 0.0027, shows a decrease in performance. While the silver nitrate filter trend line has a slope of -0.0005, meaning at least consistent performance. The lower graph is total silver concentration in effluent from silver-ceramic filter media as a function of pore volumes of flow with a long-term constant exposure to E. coli. Data is for two filter types with varying silver application methods and amounts

The top of Figure 3.4 shows the log removal of *E. coli* versus pore volumes of flow. The silver nanoparticle method of silver application shows better performance initially, but then inconsistent performance over time. This could potentially be explained by *E. coli* growth in the filter. The bacteria may cause clogging in the filter but the HPLC pump used to maintain constant influent flow rates might be increasing pressure in an attempt to keep a constant flow rate, dislodging the bacteria growth. This could cause bacteria to be released in clumps, creating low removal rates after continued use. The silver nitrate filter performed more consistently, slightly increasing in performance over time and possessing a longer effective lifespan. The lower figure shows the silver results over the same time period. As expected, the silver nanoparticle method resulted in much higher silver levels than the silver nitrate filters, with average effluent concentrations of 64 and 18 μg/L respectively. Additionally, some of the early time points for the filters made with the silver nanoparticle method feature silver levels in the effluent above drinking water standards. Silver concentrations in the effluent seem to vary some, this may be due to silver sloughing off close to a sampling time. Silver may not come out of the filter at a perfectly consistent rate, it may be releasing as pressure builds up and requires more pore channel space for flow. A mass balance of silver release over the course of the experiment shows that an average of 0.63 mg of silver have come out of the silver nanoparticle filters versus 0.29 mg of silver that have come out of the silver nitrate filters. The silver nanoparticle filters only started with 4.96 mg of silver per filter, while the silver nitrate filters had five times as much. The silver nitrate method shows up to a ten times longer lifespan with a silver loss rate about half that of the silver nanoparticle method and containing five times as much silver.

Hydraulic conductivity, *K*, was measured before and after the *E. coli* exposure to quantify clogging in the filters. Hydraulic conductivity decreased by an average of 38.89% and 75.15% for the silver nanoparticle and silver nitrate filters respectively. This decrease was expected, as bacterial growth promoted by the influent would likely cause clogging of the filter. Silver nitrate filters have more silver and therefore might be causing more microbial death in the filter. However it is important to note, that this is a relatively small change, but could affect performance in actual use.

Tritiated water was used as a conservative tracer in pulse injections both before and after the *E. coli* exposure to estimate the change in the linear velocity (v) and dispersion coefficient (D). Table 3.1 shows the model output values generated for v and D by CXTFIT from the experimental results.

Table 3.1: Linear velocity, dispersion coefficient, and hydraulic conductivity for silver nanoparticle and silver nitrate filters before and after 2 weeks of constant flow of *E. coli*.

	v (cm/min)	Δv (%)	D (cm^2 /min)	ΔD (%)	K (cm/min)	Average Porosity (%)
Silver Nanoparticle Initial	3.17 X 10^{-2}		1.52 X 10^{-2}		3.62 X 10^{-4}	33.48
Silver Nanoparticle Final	2.84 X 10^{-2}	-10.3	7.58 X 10^{-3}	-50.2	1.84 X 10^{-4}	
Silver Nitrate Initial	3.02 X 10^{-2}		9.41 X 10^{-3}		7.64 X 10^{-4}	32.24
Silver Nitrate Final	2.48 X 10^{-2}	-17.73	5.08 X 10^{-3}	-46.01	1.82 X 10^{-4}	

The linear velocity and dispersion coefficient decreased for both silver methods. The silver nanoparticle filter caused decreases of 10.3% and 50.2% for v and D respectively. While the silver nitrate disk saw a decrease of 17.73% and 46.01% for v and D respectively. A decrease in diffusion coefficient, D, would be indicative of a less tortuous path for flow. This could be due to dislodging of loose ceramic particles and nanosilver along pore channels. The output model with these v and D values and an assumed R=1 and μ=0 can be seen in Figure 3.5.

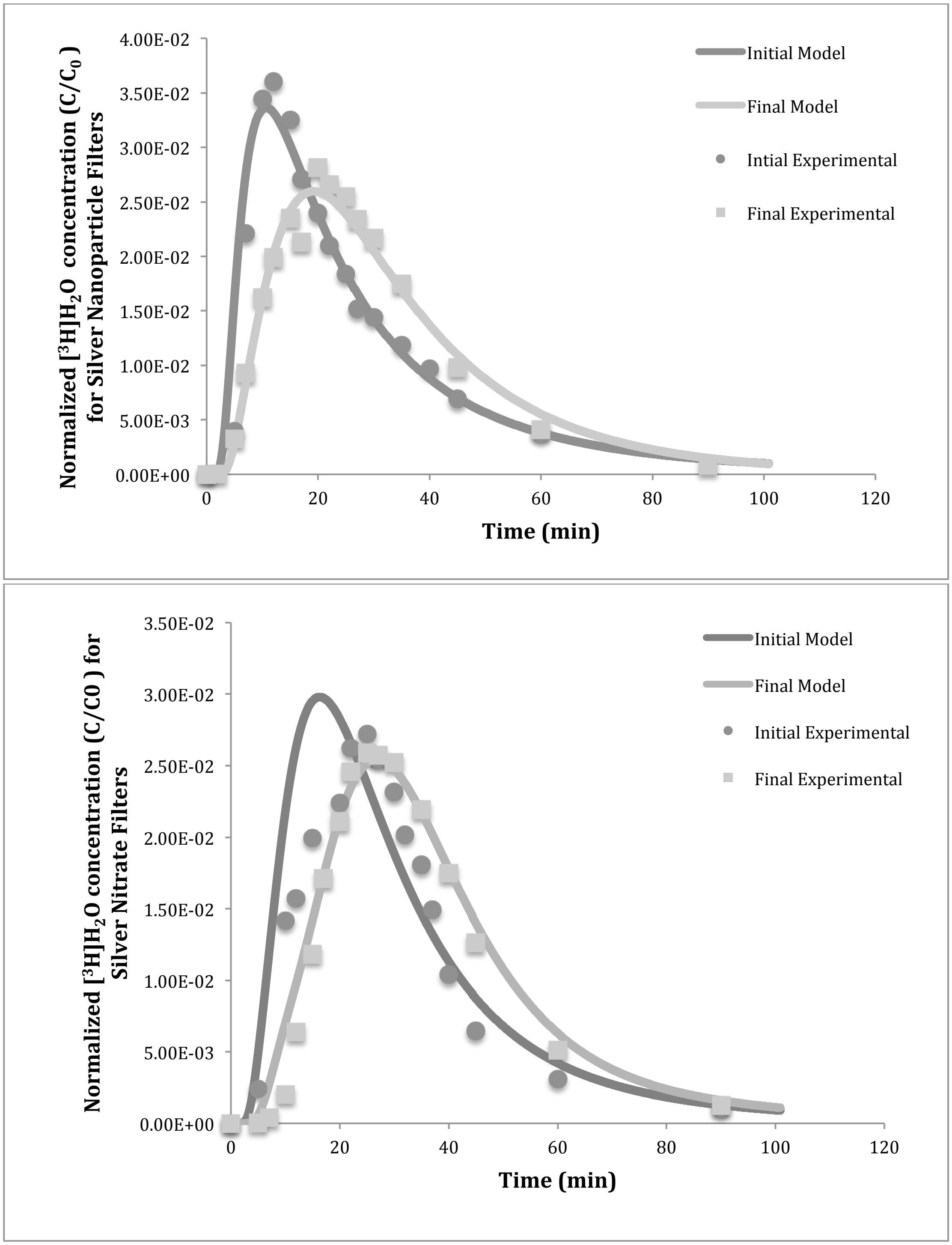

Figure 3.5. Results of [^{3}H]H_2O transport experiments and simulations through silver nanoparticle ceramic filters (upper graph) and silver nitrate ceramic filters (lower graph). Tracer experiments and simulations are shown for experiments conducted at the start and end of a two-week period of constant flow of synthetic groundwater containing *E. coli*.

The changes of *v* and *D* between initial and final measurements for silver nanoparticle and silver nitrate filters are similar, showing the silver nitrate method maintains performance of flow over time equal to the current production protocol.

3.4.3 PERFORMANCE EVALUATION AFTER 2 AND 4 WEEKS OF FLOW

This experiment was performed to further evaluate the longer-term performance of the two different silver applications in the ceramic filters. Filters had synthetic groundwater flowing through for two weeks, and then an *E. coli* pulse was injected. Then after two more weeks of synthetic groundwater flowing, another *E. coli* pulse was injected. The results of this experiment are found in Figure 3.6.

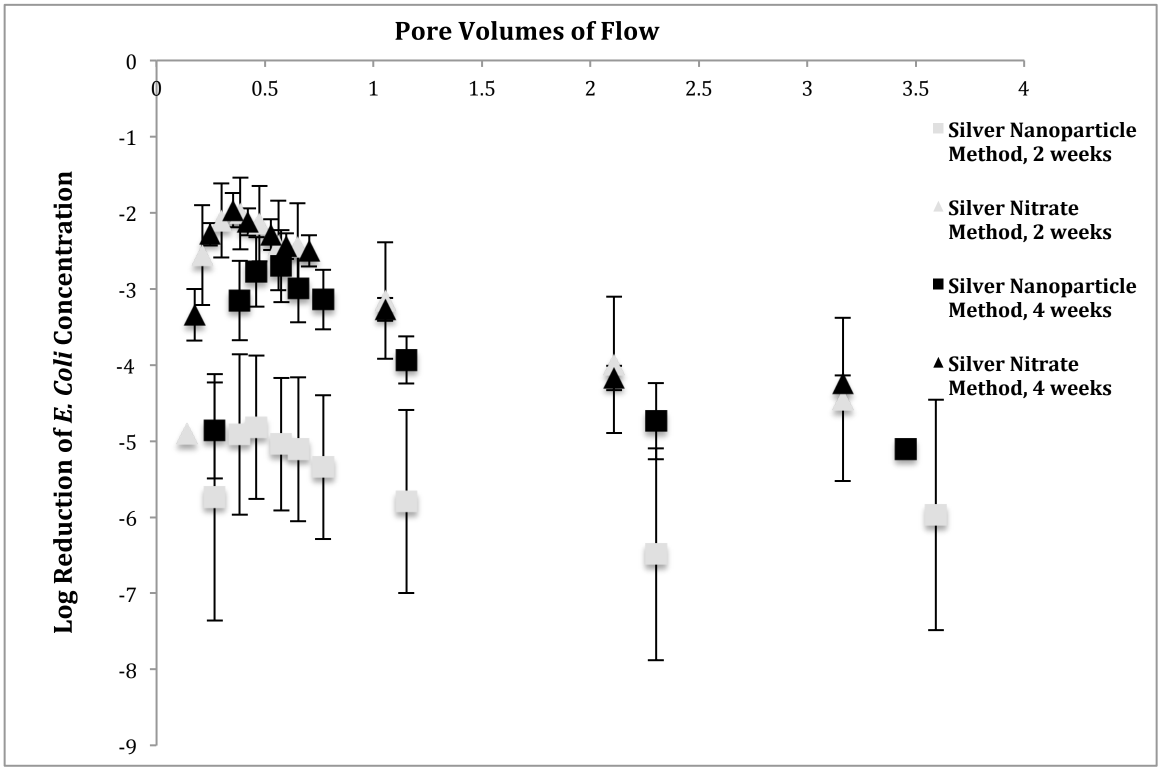

Figure 3.6. Average log change of *E. coli*, Log(C/C₀), versus pore volume after a pulse injection of E. coli over 1 min by silver application method and time of saturation. Error bars show one standard error above and below the mean.

Again, using the graphed data and error bars for analysis, the filters with silver nanoparticle method perform better initially, allowing less *E. coli* to pass through the filter during the pulse after two weeks. But after four weeks of use, the same filter showed a large decrease if efficacy. The silver nitrate filters were consistent and showed almost identical performance after two and four weeks of use.

3.5 CONCLUSIONS

The silver nitrate method presented here presents another potential benefit in that it reduces possible exposure of workers to silver-nanoparticle inhalation. A recent article by Fewtrell et al. [31] assessed the safety of using silver nanoparticles in household water treatment, and noted that one of the most significant silver exposure risks could be to workers fabricating the filters and inhaling the silver nanoparticles. They noted that occupational safety procedures in developing-world work environments may not be sufficient to protect the workers. They based their assessment of a study that observed genotoxic damage to silver workers [32].

Furthermore, filters manufactured using the silver nitrate method release lower levels of silver into the treated water and the form of the silver is Ag^{+1} (e.g. ionic silver) [21]. Some studies show genotoxic effects cause by exposure to silver nanoparticles, albeit at silver doses that are orders of magnitude greater than what is found in drinking water treated with silver-ceramic water filters. If an adult weighing 50 kg were to consume 2 liters of water per day with 0.018 mg/L of silver (the mean silver concentration in water treated with filters manufactured using the silver nitrate method evaluated in this manuscript), 0.00072 mg/kg of body weight will be consumed per day. This level is far below the conservative Tolerable Daily Intake value of 0.0025 mg/kg of body weight per day suggested by Hadrup and Lam

[33]. The levels presented in Table 3.1 of Fewtrell et al. are 0.25 to 500 mg/kg, 125 to 250,000 times greater than the doses found in water treated by our filters [31]. Studies with lower doses of silver show no genotoxic effects, and studies with ionic silver show no genotoxic effects at any reported concentration. Silver-ceramic water filters have been shown to release silver ions, not silver nanoparticles [21,34]. Fewtrell et al. [31] and the response by Lantagne et al. [34], state that studies of silver ion toxicity have shown no adverse effects. Therefore, the silver nitrate method helps to insure the microbiological safety of the treated water while releasing only extremely low levels of ionic silver (more than 5 times below the EPA and WHO suggested permissible levels). At the same time, the method reduces the risks of exposure to workers in developing-world production facilities.

Each of the experiments points to a similar result when comparing the conventional method of painting on silver nanoparticles after firing and the new method of embedding silver nitrate prior to firing in ceramic water filters. The silver nanoparticle method may perform better initially, but decreases in antimicrobial efficacy over time and commonly releases silver at rates above the drinking water standard. The silver nitrate application method produced filters that release lower amounts of silver and performed more consistently in bacterial removal over time. These results indicate that the silver nitrate application method is a viable substitute to painting on silver nanoparticles for the production of ceramic water filters. However, there are some concerns that this may not be ideal for all filter production facilities. Conventionally, filters that are manufactured will not receive the silver nanoparticle application until after passing quality control tests. With the new method of silver application prior to firing, filters that do not pass these pressure and flow rate tests, will be wasting the silver applied to them. Therefore, the new silver nitrate method of application may only be a good option to be incorporated at filter production facilities with relatively high pass rates for quality control tests. These results from experiments in clean lab conditions are promising, but are not a perfect indicator of how silver nanoparticle and silver nitrate filters would perform in expected in home use. In homes, there will be varying flow rates, turbidities, ionic strengths, temperatures and many more variables that could affect performance. The next experiments to be done are to test the application of full size filters and with real world use, rather than simplified lab conditions.

3.6 ACKNOWLEDGEMETS

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3.7 REFERENCES

1. Dillingham, R.; & Guerrant, R. L. Childhood stunting: Measuring and stemming the staggering costs of inadequate water and sanitation. *Lancet* **2004** *363(9403),* 94–95. doi:10.1016/S0140-6736(03)15307- X PMID:14726158
2. Clasen, T.; Roberts, I.; Rabie, T.; Schmidt, W.; Cairncross, S. Interventions to improve water quality for preventing diarrhoea. *Cochrane Database Syst. Rev.* **2006**, No. 3, CD004794.
3. Lantagne, D. S.; Quick, R.; Mintz, E. D. Household water treatment and safe storage options in developing countries: A review of current implementation practicies; Wilson Center: Washington, DC, **2006**.
4. Clasen, T.; Nadakatti, S.; & Menon, S.. Microbiological performance of a water treatment unit designed for household use in developing countries. *Tropical Medicine & International Health* **2006**, *11(9),* 1399–1405. doi:10.1111/j.1365-3156.2006.01699.x PMID:16930262
5. Clasen, T.; Schmidt, W. P.; Rabie, T.; Roberts, I.; Cairncross, S. Interventions to improve water quality for preventing diarrhoea: Systematic review and meta-analysis. *BMJ* **2007**, *334*, 782.
6. PATH's Safe Water Project. Perspectives: Commercial approaches to

delivering household water treatment and safe storage products and solutions to low-income households (Special Report); PATH Publications: Seattle, WA, **2012**.

7. Lantagne, D. S.; Clasen, T. F. Use of household water treatment and safe storage methods in acute emergency response: Case study results from Nepal, Indonesia, Kenya, and Haiti. *Environ. Sci. Technol*. **2012**, *46*, 11352−11360.

8. UNICEF. Promotion of household water treatment and safe storage in UNICEF WASH Programmes; UNICEF: New York, 2008.

9. Rayner, J. Current Practices in Manufacturing of Ceramic Pot Filters for Water Treatment. *(Master Thesis), Loughborough University*. 2009

10. Huang, H. I.; Shih, H. Y.; Lee, C. M.; Yang, T. C.; Lay, J. J.; Lin, Y. E. In vitro efficacy of copper and silver ions in eradicating Pseudomonas aeruginosa, Stenotrophomonas maltophilia and Acinetobacter baumannii: Implications for on-site disinfection for hospital infection control. *Water Res.* **2008**, *42*, 73−80.

11. Grier, N. Disinfection, Sterilization and Preservation; *In Silver and Its Compounds*, 3rd ed.; Block, S. S., Ed.; Lea & Febiger: Philadelphia, 1983; pp 375−389.

12. Dankovich, T. A. Microwave-assisted incorporation of silver nanoparticles in paper for point-of-use water purification. *Environ. Sci.: Nano* **2014**, *1*, 367–378.

13. Xiu, Z. M.; Zhang, Q. B.; Puppala, H. L.; Colvin, V. L.; Alvarez, P. J. Negligible particle-specific antibacterial activity of silver nanoparticles. *Nano Lett*. **2012**, *12*, 4271–4275.

14. Sankar, M. U.; Aigal, S.; Maliyekkal, S. M.; Chaudhary, A.; Kumar, A. A.; Chaudhari, K.; Pradeep, T. Biopolymer-reinforced synthetic granular nanocomposites for affordable point-of-use water purification. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 8459–8464.

15. Oyanedel-Craver, V.; & Smith, J. A. Sustainable colloidal-silver-impregnated ceramic filter for point-of-use water treatment. *Environmental Science and Technology* **2008**, *42(3),* 927-933.

16. Quang, D. V.; Sarawade, P. B.; Jeon, S. J.; Kim, S. H.; Kim, J.-K.; Chai, Y. G.; & Kim, H. T. Effective water disinfection using silver nanoparticle containing silica beads. *Applied Surface Science,* **2013,** *266,* 280–287. doi:10.1016/j.apsusc.2012.11.168

17. World Health Organization. *Guidelines for Drinking-Water Quality*; World Health Organization: Geneva, 2011.

18. Praveena, S. M.; & Aris, A. Z. Application of Low-Cost Materials Coated with Silver Nanoparticle as Water Filter in Escherichia coli Removal. *Water Qual Expo Health* 2015; doi:10.1007/s12403-015-0167-5

19. Ren, D.; & Smith, J. A. Retention and Transport of Silver Nanoparticles in a Ceramic Porous Medium Used for Point-of-Use Water Treatment. *Environmental Science and Technology* **2013** *47,* 3825-3832.

20. Lantagne, D.; Klarman, M.; Mayer, A.; Preston, K.; Napotnik, J.; & Jellison, K. Effect of production variables on microbiological removal in locally-produced ceramic filters for household water treatment. *International Journal of Environmental Health Research* **2010***, 20(3),* 171-187.

21. Ehdaie, B.; Krause, C.; & Smith, J. A. Porous Ceramic Tablet Embedded with Silver Nanopatches for Low- Cost Point-of-Use Water Purification. *Environmental Science and Technology* 2014*, 48,* 13901-13908.

22. Nunnelley, K. G., Smith, J.A., Smith, M.Y. and Samie, A., 2016, A New Method for Nanosilver Application in Ceramic Water Filters: *Proceedings of The World Environmental and Water Resources Congress*, May 2016, West Palm Beach, FL.

23. Lantagne, D. S. Investigation of the Potters for Peace colloidal silver impregnated ceramic filter; USAID 2001, p79.

24. USEPA. Guidelines establishing test procedures for the analysis of pollutants; Analytical methods for the biological pollutants in wastewater and sewage sludge; Final rule; *U. S. Federal Register*, 2007.

25. World Health Organization. *Guidelines for Drinking-Water Quality*; World Health Organization: Geneva, 1996; Vol. 2.

26. Rompre, A.; Servais, P.; Baudart, J.; de-Roubin, M. R.; Laurent, P. Detection and enumeration of coliforms in drinking water: Current methods and emerging approaches. *J. Microbiol. Methods* **2002**, *49*, 31–54.

27. Toride, N.; Leij, F. J.; Genuchten, M. T. V. The CXTFIT code for estimating transport parameters from laboratory or field tracer experiments, Version 2.0; Research Report 137; U.S. Salinity Laboratory, USDA: Riverside, CA, 1995.

28. U.S. Environmental Protection Agency. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine Organisms; EPA-821-R-02-012, Washington, DC, 2002.

29. Lui, A.; Gonzales, R. D. Adsorption/Desorption in a System Consisting of Humic Acid, Heavy Metals, and Clay Minerals. *J. Colloid Interface Sci.* **1999**, *218 (1)*, 225–232.

30. Kallman, E. N.; Oyanedel-Craver, V. A.; Smith, J. A. Ceramic Filters

Impregnated with Silver Nanoparticles for Point-of Use Water Treatment in Rural Guatemala. *Journal of Environmental Engineering* **2011***, 137*, 407-415. doi: 10.1061/(ASCE)EE.1943-7870 .0000330

31. Fewtrell, L.; Majuru, B.; Hunter, PR. A re-assessment of the safety of silver in household water treatment: rapid systematic review of mammalian in vivo genotoxicity studies. *Environmental Health* **2017**, *16:66*. doi: 10.1186/s12940-017-0279-4

32. Aktepe, N.; Kocyigit, A.; Yukselten, Y.; Taskin, A.; Keskin, C.; Celik, H. Increased DNA Damage and Oxidative Stress Among Silver Jewelry Workers. *Biol Trace Elem Res.* **2015**,*164(2),*185–91.

33. Hadrup, N.; Lam, HR. Oral toxicity of silver ions, silver–a review. *Regul Toxicol Pharmacol* **2014**, *68(1*), 1–7*.* doi: 10.1016/j.yrtph.2013.11.002

34. Lantagne, D.; Rayner, J.; Mittelman, A.; Pennell, K. Comment on “A re-assessment of the safety of silver in household water treatment: rapid systematic review of mammalian in vivo genotoxicity studies”. *Environmental Health* **2017**, *16,* 121. doi: 10.1186/s12940-017-0334-1

Chapter 4: Application of a New Method for the Deposition of Metallic Silver and Metallic Copper on Full- Size Porous Ceramic Water Filters

The work presented in this chapter resulted in a journal article in preparation.

Jackson, K. N., Smith, J.A., and Edokpayi, J. N., (Submitted) Application of a New Method for the Deposition of Metallic Silver and Metallic Copper on Full- Size Porous Ceramic Water Filters. *Environmental Engineering Science.*

4.1 ABSTRACT

The performance of a new method for the deposition of metallic silver and metallic copper in ceramic water filters was investigated. Ceramic water filters were manufactured at two PureMadi filter production facilities in South Africa. Silver or copper was applied to the filters in solution as silver or copper nitrate ($AgNO_3$ or $Cu(NO_3)_2$) and mixed with clay and sawdust. The mixture was pressed into a pot shape and fired in a kiln. During firing the ionic silver or copper is reduced to metallic nanopatches dispersed through the porous ceramic media. For comparison testing, filters were also manufactured using a conventional silver application method consisting of painting an aqueous suspension of silver nanoparticles (AgNPs) onto filters post-firing. The resulting filters were evaluated in flow-through experiments and in stagnant water experiments (to consider a worst-case scenario for silver release). Flow through experiments quantified total coliform and *E. coli* removal and metal concentration in the effluent. Total coliform and *E. coli* removal was greater for $AgNO_3$ filters (log reductions of 4.06 and 4.11) relative to AgNP filters (log reductions of 3.85 and 3.92). The $AgNO_3$ filters also resulted in lower silver concentrations in the effluent, potentially increasing the effective lifespan of the filter. Filters made with $Cu(NO_3)_2$ performed slightly worse than AgNP filters, but were still effective with log total coliform and *E. coli* removals of 3.33 and 3.54, respectively. The new method of silver application could potentially reduce production costs, improve performance and increase safety of production for workers.

4.2 INTRODUCTION

Silver-impregnated ceramic water filters are a common point-of-use water treatment technology (Brown et al. 2008 and Kallman et al. 2011). The ceramic filters have been shown to be effective at microbial disinfection and filtration without a residual taste or odor, while also being socially acceptable (Lantagne 2001a,b, Campbell 2005, Oyanedel-Craver and Smith 2008, Simonis and Basson 2012 and Zhang et al. 2012). Ceramic water filters also have a long lifespan (2-5 years) if not broken and are relatively low cost due to local production (Lantagne at al. 2006). At more than 35 filter production facilities worldwide, ceramic pot-shaped filters are produced using local labor and materials (e.g. clay, sawdust and water) (Rayner 2009 and Lantagne et al. 2010). The ceramic filter is suspended inside a plastic bucket with a spigot on the bottom for personal use. Source water is poured into the ceramic filter and percolates through to the lower reservoir where clean water is dispensed through the spigot. The relatively small pore size (mean around 10 μm) of the ceramic filter decreases turbidity and removes suspended particles (Oyanedel-Craver and Smith 2008). Silver nanoparticles (AgNPs) are applied to the filter using an aqueous suspension of the nanoparticles and a paintbrush. The silver provides effective disinfection of the source water. The silver release rate into the treated water generally results in silver concentrations below the secondary drinking water standard of 0.1 mg/L set by the USEPA (2018) and the World Health Organization (2011a). Life cycle assessments of the social, economic, and environmental impact of the filters have also shown them to be a sustainable alternative for drinking water treatment in a developing world setting (Ren et al. 2013a).

The AgNP solution is painted onto the inside and outside of the ceramic after the filter is fired (Oyanedel-Craver and Smith 2008). The high nanoparticle surface-area-to-volume ratio leads to significant bactericidal activity (Praveena and Aris 2015). The painted-on AgNPs also prevent biofilm formation in the filter (Morones et al. 2005 and Oyanedel-Craver and Smith 2008). Silver released into the treated water provides residual disinfection. This method of AgNP application is in use at many filter production facilities around the world, including the PureMadi Production Facility in Mukondeni, South Africa and the PureMadi Production Facility at Dertig, South Africa.

This method, however, has several disadvantages. First, AgNPs are typically not available for purchase in developing-world markets. Therefore, it must be imported by the production facility, which may be difficult for the local organization. Second, nanoparticles may be released from the filter, particularly in the early stages of filter use, which in turn can potentially result in silver concentrations in the treated water that are greater than the USEPA and World Health Organization drinking water standard of 100 μg/L (Ren and Smith 2013b). Metallic silver released into the treated water is less effective for disinfection than a similar amount of ionic silver. Therefore, the released nanoparticles only shorten the lifespan of the filter and do not significantly improve residual disinfection. Third, application of the aqueous nanoparticle suspension is labor intensive, requiring facility workers to manually paint the solution on the surfaces of every filter. Fourth, the use of AgNPs during the manufacturing process may also be a health concern for workers manufacturing filters, as some research suggests inhalation of silver particles may result in geno-toxic effects (Fewtrell et al. 2017; Aktepe et al. 2015).

In the previous chapter, we demonstrated a new method of silver application to ceramic porous media. For this method, silver nitrate ($AgNO_3$) is dissolved in water and uniformly combined with clay and sawdust, pressed into a ceramic disk, and fired in a kiln. During firing, the clay is sintered to form a ceramic medium, the sawdust combusts to create porosity, and the ionic silver is reduced to metallic silver nanopatches with mean diameters between 2 and 3 nm (Ehdaie et al. 2014). In subsequent filtration experiments, the ceramic media was highly effective at removing *E. coli* from challenge waters. In addition, effluent silver concentrations were significantly lower than silver-ceramic filter cylinders manufactured with AgNPs as a precursor.

Copper is also a promising water disinfectant and may be a better viral disinfectant than silver (Anyaogu et al. 2008; Esteban-Cubillo et al.2006; Ramyadevi, et al. 2012). Copper is less expensive than silver and has a higher drinking water standard (1.0 mg/L, which is ten time higher than the standard for silver) (USEPA 2018). Therefore, it may be possible to use larger amounts of copper in filters relative to silver while maintaining a similar cost and performance.

The purpose of this manuscript is to evaluate the silver-application method developed in Jackson and Smith (2018) and a similar copper-application method in full-size filters produced in real production facilities. Filters were tested in the laboratory to compare the silver-nanoparticle, silver-nitrate, and copper-nitrate application methods. Filter performance was evaluated based on treatment efficiency of natural ground-water samples spiked with *E. coli* combined with measurement of effluent silver and copper concentrations.

4.3 MATERIALS AND METHODS

Filters from two different filter production facilities managed by the non-profit organization PureMadi (www.puremadi.org) were produced and tested in this investigation. The two production facilities are located in the communities of Mukondeni (in Limpopo Province, South Africa) and Dertig (in North West Province, South Africa). The Mukondeni facility manufactured AgNP and $AgNO_3$ filters while the Dertig facility manufactured AgNP and $Cu(NO_3)_2$ filters for this study. Influent and effluent water samples for these filters were tested in for total coliform bacteria, *E. coli*, and total silver.

4.3.1 Source Water Characterization

The filters were tested using untreated natural groundwater from a residential borehole in Makhado, South Africa. Groundwater samples were characterized at the University of Venda. Electrical conductivity, pH, total dissolved solids (TDS), and salinity were measured using an EC500 EXStik multimeter (Extech Instruments Corporation, USA). Fluoride measurements were performed using an Orion STAR

A214 Fluoride Ion Selective Electrode (ThermoScientific, South Africa). Ion Chromatography was performed using a Metrohm Ion Chromatograph and trace metals were measured with a Perkin Elmer Flame Atomic Adsorption Spectroscopy. Turbidity was measured with a TB200TM Portable Turbidimeter from Orbeco-Hellige.

4.3.2 Ceramic Filters

All filters evaluated in this investigation were manufactured at one of two PureMadi filter production facilities in South Africa. The Dertig production facility is located in the North West Province of South Africa about 50 km north of the city of Pretoria. The Mukondeni production facility is located in the Limpopo Province of South Africa about 40 km southeast of the city of Makhado. AgNP and $AgNO_3$ filters were produced at the Mukondeni facility. Clay was collected from a natural clay deposit near the facility and ground in a hammer mill. White pine sawdust from a local lumber mill was sieved to 20 mesh. In order to produce 20 filters, 68 kg clay and 8 kg of sawdust were combined and mixed for 30 min in a mechanical mixer. 33 L of water were added to the clay and sawdust and mixed for an additional 30 min. The mixture was then molded into the pot-shape by using a filter press with a hydraulic jack. The filter was air-dried for 1-3 days. Filters were then fired with an electric kiln (Industrial Pyrometer Company Studio 3000) with the following temperature program: ramp at 130°C/h to 900 °C, hold 1 h, and then gradual cooling. During the firing process, the sawdust combusts to form pore channels. After cooling, the ceramic filters were pressure- and flow-tested for quality assurance. For the pressure test, the external surfaces of each filter were submerged in water for 10 seconds. If the interior of the filter remained dry, the filter passed the test. If a wet spot was observed on the inside of the filter, it failed the test (as it is possible that there is a crack or macropore that would render the filter ineffective). For the flow test, each filter was saturated with water for 24 h. Each filter was then filled completely with water and after one hour, the water level position in the filter was measured using a flow-calibrated T-bar. To pass this second test, the flow rate was required to be between 1.5 and 3.0 L/h. Filters that failed either test were destroyed.

As a final step, 300 mL of a AgNP solution made with 0.56 g of powder form collargol (70% Ag by mass) from Laboratorios Argenol S.L. (Zaragoza, Spain) dissolved in water. This solution was painted first onto the inside of the filter, then the outside of the filter, then the inside again for a total of 0.4 g of silver in each filter.

For the $AgNO_3$ method, the same natural clay and white pine sawdust were prepared as above. For a batch of 10 filters, 34 kg clay and 4 kg of sawdust were mixed mechanically for 30 min. 6.3 g or 31.5 g of 99.5% pure $AgNO_3$ (Acros Organics) was dissolved in 11 L of water, added to the mixture and mixed for 30 more min to produce filters with 0.4 or 2.0 g of Ag. This eliminates the need for painting-on silver at the end. Like above, the mixture was then molded into the pot-shape by using a filter press with a hydraulic jack. The filter was air-dried for 1-3 days. Filters were then fired as described above. During the firing process, the sawdust combusts to form pore channels and the ionic silver reduces to metallic silver. After cooling, the ceramic filters were subject to the same pressure and flow tests for quality assurance.

AgNP and $Cu(NO_3)_2$ filters were produced at the Dertig facility. To produce ten AgNP filters, 26.4 kg of terracotta clay, 8.25 kg of sawdust and 2.64 kg grog (a substance made by crushing fired filters that have failed the quality tests) were mechanically mixed for 30 mins. 20 L of water was added to the mixture and mixed for 30 mins. The filter was shaped using a mold and a press. The filters were air dried for 1-3 days before being fired in a VEL50 front door kiln. The kiln temperature program increased at 150°C/h until 600°C, then 250°C/h until 1000°C. The kiln temperature ramped down gradually until it reached 400°C, where it held for 1 h. The temperature continued to drop gradually and was left to cool for about 2 h before it was opened. Pressure and flow rates tests as described previously were performed to confirm the quality of the filters. Filters that failed quality assurance tests, were destroyed and saved for use as grog. Finally, a AgNP solution was applied to the filters as described previously for the Mukondeni filters.

To produce five $Cu(NO_3)_2$ filters, 13.2 kg of terracotta clay, 4.125 kg of sawdust and 1.32 kg grog (a substance made by crushing fired filters that have failed the quality tests) were mechanically mixed for 30 mins. Either 38 g or 76 g of 99.5% pure copper (II) nitrate from Acros Organics was dissolved in 10 L of water, added to the mixture and mixed for 30 mins. Filters were shaped using the same mold and press and air dried for 1-3 days before firing in the same kiln with the same pattern described for production at Dertig. Pressure- and flow- tests were performed as above.

Table 4.1 summarizes the characteristics of the filters used in this investigation. Figure 4.1 is a picture of a filter produced at each production facility.

Table 4.1. Summary of information for each type of filters included in this investigation.

Production Facility	**Mass of Ag or Cu in Each Filter (g)**	**Metal Used**
Mukondeni	0.4	AgNP
Mukondeni	0.4	$AgNO_3$
Mukondeni	2.0	$AgNO_3$
Dertig	0.4	AgNP
Dertig	2.0	$Cu(NO_3)_2$
Dertig	4.0	$Cu(NO_3)_2$

Figure 4.1. Filters produced at the production facilities. Left is from the Mukondeni Pottery Cooperative and the right is from the Dertig Production Facility.

4.3.3 Filters Performance Testing

All filters were tested in triplicate. Mukondeni and Dertig filters were compared over 36 and 17 days, respectively, for total coliform and *E. coli* removal efficiency. A nonpathogenic strain of *E. coli* was purchased from IDEXX Laboratories (cat. 982900700, Lot 042313) and cultured, used and stored in the same method outlined in Ehdaie et al. (2014). Natural borehole water was spiked to a concentration of ~4.6×10^4 CFU/100 mL *E. coli* with the lab-grown nonpathogenic *E. coli* to create the influent test water. 4 L of borehole water spiked with a nonpathogenic *E. coli* strain was poured into the top reservoir of each filter. After 14 h, samples were collected from the spigot from the lower reservoir and tested for total coliform and *E. coli* removal using membrane filtration. As in Kahler et al. (2016), manifold sample cups were placed in a hot water bath set to 100 °C for a minimum of 20 min for disinfection. 0.45 μm pore filter paper disks (EMD Millipore, Billerica, MA, USA) were transferred to the surface of the manifold with forceps with an aseptic technique. A100 mL water sample was passed through the filter. The filter paper was transferred to a sterile petri dish with absorbent pad. A sterile, 2 mL ampule of selective growth media solution (m-ColiBlue24, EMD Millipore) was added to the petri dish. The petri dishes were incubated at 35 °C for 23–25 h, before being counted for total coliform and *E. coli*.

Effluent samples were also taken and tested for total silver and total copper concentration via GFAA. 70% nitric acid (Fisher Chemical) was diluted to a concentration of 2% and added to samples to reduce silver ion chelation. Total silver was measured with a graphite furnace (HGA 900, Perkin-Elmer, Waltham, MA, USA) atomic absorption spectrometer (AA2100, Perkin-Elmer).

4.3.4 No-Flow Conditions

An additional set of experiments was conducted to evaluate silver concentrations in treated water for a worst-case use scenario. It was hypothesized that relatively high silver concentrations can occur when the treated water is in contact with the silver-ceramic filter media for long periods of time. This may occur if the filter is filled and then the user does not withdraw water from the filter for an extended period of time (e.g. – a family goes away from home for several days to a week).

During this time period, water is in contact with the silver-ceramic filter medium and silver may continue to be released into the treated water, potentially resulting in concentrations exceeding 100 µg/L.

Two AgNP and two $AgNO_3$ filters were produced at the Dertig Production Facility. Filters produced at Dertig were used for these experiments since they produced the highest silver levels in previous tests. These filters were placed in 22 L filter receptacles. The filters were then filled with 4 L semi-hard synthetic groundwater solution (1.20 g of $MgSO_4$, 1.92 g $NaHCO_3$, 1.20 g of $CaSO_4$ and 0.080g KCl per 20 L) (USEPA 2002) twice, according to directions for filter use. The filter receptacles were then filled to capacity with 20 L of synthetic groundwater and left stagnant for 7 days. Samples were taken daily and measured for silver levels with a graphite furnace (HGA 900, Perkin-Elmer, Waltham, MA, USA) atomic absorption spectrometer (AA2100, Perkin-Elmer).

4.4 RESULTS

4.4.1 Source Water Characterization

Results from the physico-chemical characterization of natural groundwater used for the influent of the filters can be seen in Table 4.2.

Table 4.2. Results of physico-chemical characterization of natural groundwater used in filter performance tests.

Water Quality Parameter	Concentration
Na^+	30.6 (mg/L)
K^+	0.10 (mg/L)
Ca^{2+}	32.3 (mg/L)
Mg^{2+}	22.6 (mg/L)
F^-	0.09 (mg/L)
Mn^{2+}	0.99 (mg/L)
pH	5.94
Electrical Conductivity	465 (μS/cm)
Total Dissolved Solids	326 (mg/L)
Salinity	223 (mg/L)
Turbidity	163 (NTU)
Average Coliform	3 X 10^3 CFU/100 mL
Average *E. coli*	≤1.0 CFU/100 mL

Copper, lead, cadmium, chromium, iron and nickel levels were all below the detection level of the Metrohm Ion Chromatograph.

4.4.2 Microbiological Performance Tests Of Filters

Table 4.3 presents the result of triplicate tests of the microbiological performance of filters manufactured at the PureMadi Production Facility at Mukondeni. The source water had an average coliform and *E. coli* concentration of 3 X 10^3 and ≤1 CFU/100 mL, respectively. Since a log removal rate of greater than 3 was expected, lab-grown nonpathogenic *E. coli* was added to the influent water for filter testing. This increased the total coliform concentration so that coliform would be present in the effluent for accurate removal calculations. The membrane filtration method used measured the concentration of *E. coli*, and of all other types of coliform separately. Since *E. coli* is a type of coliform bacteria, the *E. coli* concentration was added to the coliform concentration for the total coliform concentration. Log coliform and *E. coli* removals were consistently on the order of 4 with relatively low standard errors, suggesting effective and consistent performance for all filter types.

Table 4.3. Average total coliform and *E. coli* removal by filters produced at PureMadi Production Facility at Mukondeni over 36-d by each filter production method

Filter Type	**Average log Removal Total Coliform**	**Standard Error**	**Average log Removal *E. coli***	**Standard Error**
0.4 g Ag as AgNP	3.85	0.07	3.92	0.08
0.4 g Ag as $AgNO_3$	3.69	0.06	3.92	0.06
2.0 g Ag as $AgNO_3$	4.06	0.06	4.11	0.05

Figures 4.2 and 4.3 show the total coliform and *E. coli* removal (respectively) over time for filters produced at the PureMadi Production Facility at Mukondeni for each filter type tested. Performance is relatively consistent over the 36-d test period for all filter types.

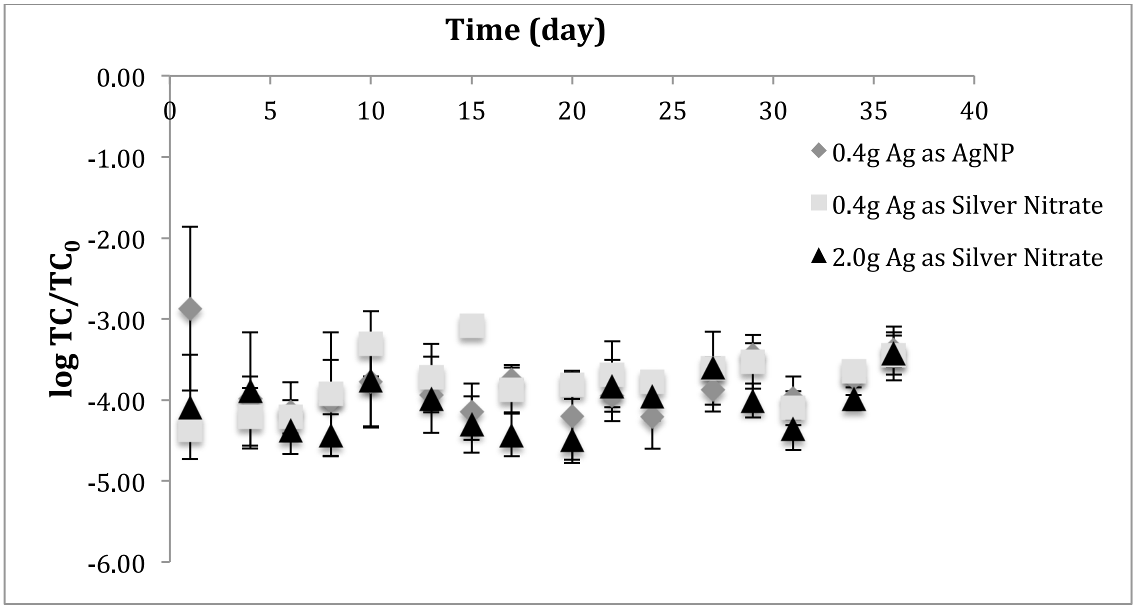

Figure 4.2. Mean values for log of total coliform concentration (TC) in the effluent over the influent (TC_0) averaged for each production method over time for filters produced at the PureMadi Production Facility at Mukondeni. Error bars indicate one standard error above and below the mean.

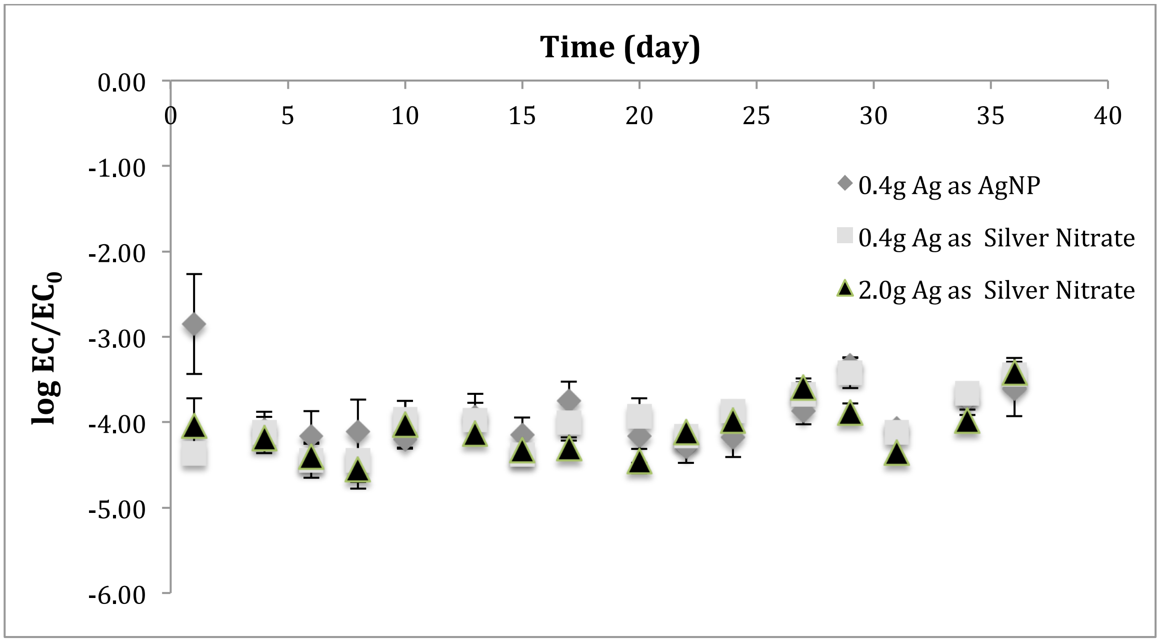

Figure 4.3. Mean values for log of *E. coli* concentration (EC) in the effluent over the influent (EC_0) averaged for each production method over time for filters produced at the PureMadi Production Facility at Mukondeni. Error bars indicate one standard error above and below the mean.

Table 4.4 presents the result of triplicate tests of the microbiological performance of filters manufactured at the PureMadi Production Facility at Dertig. The source water was the same used for the Mukondeni filters described above. Again, source water was spiked with lab-grown nonpathogenic *E. coli* for the influent water in filter testing. The same membrane filtration method was used to measure the concentration of *E. coli*, and of all other types of coliform separately. The two concentrations added together is the total coliform concentration. Log coliform and *E. coli* removals were consistently greater than 3 with relatively low standard errors, suggesting effective and consistent performance for all filter types.

Table 4.4. Average total coliform and *E. coli* removal for filters produced at the PureMadi Production Facility at Dertig over 17-d by each filter production method

Filter Type	Average log Removal Total Coliform	Standard Error	Average log Removal *E. coli*	Standard Error
0.4 g Ag as AgNP	3.71	0.08	3.76	0.07
2.0 g Cu as $Cu(NO_3)_2$	3.3	0.12	3.54	0.14
4.0 g Cu as $Cu(NO_3)_2$	3.33	0.19	3.54	0.19

Figures 4.4 and 4.5 show the total coliform and E. coli removal (respectively) over time for filters produced at the PureMadi Production Facility at Dertig for each filter type tested. Performance is relatively consistent over the 17-d test period for all filter types.

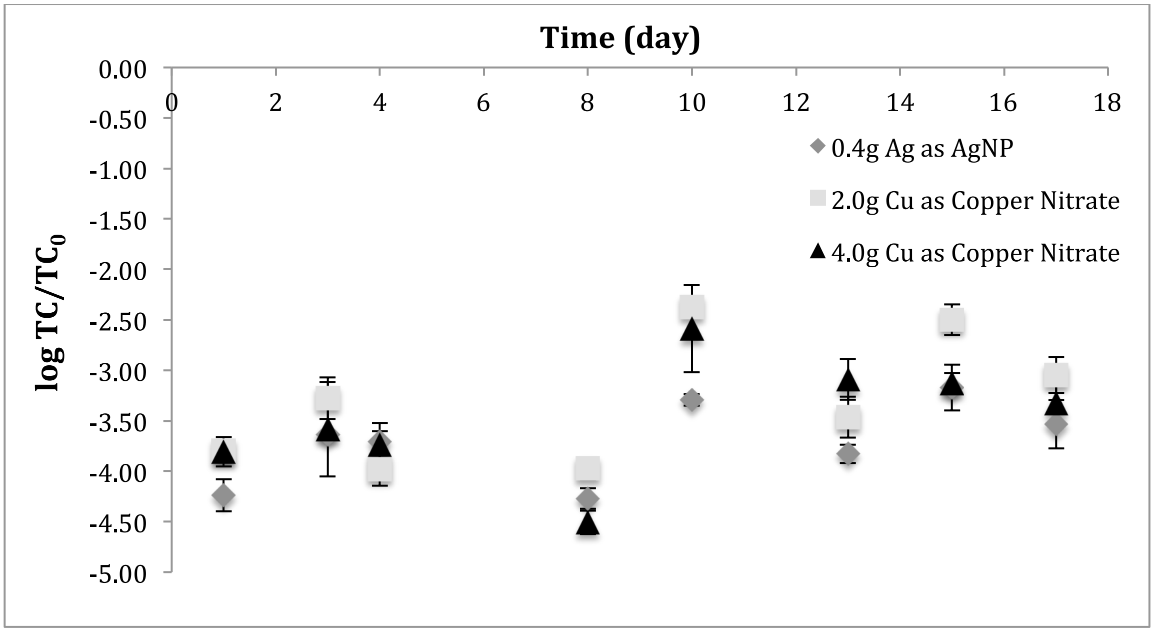

Figure 4.4. Log of total coliform concentration (TC) in the effluent over the influent (TC_0) averaged for each production method over time for filters produced at the PureMadi Production Facility at Dertig. Error bars indicate one standard error above and below the mean.

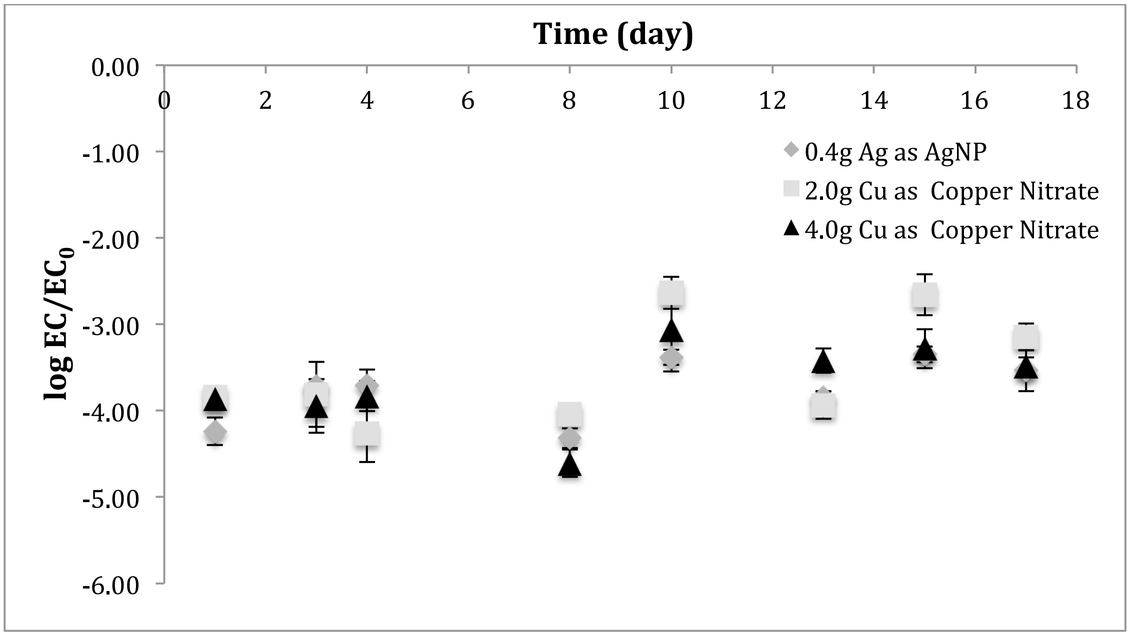

Figure 4.5. Log of *E. coli* concentration (EC) in the effluent over the influent (EC_0) averaged for each production method over time for filters produced at the PureMadi Production Facility at Dertig. Error bars indicate one standard error above and below the mean.

Figure 4.6 graphically presents the average log of total coliform and *E. coli* removal by each type of filter.

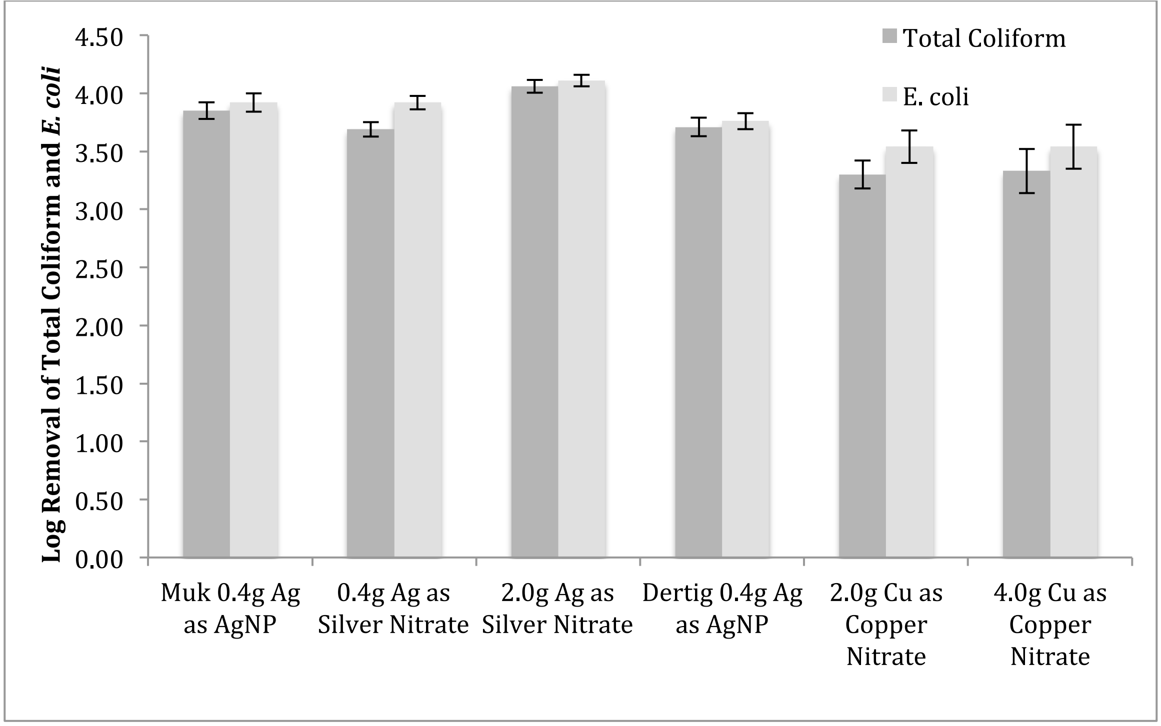

Figure 4.6. Results from fieldwork comparing full size filters for total coliform and *E. coli* removal over 36 days for Mukondeni (Muk) filters and 17 days for Dertig filters. Error bars indicate one standard error above and below the mean.

Figure 4.7 shows the average metal levels, measured via GFAA, in effluents from filters over time.

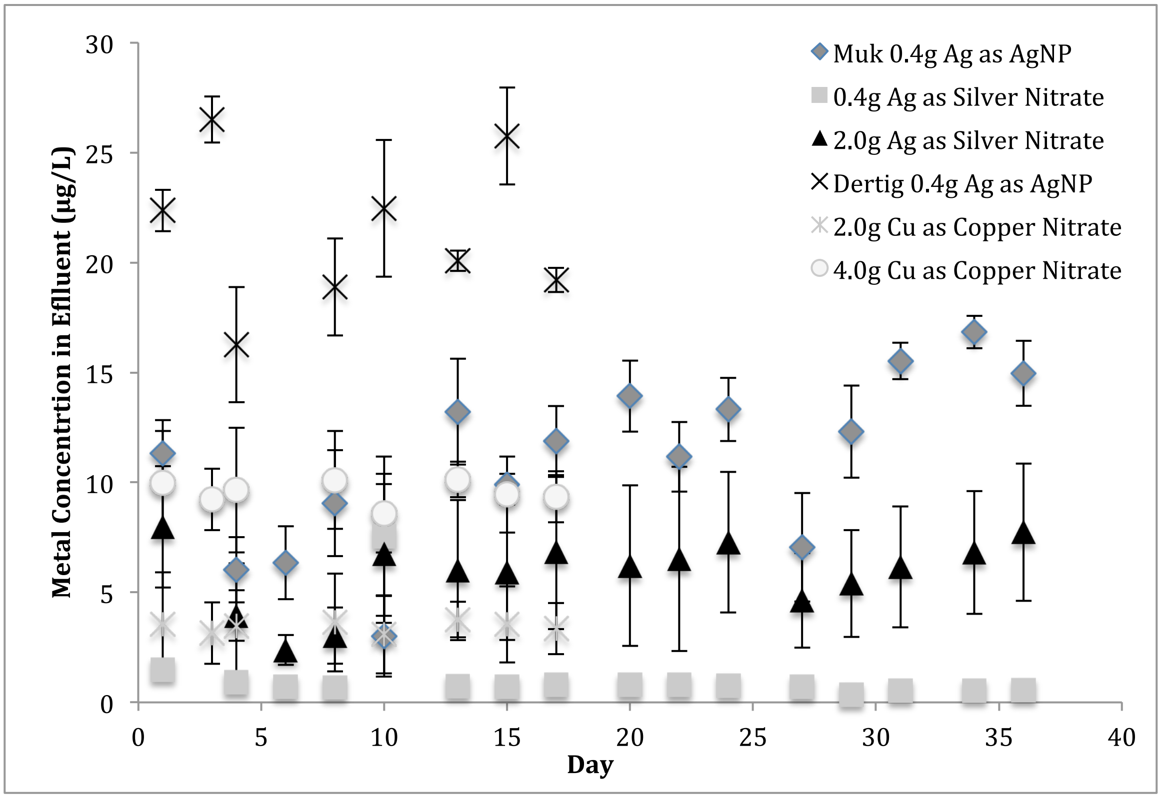

Figure 4.7. Total silver or copper levels in the effluent averaged by filter type over the timespan of testing. Error bars indicate one standard error above and below the mean.

Figure 4.8 shows the metal levels in the effluent averaged over the length of the experiment.

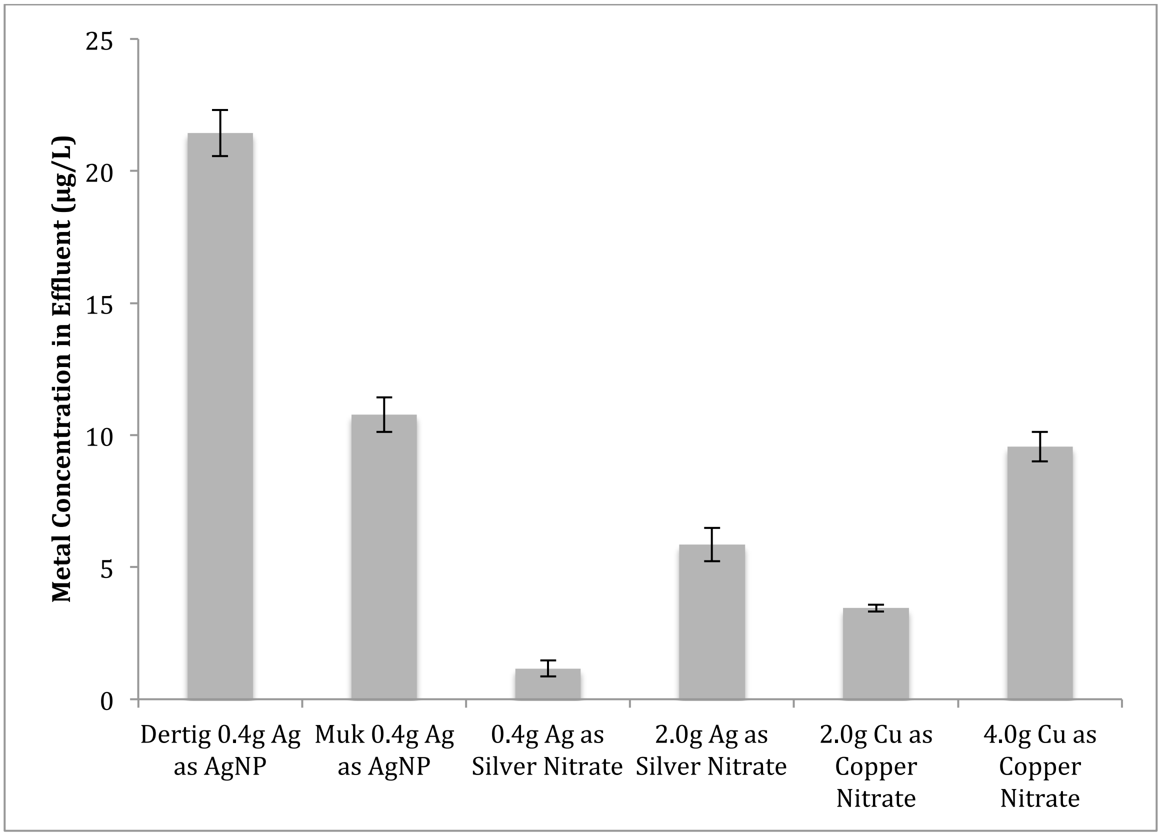

Figure 4.8. Results from fieldwork comparing full size filters for total silver or copper levels in the effluent.

4.4.3 No-Flow Conditions

Results of experiments to study a worst-case scenario with respect to silver release, where the storage container is filled with water to capacity to maintain maximum and continuous contact with the filter, are presented in Figure 4.9 below.

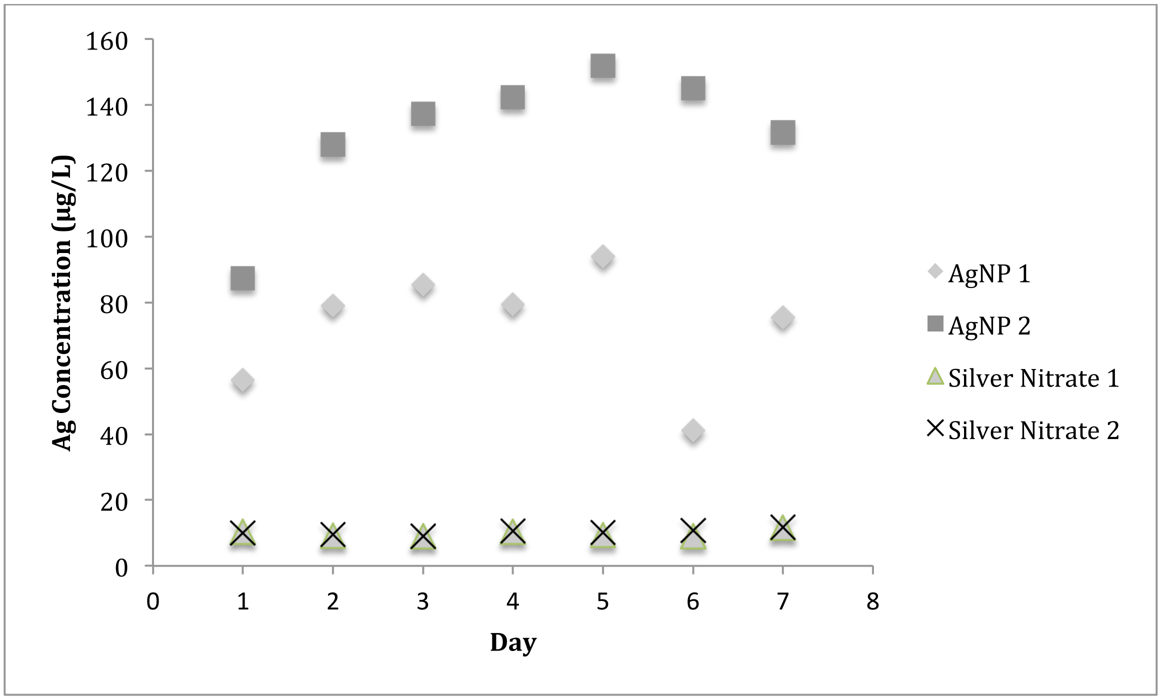

Figure 4.9. Results of duplicate experiments showing the silver concentration in effluent from filters over one week of stagnant exposure.

4.5 DISCUSSION

Incorporating $AgNO_3$ into ceramic water filters prior to firing results in filters that are equally or more effective at total coliform and *E. coli* removal relative to filters produced with painted-on AgNPs. Figures 2 and 3 show the average log removal of total coliform and *E. coli* for each filter type for each day over the 36-day span of the experiment. The different production methods result in similar levels of total coliform and *E. coli* removal, but the AgNP method results in greater variation in removal.

Table 4 shows the average log reduction of total coliform and *E. coli* for the filters produced at the Mukondeni facility. 2.0 g $AgNO_3$ filters produced the highest average log removal of total coliform and *E. coli*—with averages of 4.06 and 4.11

respectively. This represents more than 99.99% removal of both total coliform and *E. coli*, and is also in the “highly protective” performance category for the WHO (WHO 2011b). The 0.4g $AgNO_3$ filters had slightly lower average log removals of total coliform (3.69), but, had the same log removal of *E. coli* (3.92), as the AgNP filter with a lower standard error. The average log removal of total coliform for the AgNP filter was 3.85, but its error bars overlap with the bars for the 0.4 g $AgNO_3$ filters.

$AgNO_3$ filters release smaller amounts of silver into the treated water than AgNP filters. For example the mean effluent silver concentration for the Dertig and Mukondeni 0.4 g AgNP filters were 22 and 12 μg/L (respectively), compared to only 2 μg/L for the Mukondeni 0.4 g $AgNO_3$ filter (Figure 8). This corresponds to a 6-11-fold reduction in effluent silver concentration. $AgNO_3$ filters effluent silver concentrations are consistently lower over time, and even $AgNO_3$ filters made with five times as much silver had effluent concentrations lower than the AgNP filters.

The precise cause for this result unknown. However, it is likely related to the nature of metallic silver formation/deposition in the $AgNO_3$ filters relative to the AgNP filters. For the $AgNO_3$ filters, metallic silver “nanopatches” are formed reduction during the firing process. These nanopatches have a mean diameter between 2 and 3 nm and are distributed relatively uniformly throughout the ceramic porous media as detailed by Ehdaie et al (2014). Since the silver reduction occurs under dry, high-temperature conditions, the metallic silver appears to form a tight bond with the ceramic pore surface. For a ceramic tablet infused with silver in this manner, Ehdaie et al. (2014) demonstrated that only silver ions are released from the porous ceramic under stagnant water conditions. Conversely, AgNPs are applied to ceramic water filters using an aqueous suspension of the nanoparticles. The suspension is drawn into the ceramic media by capillary action during the painting process, and the nanoparticles presumably lodge in the pore space. When the filter begins to be used, it is reasonable to assume that many of these nanoparticles will be mobilized. Indeed, this has been quantified previously in the laboratory by Ren and Smith (2013a). It is also possible that some of the silver applied using the $AgNO_3$ method is isolated in pores not connected to the flow system, since the filters are pressed and fired after application of $AgNO_3$. However, this silver isolation, if it is significant, does not appear to have any significant effect on filter performance in terms of coliform bacteria or *E. coli* removal relative to AgNP filters (Figure 6).

One prior study has considered the alternative method of AgNPs in the ceramic prior to firing for a higher retention rate and longer lifespan, but this study did not test that method for bacterial removal in laboratory or field experiments (Ren and Smith 2013b). Brown and Sobsey (2010) tested the use of $AgNO_3$ in ceramic water filters in Cambodia, but applied the $AgNO_3$ after firing and did not test silver levels in the effluent. Mittleman et al. (2015) also addressed the use of $AgNO_3$ compared to AgNP, but applied both by painting on the silver after firing which actually resulted in higher silver levels in the effluent from $AgNO_3$ filter disks. Abebe et al. (2014) and Ehdaie (2015) both measured average total silver in the effluent from ceramic water filters produced in South Africa painted with colloidal silver of 11.7 μg/L and average ranges from 46.4 μg/L to 6.94 μg/L, respectively. Kallman et al. (2011) studied filters painted with AgNPs produced in Guatemala and found the average ionic silver concentration was 20 μg/L with a maximum of 90 μg/L seen during the first 12 h of use.

It is also important to note that for all the flow-through experiments conducted in this investigation, the silver concentration in the effluent filter water never exceeds 30 μg/L, which is significantly below the USEPA and WHO standards of 100 μg/L. The highest effluent silver levels were observed for the AgNP filters from Dertig 22 μg/L, Figure 8), which is significantly higher than the effluent silver concentrations observed for the Mukondeni AgNP filters (12 μg/L, Figure 8). The Dertig and Mukondeni AgNP filters both use the same silver application method, so this difference is likely caused by differences in the raw filter materials (clay and burnout material).

The $AgNO_3$ filters with 0.4 g of silver produced an average concentration of 1.2 μg/L. This is less than a tenth of the average concentration of silver in the Mukondeni AgNP filters (11 μg/L) produced with the same amount of silver. 2 g $AgNO_3$ filters produced an average concentration of 5.9 μg/L. This filter type also provided the most effective removal of coliform bacteria and *E. coli*.

Figure 9 addresses the issues found during no flow use of filters, where water may be in contact with the sides of the filters for an extended period of time before consumption. This leads to more metal wash out and higher effluent concentrations. This figure shows the silver concentrations in the effluent monitored over 7 days, with stagnant water in the filter. Filters produced at Dertig were used to establish a “worst case” scenario, since they produced the highest silver levels in previous tests. Silver concentrations are much higher in the AgNP filters than the $AgNO_3$ filters. The silver concentrations on average are 102 μg/L and 10 μg/L for the AgNP and $AgNO_3$ filters, respectively. These results show that AgNP filters can produce silver concentrations above the drinking water standard, albeit in a worst-case scenario. By contrast, $AgNO_3$ filters consistently result in silver concentrations in the treated water well below the drinking water standard. The $AgNO_3$filters silver concentrations do slightly increase over time (slopes of 0.30 and 0.12), but remain fairly stable and low across the 7-d experiment.

$Cu(NO_3)_2$ filters also demonstrated effective performance and relatively low copper concentrations in the treated water. Figures 4 and 5 show the average log removal by each Dertig filter type for each of 17 consecutive days. Table 5 presents the average log reduction of total coliform and *E. coli* for the filters produced at the Dertig facility. The $AgNO_3$ filters performed significantly better than the $Cu(NO_3)_2$ filters—with average log reduction of total coliform and *E. coli* of 3.71 and 3.76 respectively for the $AgNO_3$ filters versus 3.33 and 3.54 for the 4.0 g $Cu(NO_3)_2$ filters. The copper filters also exhibited greater variability between filters based on the larger standard errors. The filters with 2.0 g of copper and 4.0 g of copper performed almost identically, indicating higher amounts of copper do not improve filter performance of these 17-d experiments. While the copper filters performed slightly worse than those made with silver, they still removed more than 99.9% of total coliform bacteria.

Figure 7 shows the average metal levels, measured via GFAA, in effluents from filters over time—17 days for Dertig filters and 36 days for Mukondeni filters. Figure 8 shows the average metal concentrations (μg/L) for each type of filter over the extent of the experiment. The average copper concentrations of 3 and 10 μg/L in the effluent of filters produced with 2.0 or 4.0 g of copper are less than 1% of the drinking water standard. The copper filters represent an effective alternative as the cost of $Cu(NO_3)_2$ is approximately one-tenth of the cost of $AgNO_3$, and the drinking water standard is 1.0 mg/L for copper versus 100 μg/L for silver (USEPA 2018). Despite this observation, it should be noted that there is much less laboratory and field data on the use of copper in ceramic filter media relative to silver.

Application of silver or copper to ceramic water filters as $AgNO_3$ or $Cu(NO_3)_2$ prior to firing represents a promising new production method. In addition to good bacterial removal and lower silver levels, the $AgNO_3$ method offers distinct economic and safety benefits over filters made with AgNPs. $AgNO_3$is less expensive, costing only $1 per gram of silver compared to $2.71 per gram of silver in the form of AgNPs based on 2018 pricing. The result is that the same amount of silver can be used per filter at reduced the material cost, or more silver could be added to improve filter performance while maintaining the same price. The same holds true for the use of $Cu(NO_3)_2$. The painting step is also removed, saving on labor costs. $AgNO_3$ and $Cu(NO_3)_2$ are also more readily available in developing world markets relative to AgNPs.

The use of $AgNO_3$ during the manufacturing process also eliminates certain health risks for workers manufacturing filters, as some research suggests inhalation of AgNPs may result in geno-toxic effects (Fewtrell et al. 2017; Aktepe et al. 2015). The lower metal concentrations in the effluent indicate better retention of metals in the filters. This would likely increase the filter's effective lifespan. The use of copper is particularly promising, given its higher safe drinking water standard and it is less expensive, which means more copper could be used to improve efficacy against viruses while keeping a similar costs.

One drawback to note is that since $AgNO_3$ filter production requires the application of silver or copper prior to pressure and flow testing, if the filter fails to pass one or both tests, that silver or copper will be wasted.

In summary, the use of $AgNO_3$ or $Cu(NO_3)_2$ in ceramic water filters shows promising results. Lower metal concentrations with equal or better removal of total coliform and *E. coli* are ideal for decreasing the cost of production and increasing the life span of the filter. Field tests, with actual in home use with real source water are necessary to confirm before switching to the new production method. In the long run, as suggested in Ruparelia et al. (2008), a combination of silver and copper nanoparticles may produce a more complete bactericidal effect against mixed bacterial populations that would be more common in actual use.

4.6 ACKNOWLEDGEMETS

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4.7 REFERENCES

1. Brown, J., Sobsey, M. D. & Loomis, D. (2008) Local drinking water filters reduce diarrheal disease in Cambodia: a randomized, controlled trial of the ceramic water purifier. *Am. J. Trop. Med. Hyg*. 79 (3), 394–400.
2. Kallman, E. N., Oyanedel-Craver, V. A. & Smith, J. A. (2011) Ceramic filters impregnated with silver nanoparticles for point-of-use water treatment in rural Guatemala*. J. Environ. Eng.* 137 (6), 407–415.
3. Lantagne, D. S. (2001a). Investigation of the Potters for Peace Colloidal Silver Impregnated Ceramic Filter Report 1: Intrinsic Effectiveness. Retrieved from http://web.mit.edu/watsan/Docs/Other%20Documents/ceramicpot/PFP-Report1-Daniele% 20Lantagne,%2012-01.pdf.
4. Lantagne, D. S. (2001b). Investigation of the Potters for Peace Colloidal Silver Impregnated Ceramic Filter Report 2: Field Investigations.
5. Campbell, E. (2005*)* Study on Life Span of Ceramic Filter Colloidal Silver Pot Shaped (CSP) Model. http://potterswithoutborders.com/wpcontent/uploads//06/filter-longevity-study.pdf.
6. Oyanedel-Craver, V. A.; Smith, J. A. (2008) Sustainable colloidal-silver impregnated ceramic filter for point-of-use water treatment. *Environ. Sci. Technol.* 42 (3), 927−933.
7. Simonis, J. J.; Basson, A. K. (2012) Manufacturing a low-cost ceramic water filter and filter system for the elimination of common pathogenic bacteria*. Phys. Chem. Earth, Parts A/B/C*, 50−52, 269−276.

8. Zhang, H. Y.; Smith, J. A.; Oyanedel-Craver, V. (2012) The effect of natural water conditions on the anti-bacterial performance and stability of silver nanoparticles capped with different polymers. *Water Res.* 46 (3), 691–699.
9. Lantagne, D. S., Quick, R., & Mintz, E. D. (2006). Household Water Treatment and Safe Storage Options in Developing Countries: A Review of Current Implementation Practices
10. Rayner, J. (2009) *Current practices in manufacturing of ceramic pot filters for water treatment.* Loughborough University, Leicestershire, UK.
11. Lantagne, D.; Klarman, M.; Mayer, A.; Preston, K.; Napotnik, J.; & Jellison, K. (2010) Effect of production variables on microbiological removal in locally-produced ceramic filters for household water treatment. *International Journal of Environmental Health Research* 20(3), 171-187.
12. United States Environmental Protection Agency. (accessed 22 February 2018) Secondary drinking water standards: guidance for nuisance chemicals. Available online: http://www.epa.gov/dwstandardsregulations/secondary-drinking-water-standards-guidance-nuisance-chemicals
13. World Health Organization. (2011a) *Guidelines for Drinking-Water Quality*; World Health Organization: Geneva.
14. Ren, D., Colosi, L.M., Smith, J.A. (2013a) Evaluating the sustainability of ceramic filters for point-of-use drinking water treatment. *Environ. Sci. Technol*. 47, 11206e11213.
15. Praveena, S. M., & Aris, A. Z. (2015). Application of Low-Cost Materials Coated with Silver Nanoparticle as Water Filter in *Escherichia coli* Removal. *Water*

Qual Expo Health. doi:10.1007/s12403-015-0167-5

16. Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramírez, J. T. & Yacaman, M. J. (2005) The bactericidal effect of silver nanoparticles. *Nanotechnology* 16, 2346–2353.
17. Ren, D.; & Smith, J. A. (2013b) Retention and Transport of Silver Nanoparticles in a Ceramic Porous Medium Used for Point-of-Use Water Treatment. *Environmental Science and Technology* 47*,* 3825-3832.
18. Fewtrell, L.; Majuru, B.; Hunter, PR. (2017) A re-assessment of the safety of silver in household water treatment: rapid systematic review of mammalian in vivo genotoxicity studies*. Environmental Health* 16:66*.* doi: 10.1186/s12940-017-0279-4
19. Aktepe, N.; Kocyigit, A.; Yukselten, Y.; Taskin, A.; Keskin, C.; Celik, H. (2015) Increased DNA Damage and Oxidative Stress Among Silver Jewelry Workers*. Biol Trace Elem Res*., 164(2),185–91
20. Jackson, K. N. and Smith, J. A. (2018) A New Method for the Deposition of Metallic Silver on Porous Ceramic Water Filters. *Journal of Nanotechnology*, vol. 2018, Article ID 2573015, 9 pages. doi:10.1155/2018/2573015.
21. Ehdaie, B.; Krause, C.; & Smith, J. A. (2014) Porous Ceramic Tablet Embedded with Silver Nanopatches for Low- Cost Point-of-Use Water Purification*. Environmental Science and Technology*, 48*,* 13901-13908.
22. Anyaogu, K., Fedorov, A. V., & Neckers, D. C. (2008). Synthesis, characterization, and antifouling potential of functionalized copper nanoparticles. *Langmuir*, 24, 4340-4346

23. Esteban-Cubillo, A., Pecharroman, C., Aguilar, E., Santaren, J., & Moya, J. S. (2006). Antibacterial activity of copper monodispersed nanoparticles into sepiolite*. J. Mater. Sci*., 41, 5208-5212.

24. Ramyadevi, J., Jeyasubramanian, K., Marikani, A., Rajakumar, G., & Rahuman, A. (2012). Synthesis and antimicrobial activity of copper nanoparticles. *Mater. Lett*., 71, 114-116

25. Kahler, D.M., Koermer, N.T., Reichl, A.R., Samie , A. and Smith, J.A.. (2016*)* Performance and Acceptance of Novel Silver-Impregnated Ceramic Cubes for Drinking Water Treatment in Two Field Sites: Limpopo Province, South Africa and Dodoma Region, Tanzania. *Water*, 8(3), 95; doi:10.3390/w8030095

26. U.S. Environmental Protection Agency. (2002) *Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine Organisms*; EPA-821-R-02-012, Washington, DC.

27. World Health Organization. (2011b) *Evaluating household water treatment options: Heath-based targets and microbiological performance specifications*. ISBN: 978 92 4 154822 9

28. Brown, J., & Sobsey, M. D. (2010). Microbiological effectiveness of locally produced ceramic filters for drinking water treatment in Cambodia. *Journal of Water and Health*, 8(1), 1-11.

29. Mittelman, A. M., Lantagne, D. S., Rayner, J., and Pennell, K. D. (2015). *Silver* Dissolution and Release from Ceramic Water Filters. *Environmental Science & Technology ,49* (14), 8515-8522 DOI: 10.1021/acs.est.5b01428

30. Abebe, L. S., Smith, J. A., Narkiewicz, S., Oyanedel-Craver, V., Conaway, M., Singo, A., . . . Dillingham, R. (2014). Ceramic water filters impregnated with silver nanoparticles as a point-of-use water-treatment intervention for HIV-positive individuals in Limpopo Province, South Africa: a pilot study of technological performance and human health benefits. *Journal of Water and Health,* 12(2), 288-300. doi:10.2166/wh.2013.185
31. Ehdaie, B. (2015). Development of a Porous Ceramic Tablet Embedded with Silver Nanopatches for Low-cost Point-of-use Water Purification. (Doctor of Philosophy), University of Virginia.
32. Ruparelia, J., Chatterjee, A., Duttagupta, S., & Mukherji, S. (2008). Strain specificity in antimicrobial activity of silver and copper nanoparticles. *Acta Biomaterialia*, 4(3), 707-716.

Chapter 5: Inactivation of MS2 Bacteriophage and Adenovirus with Silver and Copper and Embedded in Ceramic Water Filters

The work presented in this chapter resulted in a journal article in preparation.

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5.1 INTRODUCTION

Viruses can cause gastrointestinal infections leading to sickness harmful to human health (Casteel et al. 2008, Abad et al. 1994). Adenovirus, a specific type of virus, is widespread in nature and can cause infections of the gastrointestinal tract, the respiratory tract and the eyes. Adenovirus is only second to rotavirus in terms of its significance as a pathogen of childhood gastroenteritis (Crabtree et al. 1997). It has been shown to occur in substantial numbers in raw water sources and treated drinking-water supplies due to it being excreted in human feces (WHO 2011; Crabtree et al. 1997). Adenovirus does not have a vaccine like other common viruses, like rotavirus, and is considered important by the World Health Organization because of the exceptional resistance to some water treatment and disinfection processes (WHO 2011).

Due to the large investment needed for centralized water treatment, point-of-use (POU) technologies have been suggested by the World Health Organization, where water is treated in the home, immediately before consumption (Clasen et al. 2006, Lantagne et al. 2006). POU technologies, like ceramic water filters, have been shown to be effective for total coliform and *E. coli* removal (Clasen et al. 2006, Lantagne et al. 2006, Clasen et al. 2007, PATH 2012, Lantagne and Clasen 2012, UNICEF 2008, Oyanedel-Craver and Smith 2008, Kallman et al. 2011). Ceramic water filters are a popular point-of-use technology, being produced at over 50 facilities worldwide using local labor and materials (Rayner 2009). Filters are produced using clay, a burn out material such as sawdust or rice husk, and water. After mixing and pressing into a pot shape, the filter is fired in a kiln. The burnout material combusts to form pore channels making the ceramic porous for filtration. After passing quality control tests, the filter is often painted with a silver nanoparticle solution on the inside and outside as an antimicrobial agent. This prevents fouling in the filter while also releasing silver into the effluent at levels safe for consumption for continued residual disinfection (Lantagne et al. 2006, Oyanedel-Craver and Smith 2008).

Although thoroughly tested for total coliform and *E. coli* removal, ceramic filters have not been evaluated extensively for performance against viruses (Abebe et al. 2015). One study found an average of 1.3 $\log_{10}$ reduction in MS2 by ceramic water filters treated with silver (Brown and Sobsey 2010). Viruses are particularly hard to treat using a point-of-use technology (Chen et al. 2002, Su et al. 2014, USEPA 2001, World Health Organization 2011) because their small size, 20-100 nm, makes them very hard to remove via filtration (Wu et al. 2010).

HOCl is effective for the disinfection of viruses, but as a point-of-use method, chlorine drops are not readily accepted because of change in taste, short shelf lives of the commercial drops, and the behavior change required to use it (Arnold et al 2007; Ram et al. 2007; Colindres et al. 2007). One study in Kenya found that after six months, only 33.5% of households were using the free chlorine and only 18.5% were still using the modified safe storage container (Makutsa et al. 2001). Overall, sustained and effective use of household water chlorination is usually low relative to other interventions (Sobsey et al. 2008).

Silver, used frequently for POU water treatment (including in ceramic water filters), has been shown to be most effective in the nanosize (1-10nm) for the disinfection of viruses, with the capping agent also having an affect (Elechiguerra at al. 2005, Rogers et al. 2008). Several current commercially available technologies such as the MadiDrop ™ and Silverdyne rely entirely on silver for disinfection (Ehdaie et al. 2014).

MS2 is a non-pathogenic RNA bacteriophage that infects *E. coli* (Kuzmanovic et al. 2003). It is relatively small in size with a simple composition and is easily propagated, stored and quantified (Kuzmanovic et al. 2003, Jones et al. 1991). Due to this, its harmlessness to humans, and durability, MS2 is used frequently for testing efficacy of technologies against viruses in general (Jolis et al., 1999; Lykins et al., 1994; Oppenheimer et al., 1997; Woolwine and Gerberding, 1995). Bacteriophages such as MS2 are useful surrogates for modeling the behavior of enteric viruses in water treatment processes (Grabow 2001) and have been used to model virus retention in other filtration processes (Sobsey et al. 1995; Van Voorthuizen et al. 2001; Brown and Sobsey 2010) However, the validity of using bacteriophages to model mammalian viruses (the ones of concern for drinking water) depends on the sensitivity to disinfectants, which may differ significantly (Jones et al. 1991).

Conversely, adenovirus is a potentially pathogenic, non-enveloped DNA virus commonly found where contamination with human feces or sewage has occurred (Pond 2005, Gall et al. 2015). While infections are normally acute, greater severity of illness occurs in immunocompromised individuals (Crabtree et al. 1997). Adenovirus is noted as being unusually resistant to physical or chemical treatments and adverse pH conditions, meaning the virus has prolonged survival outside of a human host (Pond 2005). Adenovirus is also resistant to both tertiary treatment and UV radiation in urban wastewater, as well as, potentially underestimated for its prevalence in environmental waters, making it even harder to treat on a household level (Thompson et al. 2003; Thurston-Enriquez et al. 2003; Crabtree 1997). However, due to the difficult nature of propagating the virus and long incubations times for detecting replication (Gall et al. 2015), adenovirus is not commonly used for evaluating point-of-use water treatment technologies.

The purpose of this manuscript is to quantify the inactivation of MS2 bacteriophage and adenovirus using silver ions and copper ions at different concentrations. This is not only to compare the effectiveness of silver and copper, but also evaluate the validity of using MS2 as a model virus. In addition, the transport of MS2 bacteriophage through a silver-ceramic and copper-water filter will be quantified.

5.2 MATERIALS AND METHODS

5.2.1 MS2 INACTIVATION

MS2 bacteriophage was grown and quantified like in Ehdaie (2015).

5.2.1.1 MATERIALS FOR MS2 DISINFECTION EXPERIMENTS

Tryptic soy broth (TSB) (Becton, Dickson, and Company, Franklin Lakes, NJ, USA (BD)) was prepared according to packaging. 30 mg of TSB was dissolved in 1 L of distilled water with heat. The dissolved solution was then autoclaved for 25 min at 121˚C. Tryptic soy agar 1.5% (TSA) (BD) was prepared according to packaging. 40 mg of TSA was dissolved in 1 L of distilled water by bringing the solution to a boil then autoclaving the solution for 25 min at 121˚C. The TSA solution was cooled to 50-60˚C and poured into agar plates. Agar plates were allowed to cool and solidify at room temperature (25˚C) before being stored in the fridge. Top agar 0.5% was prepared with TSA working stock diluted in TSB in 50-mL conical tubes and stored in the fridge.

5.2.1.2 CULTURING E. COLI AND MS2 BACTERIOPHAGE

E. coli C300 was purchased from ATCC (Manassas, VA) and used for propagation and plaque counting of MS2 bacteriophage. An *E. coli* stock was cultured by resuspending the dehydrated pellet in 1 mL of TSB. In a 250-mL flask, 100 μL of *E. coli* stock was added to 25 mL of TSB and kept in a shaking incubator at 200 rpm and 37˚C overnight. Solid bacteria culture was prepared in addition to liquid culture by inoculating a TSA plate with 10 μL of *E. coli* liquid culture and incubating the plate at 37˚C overnight. The overnight solid culture was stored at 4˚C and used within 5 d. Solid cultures were used to prepare log-phase *E. coli*. Log-phase *E. coli* was prepared by inoculating 10mL of TSB with *E. coli* C300 from solid culture and incubating in a shaking incubator for 2-4 hours at 37˚C until an OD600 reading between 0.4-0.8 was achieved.

MS2 bacteriophage was used as the surrogate to waterborne viruses in this experiment. MS2 was purchased from ATCC and the dry pellet was resuspended in 1 mL of TSB and stored at 4°C. MS2 bacteriophage is similar in size (25 nm), shape (round), and type of nucleic acids (RNA) to waterborne viral pathogens such as rotavirus, enterovirus, and norovirus, and is a commonly used surrogate in laboratory research (Borkow and Gabbay, 2005).

5.2.1.3 VIRUS BATCH REACTION EXPERIMENT

In a 50-mL conical tube, 25 mL of deionized water (DI) was inoculated with MS2 bacteriophage for a final concentration of ~$2.5X10^6$ PFU/mL. A concentrated stock solution of silver or copper nitrate dissolved in DI water was added to each tube for the appropriate concentration and kept at room temperature. Concentrations of 200 μg/L and 1.0 mg/L Cu and 20 μg/L and 100 μg/L Ag were used. Samples were taken at 8 h and 24 h and analyzed for virus inactivation. Each concentration was performed in duplicate. 25 mL of DI with 10^6 PFU/mL MS2 bacteriophage was used as the control.

5.2.1.4 VIRUS QUANTIFICATION

Quantification of MS2 bacteriophage was performed by a standard plaque counting method. Samples for each time point were treated with 60 g/L sodium thiosulfate (26.4μL per 1 mL of sample) for 2 min at room temperature to inhibit continual disinfection.

Ehdaie (2015) showed sodium thiosulfate treatment of phage did not impact virus viability. Samples were then incubated with log-phase E. coli (200 μL of E. coli per

10 μL of phage sample) for 4 min at room temperature. The phage-bacteria culture was added to 3 mL of melted 0.5% TSA top agar and immediately poured onto 1.5% TSA agar plate. The top agar was allowed to solidify at room temperature. Plates were incubated inverted at 37°C overnight. During the overnight incubation, viable viruses infected E. coli and lysed forming visible clear plaques over the bacteria lawn. The next day, clear plaques were counted to determine the number of viable viruses in solution (Schuster and Adams, 1962; Williams et. al., 2001; Easton, 2005).

5.2.2 ADENOVIRUS INACTIVATION

5.2.2.1 PREPARATION OF ADENOVIRUS STOCKS

Recombinant human adenovirus type five expressing enhanced green fluorescent protein was purchased from Vector Biolabs, Malvern, PA, USA. Iscove's Modified Dulbecco's Medium (IMDM) was used as the medium for virus replication. The 293T human tissue culture cell line was used for cultivation and quantification. Purified virus was resuspended in medium and added to 15 cm petri dishes of 293T cells. Dishes were agitated every 15 min for 90 mins before removing the virus and putting 20 mL of medium on the dish. After 3 days of incubation, the cells were ready to harvest. Virus infected cells were subjected to 3 freeze/thaw cycles with liquid nitrogen and a water bath at 37°C. Cell debris was pelleted by centrifugation at 2000 rpm for 5 min in a Sorvall RT7 centrifuge and the virus lysates were used in the inactivation experiments.

5.2.2.2 VIRUS BATCH REACTION EXPERIMENT

Virus lysate was resuspended in 20 mL of deionized water, drop wise. In a 15-mL conical tube, 4 mL of DI water was mixed with 4 mL of adenovirus for a final titer of

$5X10^5$ TCID50/100 μL. A concentrated stock solution of silver nitrate or copper nitrate dissolved in DI water was added to each tube at various concentration and the tubes were kept at room temperature. The final concentrations were 200 μg/L and 1.0 mg/L of Cu and 20 μg/L and 100 μg/L of Ag. Aliquots were taken at 8 h and 24 h and analyzed for virus inactivation in an end point titration assay. Duplicate experiments were performed for each concentration 0.8 mL of DI water with $5X10^5$ TCID50/100 μL adenovirus was used as the control.

5.2.2.3 ADENOVIRUS QUANTIFICATION

Quantification of adenovius was performed by determination of the 50% tissue culture infective dose (TCID50). Samples representing each time point were treated with 60g/L sodium thiosulfate (26.4 μL per 1 mL of sample) for 2 min at room temperature to inhibit further inactivation. Initial experiments insured that the sodium thiosulfate treatment of the sample did not impact virus viability. 24 well plates with $2.5X10^5$ 293T human tissue cells per well were used for dilutions with medium. An EVOS microscope with a 10x objective lens and the appropriate filter to visualize GFP was used to find the end point titer after one week of incubation. The last well to show cells expressing GFP was used as the end point. The use of 293T cells allowed a spreading infection, since E1 expression in these cells complements the lack of E1 in the GFP vector.

5.2.2.4 ADENOVIRUS IMAGING

For these experiments, Adenovirus was purified to improve imaging quality. Frozen pellets from the same GFP adenovirus was used as before. Two pellets were resuspended in 8 mL of phosphate buffer solution and subjected to 3 freeze/thaw cycles with liquid nitrogen and a water bath at 37˚C. Virus was then centrifuged at 2000 rpm for 5 min in a Sorvall RT7 centrifuge and put on ice. Supernatant was pooled and transferred to 12 mL high-speed centrifuge tubes. Cesium chloride was added until a density of 1.34 was achieved. 2 mL of mineral oil was added to top off the centrifuge tubes. Tubes were put in high speed centrifuge buckets and run in a Beckman Coulter Optima LC-80k Ultracentrifuge at 35000 rpm at 4˚C for 24 h. Virus was harvested in bands by puncturing the side of the centrifuge tube with a BD syringe with 18 G 1 ½ BD precision glide needle and removing the bands. Virus was then dialyzed by injecting the harvested virus into a Thermoscientific Slide-a-lyzer Dialysis Cassette. Then the cassette was soaked in a 0.9 % sodium chloride solution with 5% glycerol for four hours on a stir plate in 4˚C. Then the dialysis was repeated with fresh solution overnight. Virus was then harvested for freezing.

In a 2 mL centrifuge tube, 40 µL of purified adenovirus was diluted 1:1 with either DI or Ag or Cu dissolved in DI. Four types of reactors were tested in duplicate: Deionized water treated with sodium thiosulfate at collection time, deionized water not to be treated with sodium thiosulfate, 100 µg/L Ag, and 1.0 mg/L Cu. After 8 h, sodium thiosulfate was added to the control, Ag and Cu reactors. Samples were then taken for imaging.

Samples for imaging were prepared as in Wagner et al. (2016). For electron microscopy, the sample (3 mL) was applied to a glow-discharged, continuous carbon-coated grid for 2 min. Excess liquid was blotted off by touching the edge of the grid with filter paper. The grid was washed with 0.1 M KCl, blotted, stained with 2% (w/v) uranyl acetate for 2 min, and blotted again. Images of the negatively stained samples were recorded using a Tecnai F20 transmission electron microscope (Philips/FEI) operating at 120 kV.

5.2.3 MISCIBLE DISPLACEMENT TRANSPORT EXPERIMENTS WITH MS2

5.2.3.1 DISK PREPARATION

Ceramic filters were prepared like in Chapter 3 in Charlottesville, VA. For ceramic filters, 200-mesh Redart clay produced by Cedar Heights Clay Company and sawdust sieved to 20-mesh from a commercial lumberyard in Earlysville, Virginia (USA) were used. The clay has a particle-size distribution (PSD) of 30.7%, 54.9%, and 14.4% for particles <2, 2− 20, and >20 μ m, respectively (Oyanedel-Craver and Smith, 2008). Two types of filters were studied in this investigation: (i) silver nitrate filters and (ii) copper nitrate filters. Silver nanoparticle filters use a conventional synthesis method similar to that described by Oyandedel-Craver and Smith (2008). 168.75 g of Redart clay and 18.75 g of sawdust (total mass of 187.5 g) were mixed by hand. Then 57 mL of deionized, organic free water with the silver or copper nitrate dissolved in it, was added and thoroughly mixed by hand. This mix was then separated by hand into three portions of equal weight, placed in a 6.5-cm-diameter PVC mold and compressed at 1000 psi for 1 minute. The resulting filter was an approximately 1 cm thick disk, providing a one-dimensional simplified geometry for lab testing (Figure 5.1).

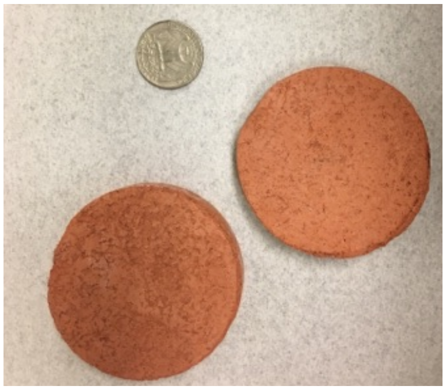

Figure 5.1. Ceramic filter disks used for laboratory testing

After air-drying for 48 h, the ceramic filters were fired in a kiln with the following temperature program: increase temperature from 20˚C at 150 ˚C/h to 600 ˚C, then increase at 300 ˚C/h to 900 ˚C, then isothermal for 3 h.

For the silver filters, 99.5% pure silver nitrate from Acros Organics was used for the ceramic filter disk fabrication. For the copper filters, 99.5% pure copper (II) nitrate from Acros Organics was used. Silver filter disks had 24.8 mg Ag, proportional to a full size filter produced at PureMadi Mukondeni Filter Production Facility in Jackson and Smith (2018). Copper filters had 248 mg Cu, again proportional to full size filters produced at PureMadi Dertig Production Facility tested in Jackson and Smith (2018).

5.2.3.2 EXPERIMENTAL SETUP

As in Jackson and Smith (2018), the cylindrical ceramic filters were loaded into a flexible-wall permeameter, holding a 10-psi pressure on the cell to ensure flow through the filter, rather than around. A high-performance liquid chromatography (HPLC) pump (Acuflow series IV), a 1.0 mL syringe and the inflow valve of the permeameter chamber were connected with a three-way stopcock. The HPLC pump maintained a constant flow rate of 0.6 mL/min to mimic the average flow of pot filters (1.5 L/h). DI water was used as the inflow solution. The effluent valve of the permeameter chamber was open to the atmosphere for collection of effluent water samples. Filters were saturated by pumping inflow solution through the filter for 24 hours prior to the experiment. After the saturation period, a 1.0 mL syringe was used for a pulse injection of 0.6 mL of 10^5 PFU/mL MS2 bacteriophage was injected over 1 min. Effluent samples were collected and treated with sodium thiosulfate to deactivate disinfection. MS2 concentration was quantified with the plaque assay method described in the disinfection experiment. Effluent samples were also collected and acidified to 2% nitric to measure copper concentrations in the effluent via Agilent 7900 Inductively coupled plasma mass spectrometry (ICP-MS). Helium gas was used as the collision gas and argon was used as a makeup gas.

5.3 RESULTS

5.3.1 MS2 INACTIVATION

At each time point, the number of viable MS2 bacteriophage in the treated sample (C) was divided by the number of viable viruses in the control (C_C) to determine log reduction. This was done to account for MS2 die off not due to silver of copper. Figure 5.2 shows the log C/C_C for MS2 phage for each metal and concentration after 8 and 24 h of exposure from duplicate batch reactors.

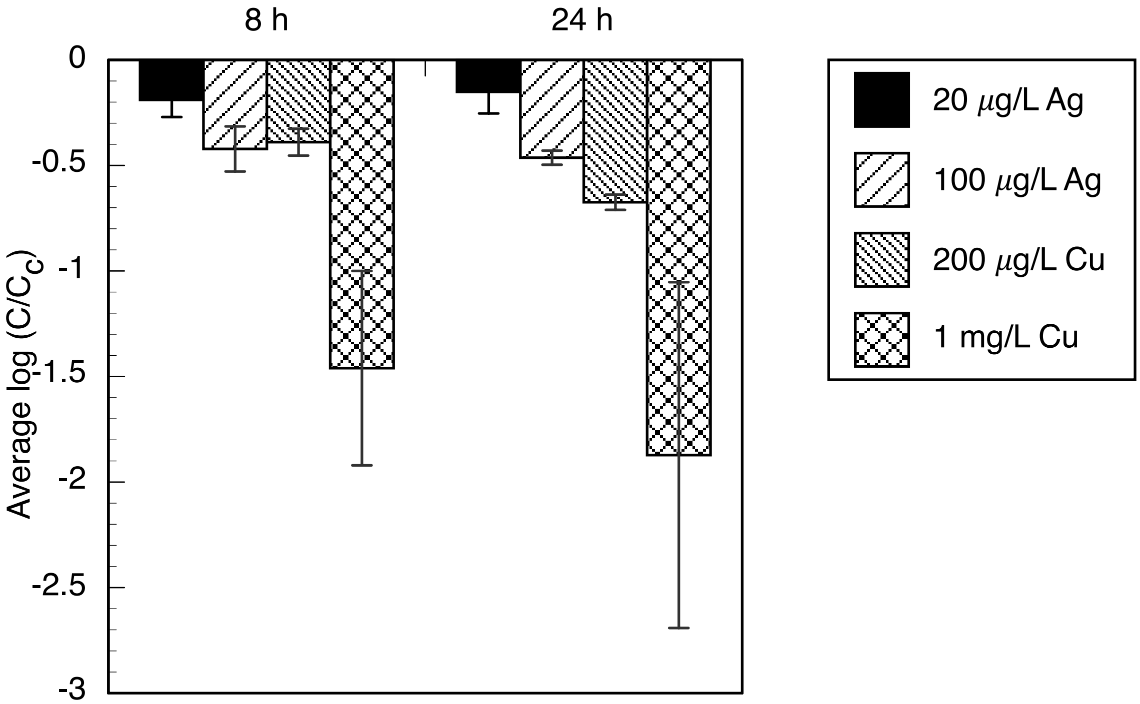

Figure 5.2. The disinfection of MS2 calculated by taking the $\log_{10}$ of the concentration in the sample (C) after 8 h and 24 h of exposure to different levels of silver and copper divided by the concentration in the control (C_C) at the same time point. Error bars indicate one standard error above and below the mean.

Table 5.1 presents the log C/C_C of MS2 phage after disinfection by the different concentrations of silver and copper after 8 and 24 h. Again, C is the concentration of viable MS2 in the sample and C_C is the concentration of viable MS2 in the control at the same time point.

Table 5.1. Log of C/C_C where C is the concentration of viable MS2 in the sample and C_C is the concentration of viable MS2 in the control at the same time point at 8 and 24 h

Metal Concentration	8h	24h
20 µg/L Ag	-0.19	-0.15
100 µg/L Ag	-0.42	-0.46
200 µg/L Cu	-0.39	-0.67
1 mg/L Cu	-1.46	-1.87

5.3.2 ADENOVIRUS INACTIVATION

At each time point, the number of viable viruses in the treated sample (C) was divided by the number of viable viruses in the control (C_C) to determine log reduction. Figure 5.3 shows the log C/C_C for adenovirus for each metal and concentration after 8 and 24 h of exposure from duplicate batch reactors.

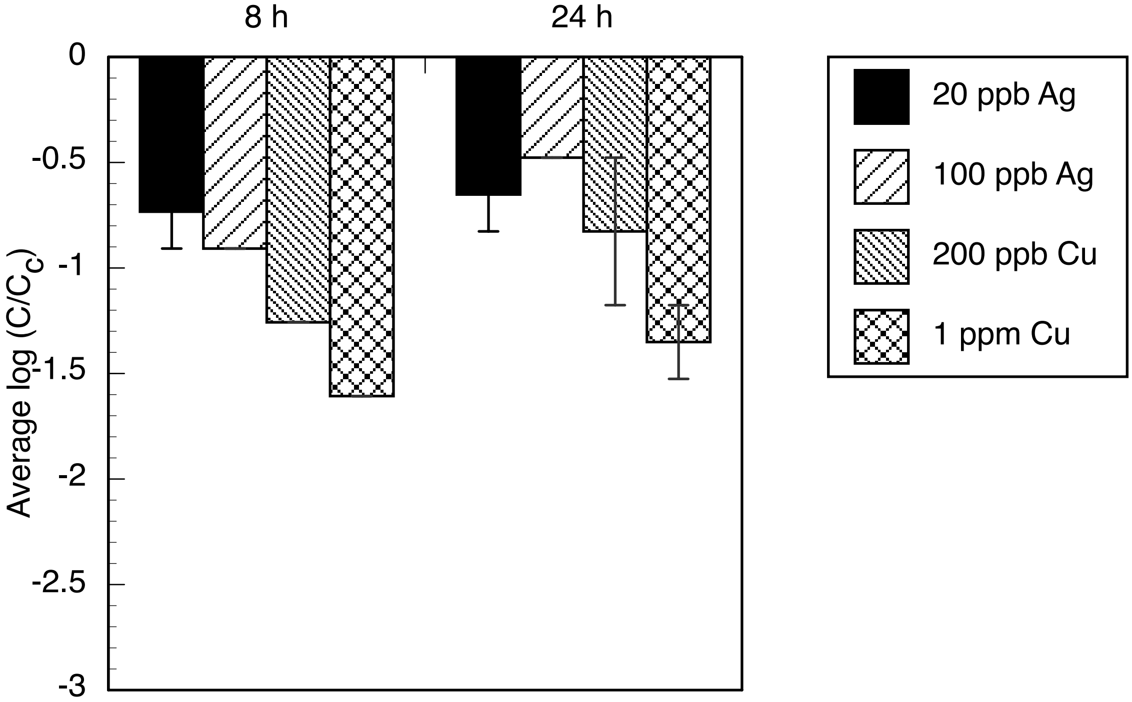

Figure 5.3. The disinfection of adenovirus calculated by taking the $\log_{10}$ of the concentration in the sample (C) after 8 and 24 h of exposure to different levels of silver and copper divided by the concentration in the control (C_C) at the same time point. Error bars indicate one standard error above and below the mean.

Table 5.2 presents the log C/C_C of adenovirus after disinfection by the different concentrations of silver and copper after 8 and 24 h. Again, C is the concentration of viable adenovirus in the sample and C_C is the concentration of viable adenovirus in the control at the same time point.

Table 5.2. Log of C/C_C where C is the concentration of viable adenovirus in the sample and C_C is the concentration of viable adenovirus in the control at the same time point at 8 and 24 h

Metal Concentration	8 h	24 h
20 ppb Ag	-0.73	-0.65
100 ppb Ag	-0.91	-0.48
200 ppb Cu	-1.26	-0.83
1 ppm Cu	-1.61	-1.35

Figures 5.4, 5.5, and 5.6 show TEM images of purified adenovirus untreated and treated with silver or copper.

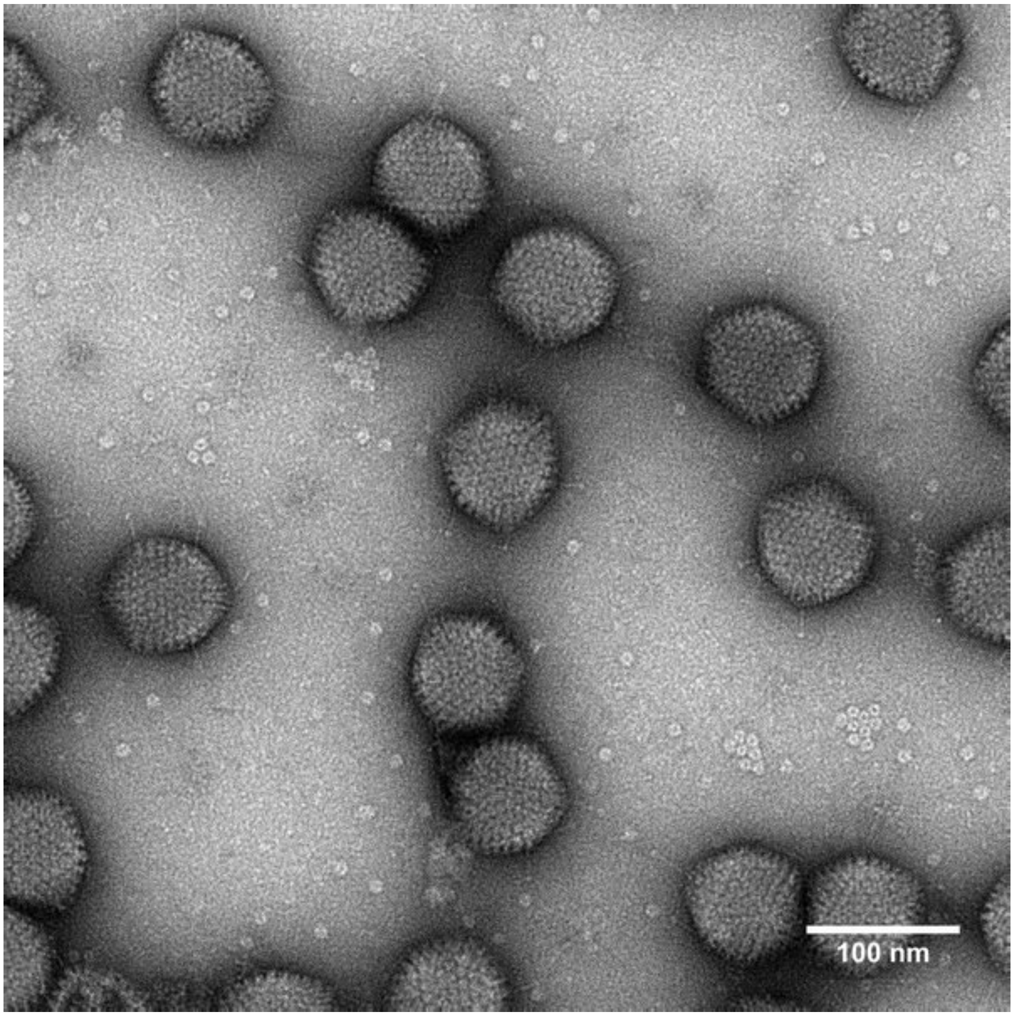

Figure 5.4. TEM image of untreated purified adenovirus.

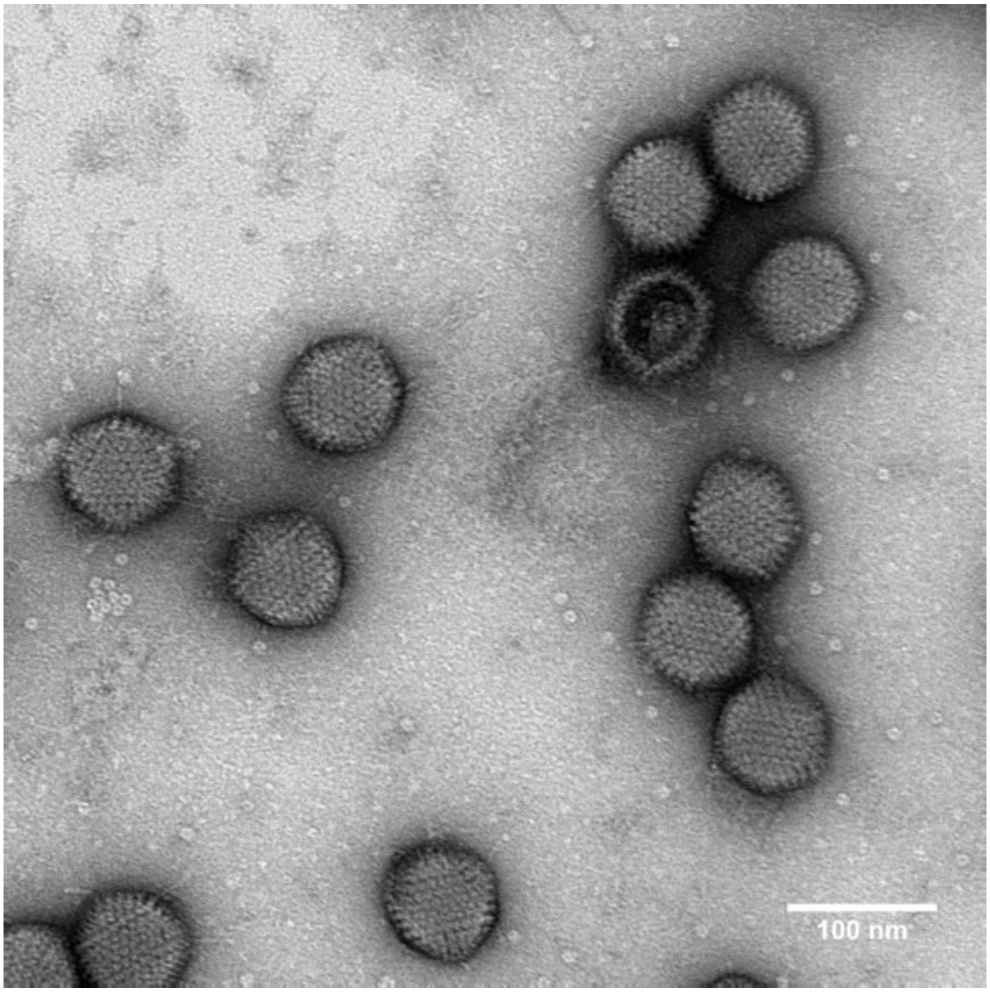

Figure 5.5. TEM image of purified adenovirus treated with 100 μg/L Ag for 8 h

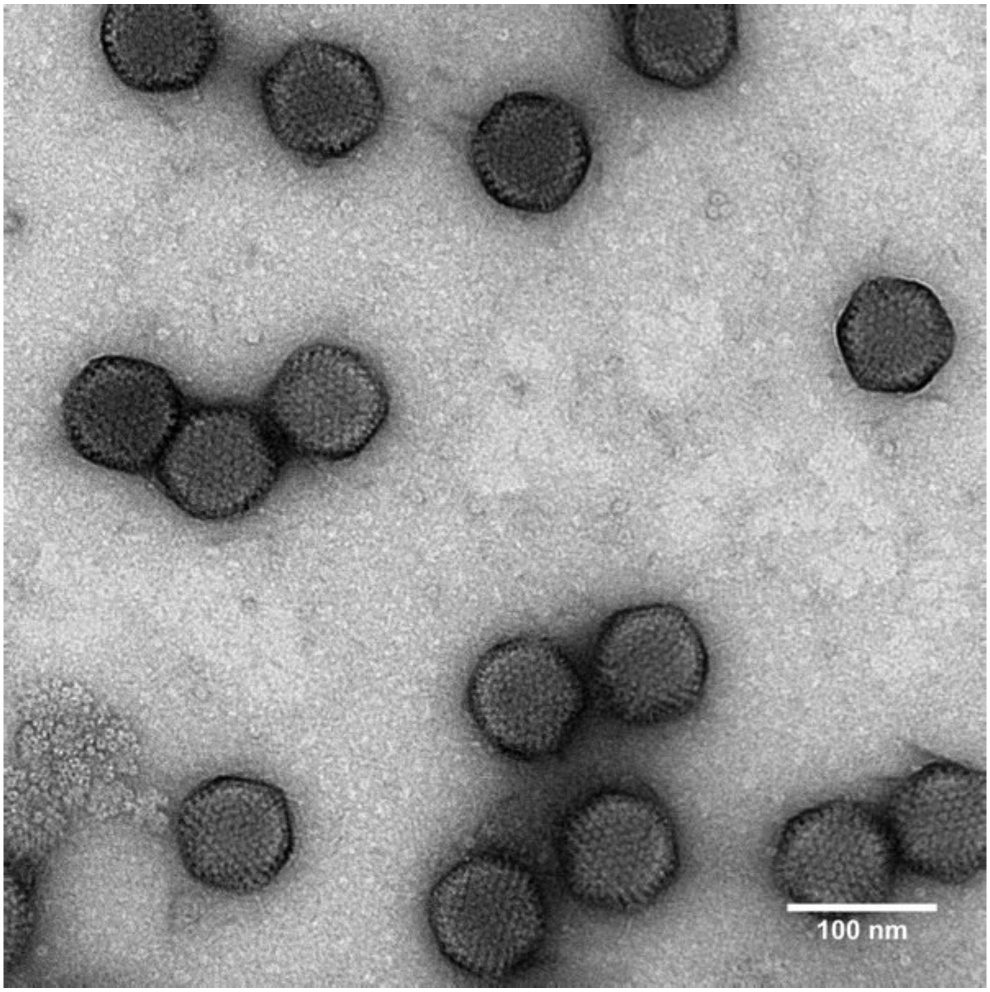

Figure 5.6. TEM image of purified adenovirus treated with 1.0 mg/L Cu for 8 h

5.3.3 MISCIBLE DISPLACEMENT TRANSPORT EXPERIMENTS WITH MS2

The results of the MS2 phage pulse injections into the ceramic filter disks are shown in Figure 5.7. Figure 5.7 shows the log removal of MS2 phage versus the pore volumes of flow. The filters embedded with copper performed slightly better, but not statistically different than the filters embedded with silver due to the large error bars overlapping. To calculate results, the effluent concentration (C) was divided by influent concentration (C_0), followed by taking the log of C/C_0.

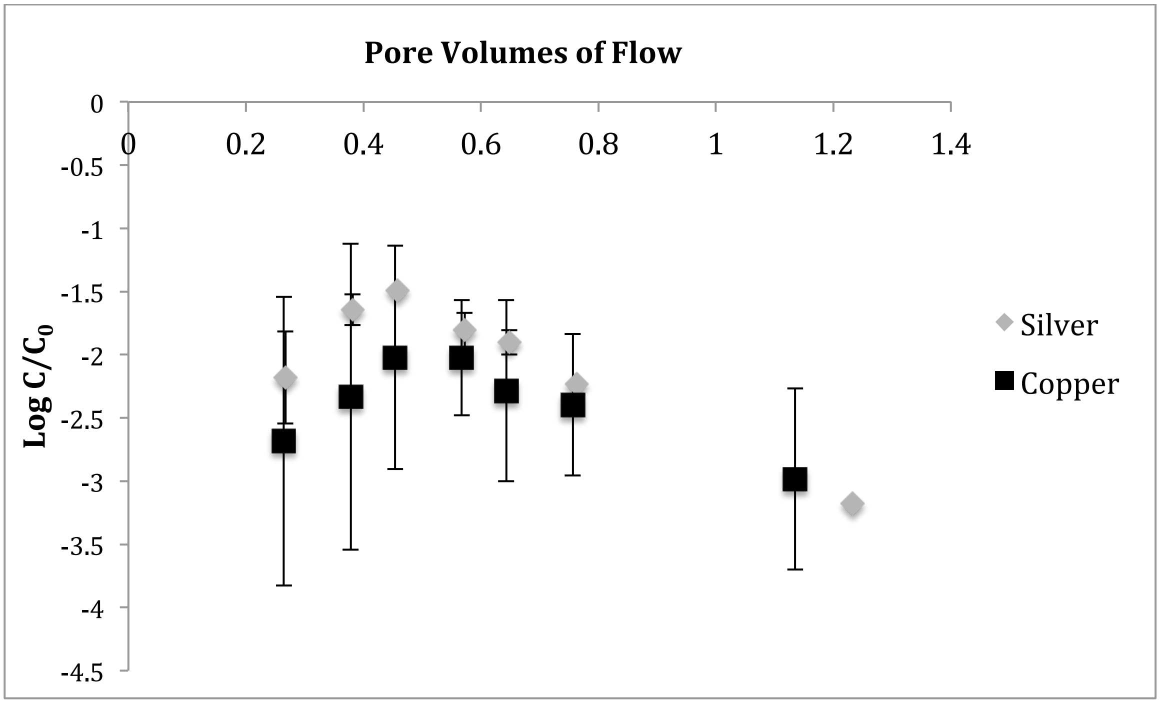

Figure 5.7. Miscible displacement curve as the result of a 0.6 mL pulse of ~10^3 PFU/10uL MS2 through ceramic filter disks made with $AgNO_3$ and $Cu(NO_3)_2$ in duplicate.

Copper concentration was also measured via ICP-MS in the filter effluents after 20 h of pumping DI through the filter. The average of the two filters was 4.46 μg/L. This is a very low level compared to the drinking water standard of 1.0 mg/L for copper (USEPA 2018).

A mass balance was used to calculate the amount of MS2 removed/deactivated by the filters. Filters embedded with silver removed on average 71.95% of the inject MS2. Filters embedded with copper removed on average 75.98 % of MS2 injected.

5.4 DISCUSSION AND CONCLUSIONS

These virus inactivation experiments performed with silver and copper show that the primary inactivation happens within 8 h. There seems to be very little additional inactivation between 8 and 24 hours. Having 8 hours of exposure time during in home treatment could be typical if water is treated in the evening for the next day. Since the concentrations are normalized to the control at the same time point, some of the log removal rates even decrease between the 8 and 24 h time points due to the die off of the control. The MS2 phage showed less removal in the control over time and the removal rates improve with higher amounts of metals and more time. The adenovirus does not follow the same trend. While both silver and copper at both concentrations result in lower levels of viable virus in the 24 h sample, the control it is normalized to also decreases in infectivity ($2.5X10^4$ to $1.13X10^4$ PFU/10 µL for MS2 bacteriophage at 8 and 24 h respectively and $4.52X10^5$ to $1.68X10^5$ TCID50/100 µL for adenovirus at 8 and 24 h respectively), causing the lower log removal levels.

At the 8 h time point, the 100 µg/L Ag deactivation is 0.42-log and 0.91-log for MS2 and adenovirus, respectively. Silver inactivated adenovirus more than twice as efficiently as MS2. While MS2 is a commonly used virus surrogate in the lab, adenovirus is an actual waterborne virus with human health concerns. Adenovirus is likely to be more representative virus and could be a better indicator for silver's performance in the inactivation of viruses for a point-of-use water treatment application. This also appears true for the lower level of copper. With 200 µg/L Cu, after 8 h there is only 0.39 log removal of MS2, while there is a 1.26 log removal of adenovirus.

When comparing the TEM images of the adenovirus treated and untreated, the visible difference appears to be in the fibers. Adenovirus infectivity is mediated predominantly by the penton and the fiber capsid proteins (Bosshard et al. 2012; Medina-Kauwe 2013). Changes to these structures could indicate the mechanism of adenovirus inactivation. The untreated sample, figure 4, shows mostly intact virus particles with the hexagonal structure with fibers visible at most of the corner pentons. However, as can be seen in figure 5, with adenovirus exposed to 100 μg/L Ag, there appears to be slightly fewer intact fibers attached to the virus structure, and even a collapsed hexon. Figure 6, the adenovirus exposed to 1.0 mg/L Cu, has the least amount of intact fibers. The number of intact fibers correlates with the inactivation observed in the pervious inactivation experiment. This suggests that potentially fiber removal may be contributing to inactivation. Further testing and imaging would need to be done to quantify the effect of fiber loss to confirm this theory. If indeed fiber removal it the mechanism of deactivation of adenovirus, viruses with similar structures could be affected the same way and should be evaluated.

Due to the small size of viruses (Wu et al. 2010), the ceramic water filter cannot remove them solely based on size exclusion. The inclusion of silver or copper would help with the removal and inactivation of viruses. The addition of the metals would help prevent virus growth in the ceramic, as well as, release metals into the effluent for residual disinfection like in experiments described above. The miscible displacement transport experiment results for the total amount of MS2 inactivated indicate that use of copper in a ceramic filter would be slightly better than silver for viral inactivation. The pulse curve for the copper filters has a slightly lower peak breakthrough with overlapping error bars, but a larger percentage of the virus is inactivated (75.98% versus 71.95%). However, in with both silver and copper the inactivation is modest. This experiment also included the immediate inactivation of the metal upon sample collection. The continued disinfection after filtration and before consumption is not included. Thusly, for real world use, the filter removal plus the continued disinfection must be considered.

From these results, it would be suggested to compare performance of full size filters made with silver and with copper for removal of adenovirus and MS2. This could confirm the doubts of MS2 as an accurate model virus. It could also confirm if the inclusion of copper, instead of silver, would improve the performance. Eventually, studies to optimize the use of silver and copper together could be performed to improve filter efficacy. More TEM imaging should be done to confirm the mechanism of inactivation, and then other viruses, similar in structure, could be identified and similarly evaluated.

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5.6 REFERENCES

1. Casteel, M. J.; Schmidt, C. E.; Sobsey, M. D. Chlorine disinfection of produce to inactivate hepatitis A virus and coliphage MS2. Int. J. Food Microbiol. 2008, 125, 267- 273.
2. Abad, F. X.; Pinto, R. M.; Diez, J. M.; Bosch, A. Disinfection of human enteric viruses in water by copper and silver in combination with low levels of chlorine. Appl. Environ. Microbiol. 1994, 60, 2377-2383.
3. K.D. Crabtree, C.P. Gerba, J.B. Rose, C.N. Haas. Waterborne adenovirus: a risk assessment. Water Sci. Technol., 35 (1997), pp. 1-6
4. World Health Organization Guidelines for Drinking-water Quality; WHO: Geneva, 2011;
5. Clasen, T.; Roberts, I.; Rabie, T.; Schmidt, W.; Cairncross, S. Interventions to improve water quality for preventing diarrhoea. Cochrane Database Syst. Rev. 2006, No. 3, CD004794.
6. Lantagne, D. S.; Quick, R.; Mintz, E. D. Household water treatment and safe storage options in developing countries: A review of current implementation practicies; Wilson Center: Washington, DC, 2006.
7. Clasen, T.; Schmidt, W. P.; Rabie, T.; Roberts, I.; Cairncross, S. Interventions to improve water quality for preventing diarrhoea: Systematic review and meta-analysis. BMJ 2007, 334, 782.
8. PATH's Safe Water Project. Perspectives: Commercial approaches to delivering household water treatment and safe storage products and solutions to low-income households (Special Report); PATH Publications:

Seattle, WA, 2012.

9. Lantagne, D. S.; Clasen, T. F. Use of household water treatment and safe storage methods in acute emergency response: Case study results from Nepal, Indonesia, Kenya, and Haiti. Environ. Sci. Technol. 2012, 46, 11352−11360.
10. UNICEF. Promotion of household water treatment and safe storage in UNICEF WASH Programmes; UNICEF: New York, 2008.
11. Oyanedel-Craver, V. A.; Smith, J. A. Sustainable colloidal-silver impregnated ceramic filter for point-of-use water treatment. Environ. Sci. Technol. 2008, 42 (3), 927−933.
12. Kallman, E. N., Oyanedel-Craver, V. A. & Smith, J. A. (2011) *Ceramic filters impregnated with silver nanoparticles for point-of-use water treatment in rural Guatemala*. J. Environ. Eng. 137 (6), 407–415.
13. Rayner, J. Current Practices in Manufacturing of Ceramic Pot Filters for Water Treatment. (Master Thesis), Loughborough University. 2009
14. Abebe, L. S., Smith, J. A., Narkiewicz, S., Oyanedel-Craver, V., Conaway, M., Singo, A., . . . Dillingham, R. (2014). Ceramic water filters impregnated with silver nanoparticles as a point-of-use water-treatment intervention for HIV-positive individuals in Limpopo Province, South Africa: a pilot study of technological performance and human health benefits. *Journal of Water and Health, 12*(2), 288-300. doi:10.2166/wh.2013.185
15. Brown, J., & Sobsey, M. D. (2010). *Microbiological effectiveness of locally produced ceramic filters for drinking water treatment in Cambodia*. Journal of

Water and Health, 8(1), 1-11.

16. Chen, X. M.; Keithly, J. S.; Paya, C. V.; LaRusso, N. F. Cryptosporidiosis. N. Engl. J. Med. 2002, 346, 1723-1731.
17. Su, Y. H.; Tsegaye, M.; Varhue, W.; Liao, K. T.; Abebe, L. S.; Smith, J. A.; Guerrant, R. L.; Swami, N. S. Quantitative dielectrophoretic tracking for characterization and separation of persistent subpopulations of Cryptosporidium parvum. Analyst 2014, 139, 66-73.
18. USEPA Cryptosporidium: Drinking Water Health Advisory. Office of Science and Technology, Office of Water 2001, EPA-822-R-01-009.
19. Wu, J.; Li, H.; Huang, X. Indigenous somatic coliphage removal from a real municipal wastewater by a submerged membrane bioreactor. Water Res. 2010, 44, 1853-1862.
20. Arnold, B. F.;.; Colford, J. M., Jr. Treating water with chlorine at point-of-use to improve water quality and reduce child diarrhea in developing countries: a systematic review and metaanalysis. Am. J. Trop. Med. Hyg. 2007, 76 (2), 354–64.
21. Ram, P. K.; Kelsey, E.; Rasoatiana; Miarintsoa, R. R.; Rakotomalala, O.; Dunston, C.; Quick, R. E. Bringing safe water to remote populations: an evaluation of a portable point-of-use intervention in rural Madagascar. Am. J. Public Health 2007, 97 (3), 398–400.
22. Colindres, R.; Mermin, J.; Ezati, E.; Kambabazi, S.; Buyungo, P.; Sekabembe, L.; Baryarama, F.; Kitabire, F.; Mukasa, S.; Kizito, F.; Fitzgerald, C.; Quick, R. Utilization of a basic care and prevention package by HIV-infected persons in

Uganda. AIDS Care 2007, 1–7.

23. Makutsa, P.; Nzaku, K.; Ogutu, P.; Barasa, P.; Ombeki, S.; Mwaki, A.; Quick, R. E. Challenges in implementing a point-of-use water quality intervention in rural Kenya. Am. J. Public Health 2001, 91 (10), 1571–3.
24. Sobsey, M. D., Stauber, C. E., Casanova, L. M., Brown, J. M., & Elliott, M. A. (2008). Point of Use Household Drinking Water Filtration: A Practical, Effective Solution for Providing Sustained Access to Safe Drinking Water in the Developing World. *Enviromental Science and Technology, 42*, 4261-4267.
25. Elechiguerra, J. L.; Burt, J. L.; Morones, J. R.; Camacho-Bragado, A.; Gao, X.; Lara, H. H.; Yacaman, M. J. Interaction of silver nanoparticles with HIV-1. J. Nanobiotechnology 2005, 3, 6.
26. Rogers, J. V.; Parkinson, C. V.; Choi, Y. W.; Speshock, J. L.; Hussain, S. M. A preliminary assessment of silver nanoparticles inhibition of monkeypox virus plaque formation. Nanoscale Research Letters 2008, 3, 129-133.
27. Ehdaie, B., Krause, C., & Smith, J. A. (2014). Porous Ceramic Tablet Embedded with Silver Nanopatches for Low- Cost Point-of-Use Water Purification. *Environmental Science and Technology, 48*, 13901-13908.
28. Deborah A. Kuzmanovic, Ilya Elashvili, Charles Wick, Catherine O'Connell, and Susan Krueger Bacteriophage MS2: Molecular Weight and Spatial Distribution of the Protein and RNA Components by Small-Angle Neutron Scattering and Virus Counting. Structure, Vol. 11, 1339–1348, November, 2003. DOI 10.1016/j.str.2003.09.021
29. M. V. Jones, K. Bellamy, R. Alcock and R. Hudson. The use of bacteriophage

MS2 as a model system to evaluate virucidal hand disinfectants. Journal of Hospital Infection (1991) 17, 279-285

30. Jolis, D., Hirano, R., and Pitt, P. (1999). Tertiary treatment using proper microfiltration and UV disinfection for water reclamation.Water Envi ron. Res. 71, 224–231.
31. Lykins, B.W., Koffskey, W.E., and Patterson, K.S. (1994). Alternative disinfectants for drinking water treatment. J. Environ. Eng. 120, 745–758.
32. Oppenheimer, J.A., Jacangelo, J.G., Laine, J.M., and Hoagland, J.E. (1997). Testing the equivalency of ultraviolet light and chlorine for disinfection of wastewater to reclamation standards. Water Environ. Res. 69, 14–24.
33. Woolwine, J.D., and Gerberding, J.L. (1995). Effect of testing method on apparent activities of antiviral disinfectants and antiseptics. Antimicrob. Agents Chemother. 39, 921–923.
34. Grabow, W. O. K. (2001) Bacteriophages: update on application as models for viruses in water. Water SA 27(2), 251–268.
35. Sobsey, M. D., Hall, R. M. & Hazard, R. L. (1995) Comparative reductions of hepatitis A virus, enteroviruses and coliphage MS2 in miniature soil columns. Water Sci. Technol. 31, 203–209.
36. Van Voorthuizen, E. M., Ashbolt, N. J. & Shafer, A. I. (2001) Role of hydrophobic and electrostatic interactions for initial enteric virus retention by MF membranes. J. Membr. Sci. 194, 69–79.
37. Pond, K., & Organization, W. H. (2005). *Water Recreation and Disease: Plausibility of Associated Infections : Acute Effects, Sequelae, and Mortality*:

published.

38. Gall AM, Mariñas BJ, Lu Y, Shisler JL (2015) Waterborne Viruses: A Barrier to Safe Drinking Water. PLoS Pathog 11(6): e1004867. doi:10.1371/journal.ppat.1004867
39. Thompson, S.S., Jackson, J.L., Suva-Castillo, M., Yanko, W.A., El Jack, Z., Kuo, J.C.C., Williams, F.P. and Schnurr, D.P. (2003) Detection of infectious human adenoviruses in tertiary-treated and ultraviolet-disinfected wastewater. Water Environment Research: a Research Publication of the Water Environment Federation, 75(2), 163–170.
40. Thurston-Enriquez, J.A., Haas, C.N., Jacangelo, J., Riley, K. and Gerba, C.P. (2003) Inactivation of feline calicivirus and adenovirus type 40 by UV radiation. Applied and Environmental Microbiology, 69(1), 577–582.
41. Ehdaie, B. (2015). *Development of a Porous Ceramic Tablet Embedded with Silver Nanopatches for Low-cost Point-of-use Water Purification.* (Doctor of Philosophy), University of Virginia.
42. Borkow, G.; Gabbay, J. Copper as a biocidal tool. Curr. Med. Chem. 2005, 12, 2163-2175.
43. Easton, T. A. Taking Sides: Clashing views on controversial environmental issues; McGraw-Hill: New York, 2005.
44. Jackson, K. N. and Smith, J. A. (2018) *A New Method for the Deposition of Metallic Silver on Porous Ceramic Water Filters*. Journal of Nanotechnology, vol. 2018, Article ID 2573015, 9 pages. doi:10.1155/2018/2573015.
45. Schuster, H.; Adams, v. M. H. Bacteriophages. Angew. Chem. 1962, 74.

46. United States Environmental Protection Agency. Secondary drinking water standards: guidance for nuisance chemicals. Available online: http://www.epa.gov/dwstandardsregulations/secondary-drinking-water-standards-guidance-nuisance-chemicals (accessed 22 February 2018).

47. Wagner, Jonathan M.; Zadrozny, Kaneil K .; Chrustowicz, Jakub.; Purdy, Michael D.; Yeager, Mark; Ganser-Pornillos, Barbie K.; Pornillos, Owen. Crystal structure of an HIV assembly and maturation switch. *eLife* **2016**;5:e17063 DOI: 10.7554/eLife.17063

48. Williams, F. P.; Stetler, R. E.; Safferman, R. S. In Procedures for detecting coliphage; USEPA Manual of Methods for Virology; U.S.E.P. Agency: Washington, DC, 2001; pp 16-1-16-9.

49. Bosshard, F.; Armand, F.; Hamelin, R; and Kohn, T. Mechanisms of Human Adenovirus Inactivation by Sunlight and UVC Light as Examined by Quantitative PCR and Quantitative Proteomics. Appl. Environ. Microbiol. February 2013 vol. 79 no. 4 1325-1332

50. Medina-Kauwe LK. 2003. Endocytosis of adenovirus and adenovirus capsid proteins. Adv. Drug Deliv. Rev. 55:1485–1496.

Chapter 6: Conclusions and Future Work

The research presented in this dissertation evaluates the performance of a novel metallic silver and metallic copper formation in ceramic media for disinfection of bacteria and viruses. The results suggest that a change in the production method of ceramic water filters could decrease the cost of production in raw materials and labor cost, while simultaneously removing the risk of exposure to potentially geno-toxic silver nanoparticles.

Each of the lab based experiments points to a similar result when comparing the conventional method of painting on silver nanoparticles (AgNP) after firing and the new method of embedding silver nitrate ($AgNO_3$) prior to firing in ceramic water filters. AgNP filters may perform better initially, but their antimicrobial efficacy decreases in over time, and they tend to produce effluents with silver concentrations above the drinking water standard. $AgNO_3$ filters release lower amounts of silver and perform more consistently in bacterial removal over time. These results from experiments in clean lab conditions are promising, but are not a perfect indicator of how AgNP and $AgNO_3$ filters would perform in expected in home use.

The surface chemistry mechanism between the ceramic and the silver nanoparticle versus the silver nanopatch, could have an effect on the differences in silver release.

Filters produced using the same type and amount of silver nanoparticles at the two productions facilities in chapter 4, have different silver effluent concentrations. This could potentially be due to the different clay source used, but could also be affected by the different firing protocols used. During the kiln firing process, the clay is sintered to form the ceramic. Different temperature patterns could affect the ceramic formation and therefore the silver-ceramic interface. Van der Waals forces could be attracting the nanosilver to the ceramic, and easily releasing during filter use. Physically, the nanopatch formation occurs on the textured ceramic surface and this most likely holds the round nanopatch in place. There are potentially chemical forces occurring between the surfaces, but these should be the same for both the silver nanoparticle and the silver nanopatch. It is also potentially possible that silver ions are attaching to the surface via ion exchange during the mixing process. If clusters of highly negative charge density exist on the surface, multiple silver atoms could be attracted. This could cause a reaction and the formation of a metallic nanopatch during the kiln heating process.

When full-size filters produced in South Africa were tested, $AgNO_3$ filters performed better than their AgNP counterparts. Total coliform and *E. coli* removal were greater for $AgNO_3$ filters (log reductions of 4.06 and 4.11) relative to AgNP filters (log reductions of 3.85 and 3.92). Filters made with copper nitrate ($Cu(NO_3)_2$) performed slightly worse than AgNP filters, but were still effective with log total coliform and *E. coli* removals of 3.33 and 3.54, respectively. Further, the $AgNO_3$ filters release smaller amounts of silver into the treated water than AgNP filters. The mean effluent silver concentration for the Dertig and Mukondeni 0.4 g AgNP filters were 22 and 12 μg/L (respectively), compared to only 2 μg/L for the Mukondeni 0.4 g $AgNO_3$ filter. The result is that not only will the AgNP filters lead to safer consumption, but may also have a longer lifespan, making them a more cost efficient option.

With regards to cost and potential savings, the $AgNO_3$ method offers distinct economic and safety benefits over filters made with AgNPs. $AgNO_3$ is around $1 per gram of silver compared to $2.71 per gram of silver in the form of AgNPs based on 2018 pricing. The result is that the same amount of silver can be used per filter and reduce the material cost, or more silver could be added to potentially improve filter performance while maintaining the same price. Filters produced with the same amount of silver, had the same average log removal of *E. coli.* Filers made with five times as much silver via silver nitrate performed better, but would increase material costs. Ideally, filters could be made with 2.5 times as much silver via silver nitrate and tested. Then the improved performance could be evaluated versus the material cost savings of using the same amount of silver. This does not account for the labor costs saved by eliminating the painting step, nor the health benefits to workers no longer being exposed to potentially geno-toxic silver nanoparticles.

Virus inactivation experiments performed with silver and copper show that the primary inactivation happens within the first 8 h, with little additional inactivation occurring between hours 8 and 24. At the 8 h time point, the 100 μg/L Ag inactivation is 0.42-log and 0.91-log for MS2 and adenovirus, respectively, meaning more than twice as much inactivation occurs with adenovirus as with MS2. While MS2 is commonly used to model virus inactivation by a point-of-use water treatment application, our results suggest adenovirus is a better indicator for silver's performance against viruses. This also appears true for the lower level of copper. With 200 μg/L Cu, after 8 h there is only 0.39 log removal of MS2, while there is a 1.26 log removal of adenovirus.

The use of silver as a disinfecting agent is limited due to the relatively low drinking water standard compared to copper. The use of copper, either as a substitute or in addition to silver, could potentially extend the effective performance of ceramic water filters. The combination of both silver and copper could be ideal for optimizing performance of the ceramic water filter with regards to bacteria, viruses, protozoa (not addressed in this dissertation) and maintaining safe metal concentrations for human consumption. Experiments of including silver and copper in one ceramic disk have not been performed, but the formation of a silver-copper alloy in ceramic could be possible. Imaging and speciation would need to be tested to confirm metal formation and size in the ceramic porous media. Additionally, the effluent metal concentrations and species would need to be identified.

With regards to other technologies, the use of copper in the MadiDrop™ or with SilverDyne could also be suggested for the same reasons mentioned above. Current research on the use of silver or copper wires in an electrolytic method for water disinfection (by C. Hill) could potentially benefit from the use a silver-copper alloy wire. Again, speciation would need to be done to ensure effective ion release from the wire.

Moving forward, the silver nitrate application method should be evaluated on a larger scale with in-home use. Silver nitrate can cause staining of skin and clothes due to the oxidation of silver ions, or even burns in high concentrations. Workers producing filters would need to be trained to ensure proper handling of silver nitrate, just as they have been trained to handle the silver nanoparticles they currently use. Field tests measuring actual use of filters with real source water are necessary to confirm the results above before switching to the new production method could be justified. An understanding of the mechanism of nanopatch and nanoparticle binding to the ceramic could help with explaining the differences in effluent concentrations. Imaging and identification of silver formation needs to be performed for silver nanoparticles filters. A better identification of silver species in the ceramic would also help with the understanding of the surface chemistry occurring and optimization of the release of ionic silver. Also, a larger understanding of silver and copper disinfection of different viruses and the common mechanism, could help with understanding the performance of many point-of-use technologies. Long-term, the use of silver and copper in combination to optimize each metal's strengths could improve point-of-use technologies.