

EVALUATION OF ANTIMICROBIAL METALS FOR ENHANCED PERFORMANCE OF
HOUSEHOLD WATER TREATMENT METHODS

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ABSTRACT

Katie Camille Friedman: Evaluation of Antimicrobial Metals for Enhanced Performance of Household Water Treatment Methods
(Under the direction of Mark D. Sobsey)

Household water treatment and safe storage (HWTS) technologies play an increasingly important role in the reduction of global burden of waterborne disease, putting the capacity to treat unsafe drinking water directly into the hands of the consumer without reliance on centralized treatment or piped water systems. HWTS technologies differ in their ability to reduce viral, bacterial, and parasitic contaminants. Microbial reduction targets for HWTS performance set by the World Health Organization (WHO) are based on acceptable risk levels and \log_{10} reductions. One approach to improve microbial reductions to meet performance targets is the incorporation of antimicrobial metals, which have been utilized for disinfection for centuries. The aim of this research was to evaluate copper, silver, and iron incorporated into ceramic water filters for improved *E. coli* and MS2 bacteriophage reductions and to determine the kinetics and magnitude of *E. coli* and MS2 inactivation with copper and silver ions as additives to water for solar disinfection (SODIS) and safe storage. The incorporation of copper, silver, and iron additives generally improved the reductions of *E. coli* by the ceramic water filters to reach the WHO Highly Protective (4 \log_{10} reduction) target for bacteria, but viral reductions remained well below the WHO Protective (3 \log_{10} reduction) target. Leached copper and silver ions in the filter effluent contributed to additional disinfection of *E. coli* and MS2 with overnight storage time. For stored water, copper and silver ions at concentrations allowable for drinking water showed

the capacity to meet WHO Protective targets for virus reductions after overnight storage due to an apparent synergistic disinfection effect between copper and silver ions. Copper and silver ion additives also significantly increased the inactivation rates of bacteria and more notably viruses in SODIS. *E. coli* reductions were evaluated on selective and non-selective media, and differences in \log_{10} reductions indicated the ability of *E. coli* to resuscitate initially after sub-lethal injury by metals and/or solar irradiation. However, this effect decreased as exposure times increased. Incorporation of antimicrobial metal additives for ceramic water filters, SODIS, and stored water requires further study but shows promise for improved microbial reductions by HWTS technologies.

To my parents in appreciation for their love and support throughout my education.

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LIST OF ABBREVIATIONS AND SYMBOLS

AgNP	Silver Nanoparticles
ANOVA	Analysis of Variance
ASR	Aquifer Storage and Recollection
ATCC	American Type Culture Collection
CFU	Colony Forming Units
CuMP	Copper Microparticles
CWF	Ceramic Water Filter
d_{50}	Median Particle Diameter
DAL	Double Agar Layer
DI	Deionized (Water)
DNA	Deoxyribonucleic Acid
D-PBS	Dulbecco's Phosphate Buffered Saline
EPA	United States Environmental Protection Agency
FeNP	Iron Nanoparticles
GV	Guideline Value
HWTS	Household Water Treatment and (Safe) Storage
LED	Light-Emitting Diode
LRV	Log Reduction Value
LSA	Lauryl Sulfate Agar
MCL	Maximum Contaminant Level
MIP	Mercury Intrusion Porosimetry
nZVI	Nanoparticle Zero-Valent Iron

NTU	Nephelometric Turbidity Units
PB	Phosphate Buffer
PFU	Plaque Forming Units
POU	Point of Use
RNA	Ribonucleic Acid
RR	Risk Ratio
SODIS	Solar Disinfection
TOC	Total Organic Carbon
TSB	Tryptic Soy Broth
TYGA	Tryptone Yeast Glucose Agar
UNICEF	United Nations International Children's Fund
WHO	World Health Organization

CHAPTER 1: INTRODUCTION AND OBJECTIVES

1.1 Introduction

Household water treatment and safe storage (HWTS) technologies play an important role in providing access to safe water where connections to treated, piped household water services are unavailable. Although often recognized as interim water treatment solutions after an emergency such as a natural disaster, HWTS technologies can serve a long-term role for communities where continuous piped water access is not possible or probable due to logistical, political, or financial challenges. HWTS technologies vary in their ability to achieve reductions of protozoa, bacteria, and viruses. Protozoa are the largest class of microbes but may be resistant to chemical treatments such as free chlorine. Bacteria are the most frequently used indicator of microbiological water quality, and many HWTS methods can achieve bacterial reductions. Viruses, the smallest class of microbes, are difficult to remove via size exclusion.

Three HWTS technologies have been selected as the focus of this dissertation. Each technology has a set of challenges to address in order to achieve reductions of microbes that are significant enough to permit attribution of possible health impact. First, the ceramic water filter (CWF) is a HWTS technology that is typically produced in-country from local clay and a burnout material, such as sawdust. By controlling the size of the sawdust that is mixed in with the clay, the manufacturer ultimately controls the size of the pore matrix that is left behind after the sawdust burns out. Although the production and therefore performance quality of CWFs varies greatly based on manufacturer, CWFs have demonstrated the ability to meet specified

reduction targets for both parasites and protozoa but continue to fail to meet virus reduction targets.

Another HWTS technology selected for this dissertation is solar disinfection (SODIS), where water is put into a transparent container and exposed to solar radiation, with disinfection achieved by both exposure to ultraviolet (UV) rays as well as increased temperature (pasteurization). However, effective microbial reductions require sufficient sun exposure for a number of hours, with more extended exposure time necessary during days with cloud coverage. Performance can also vary based on water quality parameters such as turbidity, which can decrease efficacy. Additionally, some viruses are resistant to disinfection by SODIS.

Lastly, safe storage plays an important role in households that do not have access to continuous piped water. For intermittent piped water access, whether with household or community taps, it is common practice to store water in the home to have a sufficient supply until water access is available again. Water storage is also essential for households that obtain water from boreholes, surface water, or water delivery trucks. Water storage is subject to contamination, and even water that has been treated is likely to become contaminated during household storage. HWTS technologies that are suited for use with water storage can not only treat water from unsafe sources but also prevent contamination.

The goal of this research is to evaluate the potential to improve the performance of these selected HWTS technologies with the incorporation of antimicrobial metals: silver (Ag), copper (Cu), and iron (Fe).

1.2 Objectives

Objective 1: To evaluate potential improvements in microbial reductions by testing ceramic filter disks with different concentrations of fired-in silver, copper, and iron additives for the reduction of *E. coli* bacteria and MS2 bacteriophage suspended in chemically defined model test waters for a selection of incorporated metal concentrations.

Virus inactivation remains a challenge in ceramic water filters, preventing this technology from reaching performance targets for all three classes of microbes. These studies tested potential complementary and/or alternative (to the standard of silver nanoparticles) virus inactivation-enhancing metal additives, copper and iron, to quantify their effectiveness for reductions of both bacteria and viruses and determine potential synergies. The study was organized into two phases: an initial screening study to test initial candidate concentrations of silver and copper additives, and a follow-up study with selected silver and copper concentrations in addition to the evaluation of iron additives alone and in combination with silver and copper. Flow rates and metal leaching were also monitored to determine if the filter is not only effective in microbiological reductions but also has a practical flow rate and allowable levels of leached metal ions.

Objective 2: To determine disinfection kinetics and the magnitude and potential synergies of copper and silver ions, separately and in combination, to reduce concentrations of MS2 bacteriophage from laboratory and real world test waters for both dark storage and simulated SODIS exposure.

After primary evidence of improvement in log reduction values (LRVs) of MS2 observed in ceramic filter disks with the incorporation of Ag and Cu additives, batch studies were

conducted to determine the potential of Ag and Cu ions to inactivate MS2 in stored water, with application for stored CWF effluent and more generally for safe storage of water in the household (no light exposure assumed). SODIS studies were also conducted after initial promising results of Ag-Cu ions in the dark. Characterizing disinfection kinetics of these metals, separately and together, will provide further insight into their performance as disinfection agents, reveal if synergistic effects occur for the reduction of MS2, and evaluate the contributions of leached metal ions to microbial reductions during storage time in the filtrate reservoirs of ceramic water filter systems.

Objective 3: To determine disinfection kinetics and magnitude and potential synergies of copper and silver ions, separately and in combination, for the reduction of *E. coli* concentrations in laboratory and a real world test water for simulated dark storage and SODIS conditions, including evaluation on selective and non-selective media to evaluate for potential resuscitation of injured *E. coli*

Stored water is subject to recontamination, where bacteria such as *E. coli* that are introduced to the storage container can multiply, resulting in potentially unsafe water. After initial evidence from improved *E. coli* reductions after overnight effluent storage with relatively low concentrations of leached Ag and Cu, batch disinfection studies with the maximum allowable concentrations of Ag and Cu in drinking water were conducted to determine the potential for disinfection of *E. coli*. In addition, Ag and Cu have been tested separately as SODIS additives but not in combination, so disinfection of *E. coli* by SODIS with Ag and Cu ion additives separately and together was characterized. Lastly, the ability of bacteria with sub-lethal injury to be resuscitated on nutrient media is a recognized effect. Typically, HWTS technologies

are evaluated using selective culture media, which can be harsh enough to prevent the growth of potentially resuscitated bacteria. Samples from the dark and SODIS experiments were evaluated on both selective and non-selective media to determine if there is (a) any difference between resulting \log_{10} reduction values and (b) if so, the implications for technology recommendations.

CHAPTER 2: LITERATURE REVIEW

2.1 Unsafe drinking water and role of HWTS in increasing global safe water access

Although water and sanitation coverage has increased in recent years due to various interventions, approximately 750 million people globally still lack access to an improved water source (UNICEF & WHO 2014). This number may be an optimistic estimate for those with access to safe water because improved sources such as bore holes or piped water are also subject to contamination, as are the water management processes of transportation and storage. Recent increases in coverage are mostly attributed to increased access to piped water (WHO/UNICEF 2014), but piped water is often fecally contaminated and therefore unsafe. Furthermore, many communities face significant barriers to receiving piped services of any kind whether due to lack of infrastructure, technical knowledge, or financial resources. Therefore, alternative technical solutions such as household water treatment and safe storage (HWTS) can provide safe water access, whether in the interim or the long term, without reliance on greater or reliable infrastructure or large capital investments. HWTS technologies can also be deployed relatively quickly after an extreme weather event or natural disaster.

2.2 Microbial and DALY performance targets for HWTS technologies

Improving water quality through the reduction of microbes is associated with the reduction of risk of disease. This risk is commonly represented quantitatively using disability adjusted life years, or DALYs, which represent the magnitude of health effects on a scale of 0

(good health) to 1 (death) and considers duration of time in years lived with disabilities and years of life lost due to premature death (Devleesschauwer et al., 2014). Quantitative microbial risk assessment (QMRA) is used to link microbial pathogen concentrations in drinking water to adverse health outcomes from illnesses and deaths and therefore DALYs (Havelaar and Melse, 2003). The current WHO recommendations for HWTs microbial reduction targets, found in *Evaluating Household Water Treatment Options: Health-based targets and microbiological performance specifications*, present three levels of target microbial reductions for viruses, bacteria, and protozoa that are associated with three different risk levels:

Target	Log ₁₀ reduction required: Bacteria	Log ₁₀ reduction required: Viruses	Log ₁₀ reduction required: Protozoa
Highly protective	≥ 4	≥ 5	≥ 4
Protective	≥ 2	≥ 3	≥ 2
Interim*	Achieves “protective” target for two classes of pathogens and results in health gains		
Summary of performance requirements for small-scale and household drinking-water treatment, based on reference pathogens <i>Campylobacter jejuni</i> , <i>Cryptosporidium</i> and rotavirus (see Appendix 1).			

Figure 2.1 WHO (2011) household water treatment log₁₀ reduction performance targets for three classes: bacteria, viruses, and protozoa

Highly protective targets are associated with a 10⁻⁶ DALY risk level for correct and consistent use over the span of one year. The next level, protective, is associated with a 10⁻⁴ DALY risk level. Although highly protective targets are ideal, protective targets are still

recognized to represent a significant reduction in risk of disease from microbial pathogens for those communities with a significant burden of waterborne disease (WHO 2011). Technologies that meet interim or minimally protective targets for microbial reductions are recognized as they may still result in improved health outcomes as documented by credible epidemiological field studies. The ceramic water filter is currently in this category because it meets protective targets for bacteria and protozoa and has documented epidemiological evidence of reduced diarrheal disease risk from several field studies (Hunter, 2009).

2.3 Overview of current HWTS technologies

HWTS technologies vary in treatment type, cost, effectiveness, and ease of use in developing countries. The following is a summary of currently recognized HWTS technologies:

Boiling is effective in reducing microbial pathogens but will not reduce turbidity or chemical contaminants in water. The major challenges to boiling include access to affordable fuel and the contribution to decreased air quality in the case of inefficient cookstoves and other combustion processes to boil water.

Solar disinfection (SODIS) utilizes two modes of disinfection: 1) pasteurization by elevated temperature and 2) UV disinfection by exposing water to solar radiation through a transparent receptacle. This receptacle can be commercially manufactured, such as the Solvatten unit, or locally obtained, such as a clear PET plastic soda bottle. Efficacy of this method is decreased if the water has high turbidity. This method can only be successfully employed with sufficient (~6 hours) sunlight, making implementation difficult during the rainy season.

Chlorination relies on chemical disinfection through oxidation. Chlorine is available in different chemicals and forms, ranging from dichloroisocyanuric acid tablets placed in water storage containers (e.g. Aquatabs) to sodium hypochlorite solution to be added by the consumer

(e.g. Waterguard). Safe water implementation programs also support pre-dosed dispensing units of chlorine near water access points. Chlorination is effective against most bacteria and viruses, but protozoa such as *Cryptosporidium* or *Giardia* show considerable resistance. Chlorination provides a disinfection residual, contributing to safe storage by protecting from microbial contamination, but it also imparts taste and odor, which may be objectionable to consumers and make successful implementation more difficult in some communities without previous exposure to chlorine.

Flocculation/disinfection utilizes a flocculating agent (such as alum or ferric sulfate) to physically and chemically decrease turbidity and chlorine-resistant protozoa and other microbes in combination with a chemical disinfection agent (such as dichloroisocyanurate or calcium hypochlorite) to inactivate bacteria and viruses. Users must follow specific instructions of stirring time plus settling and disinfection contact time for effective treatment.

Filtration primarily uses mechanical screening to remove microbes and particulate matter based on size occlusion. Some systems such as biosand filters also feature other filtration or microbial removal mechanisms based on biological activity within the filter matrix such as predation by or adhesion to a biofilm as well as adsorption to the biologically colonized filter media. Other example filtration systems include the LifeStraw and the Sawyer filter, both of which utilize hollow fiber membranes.

2.4 Use of metals in disinfection

2.4.1 Introduction

Historically, water has been stored in metal pots long before their disinfection processes were characterized and the mechanisms were understood. Modern studies have since verified the

antimicrobial effects of certain metals. Shrestha et al., (2010) found that copper, silver, and brass pots had bactericidal affects although storage time necessary to achieve complete disinfection by each metal type was organism dependent. Disinfection of bacteria has also been documented by other studies in copper or brass pots (Sharan et al., 2010a; Sudha et al., 2012, 2009, Tandon et al., 2007, 2005)

2.4.2 Copper and Silver Ions

Copper and silver have been in use for many centuries as antiseptic agents. The major disinfection mechanisms proposed for silver ions include the binding of silver ions to the genome (nucleic acid), interaction with the cell membrane, or inhibition of transport of electrons (Thurman et al., 1989). Silver has also been shown to bind easily with sulfhydryl groups such as those found in functional and structural proteins (Morones-Ramirez et al., 2013). Possible disinfection mechanisms for copper ions include inhibition of cell respiratory enzymes, denaturing of or binding with DNA, or generation of radicals (Thurman et al. 1989).

Multiple studies have shown effective antimicrobial action of copper ions. One study showed that water stored in a copper pot versus a glass jar with a copper wire both resulted in no culturable bacteria after 16 hours of exposure. Both systems had approximately the same copper ion concentration despite a significant \log_{10} difference in copper surface area (Sudha et al., 2009). Inactivation studies by Sagripanti et al. (1993) suggest that viruses containing RNA and/or an envelope may be more susceptible to inactivation by copper ions than DNA or non-enveloped viruses. Batch inactivation studies with copper ions showed over 1.5 LRV of MS2 bacteriophage for 0.3 mg/L and over 2 LRV for 1 and 3 mg/L at 6 hours (Armstrong et al., 2017). Malone (2012) obtained similar results for disinfection by copper and zinc ions together

at the same concentrations after 6 hours with 2.0 and 2.5 LRV for 0.2 mg/L and 2 mg/L, respectively. The efficacy of disinfection by copper ions can be reduced due to physical and chemical properties of the water such as low temperature and the presence of organic matter such as humic acid (Sharan et al. 2010a; Sharan et al. 2010b). The negative effect of organic matter was also found by Malone (2012) with an over 50% decrease in LRVs of MS2 by copper and zinc ions in combination.

However, only limited research has been published on characterizing disinfection kinetics of copper and silver ions at different concentrations and when present together in water. Studies from Yahya et al. (1992) found a combination of 400 µg/L copper ions and 40 µg/L silver ions resulted in 0.023 log₁₀ reduction/minute of MS2 bacteriophage and 0.0006 log₁₀ reduction/minute for poliovirus. These rates were significantly greater than copper or silver alone, showing an additive and potentially synergistic effect.

According to the scientific and technical literature, a common application of copper and silver ions for disinfection of water is copper-silver ionization systems, which generate ions to be circulated in systems prone to contamination, such as water heating systems or swimming pools. Most of the literature on this topic has specifically studied use of commercially produced systems to control the respiratory bacterial pathogen *Legionella pneumophila* (Biurrun et al., 1999; Chen et al., 2008; Kusnetsov et al., 2001; Lin et al., 1996; Liu et al., 1994; Miuetzner et al., 1997). Copper-silver ionization systems typically utilize low concentrations of copper and silver ions at an approximately 10:1 ratio. A study by Liu et al. (1994) showed that concentrations of 0.4 ppm copper and 0.04 ppm silver were sufficient to eliminate *L. pneumophila* from a water heating system over time.

Synergy between silver ions and light has also been observed. This may occur due to the binding of Ag ions to DNA, making the genome more vulnerable to inactivation by light (Rahn and Landry, 1973). One study found that while UV-A and visible light alone achieve negligible inactivation of *E. coli*, inactivation of 1.5 LRV with 0.642 mg/L silver ions alone after 30 minutes increased to 2.0 and 4.5 LRV when combined with visible light and UV-A, respectively (Kim et al., 2008). The same study observed a similar effect with MS2 bacteriophage, where inactivation with the same concentration of silver ions resulted in 2.5 LRV after 30 minutes but increased to 4.5 LRV and 5 LRV when the silver ions were combined with visible light and UV-A, respectively. These results indicate a synergistic effect of the combination of light and silver ions to disinfect both *E. coli* and MS2 at concentrations approximately six times that of the EPA maximum contaminant level for drinking water.

2.4.3 Copper and silver nanoparticles

Copper and silver in nanoparticle forms are also widely accepted as antimicrobial agents. Copper nanoparticles were found to significantly inactivate *E. coli* due to proposed disinfection mechanisms of adhesion of bacteria to the particles due to electrostatic charges and the formation of cavities/pits in the cell walls after contact with particles (Raffi et al., 2010). Copper ions also contribute to the disinfection capacity of copper nanoparticles, as shown by a reduction in DNA degradation of *E. coli* when copper nanoparticles were combined with EDTA, a divalent ion chelation agent (Chatterjee et al., 2014). Copper oxide nanoparticles have shown greater antibacterial activity in comparison to NiO, ZnO, and Sb₂O₃ nanoparticles (Baek and An, 2011) and have shown enhanced antibacterial properties when combined with a sub-minimum bactericidal concentration of silver nanoparticles (Ren et al., 2009). Copper nanoparticles have

also shown success as an antibacterial additive to other types of media such as vermiculite (Drelich et al., 2011).

The bactericidal effects of silver nanoparticles in water are well-documented (Choi et al., 2008; Dror-Ehre et al., 2009; Kim et al., 2007; Sotiriou and Pratsinis, 2010). There is disagreement in the literature about the disinfection mechanisms of silver nanoparticles. Proposed disinfection mechanisms include attachment to bacterial cell walls, the creation of “pits” in the cell wall, increasing cell wall permeability, and accumulation in the cell membrane (Sondi and Salopek-Sondi, 2004). Another study concluded that the dominant disinfection mechanism for silver nanoparticles 12nm or smaller in diameter was the release of silver ions, not direct particle interactions (Sotiriou et al., 2012). One study concluded that the particle-specific bactericidal activity of silver nanoparticles was negligible after testing under anaerobic conditions that prevented silver oxidation and the release of silver ions (Xiu et al., 2012). Research has also shown water chemistry can influence the efficacy of disinfection by silver nanoparticles in solution. Disinfection performance was reduced in the presence of divalent cations, presumably due to particle aggregation (Zhang and Oyanedel-Craver, 2012). Steric stabilization of silver nanoparticles allowed for effective disinfection in a variety of water chemistries while electrostatic stabilization was limited to waters with low levels of salt and organic matter (Fauss et al., 2014). The release of ions from silver nanoparticles was found to increase with increasing temperature and decrease with increasing pH or the presence of humic or fulvic acids (Liu and Hurt, 2010).

There have also been investigations of silver nanoparticle incorporation into different media for water treatment. One study tested paper with embedded silver nanoparticles that were created by in situ reduction of silver nitrate. The particle-embedded paper achieved over 6 and 3

LRV of *E. coli* and *E. faecalis*, respectively, with silver ion leaching remaining below the EPA maximum allowable concentration (Dankovich and Gray, 2011). Ionic silver has also been reduced to form nanoparticles in polymers such as polyethersulfone ultrafiltration membranes (Basri et al., 2011). Different types of ceramic substrates amended with silver nanoparticles have also been investigated. One study tested a ceramic candle water filter made from sintering silica with silver nanoparticles and found over 5 LRV of *E. coli* with effluent silver concentrations remaining below 1 µg/L (Ai et al., 2014). Another study tested diatomite and clay filters, using 3-aminopropyltriethoxysilane (APTES) as an agent to facilitate connection of the silver nanoparticles to the ceramic material and found significant biocidal activity by the prepared substrate, resulting in an approximately 5 LRV of *E. coli* in the effluent (Lv et al., 2009). Silver nanoparticles immobilized on a silica surface with minimal leaching performed more effectively than colloidal silver nanoparticles in solution or silver ions at a high concentration, leading to the conclusion that contact with the particles was the primary disinfection mechanism (Agnihotri et al., 2013).

2.4.4 Zero-valent iron nanoparticles and iron ions

Iron in the form of ferric chloride or ferric sulfate are common coagulants used in water treatment processes that work through contribution of Fe^{3+} ions, which form aqua-metal complexes and participate in hydrolytic reactions to form mono-and polynuclear species that facilitate coagulation (Crittenden et al., 2012). Iron can also exhibit antimicrobial effects in water. Virus inactivation has been observed for multiple forms of iron. Brown and Sobsey (2009) found inactivation of eluted viruses after adsorption to iron oxide-enhanced ceramic media. Zero-valent iron nanoparticles (nZVI) were more effective at inactivating MS2 in air-saturated

conditions, highlighting the role of particle oxidation and the release of ferrous ions (Kim et al., 2011a). However, particle-specific effects significantly contributed to disinfection, as illustrated by continued disinfection by nZVI in the presence of the Fe(II) chelation agent phenanthroline. nZVI have also shown bactericidal properties, which were greater under de-aerated instead of air-saturated conditions due to corrosion and surface oxidation of the nZVI (Lee et al., 2008). These studies also found Fe(II) ions could pass through the cell wall into the cytoplasm.

2.5 Ceramic water filters

2.5.1 Introduction

Ceramic water filters (CWFs) are a recommended and relatively widely-used option for household water treatment in many developing countries, especially in Latin America, Southeast Asia, and Africa. CFWs are made primarily from locally available materials, clay and organic burnout material such as sawdust, and require minimal infrastructure and manufacturing capacity, making it one of the few HWTS interventions that can be produced in-country with a local labor force, supporting local manufacturing enterprise. Numerous epidemiological studies have found that ceramic water filters can lower incidence of diarrheal disease in the field (Brown et al., 2008; Clasen et al., 2006, 2004; Du Preez et al., 2008). A recent meta-analysis found ceramic filters to have a significant protective effect that is not found in field studies of other HWTS interventions such as SODIS or free chlorine (Hunter 2009).

Silver-impregnated ceramic water filters are also employed for personal or point-of-use water treatment in developed country settings for application in outdoors and camping settings. One particular product, the Katadyn “high-class” pump filter, uses a silver-impregnated ceramic filter element that features 0.2 μm pores. The company only claims to remove viruses that are

attached to other particles. This product sells for approximately \$350, has a lifetime of ~50,000L, and has a flow rate of 1L/minute (Katadyn 2015).

Presently, there are approximately 35 operational ceramic water filter factories in Asia, Africa, and Latin America (Rayner et al., 2013a). Based on a survey of filter factories, retail prices ranged from \$8-35. Business models vary among factories. These approaches include sales to NGOs and, less commonly, direct sales through retailers or community health workers. Tested financing options range from microfinance loans or installment payment plans to subsidies (PATH 2012; P. Wilson, personal communication, January 23, 2015).

2.5.2 Current microbiological performance

Despite demonstrated health impacts, the microbiological performance of the CWF has room for improvement, especially with respect to removal and/or inactivation of viruses. *E. coli* removal reached an average of 2 LRV over 15 different laboratory and field studies and varied significantly based on testing conditions, manufacturer, and filter composition (Simonis and Basson, 2011; Van der Laan et al., 2014). Initial virus reduction investigations of the CWF found ≤ 0.5 LRV of MS2 bacteriophage (Lantagne, 2001). Long-term field tests of CWFs with and without applied silver showed no statistically significant difference for virus reduction, which was approximately 1-1.5 LRV (Brown and Sobsey, 2010). Virus removal has also been modeled with virus-sized microspheres (0.02 μ m and 0.1 μ m) and was found to be highly variable, ranging from 63-99.6% removal, and showed temporarily increased removal after the application of silver, most likely due to electrostatic effects (Bielefeldt et al., 2010).

Based on current WHO classifications for household water treatment, the ceramic water filter falls under the “Interim” or “Minimum Protection” category because it achieves the

Protective target for two classes of pathogens, specifically bacteria and protozoa, and results in health gains (WHO 2011). Improvement in virus reduction has the potential to increase the WHO classification level of the ceramic water filter from the Interim or Minimum Protection category to the Protective category and therefore potentially increase public health impacts by reducing risk levels associated with poor performance against viruses. Enteric viruses of concern include rotavirus, recognized as a major cause of diarrhea globally (Kotloff et al., 2013), with an estimated 440,000 annual deaths in children under age 5 (Parashar et al., 2006). Recent evidence also documents that noroviruses are a major cause of viral diarrhea globally and contribute substantially to child diarrhea, which is becoming more relevant as rotavirus immunization of infants and children becomes more widely available (Koo et al., 2010).

2.5.3 Initial investigation of silver application

The magnitude of silver leaching depends on the form of silver applied, the concentration applied, and the method of incorporation. In particular, it has been found that silver nitrate leaches more quickly than silver nanoparticles, and that firing-in silver nitrate reduces leaching (Ehdaie et al., 2014; Rayner et al., 2013b). The magnitude of silver leaching depends on the form of silver applied, the concentration applied, and the method of incorporation. Van der Laan et al. (2014) found that storage time, not filter retention time, was the dominant factor for *E. coli* inactivation by CWFs. One silver delivery system, named the MadiDrop, uses the leaching of silver ions from silver-impregnated ceramic tables as the primary mechanism for bacterial reduction, creating a system of slow-release of silver ions for disinfection of drinking water in a storage receptacle (Ehdaie et al., 2014).

2.5.4 Material characterization

Porosity plays multiple important roles in the CWF. First, the dominant filtration mechanism is screening/size exclusion, so pores that are smaller than the target microbes to be removed need to be present. Next, porosity determines flow rate, which is an important characteristic for implementation in the field. From a user-centered design perspective, no matter how effective a filter is, a user will not choose a filter if the flow rate is too slow. Lastly, porosity is also a determining factor in material strength. A highly porous filter might have a faster flow rate, but the CWF needs to have enough mechanical strength to support the weight of approximately 10 L of water along its rim, which is held by the top of the filtered water reservoir. Pore sizes of CWFs characterized using mercury intrusion porosimetry (MIP) were found to range from ~10nm to 100µm with an overall porosity of ~36-47%, which was linearly dependent on sawdust content (Yakub et al., 2013). Van Halem (2006) found similar porosities also using MIP to characterize filters from Cambodia, Ghana and Nicaragua, with porosity values ranging from 27 to 38%.

2.6 Solar disinfection (SODIS)

2.6.1 Introduction

SODIS is the process of putting water in a transparent container, often a polyethylene terephthalate (PET) bottle, and placing the container in direct sunlight for an extended period of time. The recommended minimum exposure time to sunlight is 6 hours (Luzi et al., 2016), although exposure times up to 2 days were needed in a January field study in Haiti to achieve complete bacterial disinfection for all bottles (Oates et al., 2003), indicating a wide range of potential required solar light exposure times. This variability is based on solar light intensity and

water quality, which can change seasonally. An estimated 4.5 million people use SODIS regularly (McGuigan et al., 2012).

2.6.2 Mechanisms and efficacy

SODIS utilizes both optical and heat disinfection through solar irradiance and thermal inactivation. High temperature has been found to be the dominant disinfection mechanism over optical disinfection at temperatures $\geq 45^{\circ}\text{C}$ (Carratalà et al., 2016; McGuigan et al., 1998), although a synergistic effect for the inactivation of *E. coli* for heat and solar irradiation has been observed (Theitler et al., 2012). The efficacy of SODIS has been evaluated for bacteria, viruses, protozoa, and fungi (Lonnen et al., 2005). Some microbes have shown resistance to disinfection by SODIS, such as *Bacillus subtilis* spores (Lonnen et al., 2005), norovirus (Harding and Schwab, 2012), and adenovirus (Carratalà et al., 2016).

Different additives have been explored to increase the efficacy and treatment kinetics of SODIS. Hydrogen peroxide is a commonly tested additive, with significantly increased inactivation rates observed for *E. coli* (Fisher et al., 2008) and *Salmonella* (Sciacca et al., 2010). Lemon and/or lime juice has also been tested as a SODIS additive due to its availability in many low resource settings and the evidence for enhanced inactivation at lower pH values (Fisher et al., 2008; Harding and Schwab, 2012).

2.6.3 Evidence for recovery of bacteria from sub-lethal solar injury

Differences in measured bacteria concentrations in the comparison of microbiological media designed to neutralize reactive oxygen species (ROS) have been reported, indicating the potential for sub-lethal injury and subsequent resuscitation after solar irradiation (Hara et al.,

2004; Khaengraeng and Reed, 2005; Mani et al., 2006; Ray, 1979). Dark storage subsequent to SODIS has been shown to increase bacterial counts after solar radiation, indicating the recovery of bacteria with sub-lethal injury (Giannakis et al., 2015; Sciacca et al., 2010).

2.7 Summary

HWTS technologies create an opportunity to provide safe water at the point- of-use, putting the user in control of water quality despite challenges such as ineffective community water treatment or dependence on contaminated surface or ground water. CWFs have advantages, such as local production and minimal required infrastructure, and disadvantages such as poor virus reduction. The use of silver in the filter, although studied to some extent, still features gaps in understanding. Knowledge of successful applications of metals as disinfection agents and their disinfection mechanisms provides a basis to justify further exploration of the incorporation of antimicrobial metals into HWTS technologies. In particular, copper and iron have demonstrated antiviral activity and will therefore serve as prototype antiviral metal additives to the standard silver additive in CWF in the following studies. For safe storage, the goal is typically to prevent contamination, but the potential for treatment and contamination prevention will be explored based on the evidence for the antimicrobial effects of copper and silver ions. Lastly, there is evidence for improved inactivation for combinations of silver and UV as well as copper and SODIS, but copper and silver have yet to be combined as SODIS additives reported in the literature.

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CHAPTER 3: EVALUATION OF COPPER, SILVER, AND IRON ADDITIVES FOR IMPROVED MICROBIAL REDUCTIONS IN CERAMIC WATER FILTERS

3.1 Introduction

Ceramic water filters (CWFs) are a widely used point-of-use household water treatment technology designed for production and utilization in low-resource settings. Numerous epidemiological field studies have shown that use of ceramic water filters can lower the incidence of diarrheal disease (Brown et al., 2008; Clasen et al., 2004; Du Preez et al., 2008), and a meta-analysis found ceramic filters to have an overall statistically significant protective health effect (RR=0.37 [0.19-0.71]) based on reduced diarrheal disease risk in epidemiological field studies that was not observed with other HWTS interventions such as SODIS or chlorine (Hunter, 2009). Despite demonstrated health impacts with use of CWFs, there are significant opportunities for improvement of the microbiological performance of the filter. *E. coli* reductions vary based on manufacturing practices and protocols (Lantagne et al., 2010), while the removal or inactivation of MS2 bacteriophages, a commonly used fecal viral indicator used to model enteric viruses in water treatment processes (Grabow, 2001), generally remains low at <1 log₁₀ reduction (Salsali et al., 2011; Van der Laan et al., 2014; van Halem et al., 2009). Based on literature values and current WHO performance levels for household water treatment, the ceramic water filter could only meet a “1-star” classification representing “targeted protection” for adequate log₁₀ reduction performance for two of the three pathogen classes, specifically bacteria and protozoa (WHO 2011).

Improving virus reduction performance to the Protective “2-star” level of 3 log₁₀ virus reduction could potentially increase public health impacts of CWFs. Waterborne transmission of enteric viruses is a significant cause of diarrheal disease as well as infectious hepatitis. In particular, rotavirus has been identified as the leading cause of diarrhea globally in children under 5 in the Global Enteric Multicenter Study (Kotloff et al., 2013), although rotavirus incidence may decrease with increasing levels of rotavirus vaccination (Koo et al., 2010). Other viruses identified as major causes of diarrheal disease include adenovirus 40/41 (Liu et al., 2016) and norovirus (Ayukekbong et al., 2015), while Hepatitis A and E viruses cause infectious hepatitis. There have been limited studies investigating methods to enhance virus removal in CWFs, particularly focused on the incorporation of iron oxides. Batch studies using crushed aluminum- and iron oxide-amended ceramic media showed increased adsorption and inactivation of MS2 and φX-174 bacteriophages in comparison with unmodified ceramic media (Brown and Sobsey, 2009). Initial findings suggested CWFs without silver had better removal of MS2 than filters with silver, potentially due to biofilm formation or blocking of adsorptive sites by silver additives (Van Halem et al., 2007); however, a follow-up study did not confirm this effect (Van der Laan et al., 2014).

Copper and silver, used individually, have illustrated effective reduction of bacteria and viruses in drinking water in multiple applications (Armstrong et al., 2017, 2016, Sudha et al., 2012, 2009; Xiu et al., 2012). In addition, copper and silver ions generated electrolytically have been utilized in combination with free chlorine for water disinfection, most commonly as ionization systems used to prevent the growth of *Legionella pneumophila* in water heating systems (Chen et al., 2008; Lin et al., 1996; Stout et al., 2015). However, there are a limited number of studies investigating the combination of copper and silver for disinfection of drinking

water (L K Landeen et al., 1989; Yahya et al., 1992a), and in particular, for application in CWFs. One initial CWF study found that a copper wire placed in the filter receptacle can improve the reduction of *E. coli*, presumably due to either the leaching of copper ions into the water or the direct contact of microbes with the copper surface (Varkey and Dlamini, 2012). Iron has also been investigated as an agent to enhance virus reduction in water treatment. Iron-coated sands utilized in biosand filters have exhibited increased virus reduction attributed to enhanced adsorption in the filtration media (Ahammed and Davra, 2011; Bradley et al., 2011). Additionally, zero-valent iron nanoparticles and associated leached iron ions were shown to inactivate bacteriophage MS2 in solution (Kim et al., 2011a).

The objectives of this research on fired-in silver, copper, and iron ceramic filter additives are to determine 1) the reduction efficacy of *E. coli* and MS2 by ceramic water filters with different concentrations and combinations of fired-in metal additives and 2) the potential contribution of leached metal ions to further disinfect the filtrate during effluent storage time.

3.2 Methods and materials

3.2.1 Metal additives

Silver nanoparticles (AgNPs) with a d_{50} of 30 nm (Ames Goldsmith, USA) were purchased pre-dispersed into water. This source of AgNPs is in use by three CWF factories and is a similar size to the colloidal silver used by many other factories (Rayner et al., 2013b; The Ceramics Manufacturing Working Group, 2011). Copper microparticles (CuMPs) with a d_{50} of 1.2 μm (Ames Goldsmith, USA) were shipped as a powder by the manufacturer. CuMPs were selected instead of Cu nanoparticles due to lower cost and increased availability, making them a more feasible option as a filter additive in low resource settings. Zero-valent iron nanoparticles

(nZVI) with an average diameter <50nm were selected as candidate iron nanoparticles (FeNPs) for the study. nZVI were purchased pre-dispersed in solution (NanoIron S.R.O., Czech Republic). Iron sulfate (FeSO_4) was locally sourced in the Dominican Republic from a chemical supply company.

3.2.2 Ceramic pot filter discs

In this research, ceramic filter disks (D=125mm) were used as a model for the ceramic pot filter. Disks were chosen due to easier transportation from the manufacturing site as well as the ability to test more metal combinations using less manufacturing material and laboratory space and less test water. The precedent for the use of disks as a model for full sized CWFs has been established in previous studies (Oyandel-Craver & Smith 2008, Rayner et al. 2013b). Ceramic filter disks were manufactured at the FilterPure (now Wine to Water) CWF production facility in Moca, Dominican Republic. Two sets of disks were produced, modeled after the factory's standard manufacturing protocol. Set 1 was designed to serve in screening studies, looking at the effect of Ag and Cu concentrations and combinations on microbial reduction performance. Set 2 included a second production of selected Ag and Cu filters from the initial screening set as well as additional filter types using Fe additives. Results from both filter sets were kept separate for data analysis and comparison.

Raw materials for the disks included locally sourced sun-dried clay and sawdust regularly used for filter manufacturing at the factory. Both materials were sieved (#35 mesh/0.762mm) before use. Disks were made in sets of 5, with each disk containing 333g of dry clay and 67g of sawdust. Dry materials were weighed and mixed in a manually operated mixer for 5 minutes at approximately 60 rpm. Next, 240 g of deionized (DI) water (48 g for each disk) was weighed,

added to the mixer and mixed for 10 additional minutes. In the case of AgNP or CuMP incorporation, two methods were employed for dispersion of the metals into the water. For Set 1, the metal powder (CuMP) or solution (AgNP) was weighed and dispersed into the DI water prior to addition to the dry mixture for 5 minutes using a rotary drill bit with a large, flat head attachment. Glycerol (2.5 ml/100ml water) was added as a dispersant for the CuMP. For Set 2, a slurry of 20% of the dry clay was created by adding the clay to the water for each set of disks while using a kitchen hand mixer. Next, the metal particles (AgNP, CuMP, and/or FeNP) were added at selected concentrations, and the solution was mixed for an additional 10 minutes. Metal concentrations were chosen as a percentage of the dry clay weight. Three orders of magnitude range of metals concentrations were used: 0.005%, 0.05%, and 0.5%, referred to as 1X, 10X, and 100X, respectively. For all filter disks, the water (with or without dispersed metals) was added to the mixed dry clay and sawdust then mixed for 20 minutes in a manual hand mixer. The wet clay was molded into balls of approximately 510g, and disks were pressed using a hydraulic press at 2200 psi in an aluminum mold designed and machined in the shop of the UNC Department of Environmental Sciences and Engineering Design Center. Disks were put on shelves to dry for 3-5 days and subsequently fired to 860°C in a wood-burning kiln with a dwell time of 1 hour and then left in the kiln overnight to cool.

Typically, colloidal silver is applied to a filter post-firing. However, a few manufacturers currently use the method of incorporating metal solutions into the clay mixture before firing so that the metal additives are fired-in as was done in this study (Rayner et al., 2013a). There is initial evidence that when fired-in, AgNPs resulted in effluent leached silver concentrations consistently well below accepted limits, even when the silver concentration was increased 10-fold from the standard recommendation (Ren and Smith, 2013). The ability to incorporate higher

metal concentrations without exceeding maximum leaching concentrations coupled with FilterPure's long-term practice of firing in metal additives informed this present study to further investigate fired-in metal additives.

3.2.3 Experimental set-up

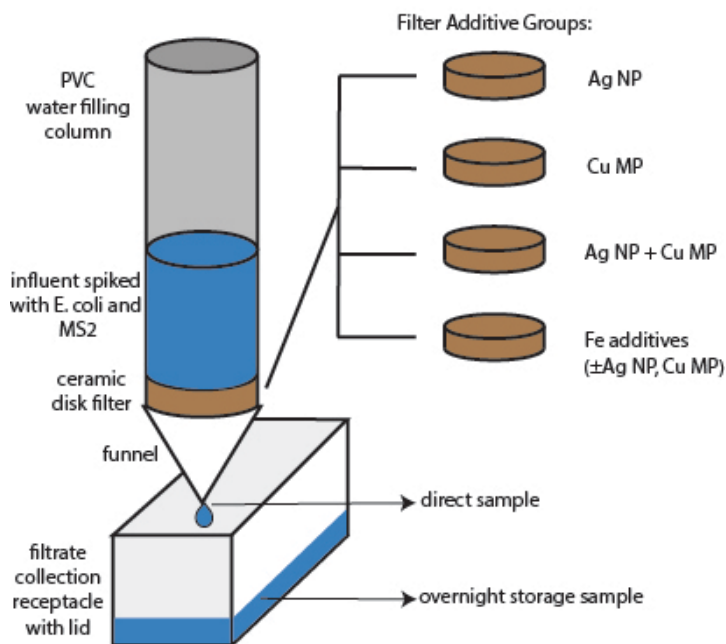


Figure 3.1 Diagram of experimental set-up used to test ceramic disk filters and schematic to illustrate groups of filter additives tested.

The testing apparatus for the disks (Figure 3.1) was constructed from PVC pipe with corresponding pipefittings containing rubber O-rings on each end. The filter disk fit snugly into the bottom O-ring of the pipe fitting, and the interior interface between the filter and the pipe was sealed with a bead of silicone adhesive to prevent leaks. In addition, the outer vertical edge of the filter disk was sealed with epoxy to ensure that all flow was directed through the full depth of the filter. The system was verified for the absence of leaks by testing the sealing system with a filter coated with a top layer of epoxy such that any water leaking could be attributed to a failure of the

silicone seal. Water was successfully contained in the column for a five-day testing period, confirming the efficacy of the silicone seal approach. A funnel was fitted to the bottom of the pipe fitting to direct the effluent into a translucent polypropylene collection receptacle with a lid.

Each testing column was initially filled with five liters of phosphate buffered water (20 mg/L KCl, 20 mg/L KH₂PO₄, 800 mg/L NaCl, 115 mg/L Na₂HPO₄) at pH 7.2 without *E. coli* or MS2, serving as an initial flush to wash out any loose clay or metal particles. Next, five liters of buffered test water spiked with microbes (10⁶ CFU/ml strain WR1 *E. coli*, 10⁷ PFU/ml of MS2 bacteriophage) was added both the day before and the day of each sampling day to ensure all residual clean water free of these test microbes was flushed through. All other days, filters received buffered water without microbes. Sampling for microbial reductions was conducted on days 1-4 (for Sets 1 & 2) and days 9 and 16 (for Set 2), resulting in filtration of 25 L (for Set 1) or 80 L (for Set 2) of water through each filter disk during the testing period. Two 5 ml samples were collected for each filter on a sampling day: 1) directly from the filtrate funnel approximately 1 h after filling the filter with spiked water and 2) from the filter effluent collection receptacle after overnight (~16 h) storage. Set 1 filters were tested at the Wetsus European Centre for Excellence in Sustainable Water Technologies in Leeuwarden, the Netherlands. Set 2 filters were tested at the Delft University of Technology (TU Delft) in Delft, the Netherlands.

Sample collection tubes contained 500 µl of a solution consisting of 18.61 g/L disodium ethylenediaminetetraacetate dihydrate (EDTA) (0.05 M) (Armstrong et al., 2016) and 250 µl of a solution consisting of 1.46 mg/L sodium thiosulfate and 1 mg/L sodium thioglycolate (Tilton and Rosenberg, 1978) to quench the copper and silver ions, respectively, and thereby prevent any

further disinfection from the metal ions in solution. After collecting the overnight sample, each receptacle was emptied and rinsed with DI water.

3.2.4 Test water preparation

WR1 strain *E. coli* (KWR Watercycle Research Institute, Netherlands) was grown overnight in buffered peptone water (10 g/L peptone, 5 g/L NaCl, 3.59 g/L Na₂HPO₄ anhydrous, and 1.5g/L KH₂PO₄) in a 37° shaking incubator at 100 rpm. The next day, the cultures were distributed into 50 ml centrifuge tubes and centrifuged for 15 minutes at 15°C, 3000 rpm. The resulting supernatant was aspirated, and the bacterial pellet was resuspended in phosphate buffered water at pH 7.2. MS2 was added into the test water from a pre-prepared refrigerated concentrated stock of 10¹¹ PFU/ml (GAP EnviroMicrobial Services, Ontario, Canada). MS2 (ATCC 15597-B1) were propagated using *E. coli* Hfr (ATCC 15597). *E. coli* Hfr was grown to log phase in tryptic soy broth in a 35°C shaking incubator. Once *E. coli* Hfr reached log phase, frozen MS2 stock was spiked into the log phase culture and incubated overnight with shaking. MS2 stock was isolated by 3000 x g centrifugation, collecting the supernatant and discarding the pelleted bacteria. MS2 stock was refrigerated and used without purification. The MS2 was not isolated from the original growth medium (TSB), but given the dilution factor (20,000X) after dosing into the test water, any components of the TSB were diluted to negligible concentrations.

Test water was prepared daily in a large polypropylene barrel by mixing DI water (5 L for each filter tested) with a concentrated phosphate buffer solution, resulting in a test water with the following buffer concentration: 20 mg/L KCl, 20 mg/L KH₂PO₄, 800 mg/L NaCl, 115 mg/L Na₂HPO₄. Test water pH was adjusted to 7.2 with HCl. Fifty mL of concentrated *E. coli* solution and 2.5 ml of concentrated MS2 stock was added to the pH adjusted test water and stirred for 1

minute followed by 5 minutes of resting time to allow for further dispersion before dispensing water from the barrel spigot into a 2 L graduated cylinder to fill the filter columns.

3.2.5 Sample analysis

Samples were serially diluted into phosphate buffered water (same composition as test water) and refrigerated until microbial analysis, specifically *E. coli* colony count and MS2 plaque assays, conducted within 24 hours. For *E. coli* analysis, methods for Filter Sets 1 and 2 were analyzed using different methods. For Set 1, *E. coli* samples were enumerated using the spot plate method. For each sample dilution, a row of three 10 µl “spots” were pipetted onto m-Lauryl Sulfate Agar plates, with three dilution rows on each plate. This method was selected to save time during assaying and reading results due to constraints with laboratory hours and lack of laboratory assistance during the first part of the study. This method was chosen with the understanding that due to the small volumes plated, the lower detection limit would be higher as a result. For Set 2, 100 µl amounts of each dilution were pipetted in duplicate onto m-Lauryl Sulfate agar plates and spread using a sterile sample spreader. After the sample was fully absorbed, plates were inverted and placed in a 37°C incubator overnight (18-24 h). Colony forming units (CFUs) were counted after incubation. For MS2 analysis, the double agar layer (DAL) method was employed according to the “Enumeration of F-specific RNA bacteriophages” protocol (ISO 10705-1) with sample dilutions plated in duplicate. Plaque forming units (PFUs) were counted after incubation.

CFU and PFU concentrations were converted to \log_{10} concentrations and \log_{10} reduction values (LRVs) were calculated, where $\text{LRV} = \log_{10} (\text{measured concentration of microbes in the influent} / \text{measured concentration of microbes in the effluent})$.

3.2.6 Statistical Analysis

Statistical analysis was conducted using GraphPad Prism. Analysis of Variance (ANOVA) analysis with the non-parametric Kruskal-Wallis test was used to determine if any filter in each additive set was statistically significantly different, and if so, the Dunn's multiple comparisons test was used to determine significant differences between direct samples from different filter types within each additive set. Significant differences between direct and overnight samples for each filter type were determined using the Wilcoxon matched-pairs signed rank test.

3.3 Results and discussion

3.3.1 *E. coli* reduction by filter disks in Set 1 (Preliminary Findings)

Filter disks from Set 1 were tested for 5 days for a total of 25 L filtered through each disk. As shown in Figure 3.2, the no-metal control filter achieved a mean *E. coli* reduction of 0.78 LRV. There was no significant change in LRV after overnight storage of the control filter effluent, illustrating no growth or die-off occurred in the control filter effluent. This confirms the performance of the no-metal filter to serve as an appropriate control. Two filter types with Ag additives, 10X Ag and 100X Ag, exhibited improved mean LRVs of 2.2 and 2.8, respectively. However, non-parametric ANOVA analysis did not show any significant differences in performance among the mean LRVs of the control and three Ag additive filters, possibly due to the small sample size. The 1X Ag filter did not show improved reductions compared to the control filter. There were slight but not statistically significant increases in mean LRVs in the effluent after overnight storage for the 10X Ag (+1.3 LRV) and 100X Ag (+0.4 LRV) filters. Overall, 10X and 100X concentrations of AgNP appeared to improve *E. coli* reductions,

although more samples would be needed to determine if there was a consistent dose-response effect between concentration of silver incorporated and reductions of *E. coli*.

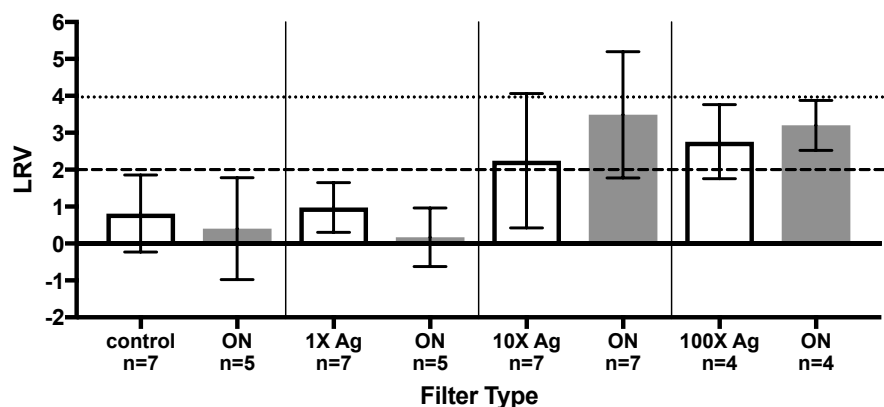


Figure 3.2 Mean LRVs of *E. coli* by Set 1 ceramic filter disks with different concentrations of AgNP additives, determined by CFUs measured in the effluent i) directly from the filter (white bars) and ii) after overnight (ON) storage in the receptacle (grey bars). Dashed and dotted lines indicate the WHO standards for protective (2 LRV) and highly protective (4 LRV) reductions of bacteria. Error bars indicate standard deviation.

Comparable to Ag additives, Cu additives at 10X and 100X concentrations resulted in a modest increase in mean LRV, with 2.2 LRV observed for both filter types (Figure 3.3), both achieving the WHO Protective target reductions. 1X Cu performance (1.3 LRV) was slightly improved compared to the control but did not meet the WHO Protective reduction target of 2 LRV. However, none of these mean LRV differences were statistically significant, possibly due to the small sample size. Comparing LRVs after overnight storage to those in immediate filtrate samples, the 10X and 100X Cu additive filters had similar LRVs that were not significantly different. However, for the 1X Cu filter, the LRV after overnight storage was a negative value that differed significantly from the 1.3 LRV of the immediate filtrate. This result suggests possible *E. coli* regrowth overnight in the receptacle. Cu is known to be an essential nutrient for *E. coli* and other aerobic bacteria, so it is possible that a low concentration of Cu in the stored effluent facilitated *E. coli* growth (Ladomersky and Petris, 2015). Overall, 10X and 100X

concentrations of CuMP showed some improvement of *E. coli* reductions with the potential to meet WHO Protective reduction targets, although more samples would be needed to confirm if there was a statistically significant antimicrobial effect.

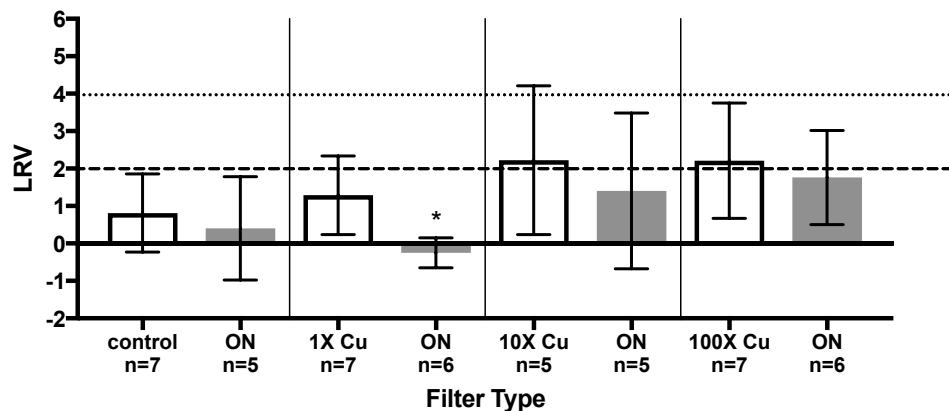


Figure 3.3 Mean LRVs of *E. coli* by Set 1 ceramic filter disks with different concentrations of CuMP additives, determined by CFUs measured in the effluent i) directly from the filter (white bars) and ii) after overnight (ON) storage in the receptacle (grey bars). Dashed and dotted lines indicate the WHO standards for protective (2 LRV) and highly protective (4 LRV) reductions of bacteria, respectively. There were no statistically significant differences between LRVs from different filter types. An asterisk (*) indicates a statistically significant difference between the mean direct and ON LRVs for that filter type. Error bars indicate standard deviation.

Ag+Cu additives in combination achieved improved mean LRVs as concentrations increased (Figure 3.4). The two filter types with the highest concentrations of Ag and Cu additives (10X Ag+100X Cu and 100x (Ag+Cu)) achieved improved mean LRVs of 4.6 and 4.8 (detection limit), respectively. These LRV improvements were significantly different increases, as determined by the Kruskal-Wallis test, and met the WHO Highly Protective performance target. Similar to mean LRVs of 10X Ag and 10X CuMP, the 10X (Ag+Cu) additive resulted in an increased mean LRV of 2.4, which met the WHO Protective performance target but was not a statistically significant increase. As with 1X Ag and 1X Cu, the 1X Ag+1X Cu filter showed an improved mean LRV (1.4) compared to the control, although not a statistically significant increase. There were also no significant *E. coli* reductions observed after overnight storage.

Overall, increased concentrations of Ag+Cu in filters resulted in increased *E. coli* LRVs, indicating a positive dose-response effect.

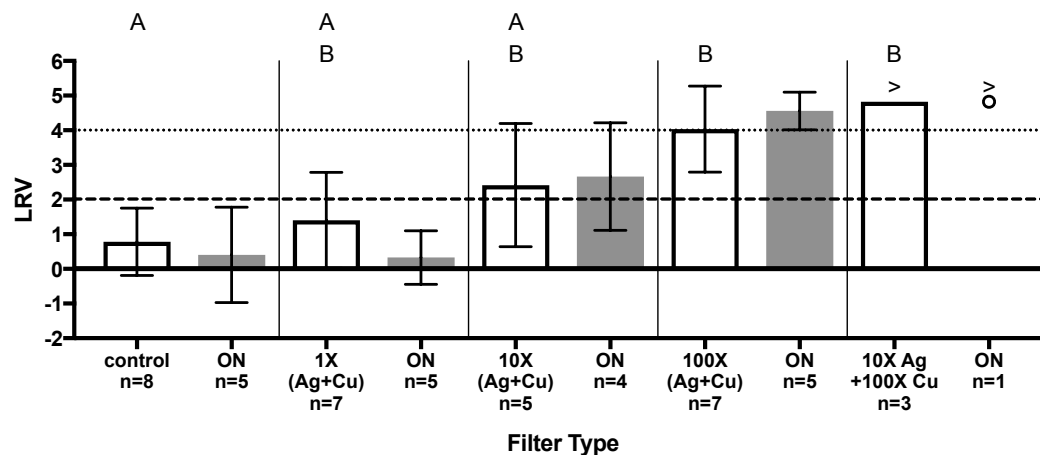


Figure 3.4 Mean LRVs of *E. coli* by Set 1 ceramic filter disks with different concentrations of AgNP and CuMP additives in combination, determined by CFUs measured in the effluent i) directly from the filter (white bars) and ii) after overnight (ON) storage in the receptacle (grey bars). Dashed and dotted lines indicate the WHO standards for protective (2 LRV) and highly protective (4 LRV) reductions of bacteria, respectively. Filter types with mean direct LRVs that are not significantly different are indicated by the same upper case letter. A greater than sign (>) indicates the detection limit was met for all samples. Error bars indicate standard deviation.

3.3.2 MS2 reduction by filter disks in Set 1 (Preliminary Findings)

The data for the control filter lacking added Ag or Cu is limited but is in agreement with many low (<1 LRV) MS2 reductions reported in the literature (Lantagne, 2001; Salsali et al., 2011; Van der Laan et al., 2014), although a field study in Cambodia found ~2 LRV (Brown and Sobsey, 2010). As shown in Figure 3.5, the control filter yielded low MS2 removal (0.36 LRV), and the reduction remained low after overnight storage (0.13 LRV). Ag additives only slightly improved MS2 reduction performance at higher concentrations, specifically 10X Ag (0.52 LRV) and 100X Ag (0.49 LRV) but did not result in statistically significant changes. Overnight storage increased MS2 reduction modestly for Ag additive filters. However, overall mean LRVs of filters with Ag additives remained below 1 LRV, including after overnight storage; this is far

below the WHO Protective performance target of 3 LRV. No statistically significant dose-response relationship was observed. Nevertheless, the small but progressive increases in LRVs with higher Ag concentrations are suggestive of a possible dose-response effect for increased MS2 reduction, with some further increase from overnight storage. Further study would be needed to determine if this possible dose-response effect is consistent and statistically significant.

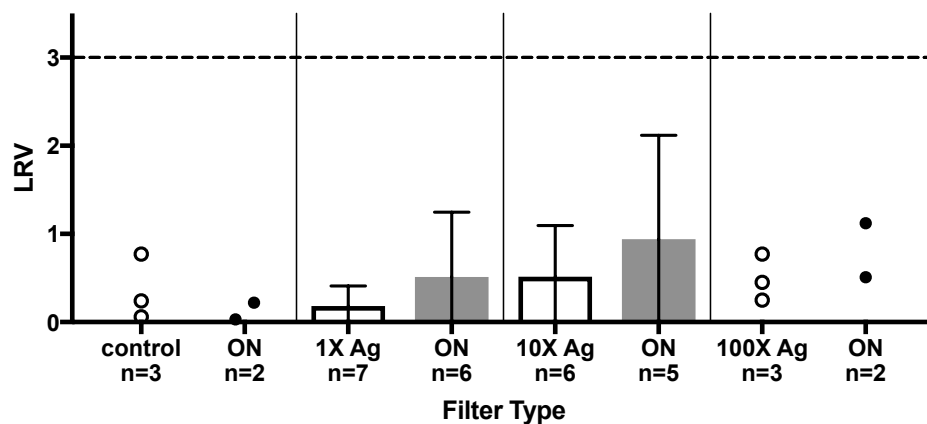


Figure 3.5 Mean LRVs of MS2 by Set 1 ceramic filter disks with different concentrations of AgNP additives, determined by CFUs measured in the effluent i) directly from the filter (white bars/circles for limited data) and ii) after overnight (ON) storage in the receptacle (grey bars/black circles for limited data). The dashed line represents the WHO Protective reduction target (3 LRV) for viruses. There were no statistically significant differences between mean LRVs of the 4 filter types or between the mean direct and ON LRVs for any filter. Error bars indicate standard deviation.

For Cu additives, mean MS2 LRVs remained below 1 (Figure 3.6). Slight but not statistically significant increases in MS2 reduction were observed after overnight storage, but further testing with a larger sample size would be needed to confirm the effect. No clear dose-response effect was observed for direct or overnight samples, and as for Ag filters, LRVs of Cu filters are well below the WHO Protective target for virus reduction of 3 LRV.

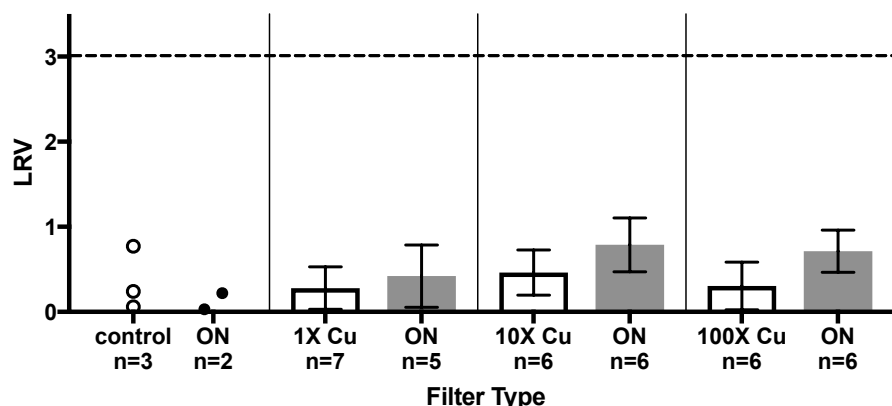


Figure 3.6 Mean LRVs of MS2 by Set 1 ceramic filter disks with different concentrations of CuMPs, determined by effluent sampled i) directly from the filter (white bars/circles for limited data) and ii) after overnight (ON) storage in the receptacle (grey bars/black circles for limited data). For the control filter and corresponding ON data, individual points were plotted due to a low number of replicates. The dashed line represents the WHO Protective reduction target (3 LRV) for viruses. Error bars indicate standard deviation.

Ag+Cu additive filters with higher metal concentrations showed increased MS2 reduction, with mean LRVs of 1.6 for 100X (Ag + Cu) and 1.2 for 10X Ag + 100X Cu, respectively (Figure 3.7). Although these LRVs reflected improved performance from the control, there were no statistically significant differences, most likely due to small sample sizes. Data was particularly limited for LRVs after overnight storage, but LRVs exceeded 2 and illustrated the potential for increased reductions to consider for future studies. No statistically significant dose-response relationship was observed. However, the increased LRVs with higher Ag+Cu concentrations are suggestive of a possible dose-response effect for increased MS2 reduction, with some further increase from overnight storage. As the numbers of replicate trials are low, further study would be needed to determine if this possible dose-response effect is consistent and statistically significant.

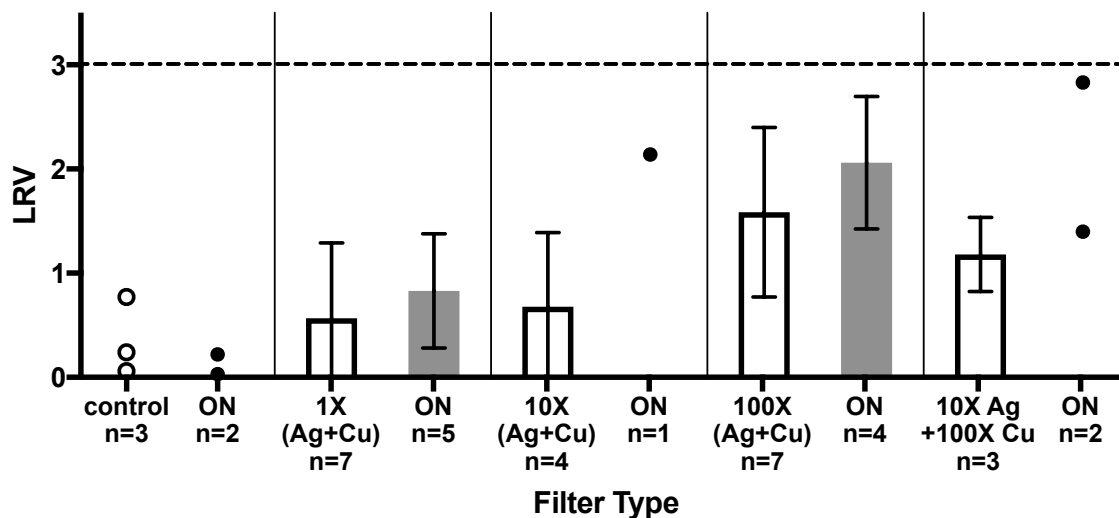


Figure 3.7 Mean LRVs of MS2 by Set 1 ceramic filter disks with different concentrations of AgNPs and CuMPs, determined by effluent sampled i) directly from the filter (white bars/circles for limited data) and ii) after overnight (ON) storage in the receptacle (grey bars/black circles for limited data). For the control filter and corresponding ON data, individual points were plotted due to a low number of replicates. Dashed line indicates the WHO standard for a protective (3 LRV) reduction of viruses. Error bars indicate standard deviation.

3.3.3 Discussion of *E. coli* and MS2 reductions by filter disks in Set 1 (Preliminary Findings)

Overall, Ag and Cu additives, particularly medium to high (10-100X) concentrations, had an apparent effect of improving mean *E. coli* LRVs. Performance of the control filter was low, with a mean *E. coli* LRV of 0.78, and all filters with Ag or Cu additives had improved mean LRVs; however, only two of the filter types achieved statistically significant improvements (100X (Ag+Cu) and 10X Ag+100X Cu). Five filter types reached the WHO Protective performance target of 2 LRV: 10X Ag, 100X Ag, 10X Cu, 100X Ag, and 10X (Ag+Cu), while two filter types reached the WHO Highly Protective performance target of 4 LRV: 100X (Ag+Cu) and 10X Ag+100X Cu. Although sample sizes were relatively small which limited the power of statistical analysis, the results from Set 1 screening study filters were used to determine the compositions for Set 2 filters to be tested in a longer study.

Table 3.1 presents a summary of results including effluent metal concentrations and flow rates. The number of samples subjected to ICP-MS analysis was limited, so early effluent samples from selected filter types were assessed with the assumption that metal effluent concentrations may start higher but decrease over time. These samples were to ensure that leached metal concentrations did not exceed the allowable concentrations set for drinking water by the EPA and WHO (Table 3.2). Observed Ag and Cu concentrations in the effluent were well below the allowable concentrations. The highest measured effluent concentrations for Ag and Cu were from the 100X (Ag+Cu) filter, although these values were only 50% and ~30% of the maximum allowable concentrations for Ag and Cu, respectively, in drinking water.

Table 3.1 Summary of Set 1 (Preliminary) Experiment Results including limited ICP-MS data on Ag and Cu concentrations in the effluent and flow rate data, which was measured one hour after 5 L was added to the water column. Ceramic pot filter flow rate estimates were calculated using equations from van Halem (2006) (Appendix).

Filter Type	Mean LRV <i>E. coli</i>	Mean LRV MS2	Concentration in the Effluent		Flow Rate (L/h)	
			Ag (µg/L)	Cu (µg/L)	Observed Disk	Estimated Pot Filter
Control	0.78	0.36	< 25	<25	0.58	2.9-3.8
1X Ag	0.98	0.18	<25	-	0.51	2.5-3.3
10X Ag	2.2	0.52	35.7	-	0.74	3.7-4.8
100X Ag	2.8	0.49	Error	-	-	-
1X Cu	1.3	0.28	-	<25	0.31	1.5-2.0
10X Cu	2.2	0.46	-	65.4	0.41	2.0-2.7
100X Cu	2.2	0.31	-	235	0.25	1.2-1.6
1X (Ag+Cu)	1.4	0.57	N/A	N/A	0.22	1.1-1.4
10X (Ag+Cu)	2.4	0.68	N/A	N/A	-	-
100X (Ag+Cu)	4.6	1.6	49.5	309	0.13	0.65-0.85
10X Ag+100X Cu	4.8	1.2	37	268	0.22	1.1-1.4

Table 3.2 Maximum concentrations of Cu, Ag, and Fe ions allowed in drinking water.

Metal	Maximum Allowable Concentration ($\mu\text{g /L}$)	Source(s)
Cu	1300	EPA MCL
Ag	100	EPA Secondary MCL, WHO GV
Fe	300	EPA Secondary MCL

Two filter types were selected for further study in Set 2 experiments: 10X Ag and 10X Ag+100X Cu and 10X Ag. The 10X Ag filter is close to the Ceramic Water Filter Manufacturing Group's recommendation (0.05 weight % in this study versus 0.03 weight % for the filter group) and was selected to continue to serve as a reference to standard practice. The 10X Ag+100X Cu filter was selected because of the initial evidence of improved LRVs for direct filter effluent samples for both *E. coli* and MS2 and limited initial evidence illustrating the potential for increased MS2 inactivation after overnight storage of the effluent. This filter type was selected over the 100X(Ag+Cu) filter because:

- 1) Both filters achieved similar performance improvements for *E. coli* and MS2
- 2) A tenfold increase in AgNP concentrations would result in a potentially unrealistic increase in cost and frequency of AgNP purchase for CWF manufacturers in low-resource settings
- 3) The flow rate of the 10X Ag+100X Cu filter (0.22 L/h) was approximately twice that of the 100X(Ag+Cu) filter (0.13 L/h). Estimated corresponding ceramic pot filter flow rates are 1.09 L/h for 10X Ag+100X Cu but only 0.65 L/h for

100X(Ag+Cu), with the latter falling below the recommendation of 1-3 L/h (The Ceramics Manufacturing Working Group, 2011) for sufficient water production

Two additional additives were selected for Set 2 filters: nanoparticle zero valent iron (nZVI) and iron sulfate (FeSO_4). Different forms of Fe have been incorporated into water treatment technologies for the improvement in virus reduction, e.g. in biosand filters (Ahammed and Davra, 2011; Bradley et al., 2011), and CWFs for removal (Brown and Sobsey, 2009; Tsao et al., 2016), and as an inactivation agent in solution (Kim et al., 2011a). However, to the authors' knowledge, Fe additives have not been tested in CWFs in combination with Ag or Cu, and nZVI has not been evaluated as an Fe additive for CWFs. Set 2 filters include two concentrations of nZVI (10X FeNP and 100X FeNP), two combinations of Ag and Fe (10X Ag+100X FeNP & 10X Ag+100X FeSO_4), and one combination of Ag, Cu, and Fe (10X Ag+10X Cu+10X Fe). Set 2 filters were manufactured in the Dominican Republic in April 2015 and tested in October/November 2015.

3.3.4 *E. coli* reduction by filter disks in Set 2

For this study, 8 filter types were tested, including a control with no metal additives (Figure 3.8). The Set 2 control had a much higher mean LRV of *E. coli* (3.1) than the mean LRV for the Set 1 control (0.78). The *E. coli* concentration remained stable after overnight storage, with a mean LRV of 3.0. These results confirmed that the filter with no metal additives served as an appropriate control and that *E. coli* concentrations in this setup were stable. A mean *E. coli* LRV of 4.2 was observed for the Set 2 10X Ag filter, meeting the WHO Highly Protective 4 LRV target for bacteria. The Set 2 10X Ag+100X Cu filter met the WHO Protective target of 2

LRV, with a mean *E. coli* reduction of 3.2 LRV. Notably, after overnight storage, the 10X Ag+100X Cu filter effluent had an increased mean *E. coli* reduction of 4.3, which was a statistically significant increase according to the Kruskal-Wallis test. This increase in *E. coli* reduction achieved with overnight storage resulted in the 10X Ag+100X Cu filter exceeding the WHO Highly Protective reduction target of 4 LRV, confirming the potential of overnight storage to contribute significantly to increased inactivation.

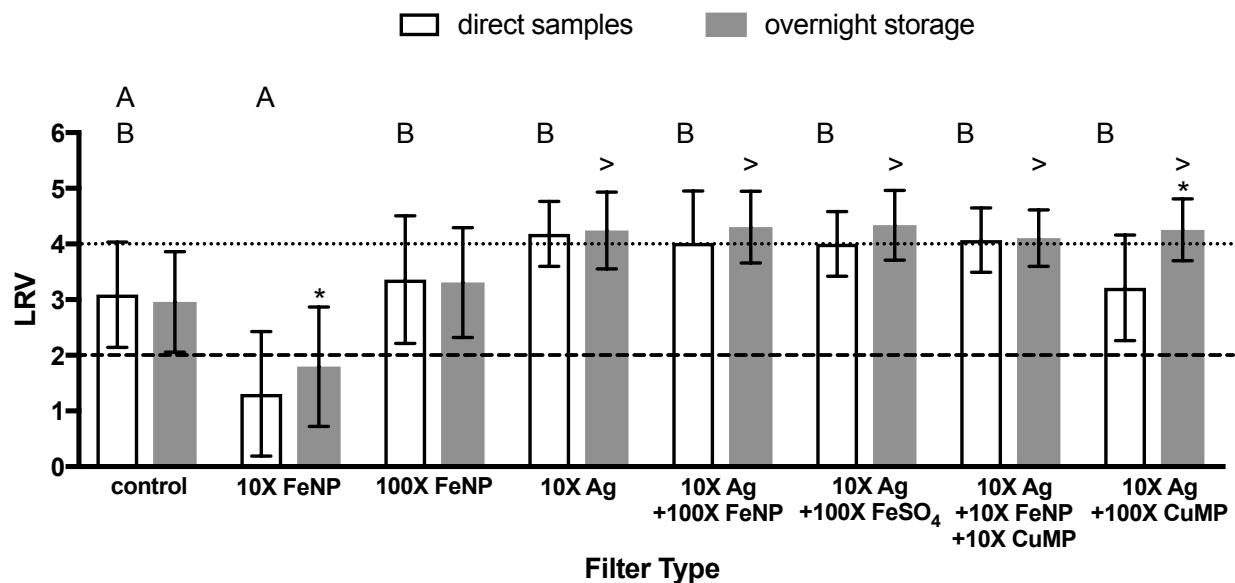


Figure 3.8 Mean LRVs of *E. coli* by Set 2 ceramic filter disks with different concentrations of metal additives, determined by effluent sampled i) directly from the filter (white bars) and ii) after overnight (ON) storage in the receptacle (grey bars). N=12, with 6 samples each from duplicate filters. Dashed and dotted lines indicate the WHO standards for protective (2 LRV) and highly protective (4 LRV) reductions of bacteria. Filter types with direct samples that are not significantly different are indicated by the same upper case letter, and a greater-than sign (>) indicates the LRV reached the detection limit. An asterisk (*) indicates a mean ON LRV that was significantly different from a mean direct LRV for the same filter.

Filters with Fe additives had varied results (Figure 3.8). With performance slightly below but not significantly different than the control, the filter with the lowest amount of iron NP incorporated (10X Fe NP) resulted in a mean LRV of 1.3, therefore falling below the WHO

Protective 2 LRV performance target. The 10X FeNP mean LRV was, however, statistically significantly worse than the mean LRVs of all filter types other than the control. All other Fe additive filters exhibited improved *E. coli* LRVs compared to the control. All three filter types containing different combinations of Fe and Ag, including the filter type with 10X Ag+10X Cu+10XFe, resulted in mean *E. coli* LRVs around 4, with many samples reaching the detection limit. This level of *E. coli* reduction meets the WHO Highly Protective standard of 4 LRV for reduction of bacteria.

3.3.5 MS2 reduction by filter disks in Set 2

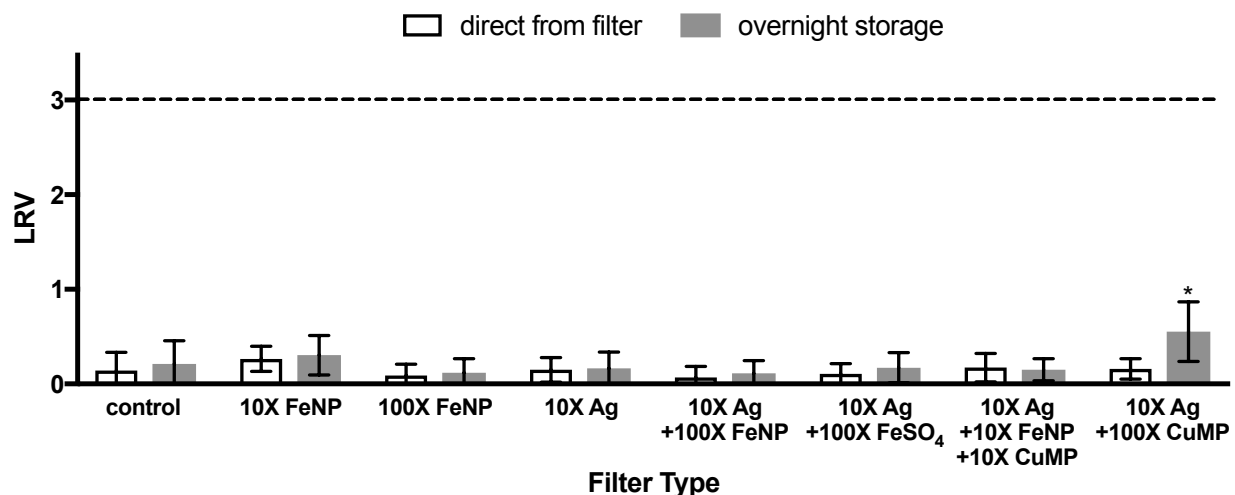


Figure 3.9 Mean LRVs of MS2 by Set 2 ceramic filter disks with different concentrations of Ag, Cu, and Fe metal additives. LRVs were determined from effluent concentrations sampled i) directly from the filter (white bars) and ii) after overnight (ON) storage in the receptacle (grey bars). N=12, with 6 samples each from duplicate filters. The dashed line indicates the WHO standard for a protective (3 LRV) reduction of viruses. An asterisk (*) indicates a statistically significant difference between the direct and ON mean MS2 LRVs. Error bars indicate standard deviation.

Overall MS2 reductions by filter disks in Set 2 were low and far below the 3 LRV WHO Protective performance target. The control filter had a mean MS2 LRV of 0.14, and none of the metal additive filters achieved a significantly different reduction (Figure 3.9). The highest mean LRV of MS2 from direct samples was 0.27, observed from the 10X FeNP filter. Mean LRVs of

MS2 generally remained stable overnight with the exception of 10X Ag+100X Cu, which had a small but statistically significant reduction increase in mean LRV (0.55).

3.3.6 Summary and discussion of *E. coli* and MS2 reductions by Set 2 filter disks

The summary of mean *E. coli* and MS2 LRVs, metal concentrations in the effluent, and flow rates for Set 2 filters is presented in Table 3.3. Overall, mean *E. coli* LRVs were relatively high, including the control, with most filter types meeting the WHO Protective (2 LRV) or Highly Protective (4 LRV) performance targets and only one filter failing to meet either of these targets (10X FeNP). In contrast, all MS2 reductions remained below 1 LRV, indicating no contribution of the Ag, Cu, and Fe metal additives to virus reductions.

Table 3.3 Summary of Set 2 filter disk results including Ag, Cu, and Fe concentrations in the effluent analyzed by ICP-MS (n=6) and flow rate data measured after 1 h of filtration. Ceramic pot filter flow rate estimates were calculated using equations from van Halem (2006) (Appendix).

Filter Type	Mean LRV <i>E. coli</i>	Mean LRV MS2	Mean Concentration in the Effluent (µg/L)			Flow Rate (L/h)	
			Ag	Cu	Fe	Observed Disk	Estimated Pot Filter
Control	3.1	0.14	1.0	0.3	4.4	0.42	2.1-2.7
10X Ag	4.2	0.15	40	-	-	0.17	0.85-1.1
10X FeNP	1.2	0.27	2.7	-	-	0.55	2.7-3.6
100X FeNP	3.4	0.088	2.2	-	-	0.33	1.6-2.2
10X Ag+100X FeNP	4.0	0.069	34	-	2.0	0.42	2.1-2.7
10X Ag+100X FeSO ₄	4.0	0.11	16	-	1.3	0.59	2.9-3.9
10X Ag+100X Cu	3.2	0.16	38	91	-	0.41	2.0-2.7
10X Ag+10X Cu +10X FeNP	4.1	0.17	11	36	1.1	0.39	1.9-2.6

The 10X Ag+100X Cu filter had statistically significant increases in both mean *E. coli* and mean MS2 LRVs after overnight storage, illustrating the ability for leached Ag and Cu ions to contribute to additional disinfection with increased exposure time. In the case of *E. coli*, the

10X Ag+100X Cu mean LRV improved from the WHO Protective (2 LRV) to Highly Protective performance target of 4 LRV for bacteria after overnight effluent storage. For MS2, although the increase was significant, the mean LRV was still below 1 LRV, far below the WHO Protective (3 LRV) reduction target for viruses. All Ag, Cu, and Fe ion concentrations in the effluent were well below maximum allowable levels in drinking water, referenced in Table 3.2.

3.3.7 Comparison of flow rate and LRV

Flow rate and LRV data were compared for both *E. coli* and MS2 Figure 3.10. A linear model was calculated in GraphPad Prism to determine if an effect was present. For *E. coli*, mean LRVs showed no correlation with flow rate. The flow rates of filters with the highest mean LRVs for *E. coli* (~4) ranged from 182-588 ml/h, while the flow rates of filters with the lowest LRVs (<2) ranged from 217-554 ml/h (Figure 3.10a). These flow rate ranges clearly overlap extensively, indicating a lack of correlation.

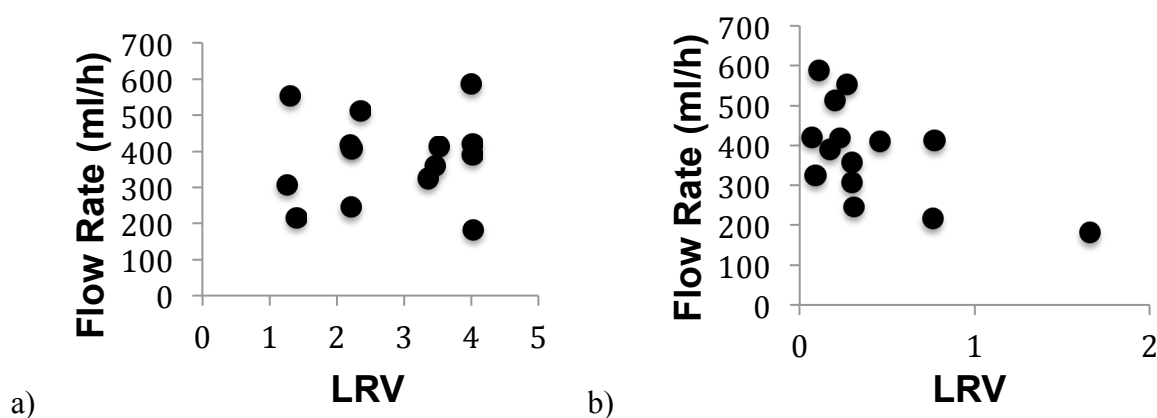


Figure 3.10 WR1 (a) and MS2 (b) data plotted with LRV (x-axis) vs. flow rate (y-axis). A calculated linear model resulted in a slope of 0.0006 (a) and -0.002 (b), indicating a lack of correlation.

Given that flow rate is often used by manufacturers as an indicator for filter quality, it should be noted that a “target” flow rate (1-3 L/h) is not necessarily correlated with reduction of

bacteria. This was recently illustrated in a study where high-flow rate filters were able to achieve target *E. coli* reduction levels (Annan et al., 2014; van Halem et al., 2017). For MS2, there was also a lack of correlation of difference between LRV and flow rate. However, the only filter that exceeded 1 LRV was the slowest filter tested (182 ml/h) (Figure 3.10b), suggesting that contact time with the most effective metal additives may play a role in virus reduction.

3.3.8 Variability between Set 1 and Set 2 filter disks

Despite best efforts to maintain consistent manufacturing methods, there were clear differences in performance between filters with identical composition in Sets 1 and 2. First, testing occurred in two different laboratories, which may have affected results despite best efforts to maintain the same experimental conditions and materials. Next, Sets 1 and 2 were produced at the same facility in the Dominican Republic but at two different times: June 2013 (Set 1) and April 2015 (Set 2). The filter clay was sourced from the same area and processed in the same way but may have had some differences that were not detected. Lastly, kilns were operated by different factory employees. So although the kilns were operated by skilled and experienced artisans who were given the same temperature profile guide, there may have been differences in the outcomes. The kiln firing is the only factor in the manufacturing process where the factory employees worked independently with occasional status checks. Further discussion of the limitations of this study is found in Chapter 7.

3.3.9 Discussion of relevant study

LRVs of Set 2 filters were comparable with a recent study evaluating Ag and Cu additives in full sized CWFs (Lucier et al., 2017) produced at the same factory with the same Ag

and Cu particle additives. All filters in the study had a mean *E. coli* LRVs meeting the WHO Highly Protective standard (4 LRV) and many Ag additive filters achieved over 6 LRV. Similar to the results presented in this chapter, the study showed that Cu additives alone were not consistently effective at improving performance of the control filter. However, there are a couple of factors that make direct comparison difficult. First, Lucier et al. only published ratios of Ag and Cu, but the exact concentration is proprietary information and therefore unavailable for comparison. Next, there were differences in the microbiological methods. There were no metal ion quenchers added, and there was no indication of potential sample storage time. So, any Ag or Cu in solution could continue to act upon the *E. coli* during sample storage and incubation. However, the phosphate buffer used as a dilution medium had a Cl concentration that was potentially high enough to result in the complexation and/or precipitation of Ag or Cu. The type of agar medium used in the spread plate method was not specified, so it is not possible to determine potential effects from a selective or nonselective medium. In addition, metal ion concentrations in the effluent were not quantified, so it is not possible to compare this aspect. Lastly, LRVs of MS2 were unusually high (>3 LRV) for all filters, including the control, which is not consistent with any of the literature cited in this dissertation. Challenges listed in the publication regarding the work with MS2 bacteriophages suggest that direct comparison is not appropriate.

3.4 Conclusions

Fired-in metal additives can improve ceramic filter performance, especially with respect to *E. coli* reduction. However, the metal additives tested did not appreciably improve the ability of CWFs to reduce virus levels in water, with <1 LRVs in filter effluents. Filter effluent from the

10X Ag+100X Cu filter showed small but statistically significant improvements in LRVs after overnight storage with low concentrations of Ag and Cu well below the EPA and WHO recommended maximum allowable concentrations. Further study of the antimicrobial activities of metal ions in water should investigate the opportunity for additional inactivation to occur either in the receptacle of a ceramic filter or in a stored water container. With the knowledge of optimal metal ion doses, next steps can include utilization of a different method to dose the metal into the household water treatment system (e.g. copper wire, water container coatings, metal-impregnated tablet, aqueous solutions, etc.). Batch disinfection studies with controlled doses of Ag and Cu ions added to stored water may contribute to further understanding of the potential increased microbial reductions resulting from extended contact time with leached or dosed concentrations of antimicrobial metals in water.

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CHAPTER 4: CHARACTERIZATION AND BATCH ADSORPTION TESTING OF METAL-AMENDED CERAMIC FILTER DISK MATERIAL

4.1 Introduction

The performance of ceramic water filters (CWFs) is dependent on the characteristics of the porous media, which must address microbial contaminants in one of three ways:

- a) remove contaminants based on size exclusion
- b) inactivate contaminants using a chemical inactivation additive (e.g. Ag)
- c) adsorb and retain and/or inactivate contaminants on the ceramic filter surface

Key material characteristics that are responsible for filter performance are determined by: the source and composition of materials, the formula of materials used in the CWF composition, the manufacturing protocol, the firing temperature and profile, the type of kiln used, and adherence to quality control protocols.

The goal of these studies was to characterize the ceramic filter media to better understand the composition and potential effects on performance. This includes general material characterization with a Scanning Electron Microscope (SEM) coupled with Energy Dispersive X-Ray Spectroscopy (EDX) and X-Ray Diffraction (XRD), in addition to batch adsorption testing to test for differences in the ability of the filter media to adsorb and potentially inactivate MS2 bacteriophage. These studies can potentially provide insight into the ceramic filter disk performance outcomes, and in particular, the lack of effective MS2 reduction with fired in Fe additives despite evidence in the literature of the anti-viral effects of Fe materials incorporated into porous ceramic filters (Brown and Sobsey, 2009; Kim et al., 2011; Tsao et al., 2016). In

particular, zero-valent iron nanoparticles (nZVI) (referenced as FeNP) and FeSO₄ were incorporated to improve log₁₀ reductions of MS2, but no improvements were observed (Chapter 3). Characterization of the ceramic filter media before and after firing will elucidate potential explanations for the inefficacy of these fired-in Fe additives in improvements of MS2 reduction.

4.2 Methods and materials

4.2.1 Batch adsorption material preparation

A subset of disk filters including AgNP and nZVI before and after firing were selected for testing. Filter samples with a higher percentage of nZVI than tested in the disk studies (5%/1000X) were included under the assumption that it would be the best case for adsorption onto nZVI. The 1000X nZVI disks were produced for a complementary master's thesis study that investigated arsenic removal. The batch adsorption procedure was replicated from Brown and Sobsey (2009). Selected ceramic filtration media from ceramic filter disks was crushed using a mortar and pestle then passed through sieves to isolate filter material particles in the 75-250 µm (200 Mesh – 60 Mesh) range.

4.2.2 Batch adsorption procedure

Test water was prepared using a tenfold dilution of Dulbecco's phosphate buffered saline (D-PBS) adjusted to pH 7.2 and MS2 added to reach 10⁶ PFU/ml. Sterile 2 ml polypropylene tubes were used as reaction vessels, with 250 mg of dried ceramic media distributed into each tube. Samples were tested in duplicate. Next, test water volumes of 1 ml were pipetted into each tube. Tubes were then mixed for 30 min by placing the tubes in a horizontal orientation on a shaker plate at 100 rpm. After 30 min of shaking, tubes were centrifuged at 8000 x g and 22°C

for 10 min. The supernatant was aspirated and serially diluted tenfold in a 1:10 dilution of D-PBS and assayed using the double agar layer (DAL) method according to “Water quality – Detection and enumeration of bacteriophages – Part 1: Enumeration of F-specific RNA bacteriophages” (ISO 10705-1). Assays were conducted in duplicate.

After all supernatant was aspirated, the pelleted ceramic media was resuspended in a virus eluent at pH 9.5 (30 g/L beef extract). The procedure was repeated with shaking, centrifugation, and aspiration, dilution, and enumeration of the supernatant to determine the concentration of potentially desorbed MS2 phages. Lastly, a second virus eluent at pH 5.5 (30 g/L beef extract, 170 g/L NaNO₃) was added to the pelleted ceramic media, and the procedure was repeated a third time followed by the enumeration of MS2 for the second virus eluent. PFUs were recorded from the starting test water and the supernatant, and log₁₀ reduction values were calculated. Percent changes in MS2 after both the pH 9.5 and 5.5 elution media were also determined to quantify the amount of MS2 recovered. Additionally, the concentration of Ag and Fe in the supernatant was analyzed by ICP-MS.

4.2.3 X-Ray Diffraction (XRD)

XRD analysis was conducted in the Department of Materials Science and Engineering at Delft University of Technology using a BrukerD8 Advance X-ray Powder Diffractometer with Bragg-Bretano geometry and a Lynxeye position sensitive detector.

4.2.4 Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy

SEM coupled with EDX analysis was conducted at the Chapel Hill Analytical and Nanofabrication Laboratory, Department of Applied Physical Sciences, University of North

Carolina. Filter samples were fractured into small pieces of cross sections and coated with a thin gold layer using a Wessington 108 Auto Suppeter coater. A Hitachi S-4700 Cold Cathode Field Emission SEM was used for imaging and EDX analysis, which resulted in information on the elemental composition of the material.

4.2.5 Micro-Computed Tomography (CT) imaging

Micro CT scanning was conducted in the Laboratory of Geoscience and Engineering, Faculty of Civil Engineering and Geosciences at Delft University of Technology in the Netherlands. A Set 1 sample of the 100X Cu ceramic filter (0.5% Cu/clay weight) was scanned and imaged to examine pore structure and distribution of Cu microparticles. The maximum resolution was approximately 1 micron. ImageJ software was used to approximate measurements such as porosity and particle size in the image.

4.3 Results and discussion

4.3.1 Batch testing of MS2 adsorption to amended ceramic filter media

The fired control filter achieved a mean LRV of 0.9, with minimal recovery of MS2 phages by elution (total of 0.06% of adsorbed MS2 phages recovered by both elutions) as detected by DAL (Table 4.1). For the dried FeNP (5%) filter, a mean LRV of 2.6 was observed, the highest reduction in this sample set. However after both elutions, ~80% of adsorbed phages were recovered by elution, indicating that the majority of adsorbed phages were not inactivated by the FeNP. Fired FeNP (0.05%/10X) had a relatively low LRV, and only ~5% of the adsorbed phages were recoverable by elution.

For the dried AgNP sample, it is unclear why the concentration of MS2 phages slightly increased over the initial adsorption experiment as well as after the first elution. It is possible that the unfired clay material and subsequent elution at basic 9.5 pH lead to the de-aggregation of suspected MS2 aggregates. The fired AgNP filter material resulted in a similar level of adsorption as the fired control and also had very low (<0.05%) recovery of adsorbed MS2 phages. Lastly, the fired 0.5% FeNP + 0.05% AgNP filter material resulted in a mean 1.6 LRV of MS2 phages, with very low recovery of adsorbed phages (<0.05%) after elution. This LRV is over 20 times the low level of reduction observed by the corresponding 100X FeNP + 10X AgNP filter (0.07 LRV, Chapter 3). Concentrations of Fe measured in the supernatant (Table 4.1) were >100X higher than observed in the filter effluent (Table 3.3). So, the concentration of Fe ions potential contributed to altered surface chemistry to support MS2 adsorption or, alternatively, there was MS2 inactivation in solution (Kim et al., 2011). Overall, leached ion concentrations of Ag in the adsorption supernatant (Table 4.1) were similar to those observed in the effluent of corresponding filtration tests (Table 3.3).

Table 4.1 LRVs observed in supernatant, and percent recovery of adsorbed phages for each of two elutions.

Filter Type	Mean LRV (n=2)	Elution 1 (pH 9.5)	Elution 2 (pH 5.5)
fired control	0.9	0.4%	0.2%
dried Fe NP (5%)	2.6	66%	13%
fired Fe NP (5%)	0.95	0.5%	0.3%
fired Fe NP (0.5%)	0.46	4.6%	0.6%
dried Ag NP (0.05%)	-0.04	-180%	1.7%
fired Ag NP (0.05%)	0.92	0.3%	0.1%
fired Fe (0.5%) + Ag NP (0.05%)	1.6	0.3%	0.1%

Table 4.2 Leached metal concentrations in supernatant determined by ICP-MS.

Filter Type	Ag (µg/L)	Fe (µg/L)
fired control	-	149
dried FeNP (5%)	-	559
fired FeNP (5%)	-	213
fired FeNP (0.5%)	-	132
dried AgNP (0.05%)	107	-
fired AgNP (0.05%)	62	-
fired FeNP (0.5%) + AgNP (0.05%)	53	268

4.3.2 XRD analysis

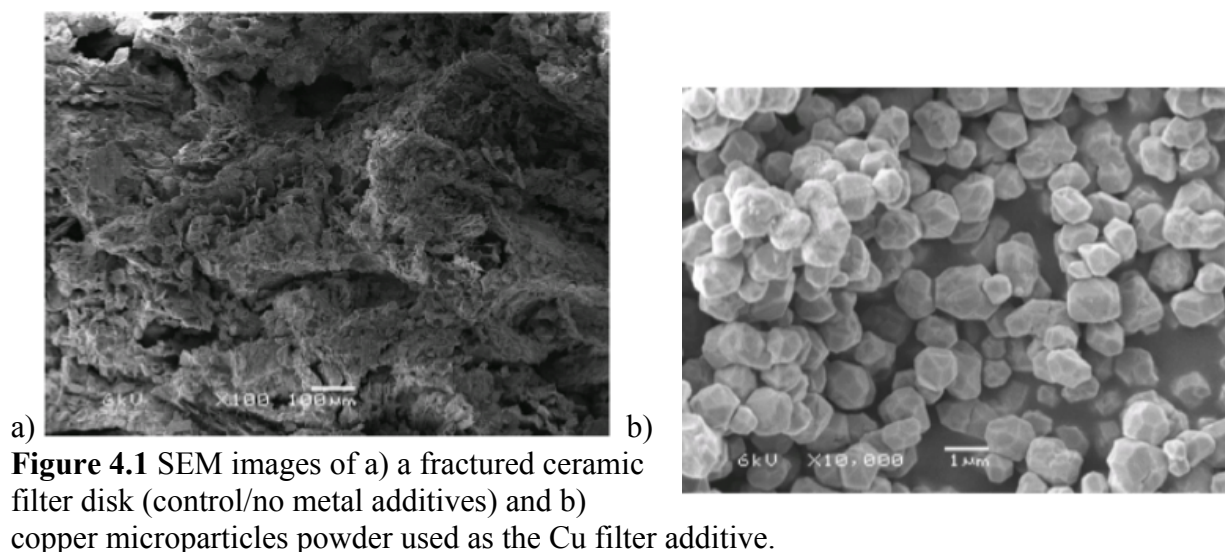
Table 4.3 Minerals detected by XRD analysis. Samples indicated with an asterisk were tested with a longer counting time per set (20 instead of 0.5 seconds/step) to increase the chance of iron detection.

		Quartz	Albite	Kaolinite	Montmorillonite	Nontronite Heated	Iron oxide hydroxide	Hematite	Iron (ZVI)
Unfired	0.05% nZVI	yes	yes	yes	yes	no	no	no	no
	5% nZVI*	yes	yes	no	yes	no	no	yes	yes
Fired	Blank*	yes	yes	no	no	no	no	yes	no
	0.05% nZVI	yes	yes	yes	no	no	no	no	no
	5% nZVI	yes	yes	no	no	yes	yes	no	no

Two minerals, quartz and albite, were identified in all samples, unfired and fired (Table 4.3). Kaolinite was also detected before and after firing in the 0.05% nZVI sample. Montmorillonite, a mineral common to clay, was present before but not after firing, indicating a transformation of this mineral. Hematite, an iron-based mineral, was detected in the unfired 5%

nZVI and the fired blank sample, both of which were subject to a longer measuring time to improve the chance of detecting iron compounds. So, hematite could potentially be detected in all samples if analyzed using the same measuring time. Zero valent iron was detected in the 5% nZVI filter and was no longer detected after firing, indicating a change to the mineral.

4.3.3 SEM-EDX analysis



Initial images of the fractured filter surface provided a first look into the interior structure. An image of the copper microparticles approximately confirmed the size given by the manufacturer (1.2 μm) and showed the generally but not perfectly round shape with a multi-faceted surface. Minerals detected in a sample of the 100X Cu filter are listed in Figure 4.2. Albite was also detected in XRD. Silica, aluminum, and iron are the most abundant elements except for oxygen, which suggests the presence of oxide forms of these elements. Figure 4.3 indicates a Cu particle identified by EDX. Given the scale for the image, the Cu particle is approximately 10 μm in size.

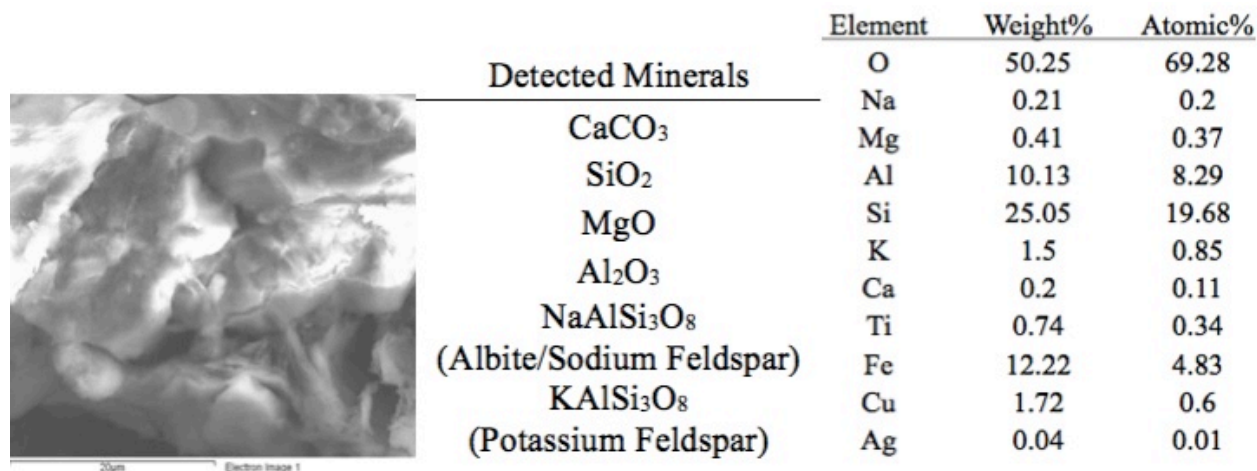


Figure 4.2 SEM imaging of 100X Cu filter with EDX results of minerals detected and elemental composition.

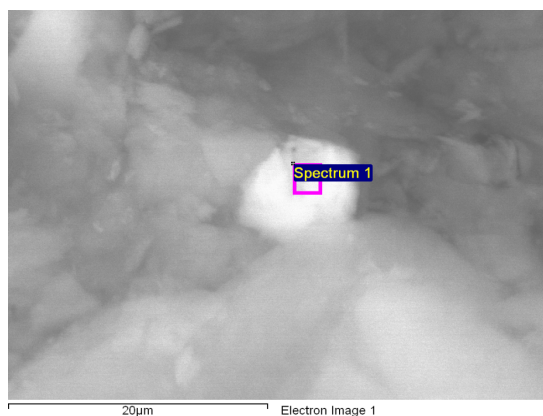


Figure 4.3 SEM imaging of 100X Cu filter with Cu particle identified.

4.3.4 Micro-CT Imaging

Imaging using Micro-CT allows for a look at a cross-section and therefore the pore network of the filter material (Figure 4.4). A threshold was set using ImageJ to try to estimate porosity by detecting all black areas in the greyscale image. The selection of the threshold is relatively subjective, but the porosity was estimated to be 35-40%. This value is in general agreement with the literature, with a range of 36-47% porosity measured using mercury intrusion porosimetry (MIP) for laboratory-made filters (Yakub et al., 2013) and 27-38% measured by MIP for filters from Cambodia, Ghana, and Nicaragua (van Halem, 2006). The sizes of the

presumed Cu particles were also estimated using ImageJ. Particle sizes were most commonly 5-10 μm , which agrees with the particle identified in Figure 4.3. A few presumed Cu clusters were in the 25-200 μm range.

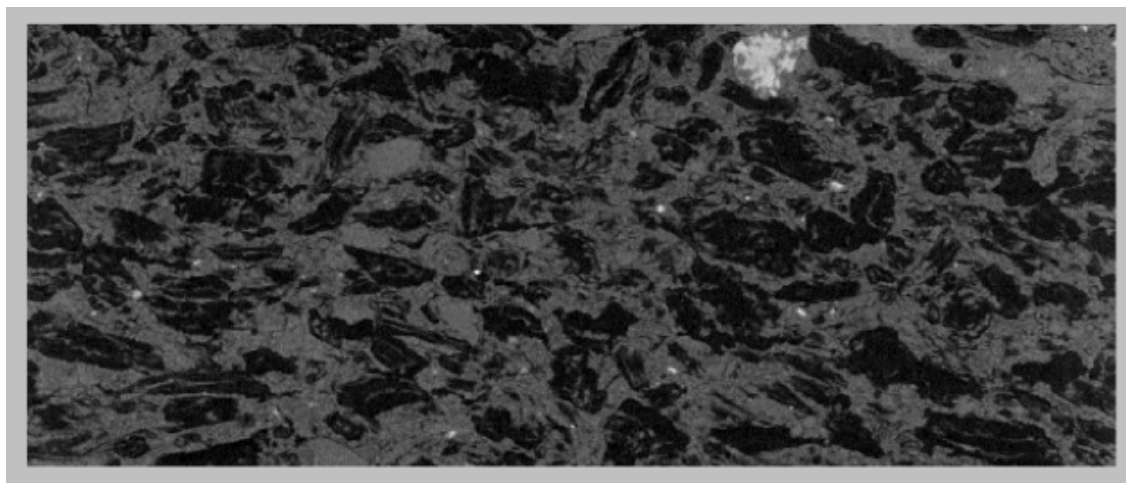


Figure 4.4 Micro CT image of 100X (0.5%) Cu filter. Pores (black) and the filter matrix (grey) and potentially Cu (white) are imaged.

4.4 Conclusions

These material characterization methods helped provide insight into the properties of the selected ceramic filter disks. Silica-based oxides (e.g. quartz) and albite were detected in the filter material by both EDX and XRD. In addition, batch adsorption tests and XRD confirmed that changes to the nZVI that occurred during firing resulted in the transformation of Fe-based minerals and the loss of the ability to adsorb MS2. Brown and Sobsey (2009) found that different Al- or Fe-oxides greatly differed in their ability to adsorb MS2, so it is possible that after firing, different Fe minerals that no longer have the ability for significant adsorption of MS2 were formed. With MicroCT imaging, it was evident that the dispersion method for CuMP (shaking container with CuMP powder in glycerol/water solution) was not effective, with many presumed

CuMP clusters estimated around 5-10 μm , one of which was confirmed using SEM-EDX, as well as some significantly larger clusters in the 25-200 μm range.

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CHAPTER 5: INACTIVATION OF MS2 BACTERIOPHAGE IN WATER BY COPPER AND SILVER IONS WITH AND WITHOUT SIMULATED SOLAR LIGHT EXPOSURE

5.1 Introduction

Human pathogenic waterborne viruses present a risk of infection and illness when contaminated water is consumed (Dongdem et al., 2009; Gall et al., 2015; Wyn-Jones and Sellwood, 2001). Rotavirus was identified as a major cause of moderate-to-severe diarrhea in infants and young children (Kotloff et al., 2013), and other studies have identified adenovirus 40/41 (Liu et al., 2016) and norovirus (Ayuokebong et al., 2015) as major causes of diarrheal disease. Waterborne viral outbreaks are often attributed to contamination of drinking water sources or distribution systems by sewage or during heavy rains (Haflinger et al., 2000; Hedberg and Osterholm, 1993). Household water treatment and safe storage (HWTS) technologies can play an important role by allowing the consumer to treat contaminated water when centralized treatment or distribution systems are either not available or delivering unsafe water (World Health Organization, 2007).

In order to produce virally safe water, household water treatment technologies must physically remove and/or physically or chemically inactivate viruses. Physical methods for virus removal include filtration through nano-scale pores for size exclusion or adsorption of viruses onto a solid phase medium. However, these physical removal methods can be challenging to implement in low resource settings. Filtration media require pores small enough to remove viruses and regular maintenance to manage filter fouling. Virus filters can be relatively

expensive and are often not readily available in low resource settings. Adsorption methods typically have no indication to the user about the efficacy of treatment, and performance can vary based on influent water quality (Bales et al., 1991). Alternatively, virus inactivation methods include chemical disinfectants, such as chlorine or antimicrobial metals, UV radiation with lamps, and solar disinfection (SODIS), which employs both UV radiation in sunlight and heat (pasteurization) for virucidal activity. Free chlorine is effective against most viruses, but proper dosing and sustained behavior change are required. Additionally, undesirable disinfection byproducts may be created (Reckhow et al., 1990), and taste acceptance can be a barrier (Luoto et al., 2011). UV lamps require electricity, relatively expensive equipment and periodic cleaning, and they often do not indicate or warn when performance declines to ineffective levels.

This study focuses on metals as chemical disinfectants in stored water and as SODIS additives because of their advantages in low resource settings, namely: 1) wide availability, 2) relatively low cost, 3) no known disinfection byproducts, and 4) no electricity or specialized equipment needed outside of a water container appropriate for safe storage or SODIS treatment.

Historically, water has been stored in metal storage vessels long before their disinfection processes were characterized and the mechanisms were understood (Alexander, 2009; Shrestha et al., 2010). Modern studies have since verified the antimicrobial effects of certain metals (Borkow and Gabbay, 2005). Disinfection of bacteria has been documented by modern studies using copper or brass pots (Sharan et al. 2010; Shresta et al. 2009; Sudha et al. 2012; Tandon et al. 2007). Copper (Cu) and silver (Ag) are currently used as antimicrobial agents in different types of water treatment technologies. Applications of Ag include ceramic water filters (CWFs) amended with colloidal silver particles or silver salt solutions (e.g., silver nitrate), carbon filters

impregnated with silver as a fouling control agent, and hospital water heating systems (Blanc et al., 2005; Karkhanechi et al., 2013; Kusnetsov et al., 2001; Li et al., 2008; Rayner et al., 2013a).

Although many drinking water studies have focused on the reduction of bacteria, there is also evidence of virus inactivation by some metals, including Ag and Cu in addition to iron (Kim et al., 2011b; Sagripanti et al., 1993) or granular activated carbon (Shimabuku et al., 2016). Ag has shown improved virus reduction when incorporated in treatment technologies such as membranes (De Gusseme et al., 2011). Cu ions have demonstrated the ability to inactivate MS2 bacteriophage in water, with inactivation increasing with dose and exposure time (Armstrong et al., 2017). However, the efficacy of disinfection by Cu ions can be inhibited due to physical and chemical properties of the water, e.g. low temperature and pH (Sharan et al., 2010a). Low concentrations of organic matter can aid in the formation of reactive oxygen species, but at high enough concentrations, organic matter can have an inhibitory effect (Carratalà et al., 2016; Sharan et al., 2010b).

Ag and Cu have also been evaluated separately as SODIS additives. Synergy with metals and exposure to UV lamps or solar light has been noted in a number of studies. Specifically, synergy between Ag ions and UV light for the inactivation of both *E. coli* bacteria and MS2 bacteriophage has been observed (Butkus et al., 2004; Kim et al., 2008). Cu has also been recognized as an additive to water to accelerate disinfection of *E. coli* and MS2 bacteriophage achieved by solar disinfection (SODIS) (Fisher et al., 2012, 2008). However, few published studies have characterized disinfection kinetics of Ag and Cu ions at different concentrations and when used in combination, particularly for virus disinfection (Abad et al., 1994; Yahya et al., 1992b).

Virus disinfection mechanisms proposed for Ag ions include the binding of Ag ions to the microbial genome or inhibition of its electron transport (Thurman et al. 1989). Ag is also recognized to have a strong affinity for sulfhydryl groups such as those found in functional and structural proteins (Thurman et al., 1989). Proposed disinfection mechanisms of Cu ions include denaturing of or binding with DNA and generation of radicals (Thurman et al., 1989). MS2 is also negatively charged at most natural water pH values, so positively charged ions such as Ag and Cu may be attracted to the surface (Thurman et al., 1989). Inactivation studies by Sagripanti et al. (1993) suggest that viruses containing RNA and/or an envelope may be more susceptible to inactivation by Cu ions than DNA or non-enveloped viruses.

It has been proposed that the use of Ag and Cu together may exhibit a synergistic disinfection effect on reductions of bacteria (Huang et al., 2008; Lin et al., 1996). The most common application of Ag and Cu ions for disinfection cited in literature is Ag-Cu ionization systems, which generate ions for circulation in water distribution systems prone to contamination, such as warm water systems or swimming pools. Most studies have focused on the ability of these systems to control *Legionella pneumophila* bacteria (Biurrun et al., 1999; Chen et al., 2008; Kusnetsov et al., 2001; Lin et al., 1996; Miuetzner et al., 1997), using systems with set metal concentrations. To the authors' knowledge, no published studies have incorporated a combination of Ag and Cu for the inactivation of viruses as 1) a combined disinfectant without light exposure or chlorine or 2) SODIS additives.

The objective of this study was to determine the MS2 disinfection kinetics of Ag and Cu ions, used separately and in combination, in two conditions of light exposure: 1) a dark, light-protected condition to simulate water stored in the household and 2) a condition with exposure to solar light to evaluate the virucidal effects of these metal additives when exposed to light. The

experiments were initially conducted in phosphate buffered reagent water and then were repeated in an environmental water sample to gather initial insight into applicability of these metal additives to solar disinfection of real-world waters. The results of this study may guide additional and/or improved applications for the incorporation of Ag and Cu into household water treatment technologies.

5.2 Methods and materials

5.2.1 Experimental design

Ag and Cu concentrations were selected to adhere to maximum allowable/recommended levels in drinking water: 0.1 mg/L Ag and 1 mg/L Cu (World Health Organization, 2011a). These metal concentrations were tested separately (Ag, Cu) and in combination (Ag+Cu) (Figure 5.1). A control with no Ag or Cu was included in all experiments. The metal conditions and control were tested in two environments: dark (foil-covered) to simulate stored water and light-exposed to simulate SODIS. For light-exposed experiments, additional metals-free controls were included: one control was open to the sunlight exposure, and one was covered in foil to prevent sunlight exposure. Tests were conducted in phosphate buffered water (PB), and SODIS tests were sampled after several light exposure times. Subsequently, SODIS tests were also conducted in an environmental water sample that was collected from an aquifer storage and recollection (ASR) water treatment plant.

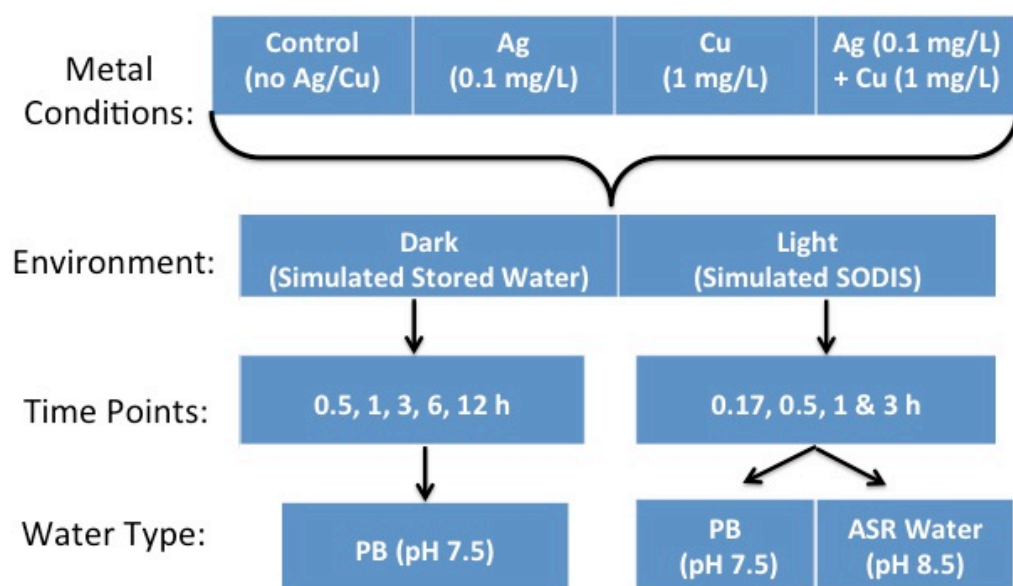


Figure 5.1 Experimental design for water disinfection with Ag and Cu metal ions with and without light exposure

5.2.4 Test water preparation

The phosphate buffered test water (PB) was composed of 1 mM phosphate buffer (39 mg/L $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 192 mg/L $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$) autoclaved and adjusted to pH 7.5 using NaOH. The environmental water sample for the additional SODIS tests was collected from the Dunea Aquifer Storage and Recollection (ASR) water treatment plant (The Hague, The Netherlands). The ASR sample was taken from the water recovered after passage through the sandy aquifer and refrigerated after collection. Table 5.1 lists the measured water quality parameters of the ASR water. Metal solutions of AgNO_3 and CuSO_4 were prepared by diluting reagent-grade 0.1 M stock solutions (Sigma Aldrich) into autoclaved deionized water, creating an intermediate dilution from which the reaction vessels were dosed in order to add volumes that were large enough to measure accurately but small enough to not alter the test water volume

significantly. Metal solutions were kept in polypropylene tubes covered with aluminum foil to prevent light exposure and prepared weekly, with stable concentrations confirmed analytically by inductively coupled plasma mass spectrometry (ICP-MS) (PlasmaQuant MS, Analytik Jena, Germany).

Table 5.1 Measured water quality parameters of Aquifer Storage and Recollection (ASR) water.

Water Quality Parameter	Concentration (units)
pH	8.5
TOC	1.83 mg/L
Turbidity	2.4 NTU
Ca	68.7 mg/L
Na	33.5 mg/L
Mg	8.85 mg/L
K	5.15 mg/L
Si	4.34 mg/L
Sr	279 µg/L
P	71 µg/L
Fe	60 µg/L
B	49 µg/L
Ba	23 µg/L

A refrigerated liquid stock of MS2 (ATCC 15597-B1) of $\sim 10^{11}$ PFU/ml was prepared by GAP EnviroMicrobial Services (Ontario, Canada). MS2 was propagated using log-phase *E. coli* Hfr (ATCC 15597) grown in tryptic soy broth (TSB). After overnight shaking at 35°C, *E. coli* was sedimented by centrifugation at 3000 x g, and the supernatant was collected and refrigerated until use. The MS2 stock first was diluted 1000-fold into buffered water ($\sim 10^8$ PFU/ml) and dosed from the dilution into the buffered test water at a ratio of 1:1000 to achieve a concentration of $\sim 10^5$ PFU/ml.

5.2.2 Experimental procedure

Volumes of 50 ml of MS2-spiked test water were distributed into 200 ml acid-washed, autoclaved glass beakers that were used as reaction vessels. For experiments without light exposure, beakers were fully covered with aluminum foil, and light exposure was minimized during sampling. At $t=0$, concentrated metal solutions (200 μl of 0.0308 mM AgNO_3 solution for Ag and Ag+Cu conditions and 250 μl 3.13 mM CuSO_4 solution for Cu and Ag+Cu conditions) were dosed into corresponding magnetically stirred reaction vessels. Samples of 2.75 ml were collected at the following time points: 0.5, 1, 3, 6, and 12 h for dark experiments and 0.17, 0.5, 1, and 3 h for light exposed experiments, giving three overlapping time points for comparison of disinfection kinetics. Upon collection, samples were neutralized with 35 μl of 0.1 M sodium thiosulfate and 275 μl of 0.05 M EDTA and mixed by vortexing. Samples were refrigerated until analysis, which occurred within 12 h. Experiments were conducted in triplicate.

5.2.3 Light exposure

For samples with light exposure, beakers were placed in a solar light simulator (Atlas Suntest XXL) fitted with a daylight filter emitting a spectrum in the range of 300-1200nm. Experiments were run with a light irradiance of 550 W/m^2 . Time points of 0.17, 0.5, 1, and 3 h correspond to fluence values of 33, 99, 198, and 594 J/cm^2 , respectively. To maintain constant temperature, reaction vessels were placed in a 20°C water bath connected to a thermostat controlled recirculating water cooler (Julabo FL 300). The reaction vessels were submerged in the water bath to the level of the water inside the beaker ($\sim 4 \text{ cm}$) and left directly open to the simulated sunlight. Samples were continually stirred via magnetic stir bar. Absorbance of both PB and ASR water was determined to be negligible due to published absorbance spectra for PB

and an environmental sample (Swiss tap water) with a similar TOC concentration to the ASR water, where the only absorbance occurred under 200nm, which is outside of the 300-1200 nm spectrum emitted by the Atlas XXL (Carratalà et al., 2016).

5.2.4 Microbial assays

Samples were serially diluted tenfold in Dulbecco's phosphate buffered saline and assayed for MS2 bacteriophage using the double agar layer (DAL) plaque assay (ISO 10705-1). The top agar layer consisted of 1 ml of sample dilutions combined with 2.5 ml molten top agar and 1 ml of log-phase WG49 *Salmonella typhimurium* culture grown in tryptose yeast extract broth (TYGB). The mixture was tempered to 45°C in a sterile glass tube, and the tubes were gently mixed then poured immediately onto prepared TYGA bottom agar plates. After solidification of the top agar layer, plates were inverted and incubated overnight at 37°C. Plaque forming units were enumerated, MS2 concentrations were calculated and resulting log₁₀ reduction values were determined by subtracting log₁₀ concentrations at time = t from initial log₁₀ concentrations at time = 0.

5.2.5 Data analysis

Data were stored and standard deviations were calculated in Microsoft Excel. Further data analysis was conducted in GraphPad Prism (Version 7.0). Linear regression models were performed on LRV versus time data to determine first-order inactivation rates for comparison between experimental variables and conditions. All data represent the average of triplicate experiments with each sample plated in duplicate.

5.3 Results and discussion

5.3.1 Inactivation of MS2 by metal additives in PB in dark conditions

Ag and Cu were tested individually and in combination in buffered water at pH 7.5 and 22°C (Figure 5.2). Under dark conditions, Ag resulted in relatively low levels of inactivation, with only ~0.35 LRV observed after 6 and 12 h. A higher level of inactivation was observed with Cu, with 1 LRV at 6 h and almost 2 LRV at 12 h. However, neither the Ag nor Cu conditions alone achieved the WHO 3 LRV protective target within 12 h. Generally, increased exposure time resulted in increased disinfection for metal-dosed conditions with the exception of Ag, for which the LRV appeared to plateau after 6 h.

Cu inactivation increased approximately linearly, with 0.40, 1.00, and 1.96 LRV observed at 3, 6, and 12 h, respectively. This observed LRV rate was approximately half of the rate observed in a similar study (Armstrong et al., 2017). However, this difference in LRV may be due to the differing experimental conditions such as use of a carbonate buffer. One study showed that the antimicrobial effects of Ag were enhanced and/or inactivated in the presence of 20 ppm of carbonate due to the binding of carbonate with peripheral proteins, decreasing an inhibitory effect and allowing increased uptake of Ag ions and resulting capsid damage (Swathy et al., 2014). Although the study was focused on Ag, the underlying concept of carbonate changing surface properties could potentially explain enhanced performance of Cu in the presence of carbonate buffer.

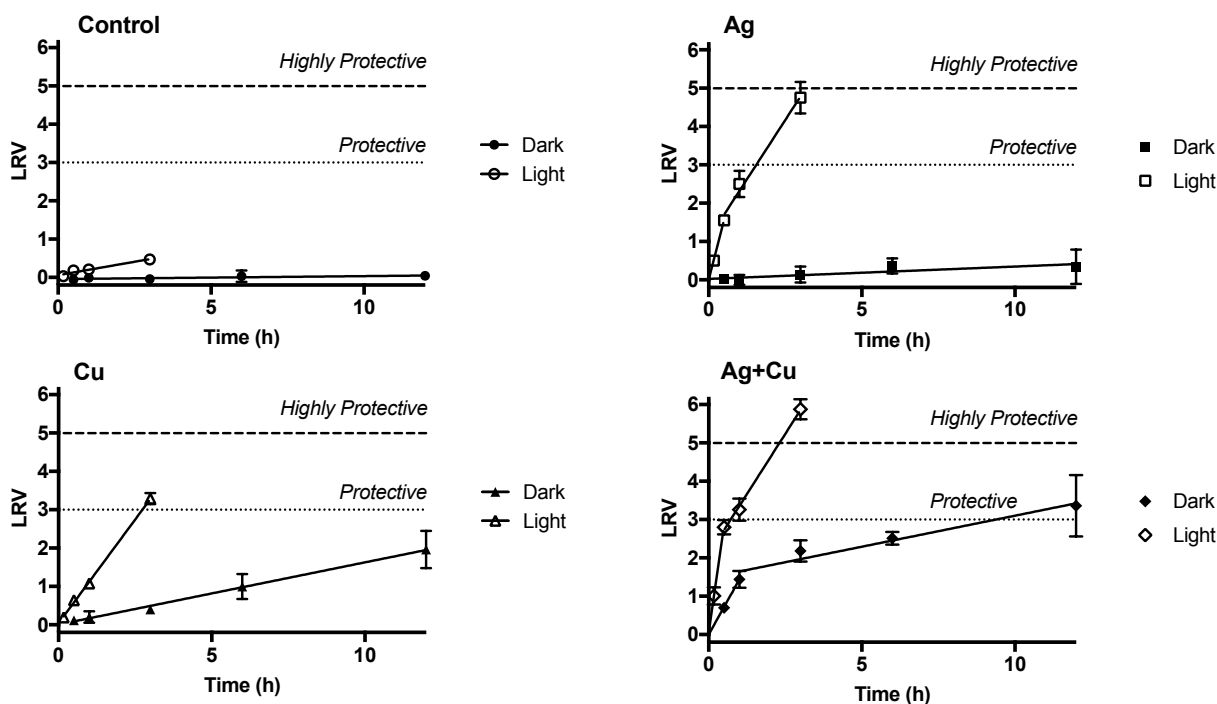


Figure 5.2 LRVs of MS2 and linear regression inactivation models for each metal condition (control, Ag, Cu, Ag+Cu) in dark (filled symbols) and solar light-exposed (open symbols) conditions. Dotted and dashed lines represent WHO target LRVs for protective and highly protective household water treatment performance, respectively.

The combination of Cu+Ag resulted in the highest LRVs, at 2.5 and 3.4 LRV by 6 and 12 h, respectively (Figure 5.2). Ag+Cu after 12 h of exposure was the only dark condition to achieve the WHO Protective target of 3 LRV. Unlike the apparent tailing observed in MS2 inactivation in the Ag only condition in PB, there was no such effect in the Cu only sample, with log-linear increases in reductions over time. When comparing the sum of the Ag only and Cu only condition LRVs to the observed Ag + Cu LRVs in PB, the combined exposure showed higher LRVs than the sum of the single metal exposure (Figure 5.2). This apparent synergistic effect is present from the first time point and reaches a peak at 3 h, where the observed Ag+Cu LRV of 2.2 was approximately four times the sum of Ag only and Cu only result of 0.54 LRV.

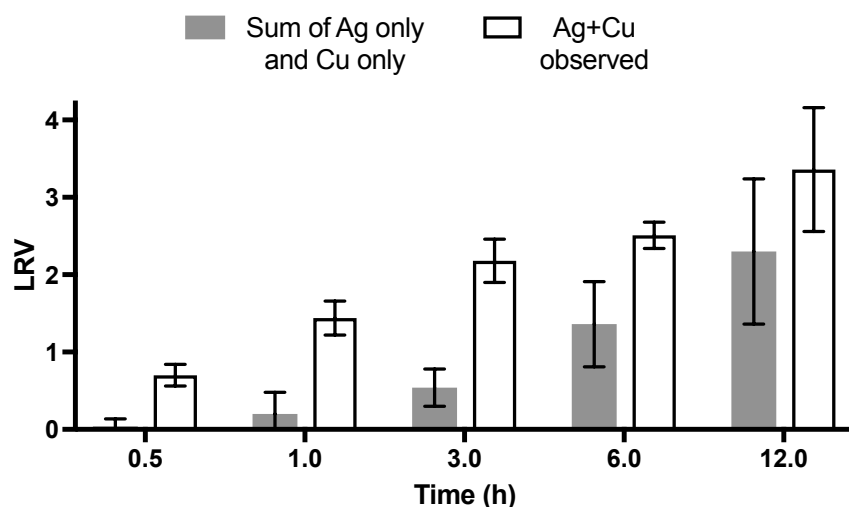


Figure 5.3 Comparison of mean MS2 LRVs in PB at 22°C over time in a dark (foil-covered) environment. The grey bars represents the sum of results from Ag and Cu trials. The white bars represents the results for the combination of Ag and Cu ions. LRVs represent the average of triplicate experiments, and error bars represent standard deviation.

5.3.2 Inactivation of MS2 by metal additives and solar light exposure in PB

Simulated solar light exposure tests were conducted to determine the amount of inactivation that occurs with light exposure in the absence of elevated temperature pasteurization by submerging the test vessels in a temperature-controlled water bath. Figure 5.2 shows the LRVs observed in each test condition at 0.17, 0.5, 1, and 3 h. Two non-metal controls were used: a dark control covered in foil to quantify any inactivation attributable to effects in the absence of light and an open control where inactivation would include the effects of light exposure. The dark control confirmed no inactivation occurred in the absence of Ag, Cu and light (data not shown). Inactivation did occur in the light control, up to 0.47 LRV at 3 h exposure. Test conditions with dosed metals and light exposure resulted in a consistent increase in LRV with increasing exposure time. The extent of inactivation with light exposure in the presence of Ag+Cu was the highest, reaching 3.3 LRV at 1 h and exceeding the WHO 3 LRV protective performance target, and reaching 5.9 LRV at 3 h, exceeding the WHO Highly Protective

performance target. Light-exposed water with Ag alone and Cu alone were less effective than the Ag+Cu combination, but they both exceeded the protective target of 3 LRV by 3 h, with 4.8 LRV for Ag and 3.8 LRV for Cu. Based on the observed rates of reaction, Ag alone and Cu alone were on a trajectory to meet or exceed the 5 LRV WHO Highly Protective performance target within 6 h.

5.3.3 Efficacy of disinfection by metals and solar light exposure in an environmental water sample

In addition to PB, SODIS tests were conducted in ASR water with 0.17, 0.5, 1, and 3 h time points of light exposure to allow for the comparison of results when natural organic matter is present (1.83 mg/L TOC) and pH is higher (8.5). ASR water LRVs observed for the control were not statistically significantly different from those observed in PB across all overlapping time points (Figure 5.4). For Ag, LRVs observed in ASR water were similar and not significantly different from those observed for PB for overlapping time points, with 2.4 and 3.0 LRV observed after 1 h, respectively.

Notably, the two conditions with Cu (Cu and Ag+Cu) both showed statistically significantly improved LRVs in ASR water starting at 0.5 h for Cu and 1 h for Ag+Cu (Figure 5.4). In the case of Cu, the observed LRV after 1 h of light exposure was increased from 1.1 in PB to 3.3 in ASR water, therefore meeting the WHO Protective target level of performance in ASR water. Additionally at 1 h, Ag+Cu LRVs further increased in ASR water from 3.3 at 0.5 h to 4.9 LRV, approaching the WHO Highly Protective performance target of 5 LRV. These results could potentially be explained by the presence of a relatively low concentration of TOC,

which can lead to the formation of reactive oxygen species (ROS) that can contribute to inactivation (Ferraudi and Muralidharan, 1981; Romero et al., 2011).

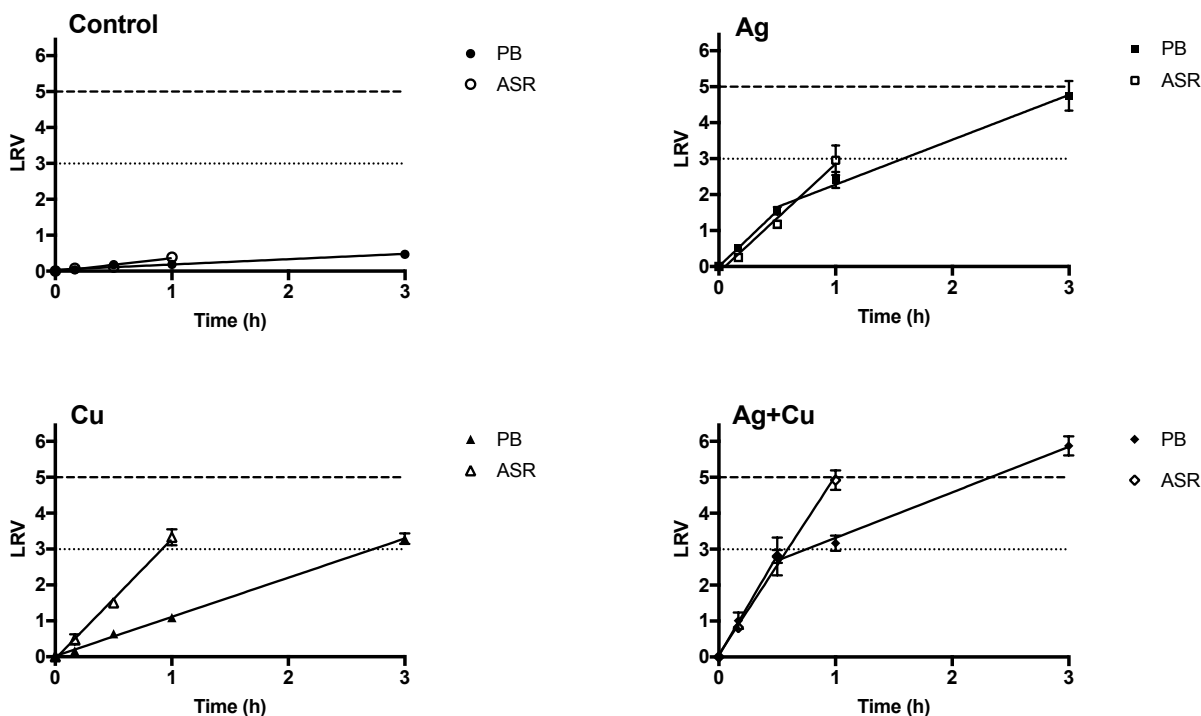


Figure 5.4 LRVs and linear models of MS2 inactivation after 0.17, 0.5, and 1 h of light exposure in two different water matrices: PB (closed symbols) and ASR (open symbols). Dotted and dashed lines represent WHO target LRVs for protective (3 LRV) and highly protective (5 LRV) household water treatment performance, respectively.

5.3.3 Comparison of inactivation rates

Using the linear regression models based on LRV versus time (Figures 5.2, 5.4), inactivation rates were calculated based on a first order reaction assumption. Inactivation rates were determined for both dark and light conditions (Table 5.2). For some conditions, the inactivation curve indicated two phases: an initial faster phase followed by a slower phase. This behavior of tailing has been observed in other inactivation experiments (Gyürék and Finch, 1998;

Hiatt, 1964; Hornstra et al., 2010). For these cases, the data were treated as two groups overlapping by one time point, and two corresponding first order inactivation rates (k_1 and k_2) were determined.

Table 5.2 First order disinfection coefficients calculated using linear regression models of dark and light data (in PB). Asterisk indicates that inactivation rate was not significantly different from zero.

Condition	Dark – PB	Light – PB	Light – ASR
	Inactivation Rate (h^{-1}) \pm SD	Inactivation Rate (h^{-1}) \pm SD	Inactivation Rate (h^{-1}) \pm SD
Control	0.0061* \pm 0.0029	0.15 \pm 0.021	0.38 \pm 0.072
Ag	0.032 \pm 0.0084	k_1 : 3.1 \pm 0.037 k_2 : 1.3 \pm 0.090	3.0 \pm 0.25
Cu	0.16 \pm 0.0051	1.1 \pm 0.020	3.3 \pm 0.12
Ag+Cu	k_1 : 1.4 \pm 0.023 k_2 : 0.16 \pm 0.026	k_1 : 5.6 \pm 0.17 k_2 : 1.3 \pm 0.10	5.0 \pm 0.27

The control inactivation rate was not significantly different from zero for the dark condition and increased slightly over zero when exposed to light (Table 5.2). For Ag, the inactivation rate in the dark was increased $\sim 100\times$ when exposed to light in both PB and ASR water for the initial inactivation phase, while this rate reduced by approximately half when tailing occurred after 0.5 h in PB. The Cu inactivation rate in PB increased over 6-fold when exposed to light, and the inactivation rate in ASR was three times that of PB in light. Both dark and light environments for Cu exhibited first-order inactivation kinetics and did not indicate tailing. For Ag+Cu, both dark and light conditions showed two distinct phases of inactivation in PB. For the dark condition, the initial inactivation rate ($k_1 = 1.40$) was much greater than the sum of the inactivation rates for Ag or Cu (0.19), confirming the previously illustrated synergistic

effect (Figure 5.3). However, after one hour, the Ag+Cu inactivation rate in PB decreased to the same as Cu (0.16). For the light exposed condition, Ag+Cu in PB initially exhibited the highest inactivation rate observed ($k_1=5.6$). However, after 0.5 h, tailing occurred, and the Ag+Cu inactivation rate in PB decreased over 4-fold ($k_1 = 1.3$) and was similar to the rate of the second phase of Ag inactivation ($k_2=1.4$). Tailing of the Ag-containing metal conditions in PB, not observed in ASR, may be attributable to the formation of Ag-phosphate complexes, which have been shown to reduce the toxicity of Ag ions even if the concentration of phosphate is well below the relevant K_{sp} (Xiu et al., 2011).

5.4. Conclusions

Concentrations of Ag and Cu allowable in drinking water are able to provide varying levels of MS2 inactivation depending on their use alone or in combination and on the presence of solar light exposure. The combination of Ag+Cu was the most effective metal additive in both dark and light conditions, exceeding 3 LRV after 12 h in the dark condition and after 1 h with the light condition. A synergistic effect was observed in two different comparisons. First, in the dark condition, the Ag+Cu condition achieved statistically significantly higher inactivation than the sum of the Ag only and Cu only results. This synergistic effect resulted in achieving the WHO Protective target of 3 LRV within 12 h without light exposure, which has potential positive implications for virus inactivation in stored water. Additionally, synergy between each of these metals individually in combination with light had been previously observed (Fisher et al., 2008; Kim et al., 2008) and was confirmed in this study. A synergistic effect between Ag+Cu and light exposure was observed, which resulted in a highly protective (>5 LRV) level of inactivation after 3 h in PB and only 1 h in ASR. Increased effectiveness of inactivation in ASR water may be due

to the presence of a low concentration of organic matter (Ferraudi and Muralidharan, 1981) or other constituents that promote the formation of antimicrobial reactive oxygen species. However, this enhanced antimicrobial effect was not observed in a previous study comparing MS2 inactivation by Cu and H₂O₂ in carbonate buffer versus Lake Geneva water (Nieto-Juarez et al., 2010). Ag was the least effective metal additive for disinfection in the dark condition, but inactivation by Ag significantly increased when exposed to light, which is consistent with the previous evidence from the literature (Butkus et al., 2004; Kim et al., 2008). Cu resulted in a less drastic but similar increase in disinfection with light exposure as has been reported in other studies (Fisher et al., 2012, 2008).

The results from this study illustrate the potential for doses of Ag and Cu as antimicrobial metals that are allowable in drinking water for treatment of stored water with relatively long contact times of at least several hours. Stored water is often subject to recontamination, so a system that doses antimicrobial metal ions into the stored water may not only provide virus inactivation but also prevent recontamination from microbial contaminants. Additionally, the results confirm the efficacy of Ag and Cu as additives for SODIS, with the greatest increase in inactivation achieved using both in combination. It should be noted that this study captures effects of solar light in a controlled setting, namely the absence of the temperature increase that would typically be observed. Therefore, further study should confirm the benefit of metal additives without temperature control to take advantage of the temperature increases that could further contribute to microbial reductions. It could be expected that in the presence of low concentrations of organic matter and increased temperature, microbial inactivation rates would increase (Carratalà et al., 2016; Romero et al., 2011). As a result, metal additives in SODIS could potentially decrease the amount of light exposure time typically required to achieve sufficient

disinfection and provide greater protection against days with lower solar light intensity. Future research should investigate if the antimicrobial effect of solar radiation and metal additives is observed at lower irradiance levels or alternative light sources and with increased turbidity or other light absorbent constituents. Another potential benefit to explore with further study would be to test if these metal additives increase inactivation rates of viruses that are known to be more resistant to SODIS than MS2 (e.g. adenovirus 40/41) (Meng and Gerba, 1996). Studies using additional pH values outside of the narrow 7.5-8.5 range should also be tested, as negative impacts on disinfection by Cu at high pH due to changes in Cu speciation have been reported (Lin et al., 2002). Finally, the potential beneficial use of light exposure of water containing Ag and Cu should be further explored for microbial disinfection applications in both point-of-use household water treatment and community water systems.

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CHAPTER 6: *E. COLI* INACTIVATION BY COPPER AND SILVER IONS IN WATER WITH AND WITHOUT SIMULATED LIGHT EXPOSURE AS MEASURED USING SELECTIVE AND NON-SELECTIVE CULTURE MEDIA

6.1 Introduction

Household water storage is a widely utilized practice to store collected water, offering convenience for water procured outside of the home as well as protection from water insecurity due to infrequent (e.g. water tanker delivery, drought-impacted surface water) or unreliable (e.g. intermittent communal or household tap) water sources. Stored water is subject to microbial contamination, including the growth of pathogenic bacteria, and can have a higher concentration of pathogenic bacteria than the source (Hoque et al., 2006; Reygadas et al., 2015; Wright et al., 2004). Household water treatment methods can be employed to reduce microbial concentrations and produce safe water at the point of use (Clasen, 2015), and numerous studies have illustrated the potential positive health impacts of some of these technologies (Hunter, 2009).

Historically, metal pots such as copper or brass were used for water storage before the disinfection properties of metals were characterized (Alexander, 2009; Shrestha et al., 2010). The oligodynamic effect of metals has been recognized in many applications (Lemire et al., 2013). Some studies have characterized bacterial disinfection by copper (Cu) and silver (Ag) ions in combination in water, most frequently to inactivate *Legionella pneumophila* in water heating systems (Chen et al., 2008; L.K. Landeen et al., 1989; Miuetzner et al., 1997). Cu and Ag have also been tested individually as additives to accelerate solar disinfection (SODIS) (Fisher et al., 2008; Kim et al., 2008).

Several studies have identified bacterial disinfection mechanisms of Ag and Cu ions. Ag has been shown to disrupt protein function by interaction with thiol groups, leading to the production of reactive oxygen species (ROS) such as OH^\bullet , increasing membrane permeability due to interference with the formation of disulfide bonds, and inhibiting replication due to interaction with DNA molecules (Feng et al., 2000; Liao et al., 1997; Morones-Ramirez et al., 2013). For Cu, disinfection mechanisms include the production of H_2O_2 , the inhibition of cell respiration enzymes, cell membrane damage, and chelation of Cu ions with the bacterial genome (Borkow and Gabbay, 2005; Taylor et al., 1989).

Differences between colony counts for samples plated on selective versus non-selective media are a well-documented outcome, especially for bacteria exposed to solar light (Fujioka and Narikawa, 1982; McDonald et al., 1983; Pulgarin and Rincon, 2007). Non-selective media can provide a less harsh environment for injured bacteria to recover and form colonies than selective media designed to inhibit growth of a subset of bacteria or fungi, and procedures have been developed to detect sub-lethal injury of bacteria (Hackney et al., 1979; Ray, 1979). However, selective media are commonly used without an effort to detect sub-lethally injured bacteria in order to determine inactivation rates and evaluate the performance of treatment technologies, even when using synthetic challenge water lacking additional microbes (World Health Organization, 2011b).

The aim of this study is to determine the inactivation rates of *E. coli* by Ag and Cu ions, separately and in combination, at allowable concentrations for drinking water in two conditions: 1) a dark, foil-covered reaction vessel used to simulate a typical stored water environment and 2) a vessel open to simulated solar light exposure. In addition, this study compares *E. coli*

enumeration by selective and non-selective media to determine the ability of injured bacteria to resuscitate and the potential effect on resulting log₁₀ reduction values (LRVs).

6.2 Methods and materials

6.2.1 Experimental Design

As in Chapter 5, Ag and Cu concentrations were selected to adhere to maximum allowable/recommended levels in drinking water: 0.1 mg/L Ag and 1 mg/L Cu (World Health Organization, 2011a). These metal concentrations were tested separately (Ag, Cu) and in combination (Ag+Cu) (Figure 6.1). The metal conditions and control were tested in two environments: dark (foil-covered) to simulate stored water and light-exposed to simulate SODIS. Dark tests were conducted in phosphate buffered water (PB), and SODIS tests were conducted in PB and an environmental water sample that was collected from an aquifer storage and recollection (ASR) water treatment plant.

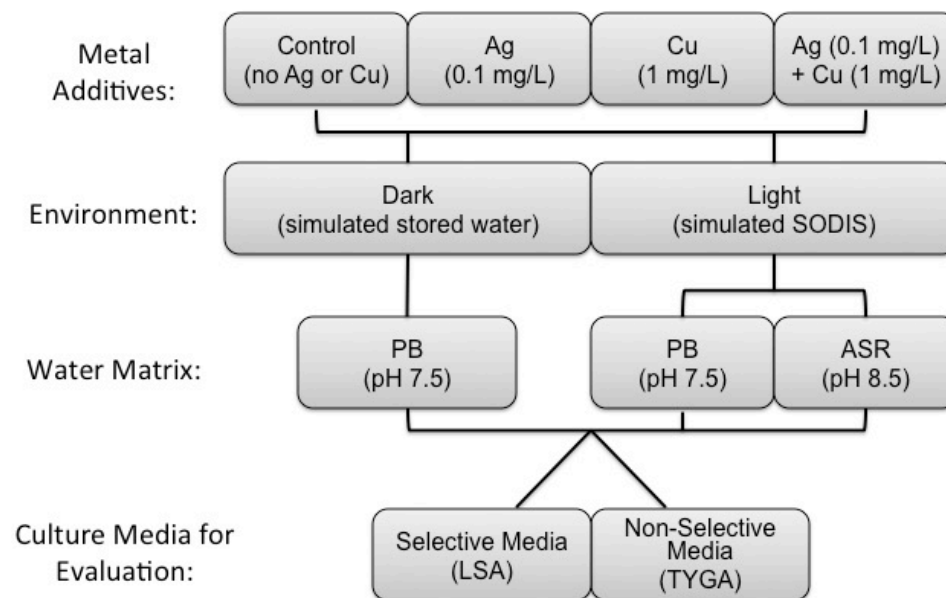


Figure 6.1 Experimental design for batch disinfection tests conducted in dark (simulated stored water) and light (simulated SODIS) conditions.

6.2.2 Test water preparation

All tests were conducted in 1 mM phosphate buffered (PB) test water (39 mg/L monosodium phosphate monohydrate, 192 mg/L disodium phosphate heptahydrate), which was prepared, autoclaved, and adjusted to pH 7.5. Additionally, SODIS tests were conducted in an environmental water sample, which was collected from the Dunea Aquifer Storage and Recollection (ASR) water treatment plant (The Hague, The Netherlands). The sample of ASR water used for this study passed through a sandy aquifer and was collected and refrigerated until use. Table 6.1 shows the measured water quality parameters of the ASR water.

Table 6.1 Measured water quality parameters of ASR water.

Water Quality Parameter	Concentration
pH	8.5
TOC	1.83 mg/L
Turbidity	2.4 NTU
Ca	68.7 mg/L
Na	33.5 mg/L
Mg	8.85 mg/L
K	5.15 mg/L
Si	4.34 mg/L
Sr	279 µg/L
P	71 µg/L
Fe	60 µg/L
B	49 µg/L
Ba	23 µg/L

Metal solutions of AgNO₃ and CuSO₄ were prepared by diluting reagent-grade 0.1 M stock solutions (Sigma Aldrich) into autoclaved deionized water, creating an intermediate dilution used to dose the reaction vessels in order to add volumes that were large enough to measure accurately but small enough not to alter the test water volume significantly. Metal

solutions were stored in polypropylene tubes covered with aluminum foil to prevent light exposure and prepared weekly, with stable concentrations confirmed analytically by inductively coupled plasma mass spectrometry (ICP-MS) (PlasmaQuant MS, Analytik Jena, Germany).

A stationary culture of *E. coli* strain WR1 was prepared from a frozen stock (KWR Watercycle Research Institute, the Netherlands) for each experiment. The culture was grown overnight in 200 ml of tryptone yeast glucose broth (10 g/L trypticase peptone, 8 g/L NaCl, 1 g/L yeast extract, 300 mg/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1 g/L glucose) at 37°C on a 100 rpm shaking plate. After incubation, the culture was centrifuged at 3000 x g for 15 minutes at 15°C. The supernatant was aspirated, and the resulting pellet was resuspended in 5 ml of phosphate buffer to create a concentrated *E. coli* solution ($\sim 10^9$ CFU/ml). The concentrated MS2 solution was dosed into the phosphate buffer in a ratio of 1:1000 (50 µl for each 50 ml of test water prepared) to create the spiked test water and mixed by inversion. The target *E. coli* concentration in the test water was 10^6 CFU/ml.

6.2.3 Microbiological evaluation methods

Samples collected at each time point were serially diluted ten-fold in PB and refrigerated until evaluation, typically within 1 h of collection. Each sample dilution was plated in duplicate on Lauryl Sulfate Agar (LSA), a selective medium, and Tryptose Yeast Glucose Agar (TYGA), a non-selective medium. The spread plate method was used, where 100 µl of a sample dilution was pipetted onto a prepared agar plate, and a sterile sample spreader was used to distribute the sample evenly across the agar plate. After the sample fully absorbed and the agar appeared to be dry, plates were inverted and incubated for 48 h at 37°C. A 48 h incubation time was chosen

because after 24 h of incubation for some conditions, additional colonies with apparent delayed growth had started to form and were difficult to enumerate without further incubation.

6.2.4 Experimental procedure

Autoclaved, acid-washed glass beakers with a magnetic stir bar were used as reaction vessels. Each beaker was filled with a 50 ml volume of *E. coli*-spiked 1 mM PB at pH 7.5 and stirred for 15 min to equilibrate before dosing metal solutions. For the dark condition, the beaker was completely wrapped in aluminum foil to protect the test water from light exposure. For the light-exposed condition, beakers were placed in a solar light simulator (Atlas Suntest XXL) fitted with a daylight filter emitting a spectrum in the range of 300-1200nm. Experiments were run with a light irradiance of 550 W/m². Time points of 10, 30, and 60 min correspond to fluence values of 33, 99, and 198 J/cm², respectively. Beakers were submerged in a 22°C temperature-controlled water bath regulated by a recirculating water cooler (Julabo FL 300). Absorbance of both PB and ASR water was determined to be negligible due to published absorbance spectra for PB and an environmental sample (Swiss tap water) with a similar TOC concentration to the ASR water, where the only absorbance occurred under 200nm, which is outside of the 300-1200 nm spectrum emitted by the Atlas XXL (Carratalà et al., 2016).

6.3 Results and discussion

*6.3.1 Reduction of *E. coli* in PB with metal additives and dark storage*

For the control condition of PB with no added metals, reductions of *E. coli* were not significantly different from 0 LRV throughout the 6 h testing period (Figure 6.2a). For exposure to Ag, inactivation occurred rapidly, but differences in LRVs were observed between the selective and non-selective media (Figure 6.2b). At 1 min of exposure to Ag, LSA indicated 1.6

LRV while TYGA indicated only 0.1 LRV. This difference was more marked at 10 min of exposure, with 3.7 LRV observed using LSA and 0.8 LRV observed using TYGA. The >4-fold difference between these LRV values indicates that *E. coli* enumeration on LSA may be an overestimate of the inactivation achieved by the treatment method. The non-selective TYGA medium may be more supportive for the recuscitation of Ag-injured bacteria that were unable to grow when plated directly on LSA. Within 30 min, enumeration of *E. coli* on LSA reached the detection limit (5.8 LRV) of the assay and therefore exceeded the WHO Highly Protective 4 LRV target. However, this performance target was not met when evaluated on TYGA until 60 min of exposure to Ag. The assay detection limit using TYGA (6.2 LRV) was reached after 360 min of exposure to Ag. The extent of difference in *E. coli* LRV when enumerated on the non-selective TYGA medium and the selective LSA medium declines and eventually disappears with increased contact time. Therefore, extensive *E. coli* reduction by Ag in PB does occur when plating on the non-selective TYGA medium, but at a slower rate than it occurs on the selective LSA medium.

LRVs observed for *E. coli* exposure to Cu in PB were significantly lower than those for Ag exposure (Figure 6.2c), with LRVs at 6 hours of 2.5 on selective LSA medium and 1.4 on the non-selective TYGA medium. Differences in LRV were observed consistently between LSA and TYGA and these differences persisted throughout the 6-hour exposure period. When assayed on selective LSA medium, *E. coli* in PB with Cu reached 2.0 LRV after 180 min of exposure, therefore reaching the WHO Protective performance target. The highest observed *E. coli* reduction on LSA was 2.5 LRV after 180 min, falling far short of the WHO Highly Protective performance target of 4 LRV. However, on TYGA medium, only 1.3 LRV was observed after 360 min, falling short of the 2 LRV WHO Protective performance target.

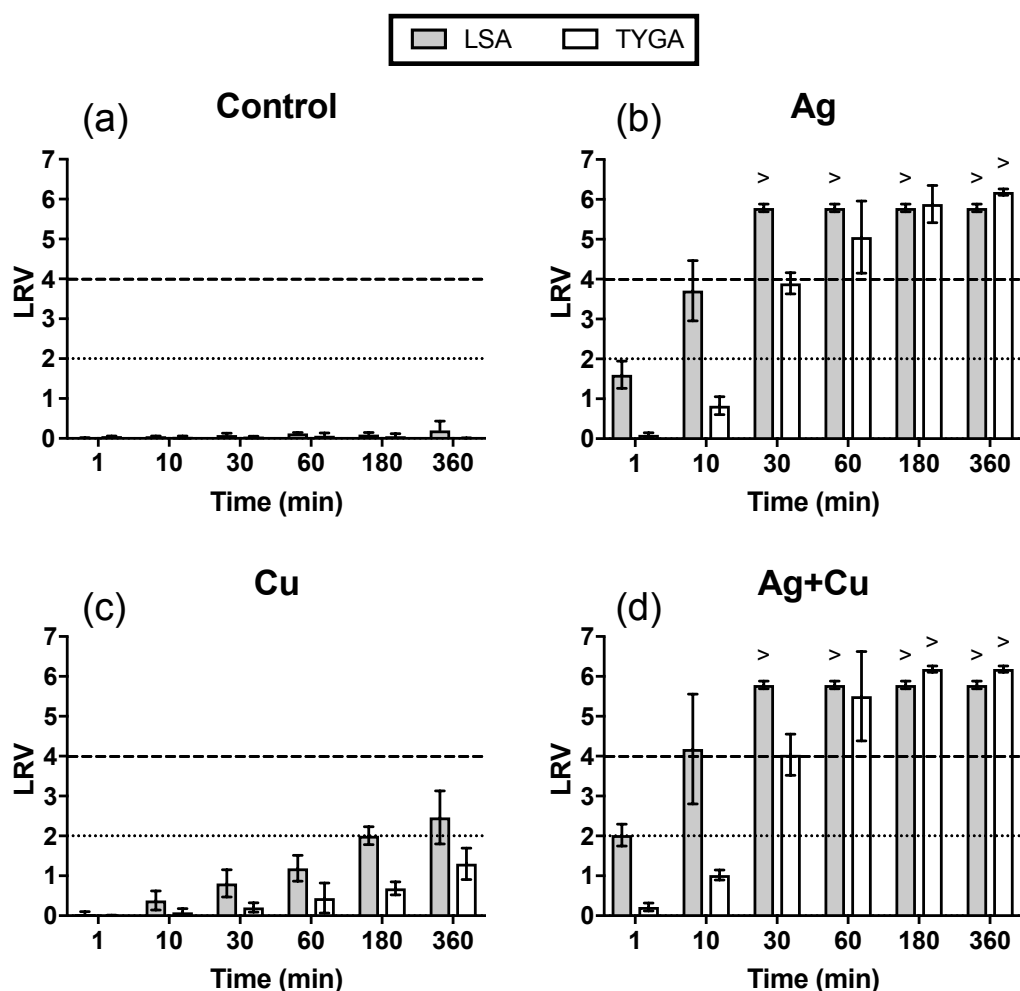


Figure 6.2 LRVs of *E. coli* with exposure to metal additives in the dark condition. Error bars represent standard deviation. The detection limit is indicated by a greater-than sign (>). The dotted (2 LRV) and dashed (4 LRV) lines represent the WHO performance targets for Protective and Highly Protective bacterial reductions.

E. coli reductions observed for Ag+Cu in PB with dark exposure condition were very similar to those observed for Ag alone (Figure 6.2d), with slight but not significantly higher LRVs observed at comparative time points. Exposure to Ag+Cu in PB resulted in achieving the WHO Protective performance target of 2 LRV within 1 min and the WHO Highly Protective performance target of 4 LRV within 10 min when enumerated on the selective LSA medium. However, when enumerated on the non-selective TYGA medium *E. coli* reductions at 1 and 10

minutes were far lower at 0.22 and 1.0 LRV, respectively, and did not meet the WHO Protective and Highly Protective performance targets until 30 min on TYGA. By 60 min and later time points, *E. coli* LRVs were extensive and similar on both culture media and exceeded the detection limits of the assay at >5.5->6 LRV at 180 minutes.

The performance differences observed in the initial rates of *E. coli* reduction in the dark by Cu and Ag alone and Ag+Cu in combination in PB over time when plated on selective and non-selective culture media are noteworthy. These observed differences in LRVs between plating on selective and non-selective culture media are especially important because they affect WHO technology performance evaluation results and could also impact technology assessments and recommendations in meeting WHO performance targets under such dark use conditions.

6.3.2 Reduction of E. coli in PB and ASR water by Ag and Cu metal ion additives with solar light exposure

Ag and Cu metal ion additives alone and combined in PB were evaluated for *E. coli* reductions at 10, 30, and 60 min of light exposure. For the control condition of no metal ions, *E. coli* reductions over time were observed with light exposure alone in both PB and ASR water (Figure 6.3). The rates of *E. coli* reduction over time in both test waters also differed significantly between enumerations on selective (LSA) versus non-selective (TYGA) media for both water matrices. *E. coli* disinfection in PB with solar light exposure was 4.9 LRV after 30 min with plating on selective LSA medium, meeting the WHO Highly Protective performance target of 4 LRV, while with plating on non-selective TYGA, only 0.81 LRV observed with plating on non-selective TYGA at the same time point. After 60 min of exposure, samples plated on selective LSA medium again exceeded the WHO Highly Protective performance target of 4 LRV while the samples plated on the non-selective TYGA medium only met the WHO

Protective performance target of 2 LRV in both test waters. This large differences in *E. coli* LRVs observed using selective LSA and non-selective TYGA media suggest that light-injured *E. coli* were resuscitated more extensively on the non-selective TYGA medium than on the selective LSA medium. These observed differences in LRVs when enumerating test bacteria on non-selective versus selective culture media, impact resulting recommendations for exposure times to meet recommended WHO performance targets, with much longer exposure times needed to meet either WHO Protective or Highly Protective levels of performance. For example, *E. coli* reductions in both PB and ASR waters met the 4 LRV WHO Highly Protective performance target by 30 min when evaluated on LSA, but the same tests evaluated using TYGA medium still fell short of this 4 LRV target after 60 min of solar light exposure and would need further exposure time to meet this target.

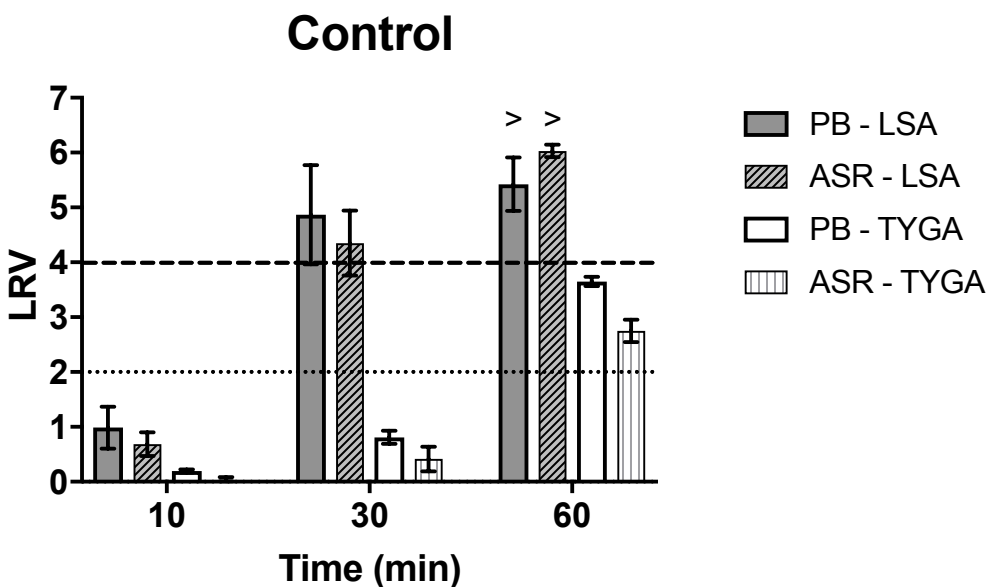


Figure 6.3 LRVs of *E. coli* with exposure light for control (no metal added) control. Error bars represent standard deviation. The detection limit is indicated by a greater-than sign (>). The dotted (2 LRV) and dashed (4 LRV) lines represent the WHO performance targets for Protective and Highly Protective bacterial reductions.

Exposure to Ag with light achieved rapid inactivation of *E. coli* in both PB and ASR water (Figure 6.4). For PB, the detection limit (5.8 LRV) was reached after only 10 min of exposure when evaluated on LSA. Evaluation on TYGA resulted in 2.4 LRV after 10 min, and 5.7 LRV was observed after 30 min, therefore achieving the WHO Highly Protective target of 4 LRV. Differences in LRVs for *E. coli* analyzed on LSA selective medium and TYGA non-selective medium were also observed for both waters initially at 10 minutes exposure and persisted to 30 minutes exposure in ASR water, after which no differences in LRVs between the two culture media were discernable.. There was a difference in the extent of *E. coli* inactivation observed with ASR water compared to PB, with LRVs significantly lower in ASR water than those in PB at 10 min on both LSA and TYGA as well as at 30 min for ASR water evaluated on TYGA. However, this importance of these observed effects is diminished by the fact that *E. coli* reduction by Ag in both water matrices (PB and ASR) and on both culture assay media (LSA and TYGA) exceeded the WHO Highly Protective performance target of 4 LRV by 30 min.

Exposure of *E. coli* in PB to Cu with light resulted in 3.5 LRV when plated on selective LSA medium but only 0.35 LRV when plated on non-selective TYGA after 10 minutes of exposure (Figure 6.4). These results are evidence that most of the injured *E. coli* were resuscitated on the non-selective medium. LRVs of *E. coli* in PB with Cu and light exposure reached the detection limit in 10 min with enumeration on selective LSA and in 60 min with enumeration on non-selective TYGA. LRVs of *E. coli* in ASR with Cu evaluated on non-selective TYGA were consistently lower than those observed in PB. This effect was the greatest at 10 min, where 2.4 LRV was observed in PB while only 0.38 LRV was observed in ASR water. However, despite the difference in LRVs, both ASR and PB water met the WHO Highly Protective 4 LRV target with enumeration on non-selective TYGA after 30 min of exposure.

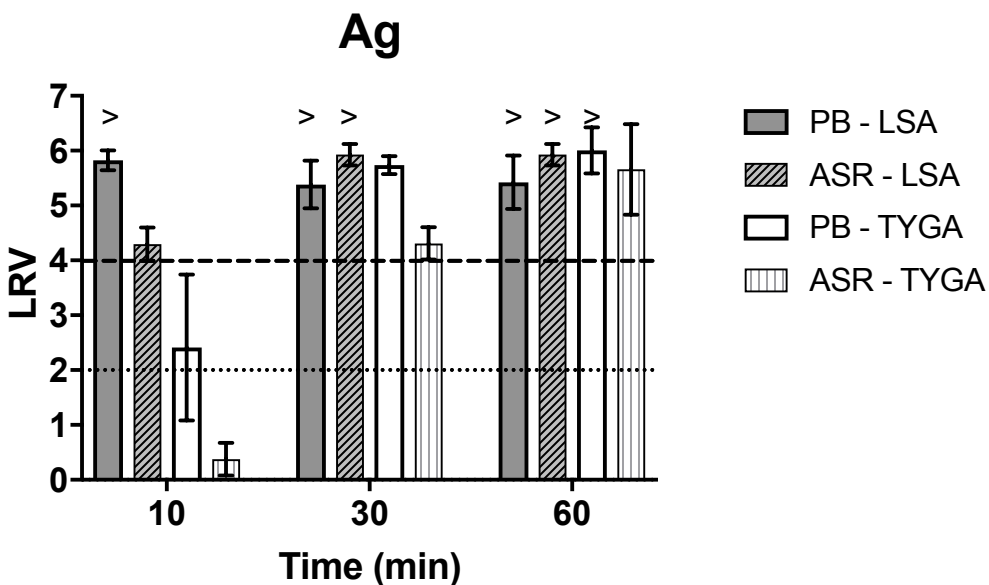


Figure 6.4 LRVs of *E. coli* with exposure to Ag and light. Error bars represent standard deviation. The detection limit is indicated by a greater-than sign (>). The dotted (2 LRV) and dashed (4 LRV) lines represent the WHO performance targets for Protective and Highly Protective bacterial reductions.

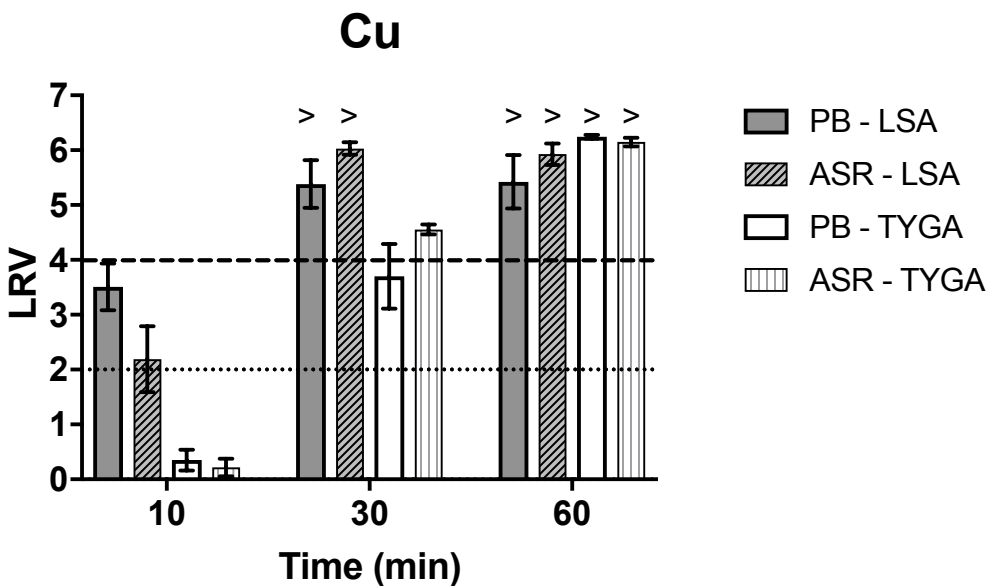


Figure 6.5 LRVs of *E. coli* with exposure to Cu and light. Error bars represent standard deviation. The detection limit is indicated by a greater-than sign (>). The dotted (2 LRV) and dashed (4 LRV) lines represent the WHO performance targets for Protective and Highly Protective bacterial reductions.

Exposure of *E. coli* in PB with Ag+Cu to solar light in PB gave very similar LRVs to Ag with light (Figure 6.6), with similar differences at 10 minutes exposure in LRVs between plating on selective LAS medium and non-selective TGYA medium and differences in LRVs between PB and ASR water on both plating media. All samples plated on LSA medium reached the assay lower detection limit and maximum LRV at 30 minutes (Figure 6.6d). For samples plated on TGYA, *E. coli* LRVs at 10 minutes of exposure in both test waters were lower than those plated on LSA, as observed previously for test waters with Ag only. However, *E. coli* in PB with Ag + CU assayed on TGYA resulted in 4.0 LRV after 10 minutes to achieve the WHO Highly Protective performance target, while for PB with Ag alone the LRV was about 2.4 and achieved only the 2 LRV WHO Protective performance target. In ASR water, initial 10-minute LRVs were lower than those in PB, as observed for waters with Ag only. Therefore, compared to PB, an initial protective or shielding effect was observed, with only 0.81 LRV measured on TGYA after 10 min of exposure compared to LRVs of 4 or more for the other three experimental conditions (PB assayed on TGYA and both waters assayed on LSA). These differences in *E. coli* LRVs indicate a significant level of resuscitation of injured *E. coli* on non-selective TGYA agar medium in the initial 10 minutes of Ag+Cu and light exposure with added protection by the ASR water.

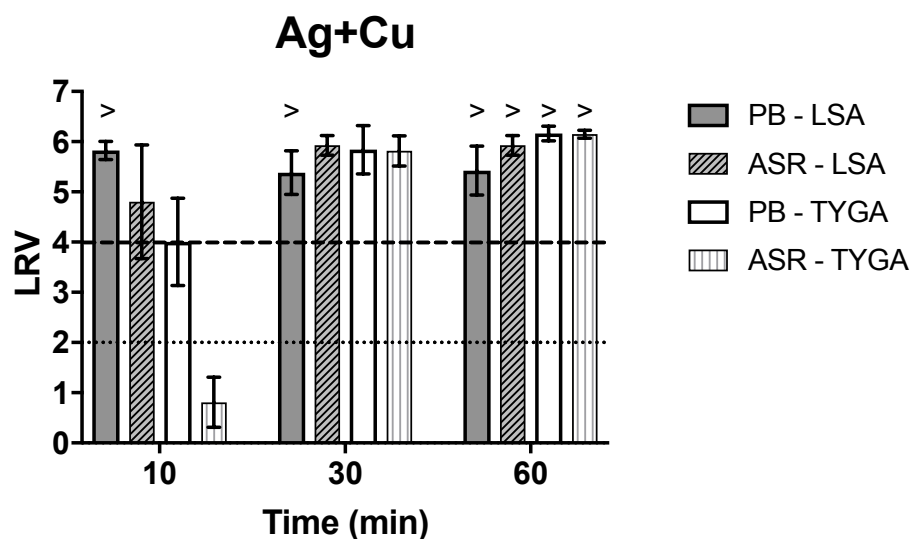


Figure 6.6 LRVs of *E. coli* with exposure to Ag+Cu and light. Error bars represent standard deviation. The detection limit is indicated by a greater-than sign (>). The dotted (2 LRV) and dashed (4 LRV) lines represent the WHO performance targets for Protective and Highly Protective bacterial reductions.

Overall, despite differences in *E. coli* LRVs observed over solar light exposure times of 10, 30, and 60 min in PB and ASR test waters alone or with added Ag only, Cu only and Ag+Cu, all test conditions achieved the WHO Highly Protective target of 4 LRV after 60 min of exposure with the exception of the metals-free control samples assayed on TGYA medium, which were 3.7 LRV in PB and 2.7 in ASR water. Given that recommendations for SODIS exposure times are typically greater than 60 min, these data show that although some light and metal-damaged *E. coli* can be resuscitated when plated on non-selective media such as TGYA in the initial periods of exposure, with enough exposure time, this resuscitation effect becomes less apparent and disappears, especially in the presence of added Ag and Cu metal ions, such that WHO LRV performance targets can be met.

6.3.3 Comparisons of *E. coli* inactivation rates in the dark (PB) and light (PB and ASR) in samples with and without Ag and Cu metal ions

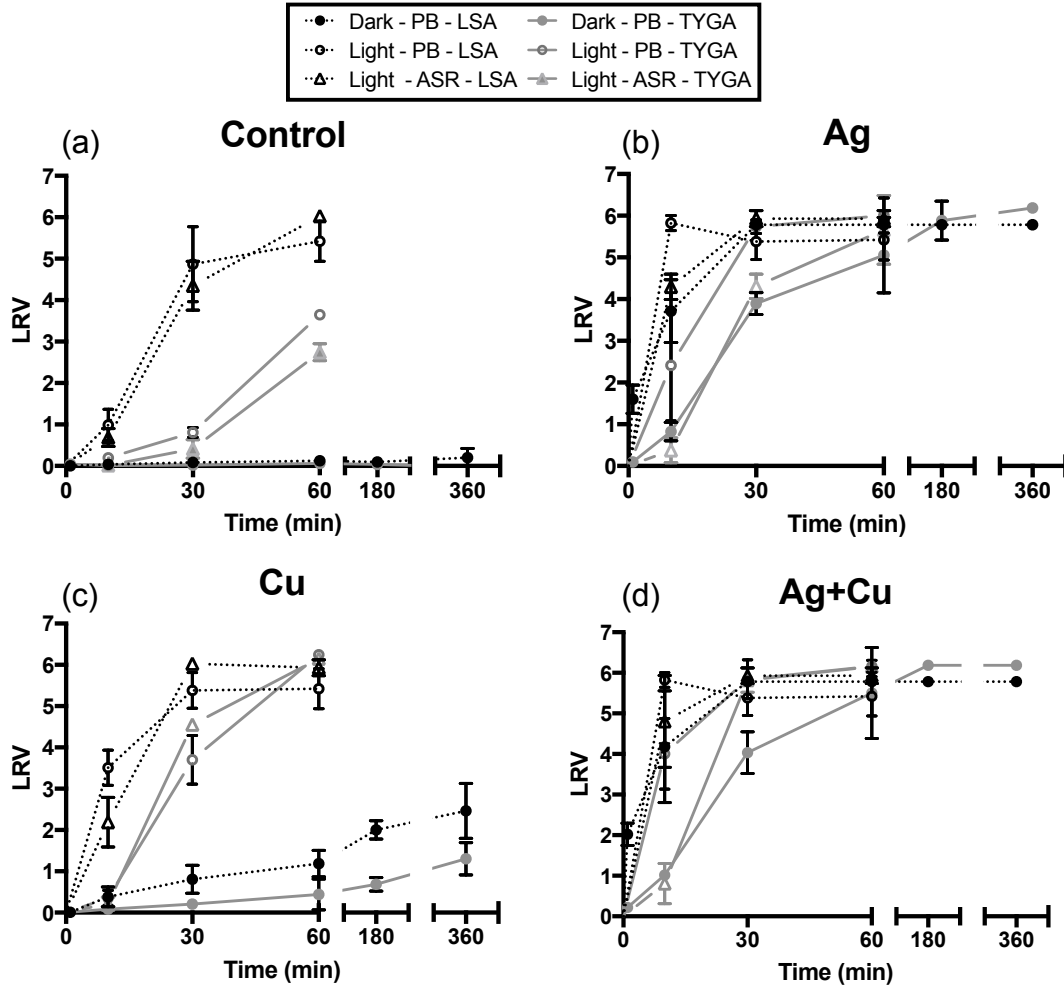


Figure 6.7 LRVs after exposure to metal ion additives with and without light exposure plotted against time, evaluated on selective (LSA) and non-selective (TYGA) media. Dotted lines connect points evaluated on LSA, and solid lines connect points evaluated on TYGA. Open symbols represent experiments conducted in light exposure, and closed symbols represent experiments conducted in the dark.

For the Ag and Cu-free control *E. coli* in PB or ASR water exposed to light, the type of evaluation media had a significant effect on the observed inactivation kinetics and LRVs (Figure 6.7a). This difference is reflected by the differences in inactivation rates measured on non-selective TYGA, which increased over time ($k_2 > k_1$) and is in contrast to the decreasing rate of

inactivation (tailing) observed in most cases (Table 6.2). It is not possible to determine if the *E. coli* inactivation rate by light decreased over time for samples assayed in selective LSA medium because at 60 min, both ASR and PB samples reached LRV detection limits due to non-detects in samples. These differences in LRVs and resulting inactivation rates between culture media suggest that light-injured *E. coli* are able to resuscitate on the non-selective medium, but this ability may diminish over increased exposure times due to increasing injury to the *E. coli*.

Table 6.1 Inactivation rates \pm standard error for all batch tests conducted. An asterisk (*) indicates the slope was not significantly different from zero. A caret (^) indicates when a slope was calculated from only two points instead of a linear regression from three or more points. Conditions where the first time point had already reached the detection limit have a greater than sign preceding the inactivation rate.

Condition	Dark – PB		Light – PB		Light – ASR	
	Inactivation Rate (h ⁻¹) LSA	Inactivation Rate (h ⁻¹) TYGA	Inactivation Rate (h ⁻¹) LSA	Inactivation Rate (h ⁻¹) TYGA	Inactivation Rate (h ⁻¹) LSA	Inactivation Rate (h ⁻¹) TYGA
Control	0.028 \pm 0.0074	-0.0089* \pm 0.005	k_I : 10 \pm 1.4 k_2 : ≥ 1.1 ^	k_I : 1.7 \pm 0.17 k_2 : 5.7 ^	k_I : 9.0 \pm 1.7 k_2 : 3.4 ^	k_I : 0.87 \pm 0.27 k_2 : 4.7 ^
Ag	19 \pm 7.0	k_I : 7.8 \pm 0.70 k_2 : 2.32 ^	≥ 35 ^	11 \pm 1.1	25 ^	k_I : 2.3 ^ k_2 : 12 ^ k_3 : 2.7 ^
Cu	k_I : 1.6 \pm 0.18 k_2 : 0.29 \pm 0.060	0.21 \pm 0.018	21 ^	7.8 \pm 2.0	13 ^	9.7 \pm 2.9
Ag+Cu	20 \pm 9.1	k_I : 8.0 \pm 0.55 k_2 : 2.9 ^	≥ 35 ^	≥ 24 ^	29 ^	12 \pm 2.5

Increased *E. coli* LRVs were observed for exposure to Ag with light at early time points compared to dark exposure (Figure 6.7b), with greater inactivation rates occurring than with dark exposure to Ag or to light alone and no Ag present. The differences in LRVs indicate an additive effect only and this was further documented by a statistical analysis. *E. coli* inactivation in LSR water with Ag and light exposure resulted in declining inactivation rates over time, which was

quantified as three different phases with $k_1 \sim k_3 < k_2$. This phenomenon was not observed for *E. coli* in PB with Ag and light exposure, for which a single rapid rate of inactivation was observed before the detection limit of the assay was reached.

For *E. coli* inactivation in PB with Cu with light exposure, there was an apparent synergistic effect (Figure 6.7c). For example, after 30 min exposure when evaluated on TYGA, Cu in the dark (0.21 LRV) plus reduction by light in PB only (0.81 LRV) resulted in a sum of 1 LRV, which would be the expected value if there were an additive effect. However, a 3.7 LRV for *E. coli* in PB with Cu and light exposure was observed, illustrating a synergistic effect between Cu and light on *E. coli* reduction. This synergistic effect is also illustrated by the *E. coli* inactivation rates, with the observed inactivation rates for Cu in both water types (PB and ASR) and with light exposure being greater than the sum of inactivation rates of Cu in the dark and the light control (no Cu) for assays on both culture media (LSA/TYGA) (Table 6.2).

For test waters with Ag+Cu, overall *E. coli* LRVs were rapid and extensive, with many time points reaching the assay detection limit when evaluated on the selective LSA medium (Figure 6.7d). Evaluation of *E. coli* inactivation in waters with Cu and AG when assayed on the non-selective TYGA medium resulted differences in inactivation kinetics in some samples. At 10 min of exposure, *E. coli* LRVs for the dark exposure in PB (1.02) and the light exposure of ASR water (0.81) were not statistically significantly different. There was potential initial inhibition of inactivation by the ASR water with Cu under both dark and light exposures. This inhibition by ASR water was also apparent in the comparison of inactivation rates with PB, with inactivation of *E. coli* by Ag+Cu in light exposure occurring at least twice as fast in PB (12 h^{-1}) as in ASR water ($\geq 24 \text{ h}^{-1}$) (Table 6.2).

6.3.4 Discussion

All experiments were run at a controlled temperature (20-22°C), but both stored water and SODIS are likely to experience higher temperatures in real-world settings. Stored water in tropical and subtropical regions may reach high temperatures, and increased temperature can be expected to increase microbial inactivation rates (Bertrand et al., 2012; Theitler et al., 2012). In a previous study *E. coli* inactivation experiments conducted in Cu vessels showed increased inactivation rates with higher temperatures and constant Cu concentration at temperatures relevant to the region in India where the tests were conducted (Sharan et al., 2010a). Because SODIS experiments were conducted at a controlled temperature of 20°C to prevent pasteurization and isolate the effects of the solar spectrum UV exposure, higher temperatures and therefore higher rates of inactivation could also be expected in a real world SODIS setup (Fisher et al., 2008; Sciacca et al., 2011). Solar radiation was dominant over UV radiation for the inactivation of *E. coli* in a lake Michigan study (Whitman et al., 2004).

The pH range tested in these experiments was narrow (7.5-8.5 pH), so further research should test waters with pH values outside of this range to reflect other potential natural water pH values. pH values of 8.5-9 were found to reduce the efficacy of Ag and Cu ionization for the treatment of *Legionella pneumophila* in a hospital hot water system (Lin et al., 2002). The authors attributed this effect to the changing complexation of Cu compounds at higher pH values, specifically the decline of the concentration of negatively charged $\text{Cu}(\text{OH})_3^-$, while Ag efficacy to be unaffected by the higher pH due to stable speciation across the relevant pH range. A study of water stored in copper pots found increased inactivation at each end of the pH range tested (pH 6 and 9), but the increased inactivation at pH 6 may have been due to increased leaching of Cu ions from the copper surface at an acidic pH value (Sharan et al., 2010a).

Selective LSA and non-selective TYGA culture media frequently resulted in significantly different LRVs for *E. coli* inactivation in water and in water with Ag and Cu ions, with significantly greater recovery of culturable bacteria on the non-selective TYGA medium during initial time periods of exposure. This resuscitation effect of injured *E. coli* has been noted in other solar light inactivation studies (Pulgarin and Rincon, 2007) and from other sources of bacterial injury including copper (Domek et al., 1984; Fujioka and Narikawa, 1982; Hackney et al., 1979). However, a study testing solar effects on *E. coli* concentrations in Lake Michigan was not able to resuscitate solar light-inactivated *E. coli* in ideal laboratory settings (Whitman et al., 2004).

6.4 Conclusions

Overall, Ag and Cu metal ion additives in both PB and ASR waters resulted in significant levels of *E. coli* inactivation both in dark and light exposed conditions. *E. coli* appeared to be much more susceptible to inactivation by Ag than Cu in the dark, with a tenfold greater inactivation rate for water with Ag than Cu. This difference between Ag and Cu effects in *E. coli* inactivation was also observed under simulated solar light exposure. However, these differences in Ag and Cu on *E. coli* inactivation rates were less significant for assays on non-selective TYGA medium than selective LSA medium, indicating the ability of Ag- and Cu-injured *E. coli* to be resuscitated to some degree during initial exposures.

Exposure to simulated solar irradiation in the absence of Ag or Cu ion additives resulted in reaching the WHO Protective level of >2 LRV when enumerated on non-selective TYGA medium and the WHO Highly Protective level of >4 LRV when enumerated on selective LSA medium after 60 min. These results illustrate the ability of UV exposure from a solar irradiation

source in the absence of increased temperature to inactivate *E. coli*. The addition of Ag, Cu, or Ag+Cu ion additives accelerated the inactivation of *E. coli*, as previously noted in other studies (Fisher et al., 2008; Kim et al., 2008). For stored water, future research should determine what level of light exposure (e.g. ambient light in the household or waterproof LED lights with light emitted at an antimicrobial wavelength) could be effective for increased inactivation. This could inform stored water treatment design, such as the use of a transparent container or the addition of an LED light source to the stored water container.

It is not always possible or practical to use non-selective media to detect fecal bacteria in water and other media, particularly in environmental samples that contain a level of background flora that would impact the accuracy of colony counts for the targeted organism. Therefore, selective media may have to be used for the evaluation of disinfection methods with the understanding that some injured bacteria may be able to resuscitate and remain viable in a real world setting despite being unable to grow on the relatively harsh conditions of a selective medium. A remedy for this problem of underestimation of cultured bacteria when using selective media is to employ repair detection dual plating methods and other resuscitation methods. In these methods the bacteria are first plated for a short time period on non-selective media that allow for resuscitation and recovery of injured bacteria. Then the culture plates with bacteria are supplemented with differential and selective agar media facilitate the detection the resuscitated target bacteria and suppress the further growth of the non-target bacteria (Domek et al., 1984; Fujioka and Narikawa, 1982; Ray, 1979). Therefore, culture-based testing should attempt to account for “false negatives” which could prematurely or misleadingly indicate sufficient treatment efficacy. Lastly, other bacteria of health concern should be considered in further

testing, as *E. coli* was shown to be more susceptible to SODIS than *Salmonella sp.*, which are frank pathogens (Sciacca et al., 2011).

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CHAPTER 7: SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

7.1 Summary of motivation and objectives

Household water treatment and safe storage (HWTS) technologies play an important role in improving water quality in the household and therefore decreasing the negative impacts of waterborne disease. HWTS technologies differ in levels of efficacy for the reduction of all three classes of microbial contaminants: parasites, bacteria, and viruses. This dissertation focused on three HWTS methods: 1) ceramic water filters 2) SODIS and 3) safe storage. Ceramic water filters have pores that are small enough to remove parasites, but bacterial reductions vary with manufacturing quality, and viral reductions are well below targets for protective health impact. SODIS is typically effective for reductions of bacteria, but some viruses are resistant, and overall treatment efficacy can vary with water quality (e.g. turbidity) and light exposure (e.g. cloudy day). Storing water in an opaque, covered container is recognized to help prevent recontamination. However, water storage does not provide effective treatment of water that is initially unsafe, and recontamination is still common.

The antimicrobial effects of Ag, Cu, and Fe have been noted in multiple water treatment applications. With the goal of improving treatment efficacy to ultimately increase health impacts of HWTS technologies, this research tested the incorporation of Ag, Cu, and Fe into ceramic water filters and Ag and Cu into SODIS and stored water treatment conditions for the enhanced inactivation/removal of viruses, modeled with MS2 bacteriophage, and bacteria, modeled with *E. coli*. Key conclusions from these research elements are presented in the following section.

7.2 Conclusions

7.2.1 Ceramic water filters with antimicrobial metal additives

- Ag, Cu, and Fe metal additives can significantly reduce *E. coli*, helping CWFs to meet the WHO Highly Protective reduction target of 4 LRV.
- Initial evidence of improved virus reductions from experiments with ceramic filter disk Set 1 for Ag and Cu additives (1.2 and 1.6 LRV observed for 100X (Ag+Cu) and 100X Ag+10X Cu, respectively) was not replicated in subsequent experiments with ceramic filter disk Set 2, with all virus reductions remaining below 1 LRV.
- Overnight storage of filter effluent containing low concentrations of leached Ag (38 µg/L) and Cu (91 µg/L) may contribute to increased microbial reductions as observed with the 10X Ag+100X Cu filter achieving statistically significant improvements in microbial reductions after 12-16 hours, specifically an additional 1 LRV observed for *E. coli* and 0.4 LRV for MS2.
- Flow rates varied between filters with different additives and ranged from 0.85-3.9 L/h when corrected to the size of a CWF. Most filters exceeded the suggested minimum flow rate of 1 L/h, however, flow rate was not correlated with LRV.

7.2.2 Batch adsorption and material characterization studies

- Ceramic filter media enhanced with iron nanoparticles (nZVI) showed >2.5 LRV of MS2 before firing, but over 65% of the bound MS2 was recovered after elution as evaluated using the DAL method, indicating nearly 2/3 of the adsorbed MS2 phages were not

inactivated. These elutable, infectious phages have the potential to reduce the performance of the filters over time.

- Fired nZVI combined with Ag showed >1.5 LRV reduction in batch adsorption studies, but this was not replicated in the ceramic filter disk study where the mean LRV was 0.07.
- XRD analysis confirmed the transformation of Fe-based crystalline minerals in nZVI additive filters, indicating a change in composition after filter firing that may be responsible for low MS2 reductions.
- Micro CT imaging showed that the method of dispersion for CuMP powder used to make the clay mix for Set 1 filters was not effective, with the presence of relatively large Cu clusters instead of a uniform and more dispersed distribution of small Cu particles throughout the porous clay matrix.

7.2.3 Reduction of MS2 in simulated dark storage and SODIS

- For simulated dark storage, Ag and Cu in combination exhibited a synergistic effect for MS2 inactivation, reaching over 3 LRV (WHO Protective reduction target) after 12 h.
- Ag only, Cu only, and Ag+Cu all exhibited a synergistic inactivation effect on MS2 with simulated solar light exposure. For dark (D) and light (L) conditions, the following LRVs were observed at 3 h: 0.1 (D) and 4.8 (L) LRV for Ag, 0.4 (D) and 3.3 (L) LRV for Cu, and 2.2 (D) and 5.9 (L) LRV for Ag+Cu. MS2 inactivation rates (h^{-1}) significantly increased by factors of 5 to as much as 10-fold of rates observed in the dark.
- For SODIS, mean LRVs of Ag only and Cu only conditions met the WHO Protective performance target of 3 LRV and the combination of Ag+Cu met the WHO Highly Protective target of 5 LRV within 3 h of exposure.

- The observed increased and more rapid inactivation of viruses in water with added metals and solar light exposure offers the potential to improve virus reductions in stored water by providing sufficient light exposure from sunlight or possibly from other light sources to achieve this effect.

7.2.4 Reduction of *E. coli* in simulated dark storage and SODIS

- For simulated dark storage, inactivation of *E. coli* by Ag was rapid, reaching the WHO Highly protective target of 4 LRV within 30 min as enumerated on selective LSA culture medium and within 60 min as enumerated on non-selective TYGA culture medium. Cu inactivation of *E. coli* was comparatively slower, with 2.5 LRV reached after 6 h as enumerated on selective LSA medium and 1.3 LRV enumerated on TYGA medium.
- All water samples with Ag only, Cu only and Ag+Cu but not the metal-free control water treated by simulated solar radiation to model SODIS and enumerated on TYGA medium met the WHO Highly Protective 4 LRV reduction target within 60 min.
- Differences in *E. coli* LRVs in water determined using selective LSA medium versus non-selective TYGA medium for enumeration indicated the ability of injured *E. coli* to be resuscitated on the non-selective medium after initial exposure to solar radiation, although this ability decreased with greater metal concentrations and/or light exposure time.
- The observed increased and more rapid inactivation of *E. coli* in water with added metals and solar light exposure offers the potential to improve bacteria reductions in stored water by incorporating sufficient light exposure from sunlight or possibly from other light sources to achieve this effect.

7.3 Limitations

The decision to produce prototype ceramic disk filters at a CWF factory was driven by the benefit of both using pre-existing manufacturing facilities and sources for materials that are used for and typical of full-sized filters in actual filter production practice as well as utilization of a realistic, low-resource manufacturing setting. However, in exchange for these benefits, there were certain limitations. First, despite best efforts to develop replicable manufacturing practices with as much precision as possible, there were some differences in filter performance, particularly between the Set 1 and Set 2 filter disk but also among individual filter disks within sets. Differences in flow rates and in corresponding LRVs were the indicators that the filter disks were not consistent in functional properties and performance. There are a few potential explanations for these differences, both between sets and among filters in each set. First, production of CWFs at the factory in the Dominican Republic typically occurs at a much larger scale and utilizes an industrial mixer to try to achieve a homogenous clay material. Given that the batch sizes for these studies were too small to utilize this machine, a simple, makeshift mixer was constructed with metal blades and a horizontal PVC pipe. Efforts were made to regularly scrape down the sides, and the clay mixture was additionally mixed by hand, but overall it was difficult to achieve the level of precision and quality control that an industrial machine or well-equipped laboratory could achieve. Next, ceramic disk filters were fired in a wood burning kiln, with Set 1 fired in a small test kiln and Set 2 fired in two batches in a medium sized kiln, still smaller than the kiln typically used to fire full sized CWFs. Although best efforts were made by the skilled kiln operators to control the temperature ramp rate and achieve a temperature

distribution as uniform as possible, this procedure is inherently difficult to control precisely and therefore replicate compared to an electronic kiln found in a laboratory.

Another limitation of the ceramic water filter study was the use of a laboratory water with a relatively high NaCl concentration (800 mg/L) without comparison with a more realistic environmental water sample. A tenfold dilution of D-PBS was chosen given its common usage as a buffered solution in microbiology. This is a deviation from what would be expected in natural waters given that the sodium concentration used (315 mg/L) is an order of magnitude higher than the concentrations typically observed in natural waters used for drinking water (<20 mg/L) (World Health Organization, 1996). However, at this early stage of the dissertation research, there was an oversight about the complexation of Cl^- with Ag and Cu. One potential effect of the high NaCl concentration is the decrease of Ag and Cu ions remaining in solution, whether in the ceramic filter pores or in the filter effluent, and thereby less available to contribute to disinfection. Also, there may have been unintended effects on the surface of the metal particles embedded in the filter, for example the formation of an external AgCl layer on a AgNP that could inhibit the release of Ag ions into the bulk solution. For batch experiments in Chapters 5 and 6, a dilute (1 mM) phosphate buffer was chosen to limit any undesired complexation of Ag or Cu ions.

For simulated storage and SODIS experiments in Chapters 5 and 6, the environmental water sample had low TOC and turbidity concentrations that may not be representative of many natural waters, especially more highly contaminated surface waters as often found in the developing world and used as drinking water sources. Metal ions could associate with the additional organic matter and/or suspended particles in such waters, reducing their ability to contribute to disinfection. In general, the magnitude of the observed disinfection results should

be confirmed in more challenging environmental water samples to determine applicability to household water treatment systems using such poor quality waters as sources.

7.4 Recommendations for future work

Further materials science research is needed to optimize the fired-in addition of Ag, Cu, and Fe to CWFs. One aspect for further consideration is the optimization of leached metal concentrations. Water quality parameters have been shown to affect the rate of metal leaching, with divalent ions such as Mg^{2+} increasing Ag ion leaching due to ion exchange reactions resulting in the displacement of Ag ions (Mittelman et al., 2015). Higher ionic strength, tested with the addition of NaNO_3 , and a lower pH value of 5 also increased Ag ion leaching in the same study. However, in a study with filter samples tested from three filter factories in different countries, the effects of influent water quality on Ag leaching were not consistent (Rayner et al., 2013b). In addition, certain material parameters should also be explored to optimize metal leaching.

One approach to evaluate factors affecting metal leaching is to test the effects of different firing temperature profiles, which may impact the association of the metal particles with the ceramic media and therefore have an effect on leached metal concentrations. The role of porosity, both overall percentages and pore size distributions, can be studied to optimize manufacturing to promote optimal metal leaching. These materials science studies were beyond the scope of this research. However, Chapter 3 results merit further study because they document antimicrobial effects from very low concentrations of leached metals in stored filter effluent that could potentially become higher if leached metals concentrations were higher.

Another alternative to consider is a metal coating as opposed to fired-in metal additives. Colloidal Ag/AgNP are commonly applied to CWFs post-firing in most CWF factories, so the potential for a Cu coating could be explored. Ionic Ag, typically in the form of AgNO₃, has been shown to leach more rapidly than Ag from AgNP in ceramic filters (Mittelman et al., 2015). If the goal were to increase leaching of Cu compared to fired-in effluent concentrations, a coating of an ionic solution may be beneficial. Alternatively, Cu nanoparticles could serve as an additional metal additive to AgNP, but leaching kinetics would need to be studied.

Further study of the approach of other methods to dose metals, such as copper wire, is warranted. Initial evidence showed increased inactivation of coliforms after storage in a receptacle with a piece of copper wire (Varkey and Dlamini, 2012). This study could be repeated with the goal of more specific characterization of the benefits of the copper wire. This would need to include: quantification of the leached Cu concentration in the effluent, the effect of storage duration, the use of metal quenchers at the time of sampling, and the addition of a virus such as MS2 bacteriophage to see if effects observed in Ag+Cu batch studies could be replicated in a filter receptacle.

There are challenges for the successful implementation of HWTS technologies beyond achieving microbial reduction targets. In order for a HWTS technology to achieve health impact, the user must practice correct, consistent, and continuous use of it (World Health Organization, 2011b). Estimates show that even just 10% of unsafe water consumed outside the home can reduce health impacts of HWTS technologies up to 96% (Brown and Clasen, 2012). Multiple studies have shown that a low level of long-term adherence to HWTS technologies resulted in reduced estimated or observed health improvements (Brown and Clasen, 2012; Clasen et al., 2015; Enger et al., 2013; Mäusezahl et al., 2009). Technologies that require little to no behavior

change may have a higher long-term adherence rate. Given that most households without a continuous piped water supply typically store water in the home, treatment technologies that can be integrated directly into storage containers present an opportunity for a minor but potentially beneficial adaptation to an existing practice.

Aside from traditional use of copper or brass storage containers, which have shown to be effective for the reduction of bacteria, a number of approaches have been tested for the incorporation of antimicrobial metals into safe storage. One study found that bacterial reductions after ceramic filtration further increased with the presence of a piece of copper wire in the receptacle (Varkey and Dlamini, 2012). This result should be replicated in a more rigorous study that measures the Ag and Cu concentrations in the effluent and uses metal quenchers at the time of sample collection to better quantify antimicrobial effects during water storage. Another approach uses a silver-embedded ceramic tablet that is designed to sit in a water storage receptacle, which achieved 3.8 LRV of *E. coli* after 6 h of exposure (Ehdaie et al., 2014). This approach can be further investigated with ceramic tablets embedded with both Ag and Cu. Given the MS2 reductions observed after 12 h of exposure to Ag and Cu ions in this research (Chapter 5), a method that doses both Ag and Cu could potentially reduce viruses in addition to bacteria in stored water. However, the viral disinfection capacity of Ag and Cu should be evaluated using representative human enteric viruses, as the differing properties and morphologies of enteric viruses can affect their survival and susceptibility to disinfectants.

SODIS with Ag and Cu additives in combination should be repeated with challenge water that includes parameters such as higher turbidity and different concentrations of organic carbon. Also, the enhanced inactivation of MS2 with metals plus solar light exposure should be confirmed with viruses more resistant to SODIS such as human adenoviruses (Carratalà et al.,

2016). Given the synergistic effect observed with metals plus solar light exposure for the reductions of *E. coli* and especially MS2, further experiments should determine whether ambient light may also enhance disinfection by metals. The use of alternative light sources that may also have the ability to increase and accelerate bacteria and virus reductions in stored water as was observed in this study should also be pursued, possibly with the use of waterproof LEDs. This could inform safe storage design, such as combining metal dosing technologies with a transparent water storage container to improve exposure to sunlight or use of another effective light source such as waterproof LEDs in the stored water.

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APPENDIX

Explanation of flow rate scale-up from disk to full sized ceramic pot filter

Source: van Halem, D. (2006). *Ceramic silver impregnated pot filters for household drinking water treatment in developing countries*. Delft University of Technology. Master's Thesis.

Description of total discharge through the filter, where

- Q is the flow rate (m^3/s)
- k is the hydraulic conductivity (m/s), calculated using Darcy's law and flow rate measurements
- t_f is the thickness of the filter wall (m)
- t_b is the thickness of the bottom of the filter (m)
- r_1 (top) and r_2 (bottom) are the radii of the filter (m)
- h_w height of the water (m)

$$Q_{\text{filter}} = \frac{k}{t_f} 2\pi \left(\frac{(r_1 - r_2)}{6L} h_w^3 + \frac{1}{2} r_2 h_w^2 \right) + \frac{k}{t_b} \pi r_2^2 h_w$$

To simplify the calculations, the height of the water was estimated. For fast flow rates, this may be an over estimate, and for slow flow rates, this may be an underestimate. For an 8-10 L volume filter, the water height was estimated to be 70-80% of the total water height after 1 h. The estimated full sized filter flow rate range is presented in Tables 3.1 and 3.3.