Improving Silver-Ceramic-Based Point-of-Use Water Treatment with Novel Copper Addition and Commercial Water Filters

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Abstract

In 2020, 1 out of 4 people did not have safely managed drinking water in their homes. Household water treatment is a low-cost method to decrease the pathogen load in drinking water to reduce instances of waterborne disease. A household water treatment product, the MadiDrop+, uses silver to achieve a 4-log reduction of bacteria, but requires 8 hours of contact time and does not remove turbidity from the water. Additionally, silver only achieves around a 1-log reduction of viral pathogens. This research addresses the shortcomings of the MadiDrop+ technology with two approaches: (1) combining the MadiDrop+ with household water filters and (2) incorporating copper into the MadiDrop+.

For the first part of this research, the bacterial reduction of several water filters with and without a silver-embedded ceramic tablet (MadiDrop+) in both the laboratory and household settings was evaluated. In laboratory tests, after 24 hours, Kohler Clarity filters with MadiDrop+ halves split between upper and lower reservoirs removed 6.0-log *E. coli* compared to filters alone that removed 2.7-log *E. coli*. After 2 hours, Rama Water carbon filters with MadiDrop+ halves split between reservoirs removed 3.3-log *E. coli* compared to filters alone that removed 1.7-log *E. coli*. After 2 hours, ceramic pot filters with a MadiDrop+ in the lower reservoir removed 3.9-log TCB compared to filters alone that removed 2.8-log TCB. In households, effluent TCB (CFU/100 mL) was between 0 – 12, 1 – 36, and 509 – 5,916 when the MadiDrop+ was in the lower reservoir, split between reservoirs, and not present in Kohler Clarity filters, respectively. Silver levels were ≤ 100 µg/L, the drinking water limit set by the U.S. EPA. The addition of silver via MadiDrop+ either wholly in the lower reservoir or split between upper and lower reservoirs of household water filters improved bacterial reduction in both laboratory and household settings.

In tandem, this dissertation developed and evaluated several approaches to achieving sustained release of copper into water. Copper has been shown to have antibacterial, antiviral, and antifungal effects in drinking water to combat microbial contamination and thus decrease instances of waterborne disease. Studies have shown 2.5- and 1.8-log reductions of *E. coli* and MS2 bacteriophage, respectively, after 6 hours of contact time with 300 µg/L copper. This is well below the drinking water limit of 1,300 µg/L. This research sought to develop a copper-based household water treatment product that could consistently release ~300 µg/L copper into 10 L of water daily for at least 1 week. The first approach was to embed copper into ceramic tablets. We varied the copper salt, mass of copper, firing temperature, and firing environment. Copper-embedded ceramic tablets generally released high amounts of copper in the first few days of use followed by gradual or drastic declines over the following days. The second approach was immersing copper metal in water. We evaluated copper release from 3 copper metal products that vary in surface area: a sheet (lowest surface area), a mesh, and a screen (highest surface area). Unlike the copper-embedded tablets, none of the metallic copper interventions had initial spikes or drastic drops in copper release over 8 days of use. The copper sheet consistently released 70-129 µg/L copper over 8 days but was more than 3 times the cost of other interventions. The copper mesh and copper screen released 145-256 µg/L and 188-333 µg/L copper, respectively, over 8 days of use and cost $0.75

and $2.00 per 10 grams, respectively. Given consistency of copper release, target range of copper concentrations, and low cost, the copper mesh and copper screen were further evaluated in combination with the MadiDrop+.

We found that wrapping copper mesh around the MadiDrop+ decreased daily silver concentrations from 47 - 82 µg/L to 1 - 6 µg/L. Folding the copper screen into a 2x2 inch area reduced daily copper concentrations in the water from 203 - 293 µg/L to 20 - 31 µg/L. Placing ten grams of unfolded copper screen and the MadiDrop+ in the same container but not wrapped around each other provided an average of 174 - 325 µg/L copper and 60 - 141 µg/L silver daily for the first 15 days of use. Copper concentrations remain between 149 - 365 µg/L for 92 days of use.

Lastly, the MadiDrop+ and copper screen, coined MadiDrop+Cu, removed >6-log *E. coli* and >3-log MS2 Bacteriophage after 8 and 24 hours of contact time, respectively. Combining the MadiDrop+Cu with chlorinated polymer gels achieved the greatest viral disinfection, reaching 4.1-log removal of MS2 Bacteriophage. MadiDrop+Cu achieves 1-star performance of the World Health Organization scheme for household water treatment. The results collected in this dissertation support the feasibility and benefit of commercializing MadiDrop+Cu which achieves greater disinfection of drinking water than the MadiDrop+ alone. The next steps in this research are to continue testing longevity of the copper screen, how long-term use affects the disinfection properties, and a practical design for using the MadiDrop+Cu.

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Research Outcomes

Sections of Chapter 2 were published in a book chapter in "Water Purification: Processes, Applications and Health Effects", in 2022. Chapter 3 was accepted by the Journal of Environmental Engineering in February 2023 and is in production. Results from chapters 4 and 5 are being prepared as a single manuscript to be submitted to a peer-reviewed journal. A list of presentations disseminating this dissertation research is also provided.

List of Publications

Harris, J.D.; Davis, J.; Reese, M.; Mannzhi, M.; Tshidumo, M.; Edokpayi, J.N.; Smith, J.A. Improving Antibacterial Performance of Household Water Filters with a Silver-Embedded Ceramic Tablet. *Journal of Environmental Engineering,* 2023. https://doi.org/10.1061/JOEEDU/EEENG-7264

Harris, J.D.; Estrella-You, A.; Smith, J. A. Development and Evaluation of a Novel Copper- and Silver-Based Household Water Treatment. *In preparation,* 2023.

Estrella-You, A.; **Harris, J. D.**; Singh, R.; Smith, J. A. Chapter 1. Inactivation of Waterborne Pathogens by Copper and Silver Ions, Free Chlorine, and N-Chloramines in Point-of-Use Technology: A Review. In *Water Purification: Processes, Applications and Health Effects*; Nova Science Publishers, 2022.

Hill, C. L.; **Harris, J. D.**; Turner, S. S.; Wason, K. L.; Gaylord, A. P.; Hatley, M. G.; Hardcastle, L. T.; Roberts, I. T.; You, J. Y.; Renneker, K. O.; Edokpayi, J. N.; Smith, J. A. Field and Laboratory Assessment of a New Electrolytic Point-of-Use Water Treatment Technology. *Water* 2022, *14* (7), 1077. https://doi.org/10.3390/w14071077

Glover, C. F.; Miyake, T.; Wallemacq, V.; **Harris, J. D.**; Emery, J.; Engel, D. A.; McDonnell, S. J.; Scully, J. R. Interrogating the Effect of Assay Media on the Rate of Virus Inactivation of High-Touch Copper Surfaces: A Materials Science Approach. *Advanced Materials Interfaces* 2022, *9* (17), 2200390. https://doi.org/10.1002/admi.202200390.

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List of Presentations

Harris, J.D. *MadiDrop+ Improves Disinfection Performance of Point-of-Use Water Filtration System*. Virtually presented findings at the University of Virginia Engineering Research Symposium. April 2021

Harris, J.D. *Silver-Embedded Tablet Increases Bacterial Removal by Ceramic and Carbon Point-of-Use Water Filters*. WaterJam. September 15th, 2021

Harris, J.D. *Increasing the Disinfection Potential of a Point-of-Use Water Treatment Technology*. SEAS Trustees Board Annual Meeting. October 15th, 2021

Harris, J.D. *MadiDrop+ Improves Household Water Filters in Lab and Field Environments*. Presentation to federal Brazilian delegates in the Special Secretariat of Indigenous Health (SESAI). October 5th, 2022

Harris, J.D. *Improving Silver-Based Household Water Treatment with a Novel Copper Addition*. University of Virginia Engineering Research Symposium. March 2023

Chapter 1: Introduction

In 2020, 1 out of 4 people did not have safely managed drinking water in their homes (WHO, 2021). Drinking untreated water can transmit waterborne diseases such as cholera, dysentery, and typhoid. A common symptom of waterborne disease, diarrhea, is the second leading cause of death in children under 5 years old (WHO, 2017). Diarrheal disease is especially prevalent in under-resourced communities where centralized water treatment is inconsistent, unmonitored, or unavailable (Kahler et al., 2016). In the absence of centralized water treatment, household water treatment (HWT) can reduce the pathogen load in drinking water to decrease instances of diarrhea and death (CDC, 2018).

Chlorine-based disinfection is a common HWT in under-resourced settings due to its effectiveness and short contact time. However, chlorine changes the taste and odor of water and has the potential for harmful disinfection by-products (Lantagne et al., 2010). Users may discontinue their household chlorination if they perceive a change in taste/odor, limiting its effectiveness (Mitro et al., 2019). Alternatively, silver and copper do not change the taste or odor of drinking water and do not create disinfection by-products.

Chapter 2 provides an extensive literature review of silver and copper as disinfectants in drinking water. We present available literature on the kinetics and mechanisms of silver-based and copper-based disinfection in water, categorized between bacteria and viruses. The toxicity of silver and copper to humans and in waterways is discussed. Lastly, papers that have found synergistic disinfection of bacterial and viral pathogens from the combination of silver and copper are reviewed. Information from Chapter 2 is often called upon to discuss findings in the following chapters.

Chapters 3, 4, and 5 share a common goal to improve the MadiDrop+, a product developed at the University of Virginia that uses silver as a chemical disinfectant. The MadiDrop+ is a 52-gram porous ceramic tablet (8 x 3 x 1.4 cm) embedded with metallic silver patches by a proprietary method. The tablet is placed into 10-20 liters of water and gradually releases silver ions for a 3-4 log reduction of coliform bacteria after 8 hours (Singh et al., 2019).

A single MadiDrop+ can be used daily for one year to treat 7,000 liters of water between $0.0008-$0.002 (USD) per liter. Comparatively, chlorine tablets can cost between $0.001-$0.01 per liter of water treated (Sobsey et al., 2008). Boiling water can cost users between $0.005-$0.049 per liter, depending on the fuel source (charcoal, electricity, gas, wood, etc.) (Clasen et al., 2008; Psutka et al., 2011). The MadiDrop+ offers treatment at a lower cost than other disinfection based HWT.

Another method of HWT that does not require an external energy source is gravity-fed filtration. There is a diverse market for household water filters that varies in cost and efficacy at removing contaminants. A user pours untreated water into the upper reservoir of the filters, which passes through the filter medium to reach the lower reservoir, where treated water is stored. There is no mechanism in place to prevent recontamination of stored water. A 2013 study in Fiji found

that 76% of households with water filters still had bacterial contamination in their stored water, suggesting that recontamination is a common concern for filtration treatment (Kohlitz et al., 2013).

Filtration alone can remove turbidity and some pathogens but is susceptible to recontamination of stored water. The MadiDrop+ provides residual disinfection to stored water but requires 8 hours of contact time and does not decrease turbidity. Chapter 3 of this dissertation evaluates the benefits of combining the MadiDrop+ tablet with several commercially available filters in both laboratory and household settings. First, researchers evaluated the effect of MadiDrop+ location in Kohler Clarity, Rama carbon, and ceramic pot filters in the laboratory setting. The Kohler Clarity filter and MadiDrop+ were then evaluated in 36 households in a rural community of Limpopo, South Africa for four weeks. The bacterial removal, silver release, and turbidity were measured to determine any water quality benefits of adding a MadiDrop+ to household water filters.

Chapters 4 and 5 of this dissertation focus on achieving the World Health Organization (WHO) performance criteria for HWT. The WHO has rigorous pathogen-removal standards for HWT where performance classifications are assigned to technologies that can reduce specified amounts of bacteria, viruses, and protozoa from drinking water (Table 1.1). Certification from the WHO is important for aid organizations and users to trust a water treatment product. More importantly, it is a designation that a technology removes disease-causing pathogens in attempts to reduce waterborne disease.

Table 1.1 The World Health Organization classifies household water treatment technologies into three levels of performance: 1-star, 2-star, and 3-star (World Health Organization, 2019).

Performance classification	**Bacteria** ($\log_{10}$ reduction required)	**Viruses** ($\log_{10}$ reduction required)	**Protozoa** ($\log_{10}$ reduction required)	**Interpretation** (with correct and consistent use)
★★★	≥ 4	≥ 5	≥ 4	Comprehensive protection
★★	≥ 2	≥ 3	≥ 2	
★	Meets at least 2-star (★★) criteria for two classes of pathogens			Targeted protection
–	Fails to meet WHO performance criteria			Little or no protection

Silver ions are effective against bacteria, but only achieve around a 1-log reduction (i.e., 90% reduction) of protozoa and viruses (Ehdaie et al., 2020; World Health Organization, 2018). There is evidence that combining silver with copper results in synergistic activity against more classes of pathogens (Arakawa et al., 2019; Chen et al., 2008; Soliman et al., 2020; Vaidya et al.,

2017). Like silver, copper does not change the taste of water when below its' drinking water limit (Zacarías et al., 2001). Chapters 4 and 5 sought to develop a product that could release both silver and copper into drinking water to increase disinfection properties from the MadiDrop+ alone.

Chapter 4 explores methods for achieving sustained release of copper into water. We took two approaches for this objective: (1) embedding metallic copper into the pores of a ceramic tablet and (2) evaluating the oxidation of copper directly from pieces of copper metal in water. The most appropriate methods for releasing copper, a copper mesh and a copper screen, were paired with the MadiDrop+ in Chapter 5. Chapter 5 demonstrates the effects of folding the copper screen and proximity between mesh and MadiDrop+ on copper and silver release in water. The most appropriate solution, the copper screen with the MadiDrop+, was further evaluated for copper and silver release for 90+ days of use in 10 liters of water. The MadiDrop+ and copper screen, coined MadiDrop+Cu when used together, were tested individually and in combination against *Escherichia coli* and MS2 Bacteriophage after 8 and 24 hours of contact time. Additionally, a chlorinated polymer gel was developed and evaluated for bacterial removal in Estrella-You (2023). was tested in combination with the MadiDrop+Cu for disinfection of MS2 Bacteriophage.

Chapter 2: Literature Review - Inactivation of Waterborne Pathogens by Copper and Silver

2.1 Silver as a disinfectant

Silver has been used for thousands of years as a disinfectant for bodily wounds and food and water storage (Silvestry-Rodriguez et al., 2007). In recent years, silver ions and silver nanoparticles (AgNPs) have been studied for their disinfectant properties in drinking water (Cameron et al., 2016; Dankovich and Gray, 2011; You et al., 2011). AgNPs benefit from high surface area to volume ratio, enhancing antimicrobial activity (Hamad et al., 2020).

The U.S. EPA and WHO recommend a daily drinking water limit of 100 μg/L of silver, lower than the limits for copper or chlorine. However, silver concentrations below the drinking water limit have strong antibacterial properties against both gram-negative and gram-positive bacteria (Singh et al., 2019; World Health Organization, 2018; Zahoor et al., 2021). Evidence is still limited for silver's inactivation of viruses and protozoa in drinking water (World Health Organization, 2021).

The following review discusses existing literature on the inactivation kinetics and mechanisms of silver on bacterial and viral species. We also discuss the toxicity of silver on waterways and human health.

2.1.1 Inactivation kinetics with silver

Many studies have hypothesized a common biocidal mechanism that depicts the binding of silver ions with the negatively charged cell wall and blocks energy transfer which results in cell death. But the rate of inactivation varies with the type of disinfectant used for different microorganisms. In general, the microbial population inactivation kinetics by a disinfectant is determined from the reaction rate constant (*k*) (Jacobs, 1960). A study by Nawaz et al. (2012) explored rainwater as a potential source of potable water using silver in a cost-effective way (concentration of silver varied from 10–100 μg/L) to disinfect *E. coli* and *P. aeruginosa.* Experiments were conducted for 168 h with different time intervals to evaluate the parameters including the inactivation rate. The factors involved in the inactivation of microorganisms are biocide concentration, the temperature of exposure, pH, and organic matter. The average values of the inactivation rate constants were calculated by using equation (2). At low concentrations of silver (10–20 μg/L) neither *E. coli* nor *P. aeruginosa* was completely inactivated. The reaction rate for *P. aeruginosa* at this concentration of silver was rapid as compared to *E. coli*. The graphical representation of log inactivation of *E. coli* and *P.* aeruginosa versus time is following Chick's findings (see Figure 2.1 (a) and (b), respectively). According to Chick, the logarithmic trend of bacterial inactivation versus time will be a straight line (Chick 1908). Higher '*k*' values indicate the sensitivity of microorganisms to the increased concentration of biocide (Yoon et al. 2007), which results in a decrease in the survival rate. *E. coli* looks more sensitive to an increase in the concentration of disinfectant than *P. aeruginosa* (See Table 2.1). However, at lower concentrations of silver (10–20 μg/L) the reaction rate was slower for *E. coli*. The rapid inactivation rate of *P. aeruginosa* could be associated with less CFU of this microorganism which results in more silver contact being available for *P. aeruginosa* than *E. coli*.

Various studies have tested different means of viral inactivation (See Table 8) with log reduction and required contact time. It is evident (See Table 8) that the greater the rate constant, the shorter the contact time needed to achieve the same log reduction. Shimabuku et al. (2018) demonstrated that granular activated carbon (GAC) modified with silver and copper oxide enhanced inactivation capacity, and the rate constant (*k*).

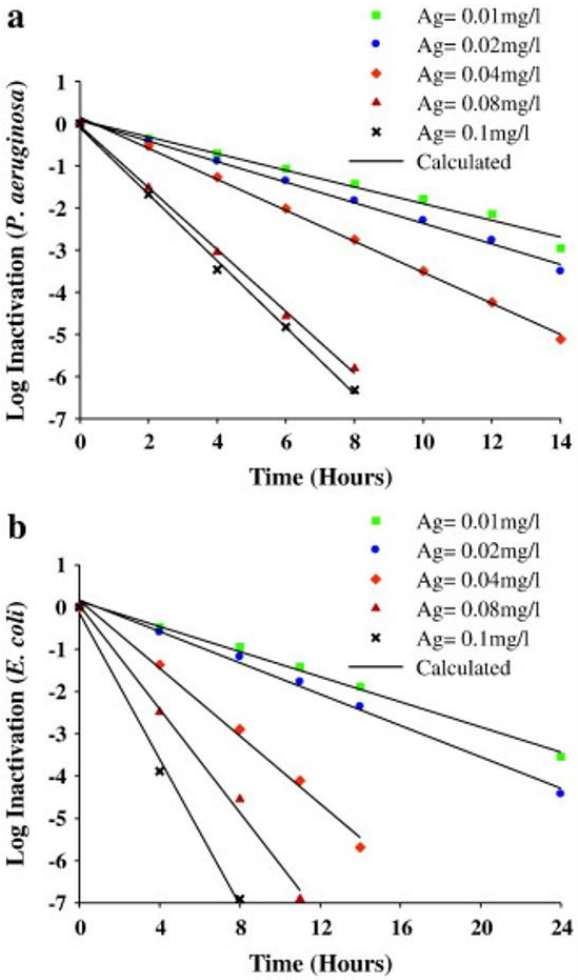


Figure 2.1. Trend of (a) *P. aeruginosa* and (b) *E. coli* log inactivation vs. time (experimental vs. calculated results). Reprinted with permission from Nawaz et al. (2012). Copyright 2012 Elsevier.

Bacteriophages T4 are well-known model agents used in many research studies which can serve as viral indicators to evaluate the effectiveness of biocidal agents/treatment technologies. Inactivation kinetics were described by the Chick-Watson linear model where viral inactivation was calculated as a function of the elapsed time of the experiment (Mamane, Shemer, and Linden 2007; Shimabuku et al. 2018). Agnihotri, Mukherji, and Mukherji (2019) investigated disinfection potential and silver release of immobilized-AgNPs and chloridized silver surfaces for simulated lake water. The efficacy of immobilized AgNPs was checked by altering the water quality parameters in model lake water.

Table 2.1. *k* and R^2 values for *P. aeruginosa* and *E. coli* at various concentrations of disinfectant (silver) (Nawaz et al. 2012)

Silver Concentration (µg/L)	*k* (1/h)		R^2 [6]	
	P. aeruginosa	***E. coli***	***P. aeruginosa***	***E. coli***
10	0.1979	0.1486	0.9818	0.9931
20	0.2440	0.1856	0.9954	0.9932
40	0.3677	0.4009	0.9980	0.9933
80	0.7335	0.6132	0.9984	0.9943
100	0.7891	0.8635	0.9975	0.9949

[6] Determination coefficient R^2: proportion of variability in a data set that is accounted for by the statistical model.

Table 2.2. Viral inactivation studies with different adsorbents (Shimabuku et al. 2018)

Study	Pathogen	Adsorbent	Achieved log reduction	Contact Time (min)
Zodrow et al. 2009	Bacteriophage MS2	Ag^+ ions	4.0	90
Sadeghi et al. 2013	Bacteriophage PRDI	Sand	4.0	600
Minoshima et al. 2016	Bacteriophage Qβ	Ag_2O	3.3	60
		$AgNO_3$	1.7	60
		Ag_2S	1.3	60
Shimabuku et al. 2018	Bacteriophage T4	GAC/NP6	4.0	11.01

The disinfection experiments were conducted over a much larger alkalinity range up to 600 mg/L than the typical range of alkalinity (30–200 mg/L globally) in drinking water to address the entire permissible limits for alkalinity. It was observed that even at the highest concentration of alkalinity (600 mg/L), the time to achieve complete disinfection was delayed marginally (10 min). When compared with the disinfection profile of chloridized Ag plate, higher alkalinity values caused an increase in disinfection time (165 to 195 min). Even though complete disinfection was achieved for all the alkalinity range, it did not affect the extent of disinfection regardless of the form of silver used for the disinfection. However, except for the low alkalinity values, i.e., 75 and 150 mg/L, an increase in alkalinity progressively reduced silver release into the water, which resulted in a marginal impact on antimicrobial activity. For different alkalinity values, silver release from immobilized AgNPs was found to be in the range of 15 to 22 μg/L at 150 min whereas, for the chloridized silver plate, aqueous silver concentration was found in the range of 91–116 μg/L after 240 min. Authors concluded that regardless of high alkalinity, there was a similar bactericidal effect of immobilized AgNPs, which supports the hypothesis that disinfection is not merely achieved through the release of silver ions.

2.1.2 Mechanisms of bacterial inactivation with silver

AgNPs can themselves kill bacteria or can release silver ions that kill bacteria. The AgNPs can anchor to the cell surface and cause cell membrane denaturation after accumulating in the pits that they form on the cell wall (Yin et al., 2020). Due to their nanoscale size, AgNPs can penetrate bacterial cell walls and disrupt the cell membrane. The disruption of the cell membrane can rupture organelles or cause cell lysis. The AgNPS can then suppress respiratory enzymes once inside the bacterial cell (Chatterjee et al., 2015). It is also suggested that the electrostatic attraction between negatively charged bacterial cells and positively charged nanoparticles is crucial for the antibacterial properties of AgNPs (Sondi and Salopek-Sondi, 2004).

At high concentrations, silver ions inhibit enzymatic activity of bacterial cells, reacting with electron donors like sulfhydryl groups (Dibrov et al., 2002). For a gram-negative bacterium like *Vibrio cholerae*, lower concentrations of silver ions induce a proton leakage through the cell membrane (Dibrov et al., 2002). Silver ions bind to negatively charged components in proteins and nucleic acids, thereby causing structural changes in bacterial cell walls, membranes, and nucleic acids that affect viability. In particular, silver ions are thought to interact with functions such as thiol groups, carboxylates, phosphates, hydroxyls, imidazoles, indoles, and amines (Kumar et al., 2005).

Like AgNPs, silver ions can disrupt the bacterial envelope and enter the cell where they deactivate respiratory enzymes. The silver ions generate reactive oxygen species (ROS) in the cell and interrupt adenosine triphosphate (ATP) production (Kumar et al., 2005). ROS are responsible for disrupting DNA replication. It is also proposed that silver ions can inhibit the synthesis of proteins by denaturing ribosomes in the cytoplasm (Yin et al., 2020) or that silver binds to functional groups of proteins, resulting in protein denaturation (Sondi and Salopek-Sondi, 2004).

2.1.3 Mechanisms of viral inactivation with silver

A number of studies have demonstrated the antibacterial potential of silver nanoparticles, but little is known about the antiviral efficacy of these nanoparticles and so the mechanism for the inactivation of viruses by silver ions is also unclear. Silver has proven to be effective against several types of viruses including human immunodeficiency virus (HIV), hepatitis B virus, herpes simplex virus, respiratory syncytial virus, and monkeypox virus and several respiratory pathogens, including adenovirus, parainfluenza, and influenza. It is believed that the inactivation involves a modified site-specific Fenton mechanism, generating hydroxyl radicals near the target site (Choi and Hu 2009; Yahya, Straub, and Gerba 1992). Antiviral efficacy of silver ions involves the interaction of thiol groups in viruses' proteins and that may damage the proteins. This further interrupts the transfer of electrons, which restrict cell division (Shimabuku et al. 2018).

The antimicrobial effects of AgNPs are due to a large surface area to volume ratio which generates more efficient contact with microorganisms. This also enhances interactions with microbial proteins. Two separate mechanisms to interfere with viral replication have been proposed by the antiviral ability of AgNPs. One is by binding via sulfur-bearing residues on surface glycoproteins. This can prevent the attachment and entry of the virus into the host cell (Lara et al. 2010; Morris et al. 2019). The second mechanism involves AgNPs crossing the cell membrane and effectively blocking cellular factors necessary for the proper assembly of viral progeny (Khandelwal et al. 2014; Morris et al. 2019).

2.1.4 Silver Toxicity

The role of silver in medicine and water treatment has been studied for centuries. The threat of silver release in the environment, specifically into water streams, is a concern and a reason to study the fate and transport of silver nanoparticles in the environment. These engineered nanoparticles are used for several applications, but when released into water bodies, can transform into different species and have environmental impacts. The environmental risks could be analyzed by assessing environmental release, transport, bioavailability, and toxicity of metal nanoparticles from point and nonpoint sources (Nowack and Bucheli 2007). The interaction between toxic organic compounds and nanoparticles can reduce the toxicity and create an inert system for living organisms (M. Rai, Yadav, and Gade 2009; Simonet and Valcárcel 2008). On the other hand, silver nanoparticles, when oxidized to form Ag_2O, release Ag^+ ions after dissolution which has potential biological impacts, and this ionic silver is responsible for toxicity (Choi and Hu 2009; Marimuthu et al. 2020). Also, post wastewater treatment poses a significant risk when silver nanoparticles are released into the water stream and are reported to be toxic due to the inhibition of nitrification. Flores-López, Espinoza-Gómez, and Somanathan (2019) reported the oxidative stress resulted from an imbalance between the production of ROS and their degradation by the cell. Figure 2.2 summarizes the harmful effects of oxidative stress on important cellular structures and impacts on human health including oxidation of protein, lipid, mitochondria, damage to DNA, and carcinogenicity.

In a recent study using continuous assessment of ecological DNA for over a year, the long-term effects of silver nanoparticle induced toxicity revealed that silver nanoparticles drastically affect the soil microbial community even at low concentrations (Grün et al. 2018). Another recent study compared the uptake of silver nanoparticles by fish cells and algae. The findings suggest that the algal cell wall was impervious to nanoparticles, but uptake happened through endocytosis in

fish cells. The exposure to silver nanoparticles affects extracellular and intracellular membrane-bound proteins in algae and fish cells, respectively (Marimuthu et al. 2020; Yue et al. 2017).

Figure 2.2. Harmful effects of oxidative stress (resulted from an imbalance between the production of ROS and their degradation by cell) on important cellular structures and effects on human health (oxidation of protein, lipid, mitochondria, damage to DNA, carcinogenesis). Reprinted with permission from Flores-López, Espinoza-Gómez, and Somanathan (2019). Copyright 2019 John Wiley & Sons.

The effect of nanoparticles on the gastrointestinal tract have been studied with in vitro cell culture models and in vivo studies in model animals. A study by Khare et al. (2020) evaluated the use of an ex-vivo human tissue model to examine the sex dependent and inflammatory effects of nanomaterials on the intestinal tract. Ex vivo refers to the utilization of freshly excised human intestinal tissues which were excised from human ileal tissue during routine surgical procedures. Extracted protein and RNA can then be analyzed to evaluate changes in cytokine levels and mRNA expression for genes related to epithelial cell junctions. This study used ex vivo intestinal ileal explants excised from human subjects as a model to examine the inflammatory effects of nanomaterials on the intestinal tract. Authors observed sex-dependent changes in cell junction gene expression after treatment with silver nanoparticles (AgNPs). Significant changes in the inflammatory response, as measured by cytokine expression, were only observed for smaller AgNPs (10 nm and 20 nm). Authors selected these samples for further investigation into mRNA expression of intestinal permeability-related genes. It was reported that the smallest AgNPs (10

nm) seemed to affect cell junction gene expression most adversely. Analysis of cytokine expression data indicated that intestinal tissue of male and female subjects responded differently to AgNP treatment, with male samples showing significantly elevated granulocyte-macrophage colony-stimulating factor (GM-CSF) after treatment with 10-nm and 20-nm AgNPs for 2 hours and significantly elevated RANTES (regulated on activation, normal T cell expressed and secreted) after treatment with 20-nm AgNPs for 24 hours. Authors concluded that the ex vivo tissue model needs further evaluation to determine the impacts of nanomaterials and other xenobiotic compounds on human intestinal mucosa. Silver (in nanoparticle form or in ion form) can penetrate this defensive layer in several ways, raising questions about the safety of some consumer goods containing AgNPs. Further research is needed in this area.

Fortunately, silver nanoparticles have little reported toxicity towards humans (Le Ouay and Stellacci 2015; Marimuthu et al. 2020; M. Rai, Yadav, and Gade 2009). The only known human health condition resulting from long-term exposure to silver is argyria. Argyria is a rare, irreversible discoloration of the skin, eyes, nails, and/or gums (Baek and Cohen, 2022). Argyria develops after chronic silver ingestion or inhalation. It is not a life-threatening condition but is cosmetically undesirable (Lansdown, 2006). A lifetime ingestion of 10 g of silver can be considered a no-observable-adverse-effect-level (NOAEL) for humans based on available epidemiological and pharmacokinetics data (Nawaz et al. 2012). The daily drinking water limit of 100 µg/L was determined to be half of the NOAEL over a 70-year period, assuming a drinking-water intake of 2 liters/day (World Health Organization, 2017a).

2.2 Copper as a disinfectant

Like silver and gold, copper is a noble metal. Unlike many water disinfectants, copper is an essential trace element for human health, aiding in the formation of red blood cells, leukocytes, and enzymes required for growth, development, and maintenance (Arakawa et al., 2019; Cervantes et al., 2013; Gaetke and Chow, 2003). In the 5th or 6th millennium B.C., humans found usefulness in copper's metallic form because it did not require smelting (Grass et al., 2011). Since then, humans have discovered attractive industrial properties of copper including high thermal and electrical conductivity, corrosion resistance, malleability, and alloying ability, making it a common-found element in electronics manufacturing and engineering (Barceloux and Barceloux, 1999).

Although today copper alloys are predominantly known for applications in electronics, copper has a historical biocidal property. Whether in ionic or compound form, copper was used throughout centuries in many cultures as a disinfectant for human tissue, solids, and liquids. Written between 2600 and 2200 BC, Smith's Papyrus is one of the oldest books ever known and contains the first record of disinfection by copper. The Egyptian text lists medicinal applications of copper like sterilizing wounds and treating drinking water (Konieczny and Rdzawski, 2012). Going forward, there is evidence that ancient Greeks, Indian Hindus, the Roman Empire, early Phonecians, Aztecs, and American pioneers all incorporated copper into health-related applications (Borkow and Gabbay, 2009).

By 1932, commercially available antibiotics became the forerunner in medical settings, decreasing the use of copper for medicinal purposes (Grass et al., 2011). However, there has been a surge in research for using copper to inactivate microorganisms to overcome recent antibiotic resistance. In comparison to other disinfectants, such as chlorine-based or silver compounds, more copper may be required to achieve the same level of disinfection. Unlike most chlorine

compounds, silver and copper do not change the taste or odor of drinking water. While silver is mainly effective in killing gram negative bacteria, copper is effective on both gram negative and gram positive bacteria (Cervantes et al., 2013; Drelich et al., 2017), has an antiviral effect on enteric viruses (Abad et al., 1994; Sudha et al., 2011) and has been shown to inactivate fungi such as yeast (Cervantes et al., 2013). Lastly, copper is about 10 times less expensive than silver, making it a viable alternative or addition to POU drinking water treatment systems.

The toxicity of copper on microbes will be detailed later in this chapter. However, instances of acute or chronic ingestion of especially high concentrations of copper have proven to be toxic to humans and certain animals. Toxicity can occur from accidental exposure, an occupational hazard, environmental contamination, or genetic defects in copper metabolism. The United States National Research Council Committee on Copper in Drinking Water published a chapter detailing the health effects of excess copper (National Research Council, 2000). Acute toxicity is commonly observed in cases of suicidal intent with ingestion of copper compounds. Systemic effects are observed following acute ingestion of more than 1g of copper salts, such as copper sulfate. The estimated lethal dose of Cu in an untreated adult can range from 10–20 g (Gaetke and Chow, 2003). Although studies have been published detailing reactions to acute copper toxicity, ranging from abdominal pain to death, the results still suffer from limitations. Confounders such as microbial water contamination are not explored thoroughly enough to distinguish the reaction between acute copper toxicity and pathogenic contamination. Additionally, the concentrations of copper are not reliably recorded in all studies.

When copper enters the human bloodstream, the first site of deposition is the liver (Gaetke and Chow, 2003). Therefore, chronic copper toxicity is most commonly manifested in liver diseases. There are rare medical conditions, such as Wilson's disease, that are genetically inherited and inhibit bodily copper excretion. Patients of Wilson's disease are predisposed to toxic effects of excess copper. Neurotoxicity from copper seems to only appear in patients with Wilson's disease (National Research Council, 2000).

Today, the United States Environmental Protection Agency (U.S. EPA) has established several maximum contaminant level goals (MCLGs) on disinfectants, inorganic chemicals, and organic chemicals in drinking water. Concentrations below the MCLGs have no known risk to public health (US EPA, 2020). The daily MCLG for copper is 1,300 µg/L, over ten times higher than that of silver. However, copper's disinfection potential in aqueous solution varies by the concentration of copper in water, contact time, type of microorganism and water chemistry.

Both engineering and medical journals have published studies using forms of copper as POU water treatment technologies. Several laboratory-based studies have tested the inactivation of microorganisms after storing contaminated water in copper containers (Cervantes et al., 2013; Sudha et al., 2012, 2011, 2009). Other studies investigated the disinfection efficacy of filtering water through paper containing copper nanoparticles (CuNPs) (Dankovich et al., 2016; Dankovich and Smith, 2014) or embedding stones with copper sub-microparticles that release copper in water over the course of a year (Drelich et al., 2017).

(Cervantes et al., 2013) and (Sudha et al., 2012, 2011, 2009) inoculated water with microorganisms and compared the rate of disinfection in copper containers versus a non-metallic container, such as glass or PVC. (Cervantes et al., 2013) tested the effects of copper containers with two series of experiments. Both series tested for an extensive list of gram negative and positive bacteria, as well as yeasts, found in Table 6. Series 1 and 2 exposed the organisms to the test material for 48 hours and recorded microbial activity over time. Most organisms in both series showed reduced viability after 30 minutes of contact with copper, compared to stable or increased

viability in PVC or Pyrex glass. All microorganisms in copper, except *E. faecalis* in series 1, were non-viable at 48 hours.

The concentrations of copper in each series of experiments were not reported in (Cervantes et al., 2013). The results of this study are a case for the use of copper pipes in healthcare facilities to disinfect common microorganisms found in hospitals. However, further research should be conducted to test the efficacy of copper piping when considering flow rate, pressure, turbulence, and other environmental variables.

(Sudha et al., 2009) reported the effects of storing three bacteria in copper pots, glass bottles with a copper coil, and glass bottles without copper present. The copper coil device was developed by the authors. Sterilized distilled water was inoculated with 500-1,000 CFU/mL and introduced to the three test materials. After 16 hours of incubation in the containers, all three pathogens tested were not culturable in the water treated with a copper pot or copper coil. The control glass containers saw an increase of 1-2 log after the 16 hours of incubation. Both the resulting pH and copper contents were within the WHO standards for drinking water. The copper pot and copper coil proved to be efficient with disinfection of bacterial contamination. The coil is one-tenth the cost of the copper container, suggesting that the copper coil would be an appropriate point-of-use device in a regular plastic container. Although these experiments with the copper coil were performed in 1 L of water, the authors claim that the surface area of the device can be scaled for the amount of water treated. A shortcoming of this study is that it does not test for the effect of turbidity or water chemistry on the effectiveness of either device. Field trials need to assess the performance of devices in households with appropriate participants.

(Sudha et al., 2011) tested for the antiviral effect of copper pots storing drinking water. The authors introduced the virus in aqueous suspension to 2L copper pots and 1L glass bottles for controls. The inoculated containers were left for 16 hours. Granular activated charcoal (GAC) was used to concentrate the virus from the water sample. The viral count in each water sample was estimated by plaque titration. Parameters such as pH of test water, virus concentration, and content of aqueous copper are summarized in Table 5.

Table 2.3: Parameters for two time points, 0 and 16 hours, are reported for copper and glass containers inoculated with Rotavirus. Data adapted from (Sudha et al., 2011) with permission from the copyright holders, IWA Publishing.

Time (hours)	Copper Container			Glass Container		
	pH	PFU/100 mL	Copper (µg/L)	pH	PFU/100 mL	Copper (µg/L)
0	7.02	2.25×10^{9}	-	7.02	2.25×10^{9}	-
16	8.76±0.03	Undetectable	447.25±4.78	8.5±0.2	8×10^{8}	Undetectable

Although the concentration of copper in the water is below the EPA's daily MCLG of 1,300 µg/L, the pH of the water stored in the copper container is slightly high at 8.76. National Secondary Drinking Water Regulations (NSDWRs), or secondary standards, are non-enforceable guidelines set by the EPA for contaminants that may have cosmetic or aesthetic effects in drinking water, such as pH. The acceptable range for pH of drinking water is between 6.5-8.5 (US EPA, 2015). It is unclear why the pH of the water in copper containers jumped from 7.02 to 8.76 in this paper, when (Sudha et al., 2012, 2009) performed similar experiments without the same increase

in pH. Additionally, to study the optimal contact time before consumption, a study needs to show the relationship of viral disinfection over time for this specific technology.

(Sudha et al., 2012) performed a similar experiment as (Sudha et al., 2009), but used groundwater instead of sterile distilled water. Distilled water is slightly acidic and might enhance copper leaching. This paper uses groundwater, slightly more basic, and some different pathogens to gain more evidence of the effectiveness of a copper pot for disinfecting drinking water with varying physiochemical properties. Groundwater from Bangalore was autoclaved then inoculated to ~50,000 CFU/100 mL. 2 L of inoculated water was poured into copper pots and 1 L of inoculated water was poured into the glass bottles. After 16 hours of incubation at 26°C, there was a decrease in copper release when using groundwater instead of distilled water (dropped from 426 to 177 µg/L). Regardless, no bacteria could be cultured or detected from the water stored in copper containers after 16 hours. In the glass control bottles, the quantity of bacteria either remained the same or slightly increased after 16 hours. Alkalinity, hardness, turbidity, chlorides, and sulfates all remained unchanged in copper and glass containers before and after inoculation. All physiochemical parameters tested complied with Bureau of Indian Standards and/or WHO limits. Although there was also a slight increase in the pH of the water using the copper container (7.83 to 7.93), the resulting pH complies with the EPA's secondary standard. A shortcoming of this study is the author's inability to confirm if the bacteria transformed into the viable but not culturable (VBNC) state. Further testing to confirm VBNC in the treated water needs to happen. Additionally, more research needs to be conducted to test copper containers' antimicrobial properties with less contact time and other waterborne pathogens such as protozoa.

Incorporating copper nanoparticles (CuNPs) into paper filters is another instance of copper POU technology in literature. After preparing sheets of CuNP paper in the laboratory in 2014, Dankovich et al. tested their effectiveness in disinfecting bacteria from a drinking water source in South Africa in 2016 (Dankovich et al., 2016; Dankovich and Smith, 2014). The 2016 study focused on two streams in the Luvuvhu stream catchment in the Venda region of Limpopo province, South Africa. The "rural" and "urban" streams are described in Table 6. Water samples from both sources were collected and analyzed over the course of a month for viable total coliform, *E. coli*., pH, and turbidity. Authors passed 2-3 L of sample water through paper with varying thickness containing either copper or silver nanoparticles. The CuNP filter papers provided over a 3-log reduction of *E. coli* and total coliform in the "urban" water source. In the "rural" water source, there was over a 2-log reduction of total coliform and there was less than 1 CFU/100 mL of *E. coli* in the effluent. The average turbidity of the test waters decreased from 8.1 NTU to 4.9 NTU. Average pH values for both water sources was 6.8. Although this proved an effective and inexpensive method for disinfecting 2-3 L of contaminated water, it would need further research and development to increase the volume of water treated. It is not sustainable to use a new paper filter for every 2-3 L of water, but it may be appropriate in a short-term, water-stressed scenario.

Table 2.4: Summary of settings in laboratory studies that use copper in POU water treatment technologies

	Sudha et al., 2009	**Sudha et al., 2011**	**Sudha et al., 2012**	**Cervantes et al., 2013**	**Dankovich et al., 2016**	**Drelich et al., 2017**
Technology	Copper pots and copper coils	Copper pots	Copper pots	Copper containers	Papers embedded with copper nanoparticles	Porous ceramic stones decorated with copper sub-microparticles inside the pores

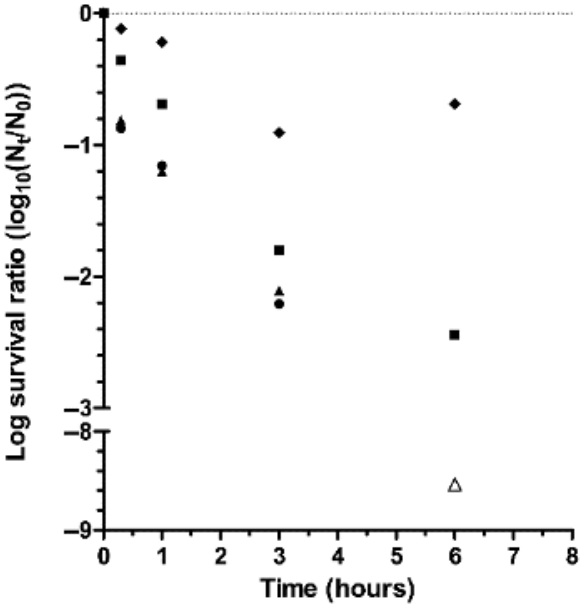
Dose	Between 405 to 460.47 µg/L	447.25 ± 4.78 µg/L	177 ± 16 µg/L	Not evaluated	1) "Rural": 222 µg/L 2) "Urban": 488 µg/L	50-200 µg/L
Source water	Sterilized distilled water inoculated with quantified cultures of bacteria	Reagent grade test water	Groundwater from Bangalore	• Series 1: saline solution with a final turbidity of 0.5 McFarland units • Series 2: sterile water	1) The "rural" stream: Cement-lined irrigation canal diverted from the Tshala stream in a rural village. Irrigation canal is used as the primary source for drinking water in the community. 2) The "urban" stream near the University of Venda which ultimately flows into the main municipal drinking water source for Thohoyandou, the Nandoni Dam Reservoir. Stream was polluted by raw sewage from a nursery school upstream.	Tap water
Pathogens analyzed	*Escherichia coli*, *Salmonella* Typhi, and *Vibrio cholerae*	Rotavirus strain SA11	*Vibrio cholerae* O1, *Shigella flexneri* 2a, enterotoxigenic *Esherichia coli*, enteropathogenic *E. coli*, *Salmonella enterica* Typhi, and *Salmonella* Paratyphi	• 5 gram-negative bacilli: Klebsiella *pneumoniae*, *Enterobacter aerogenes*, *Escherichia coli*, extended-spectrum β-lactamase producing [ESBL+] K *pneumoniae*, ESBL+ *E coli* • 4 gram-positive cocci: *Enterococcus faecalis*, MRSA, methicillin-sensitive *S aureus*, coagulase-negative *Staphylococcus* • 2 nonfermenting gram-negative bacilli: *Pseudomonas aeruginosa* and *Acinetobacter baumannii* • 3 species of *Candida* yeasts: *C albicans*, *C krusei*, and *C tropicalis* • Three additional organisms: *S aureus* ATCC 25923, *E coli* ATCC 25922, and *P aeruginosa* ATCC 27853.	Viable total coliform and *E. coli*	*Staphylococcus aureus* (gram-positive) and *Klebsiella pneumoniae* (gram-negative)
Other analysis	pH	pH	pH, turbidity, alkalinity, total dissolved solids (TDS), hardness, content of chlorides and sulphates	n/a	Turbidity was measured with a turbidimeter. Filter paper permeabilities were calculated from flow rates.	Scanning electron micrographs to quantify porosity and X-ray diffraction analysis to identify ceramic constituents.

Lastly, a laboratory study in 2017 formulated, characterized, and reported on antimicrobial performance of stones embedded with copper sub-microparticles in water for several months (Drelich et al., 2017). The stones were formed from clay then soaked in a copper sulfate solution for 5 days before being reduced in a hydrogen atmosphere. After preparation, the stones were submerged in tanks containing either 3.79 L or 1.90 L of tap water at either 20 or 50°C. Water samples were taken every 24 hours (except on weekends) and then emptied and refilled. For 410 uses, the stones released a steady concentration of copper below the MCLG. After 1 hour of contact time, the ceramic demonstrated a 1.5 log kill of *Staphylococcus aureus* and 1.3 log kill of *Klebsiella pneumoniae* and an almost complete disinfection of both pathogens at 3 hours of contact

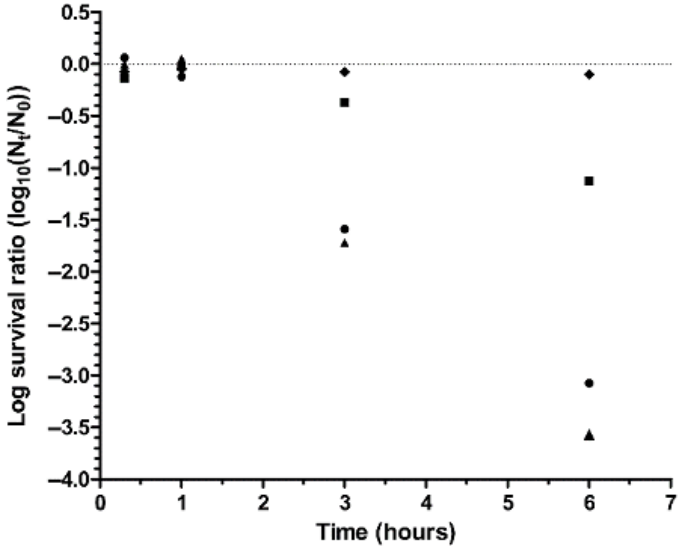
time (>99.9% reduction). The ceramic stones developed and tested in this study suggest a promising technique for treating drinking water in a low-resource setting. More testing needs to be done to increase the volume of water treated from 4 L for a field setting.

2.2.1 Bacterial inactivation kinetics with copper

Armstrong, Sobsey, and Casanova (2016) characterized the inactivation kinetics of two gram-negative bacteria by copper ions in water: *Escherichia coli* and *Pseudomonas aeruginosa*. The authors observed copper to inactivate the two bacteria at doses between 300 and 1,000 μg/L. *E. coli* was inactivated more rapidly than *P. aeruginosa*. Copper at 1,000 μg/L achieved 99.9% inactivation of *P. aeruginosa* and 99.9999997% inactivation of *E. coli* over 6 hours (See Figure 2.3).



(a)

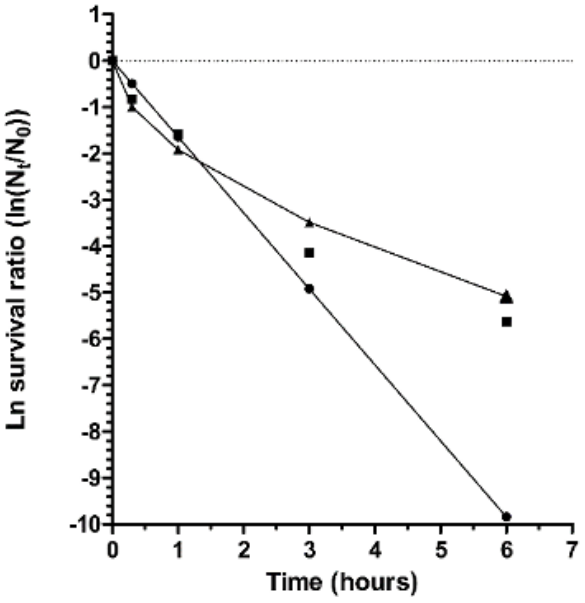


(b)

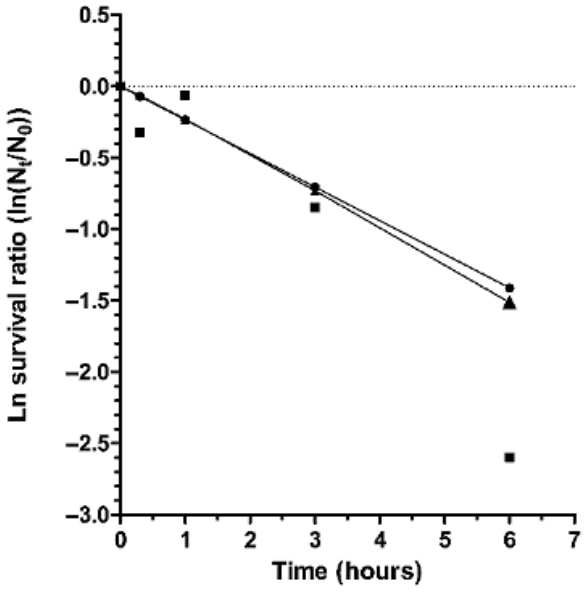
Figure 2.3. Inactivation of (a) *E. coli B* and (b) P. aeruginosa at various copper ion doses where diamonds = 100 μg/L, squares = 300 μg/L, circles = 1,000 μg/L, triangles = 3,000 μg/L, open symbols = limit of detection.

Reproduced from Armstrong, Sobsey, and Casanova (2016) with permission from the copyright holders, IWA Publishing.

Authors evaluated goodness-of-fit for the Chick-Watson (CW) and Hom models when compared to observed inactivation data. The Hom model was included to account for changes in microbial concentration over time if inactivation strayed from first-order kinetics.



(a)



(b)

Figure 2.4. Observed vs. predicted inactivation curves for (a) *E. coli* and (b) P. aeruginosa at 300 µg/L [dissolved ionic Cu]. (observed data = squares; predictions from Chick-Watson model = circles; predictions from Hom model = triangles). Reproduced from Armstrong, Sobsey, and Casanova (2016) with permission from the copyright holders, IWA Publishing.

As seen in Figure 2.4, CW predicts inactivation trends accurately for *P. aeruginosa*. Alternatively, CW deviates from observed data for E. coli inactivation. Authors speculate this is most likely due to the models' inability to account for tailing of *E. coli* inactivation after 3 hours. Water chemistry may impact inactivation kinetics for copper. Singh, Edokpayi, et al. (2019) tested the response of *E. coli* by copper ions in two water sources: synthetic groundwater (SGW) and natural surface water (NSW). The authors found that the specific lethality of copper ions is higher, and inactivation is faster, in SGW than NSW. Additionally, the CT for Cu to inactivate 99% of *E. coli* was almost double for NSW compared to SGW. One reason for the difference in inactivation between test waters is the presence of organic sediments and other cationic and anionic species in NSW which are not present in the SGW. Changes in organic matter and water constituents such as Ca^{2+}, PO_4^{3-}, Cl^-, hardness, and total solids may reduce the inactivation efficiency of *E. coli* by metals. Interestingly, maximum disinfection by copper ions occurred when the pH was 4. This phenomenon is affirmed by a study in 2017 that found the increased toxicity of CuO NPs in acidic pH was caused by the release of Cu^{2+} (Hsueh, Tsai, and Lin 2017).

2.2.2 Viral inactivation kinetics with copper

Bacteriophage MS2 is commonly used as a surrogate for human enteric viruses when studying inactivation kinetics. Armstrong, Sobsey, and Casanova (2017) published a study examining the disinfection kinetics of MS2 by copper ions in water. Authors found that disinfection of MS2 increased with increasing doses of copper. After 6 hours of contact time, doses of 300, 1,000 and 3,000 μg/L achieved at least a 1.8 $\log_{10}$ reduction. The lowest dose, 100 μg/L, saw a maximum of 0.5 $\log_{10}$ reduction throughout the 24 hours of the experiment. Daily MCLG prohibits the dose of 3,000 μg/L. Therefore, a dose of 300-1,000 μg/L copper would be appropriate for POU disinfection of MS2. However, inactivation of MS2 by copper occurs between 6 and 24 hours, a longer contact time than hypochlorite to achieve the same level of inactivation.

M. Y. M. Soliman et al. (2020) evaluated the physical and chemical parameters that affect copper antiviral activity. Inactivation kinetics of MS2 treated with 5,000 μg/L copper in acidic (pH 6), neutral (pH 7), and alkaline (pH 8) conditions followed the first order CW model. The slowest kinetics were observed at pH 8, most likely from a reduction in free ionic Cu^{2+} and dissolved Cu. Authors found that changes in solution pH had a clear effect on speciation of Cu and availability of Cu^{2+} ions.

Granular activated carbon (GAC) is used as an adsorbent for contaminants in drinking water, such as pesticides and organic matter (Golea et al. 2020). However, as GAC does not primarily disinfect water from pathogenic organisms, Shimabuku et al. (2018) examined modifications to address microbial contamination. Authors modified GAC with CuO nanoparticles and reported the inactivation kinetics of Bacteriophage T4, an indicator virus, for several scenarios. Two concentrations of copper, on a weight-by-weight percent, were tested with three doses of carbon. In the 300-minute experiment, the GAC without modification saw a maximum of 0.29 log reduction in virus. The GAC with 0.5% w/w copper had the same inactivation as the GAC without modification at 1 mg/mL of carbon. On the other hand, the GAC with 1% w/w copper had a significantly greater inactivation of 1.99 log, suggesting potential use for microbial disinfection of drinking water. The Chick-Watson model parameters for all six scenarios and the control GAC are detailed in Table 6.

Table 2.5. Bacteriophage T4 inactivation fitting parameters by Chick-Watson model (Shimabuku et al. 2018)

Modification	**Carbon dosage/NPs (mg/mL)**	**Parameters**			
		Rate constant, *k* (min^{-1})	**R^2** [5]	**Log_{10} removal**	**Elapsed time required to reach 4 log removal (hours)**
Not modified	1	0.00062	0.680	0.11	107.53
	3	0.001	0.809	0.21	53.33
	5	0.0013	0.915	0.29	51.28
0.5% w/w Cu	1	0.00063	0.680	0.11	105.82
	3	0.00307	0.971	1.61	7.17
	5	0.00370	0.980	1.82	6.80
1% w/w Cu	1	0.0055	0.951	0.90	12.12
	3	0.0325	0.936	1.50	8.72
	5	0.0831	0.923	1.99	7.47

[5] Correlation coefficients (R^2) indicate that the experimental data were well described by the Chick-Watson model

2.2.3 Mechanisms of bacterial inactivation with copper

Scientific authors speculate on the exact mechanism of bacterial inactivation by copper. (Domek et al., 1984) found that *E. coli* cells injured by copper in water showed a decreased use of oxygen and were likely to depend on fermentation pathways during recovery. The injured cells' reliance on fermentation suggests that copper may inhibit respiratory function in the cell membrane of bacteria. A few years later, (Thurman et al., 1989) added that copper may disrupt respiratory enzyme structure and function by binding thiol or other groups on protein molecules.

Additionally, copper can reversibly denature DNA in low ionic strengths by competing with hydrogen bonding. Copper ions can bind to helical structures and disorder them by cross linking between strands. Although factors such as pH, competition for binding sites, kinetic limitations, ability to form bonds, and stacking of nucleic acids can influence the copper ions ability to interact with the DNA molecules (Thurman et al., 1989).

(Liu et al., 1994) brings up that Cu^{2+}, being a positive ion, forms electrostatic bonds with negatively charged sites on the organisms' cell wall. These electrostatic bonds distort cell wall permeability, and paired with protein denaturation, leads to cell lysis and death. (Straub et al., 1995) adds that copper ions may also bind to nucleic acids in bacterial cells, catalyzing the formation of radicals, which split chemical bonds.

More recently, (Arakawa et al., 2019) commented on the interaction of CuNPs and bacteria in water. The authors claim that CuNPs produce free radicals, generating oxygen species that are highly reactive with the sulfur and phosphorus compounds in the bacterial cell membrane. Inhibiting transport of substances in the cell denatures the nucleic acids, disrupting its replication.

Although their experiments were not performed in water, (Hsueh et al., 2017) investigated the mechanism of toxicity of CuO nanoparticles. Authors claim major determinants of toxicity are the size and morphology of the CuO nanoparticles. In general, nanoparticles interact similarly with bacteria as copper ions: an increase in reactive oxygen species degrade cell membrane and thus cellular viability. Additionally, authors observed the nanoparticles increase membrane permeability and then enter cells to cause protein toxicity. It is an ongoing debate whether the nanoparticles themselves cause this aforementioned damage, or whether they leach Cu^{2+} ions that perform the inhibition. It is possible that the nanoparticles release Cu^{2+} ions which enter the cell to produce reactive oxygen species, then are deionized to CuO. The authors conclude that CuO NP toxicity must result from release of Cu^{2+} ions in lower pH.

2.2.4 Mechanisms of viral inactivation with copper

The mechanism of viral inactivation by a disinfectant must prevent reproduction in a susceptible cell. It is supposed that copper (II) may complex mRNA to inactivate viruses (Thurman et al., 1989). A more well-known mechanism for copper's relationship with viruses is described by a modified, site-specific Fenton reaction, where copper ions bind to viral macromolecules. This is then reduced by O_2 radicals, where it is reoxidized by H_2O_2, yielding OH radicals that cause the biological damage (Samuni et al., 1984). Since copper ions can repeat cyclic redox reactions, many OH radicals can be formed near the same site, multiplying damage for one target. The OH radicals may affect the peptide backbone of the capsid proteins of the virions (Abad et al., 1994).

(Abad et al., 1994) conjectured that metal ions may bind electron donor groups onto proteins or nucleic acids. They noticed that some viruses, like hepatitis A virus and other picornaviruses, have an inherently more stable molecular structure than others, leading to variation in inactivation efficacy by copper ions. (Li and Dennehy, 2011) propose that copper directly damages cellular lipid membranes after observing that viruses with RNA and lipid envelopes are more sensitive to copper. (Soliman et al., 2020) exposed MS2 to copper ions and found the morphology to stay intact, but there was evidence of penetration into the capsid of the virus. However, the exact structural damage could not be confirmed with the images they presented.

A study in 2016 observed the effect of several copper compounds on viruses with and without envelopes (Minoshima et al., 2016). HA and NA are proteins on viral envelopes that are significant for the spread of a virus. Both proteins have a stable structure due to many disulfide, or S--S, bonds. Authors found that exposure to Cu_2O and $CuCl_2$ inhibits the HA and NA proteins,

respectively, on influenza virus envelopes, inactivating the viruses' ability to infect host cells. Bacteriophage Qβ has capsid proteins on its surface instead of a viral envelope. Similarly, denaturation of the surface proteins can prevent the virus from infecting host cells. Of all the copper compounds tested, only Cu_2O was able to disinfect bacteriophage Qβ. This suggests that Cu_2O can denature protein structures on viral surfaces regardless of the presence of an envelope. See Table 2.6 for the summary of antiviral activity.

Table 2.6: Summary of antiviral activity of copper compounds against influenza virus and bacteriophage Qβ (Minoshima et al., 2016)

Copper compound	Virus with envelope (Influenza virus)	Virus without envelope (Bacteriophage Qβ)	HA	NA	S---S bonds cleavage
Solid-state Cu(I) compounds (Cu_2O)	Strong inactivation	Strong inactivation	Denaturations at low concentration	Denaturation at high concentration	Weak
Solid-state Cu(II) compounds (CuO)	No effect	No effect	No denaturation	No denaturation	No
Water-soluble Cu(II) compounds ($CuCl_2$)	Inactivation	No effect	No denaturation	Denaturation	Strong

2.2.5 Copper toxicity

Copper is an essential element for human health, aiding in the formation of red blood cells, leukocytes, and enzymes required for growth, development, and maintenance such as cytochrome c oxidase, tyrosinase, p-hydroxyphenyl pyruvate hydrolase, dopamine beta hydroxylase, lysyl oxidase, and Cu-zinc superoxidase dismutase (Arakawa et al. 2019; Gaetke and Chow 2003). However, instances of acute or chronic ingestion of especially high concentrations of copper have proven to be toxic to humans and certain animals. Toxicity can occur from accidental exposure, an occupational hazard, environmental contamination, or genetic defects in copper metabolism.

The United States National Research Council Committee on Copper in Drinking Water published a chapter detailing the health effects of excess copper (National Research Council, 2000). Acute toxicity is commonly observed in cases of suicidal intent with ingestion of copper compounds. Systemic effects are observed following acute ingestion of more than 1g of copper salts, such as copper sulfate. The estimated lethal dose of Cu in an untreated adult can range from 10–20 g(Gaetke and Chow, 2003). Although studies have been published detailing reactions to acute copper toxicity, ranging from abdominal pain to death, the results still suffer from limitations. Confounders such as microbial water contamination are not explored thoroughly enough to distinguish the reaction between acute copper toxicity and pathogenic contamination. Additionally, the concentrations of copper are not reliably recorded in all studies.

When copper enters the human bloodstream, the first site of deposition is the liver(Gaetke and Chow, 2003). Therefore, chronic copper toxicity is most commonly manifested in liver diseases. There are rare medical conditions, such as Wilson's disease, that are genetically inherited and inhibit bodily copper excretion. Patients of Wilson's disease are predisposed to toxic effects of excess copper. Neurotoxicity from copper seems to only appear in patients with Wilson's disease(National Research Council, 2000).

Lastly, copper's ability to deactivate microorganisms in water is undesirable in the context of wastewater treatment plants (WWTPs). A study in 2011 found that copper (II) can inhibit fermentative bacteria, aerobic glucose-degrading heterotrophs, and nitrifying and denitrifying bacteria, all of which biologically treat wastewater to remove organic nutrients (Ochoa-Herrera et al. 2011). Drinking water is inextricably related to wastewater. An excess of copper consumed will be excreted in human waste. If copper is used more widely in POU water treatment, it must be carefully managed to not enter into traditional WWTPs.

2.3 Copper and silver: synergistic disinfection

According to Chen et al. (2008) and Liu et al. (1994) the combination of electrolytically generated copper and silver ions can eradicate *Legionella pneumophila* in the water distribution systems of hospitals. The studies achieved this by either treating only the hot recirculation line or both the hot and cold lines. Metal levels in the treated water were maintained below the EPA standards for drinking water (1,300 µg/L for copper and 100 µg/L for silver) in >99% of samples. More specifically, Liu et al. (1994) applied doses of 400–1,400 µg/L copper and 40–140 µg/L silver and reduced the colonized sites from 65% to 0.8% in 6 months. Only 1 water sample (<1% of all samples collected) exceeded the EPA standards. Likewise, Chen et al. (2008) dosages were: 52–212 µg/L copper and 5–22 µg/L silver, which reduced the colonized sites in 7 months from 32% to 0% in the wards and from 34% to 5% in the intensive care units. Copper and silver mean effluent concentrations were 160 µg/L and 14 µg/L respectively (Chen et al., 2008; Liu et al., 1994).

Soliman et al. (2020) found Cu^{2+} and Ag^{+} to have a synergistic relationship in deactivating MS2 when the pH was ≥ 7. Combining 500 µg/L Ag and 5,000 µg/L Cu resulted in a higher log removal of MS2 than the sum of the log removals from each disinfectant separately for pH values of 7 and 8. At a pH of 6, synergism between Cu^{2+} and Ag^{+} did not occur. It is important to note that the researchers were using concentrations of each disinfectant above the MCLGs set by the EPA for drinking water. Further experiments to test the same parameters with lower concentrations of metals would be useful to inform POU technologies.

Modification of granular activated carbon (GAC) can increase disinfection efficiency in drinking water treatment. Arakawa et al. (2019) modified GAC with silver nanoparticles (AgNPs) only, copper nanoparticles (CuNPs) only, and a combination of Ag/Cu NPs. They tested each modification for removal of *E. coli*. GAC with AgNPs, CuNPs, and Ag/Cu NPs achieved reductions of 0.56 $\log_{10}$, 1.31 $\log_{10}$, and 6.4 $\log_{10}$, respectively. This is evidence of a synergistic relationship between the two metals in modified porous material.

A laboratory study by Lucier, Dickson-Anderson, and Schuster-Wallace (2017) determined the efficacy of silver and copper infused ceramic filters for the inactivation of *E. coli* and MS2 bacteriophage in drinking water. The researchers employed ceramic filters with various amounts of Ag and Cu nanoparticles fired in during the manufacturing process in the Dominican Republic (i.e., the nanoparticles were mixed with the raw materials before the firing process). The reference filter (100% Ag) represented the filter that was being manufactured and distributed in the Dominican Republic (specific amounts and sizes of sawdust and silver are proprietary). Mean flow and water filtration capacity were 0.444 to 1.823 L/h and 6 L, respectively. Twelve different nanoparticles combinations were evaluated with 23 ceramic filters (all except 100% Cu + 25% Ag had filter replicates). One of the findings of the study was that with respect to *E. coli* inactivation,

filters infused with only Ag achieved an additional 2 log removal over the blanks (filters without any Ag or Cu), Cu only, and mixed Cu/Ag filters (except for the combination 100% Cu + 50% Ag; See Figure 2.5). Therefore, replacing most of the Ag with Cu to reduce costs does not appear to confer significant benefits in terms of log removal for *E. coli*. Also, with respect to MS2 deactivation, neither 100% Ag nor 100% Cu provided additional removal benefits over those of the blank filters. Meanwhile, the overall mean effluent turbidity ranged from 1.0 to 27.6 FTU. Only one filter (50% Ag) met the WHO general standard of <1 NTU for drinking water, and 3 filters (75% Ag, 100% Cu + 25% Ag, and 100% Cu + 50% Ag) met the WHO standard of <5 NTU when treatment options were very limited. Thus, to avoid significant variations in effluent quality (i.e., turbidity and log removal) the researchers suggest reducing inconsistencies in pore size and nanoparticle distribution between filters.

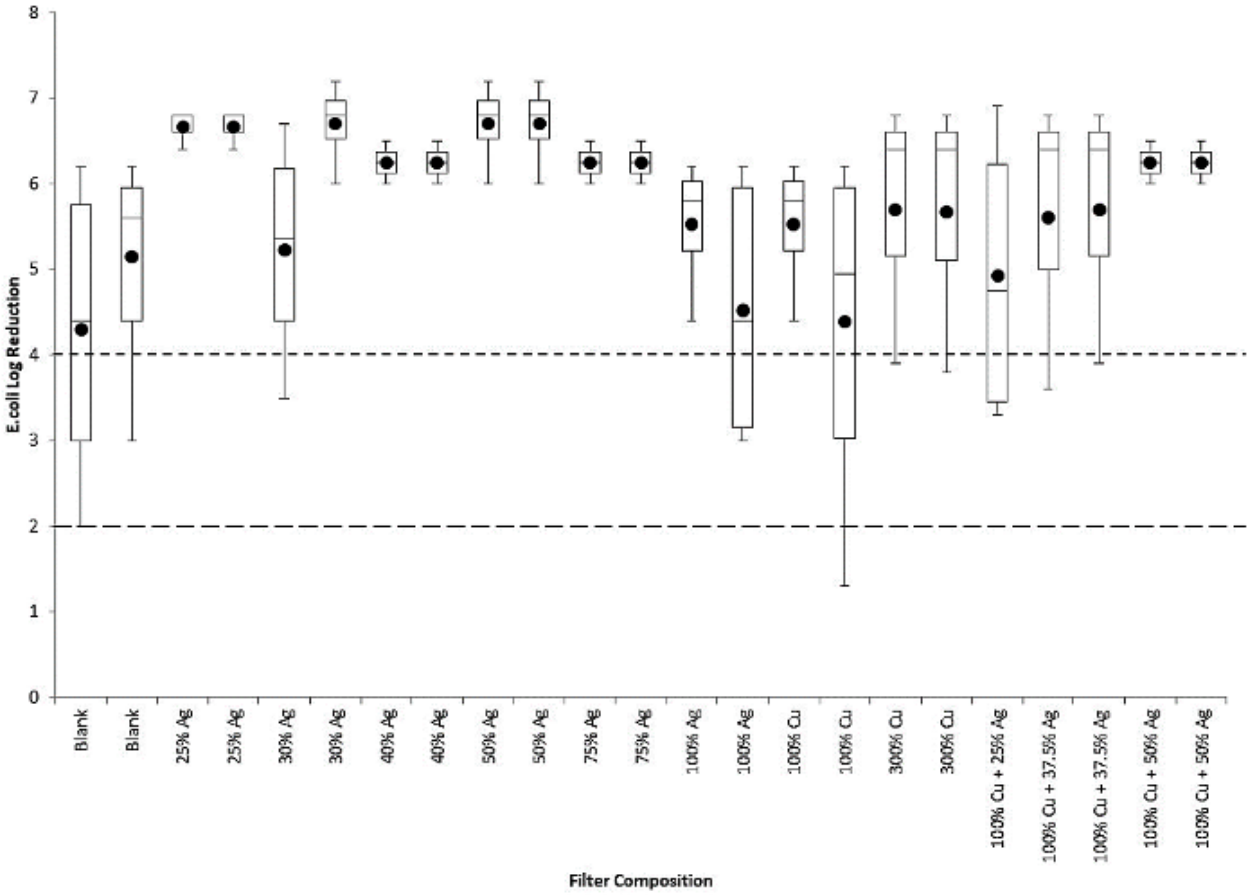


Figure 2.5 *E. coli* log removal in filter effluent samples. The short, dashed line shows the WHO standard for minimum log reduction under highly protective conditions (i.e., limit the burden of disease from drinking water to 10^{-6} disability adjusted life year, DALY, per person), while the long-dashed line shows the WHO standard for minimum log reduction under limited protection conditions (i.e., limit the burden of disease due to drinking water to 10^{-4} DALY per person). Reproduced from Lucier, Dickson-Anderson, and Schuster-Wallace (2017) with permission from the copyright holders, IWA Publishing.

Chapter 3: Improving Antibacterial Performance of Household Water Filters with a Silver-Embedded Ceramic Tablet

3.1 Abstract

In 2020, 1 out of 4 people did not have safely managed drinking water in their homes. Household water filters can reduce microbial load but are susceptible to recontamination. This study evaluated the bacterial reduction of several water filters with and without a silver-embedded ceramic tablet (MadiDrop+) in both the laboratory and household settings. In laboratory tests, after 24 hours, Kohler Clarity filters with MadiDrop+ halves split between upper and lower reservoirs removed 6.0-log *E. coli* compared to filters alone that removed 2.7-log *E. coli*. After 2 hours, Rama Water carbon filters with MadiDrop+ halves split between reservoirs removed 3.3-log *E. coli* compared to filters alone that removed 1.7-log *E. coli*. After 2 hours, ceramic pot filters with a MadiDrop+ in the lower reservoir removed 3.9-log TCB compared to filters alone that removed 2.8-log TCB. In households, effluent TCB (CFU/100 mL) was between 0 – 12, 1 – 36, and 509 – 5,916 when the MadiDrop+ was in the lower reservoir, split between reservoirs, and not present in Kohler Clarity filters, respectively. Silver levels were ≤ 100 µg/L, the drinking water limit set by the U.S. EPA. The addition of silver via MadiDrop+ either wholly in the lower reservoir or split between upper and lower reservoirs of household water filters improved bacterial reduction in both laboratory and household settings.

3.2 Background

In 2020, 1 out of 4 people did not have safely managed drinking water in their homes (WHO, UNICEF 2021). Drinking untreated water can transmit waterborne diseases such as cholera, dysentery, and typhoid. A common symptom of waterborne disease, diarrhea, is the second leading cause of death in children under 5 years old (WHO 2017). Diarrheal disease is especially prevalent in under-resourced communities where centralized water treatment is inconsistent, unmonitored, or unavailable (Kahler et al. 2016). In the absence of centralized water treatment, household water treatment (HWT) can reduce the pathogen load in drinking water to decrease instances of diarrhea and death (CDC 2018).

Chlorine-based disinfection is a common HWT in under-resourced settings due to its effectiveness and short contact time. However, chlorine changes the taste and odor of water and has the potential for harmful disinfection by-products (Lantagne, Cardinali, and Blount 2010). Users may discontinue their household chlorination if they perceive a change in taste/odor, limiting its effectiveness (Mitro et al. 2019). Alternatively, silver does not change the taste or odor of drinking water and does not create disinfection by-products.

A product developed at the University of Virginia called the MadiDrop+ uses silver as a chemical disinfectant. The MadiDrop+ is a 52-gram porous ceramic tablet (8 x 3 x 1.4 cm)

embedded with metallic silver patches by a proprietary method. The tablet is placed into 10-20 liters of water and gradually releases silver ions for a 3-4 log reduction of coliform bacteria after 8 hours (Hill et al. 2020).

A single MadiDrop+ can be used daily for one year to treat 7,000 liters of water between $0.0008-$0.002 (USD) per liter treated. Comparatively, chlorine tablets can cost between $0.001-$0.01 per liter of water treated (Sobsey et al. 2008). Boiling water can cost users between $0.005-$0.049 per liter, depending on the fuel source (charcoal, electricity, gas, wood, etc.) (Psutka et al. 2011; Clasen et al. 2008). The MadiDrop+ offers treatment at a lower cost than other disinfection based HWT.

Another HWT that does not require an external energy source is gravity-fed filtration. There is a diverse market for household water filters that varies in cost and efficacy at removing contaminants. This study evaluated the Kohler Clarity ceramic cartridge filter, Rama carbon cartridge filter, and PureMadi ceramic pot filter. A user pours untreated water into the upper reservoir of the filters, which passes through the filter medium to reach the lower reservoir, where treated water is stored. There is no mechanism in place to prevent recontamination of stored water. A 2013 study in Fiji found that 76% of households with water filters still had bacterial contamination in their stored water, suggesting that recontamination is a common concern for filtration treatment (Kohlitz et al. 2013).

A 2017 study evaluated the microbiological performance of a ceramic filter, a silver-embedded ceramic tablet, and the combination of the two in households for one year (Ehdaie et al., 2017). Authors found the filter-tablet combination performed the best against bacteria. Samples from the filter-tablet combination were free of *E. coli* after 1 year and average percent reduction in *E. coli* was 100%. Their findings suggest that the secondary treatment by the silver-embedded ceramic tablet improved the microbiological performance of ceramic water filters. However, the tablet used in the study is not commercially available and has different manufacturing protocols than the MadiDrop+.

Filtration alone can remove turbidity and some pathogens but is susceptible to recontamination of stored water. The MadiDrop+ provides residual disinfection to stored water but requires 8 hours of contact time and does not decrease turbidity. This is the first study that evaluates the benefits of combining the MadiDrop+ tablet with several commercially available filters in both laboratory and household settings. First, researchers evaluated the effect of MadiDrop+ location in Kohler Clarity, Rama carbon, and ceramic pot filters in the laboratory setting. The Kohler Clarity filter and MadiDrop+ were then evaluated in 36 households in a rural community of Limpopo, South Africa for four weeks. The bacterial removal, silver release, and turbidity were measured to determine any water quality benefits of adding a MadiDrop+ to household water filters.

3.3 Materials and methods

3.3.1 Laboratory experiments

MadiDrop+ tablets were purchased from Silivhere Technologies, Inc. (Charlottesville, VA). Kohler Clarity ceramic cartridge filters (K-20270) were purchased from Water Mission (Charleston, SC). Rama carbon cartridge filters were purchased from Rama Water Filters (Chennai, India).

Escherichia coli (*E. coli* C300, American Type Culture Collection) was cultured using sterilized LB broth. LB broth was made with 0.5 g of yeast extract, 0.5 g of sodium chloride, 0.25 g of Bacto-Tryptone, and 50 mL of deionized water (DI). The *E. coli* culture was incubated at 37℃ overnight while shaking at 200 RPM. Overnight cultures were centrifuged for 20 minutes at 2,500 RPM. The supernatant was discarded, and *E. coli* was resuspended in a 10 mM phosphate buffer (0.056 g dipotassium phosphate, 0.024 g monopotassium phosphate, and 50 mL DI). This was stored at 4℃ and used within 5 days. For long-term storage at -20℃, cultures were diluted with 40% glycerol.

Due to proprietary manufacturing processes, the silver should be evenly distributed throughout the MadiDrop+ tablet and thus a half tablet should contain half as much silver as a whole tablet. Kohler Clarity filters were tested with each of the following: a whole MadiDrop+ in the upper reservoir, a whole MadiDrop+ in the lower reservoir, half of a MadiDrop+ in both the upper and lower reservoirs, and no MadiDrop+ (Fig. 3.1). The Rama carbon filters were tested with a half MadiDrop+ in the lower reservoir, half of a MadiDrop+ in both the upper and lower reservoirs, and no MadiDrop+ (Fig. 3.2). The two filter types were not tested with identical MadiDrop+ combinations to gain a breadth of data on how MadiDrop+ size and location affects filter disinfection.

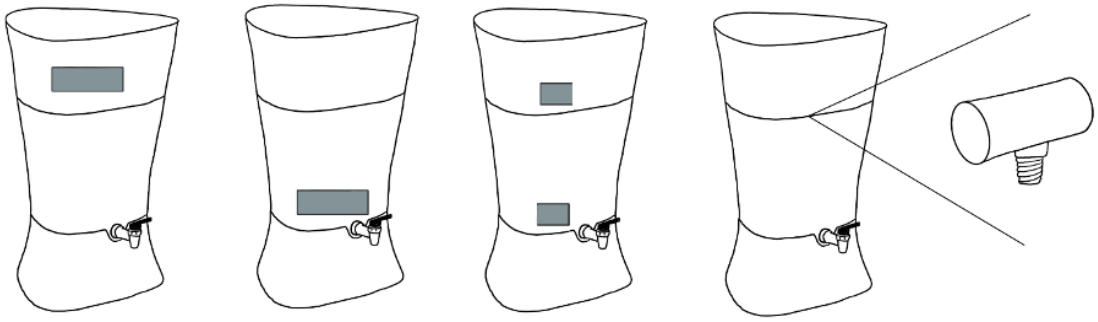


Fig. 3.1. Kohler Clarity ceramic cartridge filter and MadiDrop+ combinations tested for removal of *E. coli* in the laboratory setting. Ceramic cartridge is enlarged for visual aid.

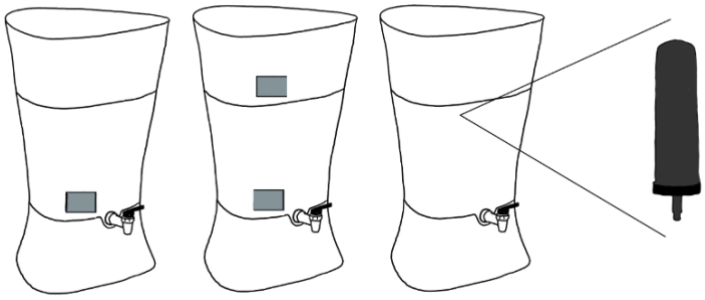


Fig. 3.2. Rama carbon filter and MadiDrop+ combinations tested for removal of *E. coli* in the laboratory setting. Carbon cartridge is enlarged for visual aid.

Prior to testing, plastic components of the filters were cleaned with detergent and water. Filter cartridges were saturated with 5 liters of synthetic groundwater (SGW) and tested for *E. coli* and total coliform bacteria to ensure sterility. SGW consisted of 1.2 g of MgSO4, 1.92 g NaHCO3, 0.08 g KCl, and 1.2 g of CaSO4 per 20 L in a plastic container (US EPA, 2002).

The upper reservoirs of the filters received 10 liters of SGW inoculated with 10^7 most probable number (MPN)/100 mL of *E. coli*, except for the Kohler Clarity filters with a MadiDrop+ in the lower reservoir, which received 10^9 MPN/100 mL. Effluent was sampled from the filter spigot after 2, 4, 8, 12, and 24 hours. An additional sampling at 1 hour was collected for the Kohler Clarity filters with a MadiDrop+ in the lower reservoir.

Samples for silver analysis were acid digested with nitric acid on the same day of collection. Silver was measured with inductively coupled plasma mass spectrometry (ICP-MS). Only samples collected from the highest performing filters with MadiDrop+ were analyzed for silver concentration to ensure silver was not exceeding the drinking water limit.

Samples for bacterial analysis were treated with 60 g/L sodium thiosulfate (26.4 μL per 1 mL sample) upon collection to stop disinfection. *E. coli* was quantified with the IDEXX Colilert Test and Quanti-Tray/2000, a method approved by the U.S. EPA ("Colilert - IDEXX US," n.d.; US EPA, 2017). Log reduction of *E. coli* was calculated by subtracting the log of the *E. coli* in the effluent at each time point from the log of the *E. coli* at the beginning of the experiment. Filter and MadiDrop+ scenarios were tested in duplicate.

3.3.2 Ceramic pot filter and MadiDrop+

Ceramic pot filters were purchased from PureMadi (Hammanskraal, South Africa, www.puremadi.org) without silver and delivered to the University of Venda for testing. Surface water from a local river in Thohoyandou, South Africa was collected and tested for *E. coli* and total coliform bacteria (TCB) using membrane filtration.

A total of 100 mL of sample, or diluted sample, was passed through a 0.45-μm Millipore membrane filter using a vacuum pump. The membrane filter paper was then placed in a sterile petri dish with Millipore m-Coliblue24 growth media and incubated for 24 hours at 37°C (US EPA, 1999). After 24 hours, the petri dishes were counted for TCB, indicated by a red colony, and *E. coli*, indicated by a blue colony. The membrane filtration apparatus was sterilized in between each use by submerging it in boiling water for a minimum of one minute as recommended by the Centers for Disease Control and Prevention (CDC, 2022). The apparatus was allowed to cool before the next sample was analyzed. One hundred mL of deionized water was measured for TCB and *E. coli* at the beginning, middle and end of each day as a control.

Ceramic pot filters either received a MadiDrop+ in the upper reservoir, a MadiDrop+ in the lower reservoir, MadiDrop+ halves split between the upper and lower reservoirs, or nothing (Fig. 3.3). Filters received 10 liters of the surface water at time zero. Effluent was sampled from the provided spigots after 2, 4, 8, 12, and 24 hours. This test was performed using water samples collected in the dry season and water samples collected in the rainy season.

Fig. 3.3 Ceramic pot filters and MadiDrop+ combinations tested for *E. coli* and Total Coliform bacteria in 10 L of surface water over 24 hours.

3.3.3 Participant enrollment and household study design

The protocol for this study was approved by the University of Virginia Institutional Review Board for Social and Behavioral Sciences (IRB-SBS #4896) and the University of Venda Research Ethics Committee (FSEA/22/ES/15/2208). The study was conducted in the Dzimauli community in Limpopo, South Africa in June 2022. The site was chosen because it was previously found that only 15% of households treat their drinking water in Dzimauli (Hill et al., 2020). Thirty-six randomly selected households were enrolled in the study. Community participants were eligible if the head of the household was at least 18 years of age, and they did not have chlorinated water piped into the home. They were then asked to participate, and verbal consent was obtained. A baseline questionnaire was conducted to obtain demographics and drinking water information. Afterwards, the household randomly received one of three interventions: 1) a water filter only, 2) a water filter with a MadiDrop+ in the lower reservoir, or 3) a water filter with MadiDrop+ halves split between the upper and lower reservoirs (Fig. 4). A total of 12 households were in each of the three intervention groups. The Kohler Clarity filter and MadiDrop+ were chosen as the interventions in this community because they are sold in South Africa.

With assistance from the interpreter, the participants were given a demonstration of how to assemble and use the water filter and, if in the appropriate intervention group, the MadiDrop+ tablet. Participants were instructed to continue storing water the way they were prior to receiving the filter and tablet. At the conclusion of the study, each household was given the water filter and a MadiDrop+ tablet.

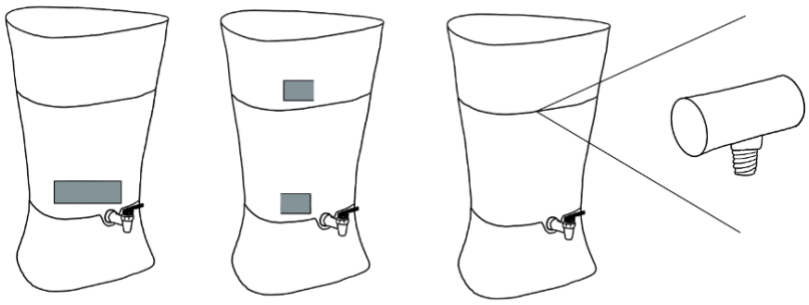


Fig. 3.4 Kohler Clarity filter interventions tested in households in Limpopo, South Africa. Ceramic cartridge is enlarged for visual aid.

3.3.4 Household sample collection and testing

Sterile Whirlpak stand up sample bags were used to collect and transport 500 mL of each sample. Samples were stored in coolers with ice during transportation from sample site to the laboratory and analyzed within 6 hours of collection. Influent samples were taken from the water source where participants filled their water filter to represent the untreated water. Effluent samples were taken from the spigot of the water filter to capture treated water. Water samples were collected once weekly for each household over four weeks. Both the influent and effluent water samples were analyzed for TCB, *E. coli*, turbidity, and total silver concentration.

TCB and *E. coli* were quantified using membrane filtration, as described previously. Water samples were measured for turbidity using the Thermo Scientific Eutech TN-100 Turbidimeter. The turbidimeter was calibrated at the beginning of each day using standards provided by the kit. Ten mL of each sample was poured into a glass vial, and the glass vial was wiped before each reading to remove any smudges or scratches. Triplicate measurements of turbidity were collected for each sample. The glass vials were rinsed with deionized water three times in between each sample.

The silver concentration of each sample was determined using a RapidSilver Visual Test Kit (Hach) to confirm that the samples did not exceed the 100 μg/L drinking water standard. The test kit used visual comparison to give a range of the silver concentration. One hundred mL of the sample was poured into the mixing bottle provided by the kit with the contents of one Hach RapidSilver Reagent powder pillow. The mixing bottle was sealed and shaken vigorously for one minute. The mixture was poured into the syringe with the attached filter holder and pushed through the filter. The filter was removed from the holder and compared to the comparator chart provided by the kit. If the filter color was not in the 0-50 μg/L range of the comparator chart, the sample was diluted 1:1 with deionized water and re-measured. The mixing bottle was rinsed with deionized water three times in between each use.

3.4 Results

3.4.1 Laboratory Evaluation of MadiDrop+ Location in Filters

The MadiDrop+ was added to Kohler Clarity, Rama Carbon, and PureMadi filters in different filter locations and evaluated for bacterial removal compared to the filters alone. The carbon and ceramic cartridge filters were inoculated with *E. coli* in synthetic groundwater. The ceramic pot filters were filled with local surface water in Thohoyandou, South Africa that had naturally occurring *E. coli* and TCB. All laboratory testing was conducted over 24 hours to simulate a day of use.

3.4.2 Kohler Clarity and Rama Carbon filters

Kohler Clarity filters with a MadiDrop+ in the lower reservoir were spiked with 10^9 MPN/100 mL *E. coli* and removed 6.2-8.4 log *E. coli* over 24 hours. Filters without MadiDrop+ that received 10^9 MPN/100 mL *E. coli* removed 4.5-8.1 log *E. coli* over 24 hours. Filters with a MadiDrop+ in the upper reservoir or split between reservoirs received 10^7 MPN/100 mL *E. coli* and removed 3.2-4.3 and 4.9-6.0 log *E. coli*, respectively, over 24 hours. Filters without MadiDrop+ that received 10^7 MPN/100 mL *E. coli* removed 2.7-5.1 log *E. coli* over 24 hours. Since log removal is a function of the initial concentration of *E. coli*, the higher log removal values

observed in Fig. 3.5A compared to 3.5B and 3.5C could be attributed to the higher initial concentration of *E. coli* in Fig. 3.5A.

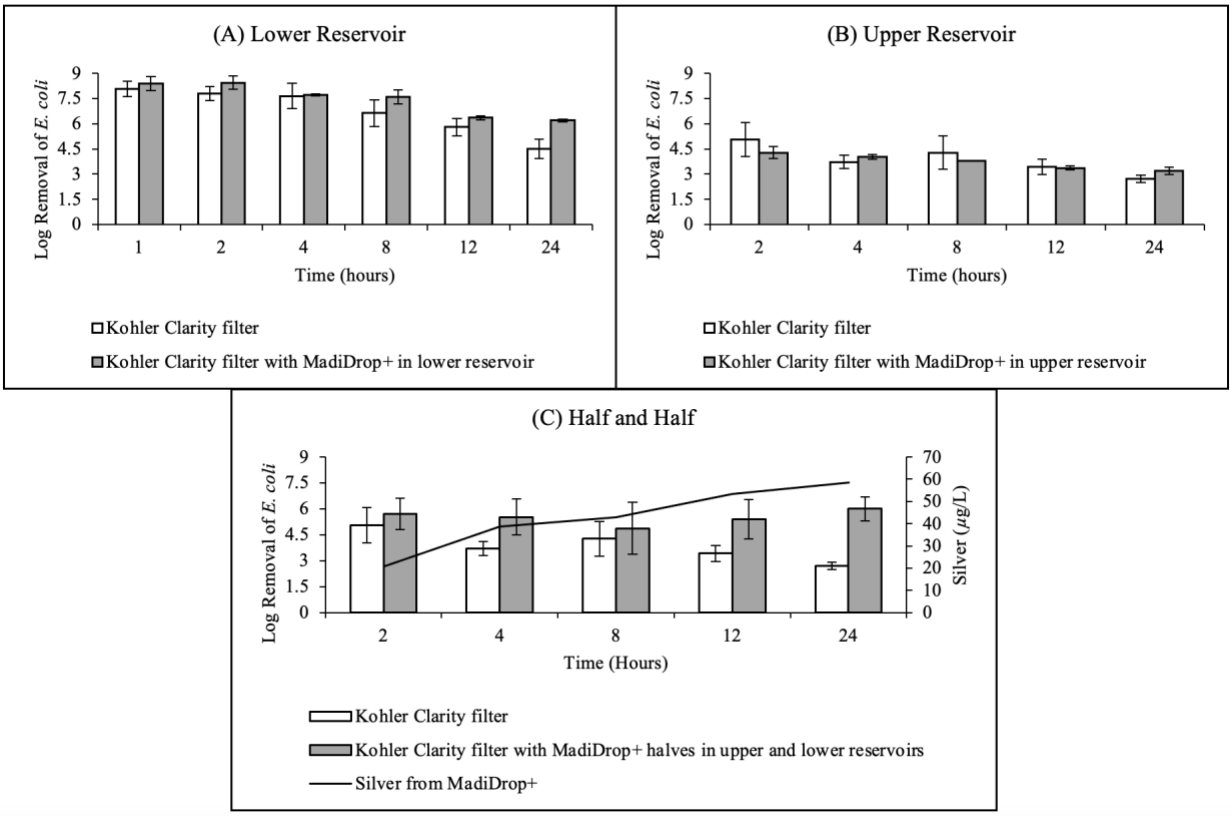


Fig. 3.5 Log removal of *E. coli* from Kohler Clarity filters only (white bars) and filters with a MadiDrop+ tablet (gray bars) in the lower reservoir (A), upper reservoir (B), or split between reservoirs (C). Filters in (A) were spiked with 10^9 CFU/100 mL *E. coli* while filters in (B) and (C) were spiked with 10^7 CFU/100 mL *E. coli*. The secondary axis in (C) shows the concentration of silver in the effluent of filters with MadiDrop+. Error bars represent standard error.

Kohler Clarity filters with a MadiDrop+ in the lower reservoir or split between reservoirs had higher average log removal values than filters without the MadiDrop+ (Fig. 3.5A, C). However, the overlap of error bars in Fig. 3.5A from hours 1-12 and Fig. 3.5C at 2 and 8 hours suggests similar, but not equal, disinfection between filters with and without MadiDrop+ at those time points. By 24 hours, filters with MadiDrop+ in the lower reservoir achieved greater disinfection without overlapping error bars with the control filters (Fig. 3.5A). The filters with MadiDrop+ split between reservoirs show higher log removal without overlapping error bars at hours 4, 12, and 24 (Fig. 3.5C).

Kohler Clarity filters with a MadiDrop+ in the upper reservoir removed less *E. coli* than filters alone at 2, 8, and 12 hours (Fig. 3.5B). Adding a whole MadiDrop+ to the upper reservoir of the Kohler Clarity filter did not consistently improve the removal of *E. coli* over 24 hours.

Fig. 3.5A and 3.5B show a trend of decreasing log removal over time, regardless of MadiDrop+ presence in the filter. Similarly, in Fig. 3.5C, the filters without MadiDrop+ generally achieve less log removal over time. Only filters with MadiDrop+ halves in both reservoirs maintain a constant range of log removal over 24 hours (Fig. 3.5C). Overall, the average log removal values are higher for filters with the MadiDrop+ in Fig. 3.5A and C, suggesting the presence of silver

enhances the antimicrobial potential of filters when in the lower reservoir or split between upper and lower reservoirs. The filters with MadiDrop+ halves released silver between 20-60 μg/L, below the drinking water limit of 100 μg/L.

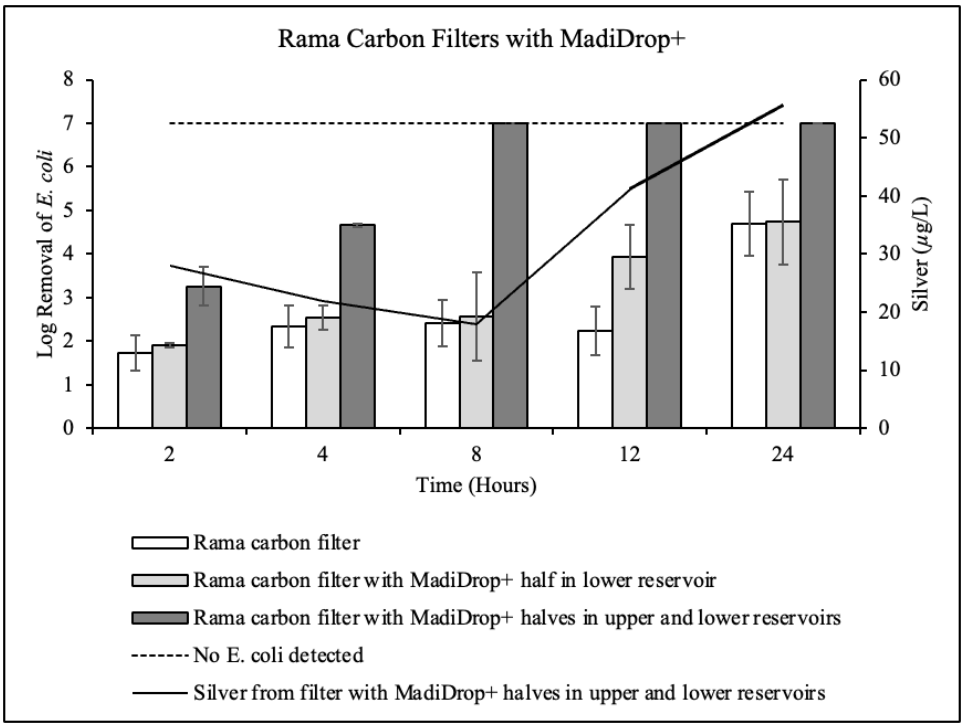


Fig. 3.6 Log removal of *E. coli* from carbon filters only (white bars), carbon filters with half of a MadiDrop+ tablet in the lower reservoir (light gray), and carbon filters with MadiDrop+ halves split between upper and lower reservoirs (dark gray). The secondary axis shows the concentration of silver measured in filters with MadiDrop+ halves split between reservoirs. Error bars represent standard error.

Log reduction of *E. coli* was similar for Rama carbon filters with or without half of a MadiDrop+ in the lower reservoir, except for 12 hours (Fig. 3.6A). However, after only 2 hours, Rama carbon filters with MadiDrop+ halves in both reservoirs removed 3.3-log *E. coli* compared to filters without MadiDrop+ that removed 1.7-log *E. coli* (Fig. 3.6B). By 8 hours, there was no *E. coli* detected in the effluent of filters with MadiDrop+ halves. Providing silver in both reservoirs of Rama carbon filters allowed for significantly more disinfection than silver only in the lower reservoir or no silver at all.

3.4.3 Ceramic pot filters

Over 24 hours, ceramic pot filters with a MadiDrop+ in the lower reservoir removed 3.5-4.1 log TCB compared to filters without MadiDrop+ that removed 2.1-2.9 log TCB (Fig. 3.7). Filters with MadiDrop+ halves split between reservoirs removed 3.1- to 3.5-log TCB during the first 12 hours, then decreased to a 2.2-log reduction at 24 hours. Adding a MadiDrop+ to the upper reservoir of the filter did not improve TCB reduction compared to filters alone. The highest TCB log reductions were achieved by filters with a MadiDrop+ in the lower reservoir at all time points.

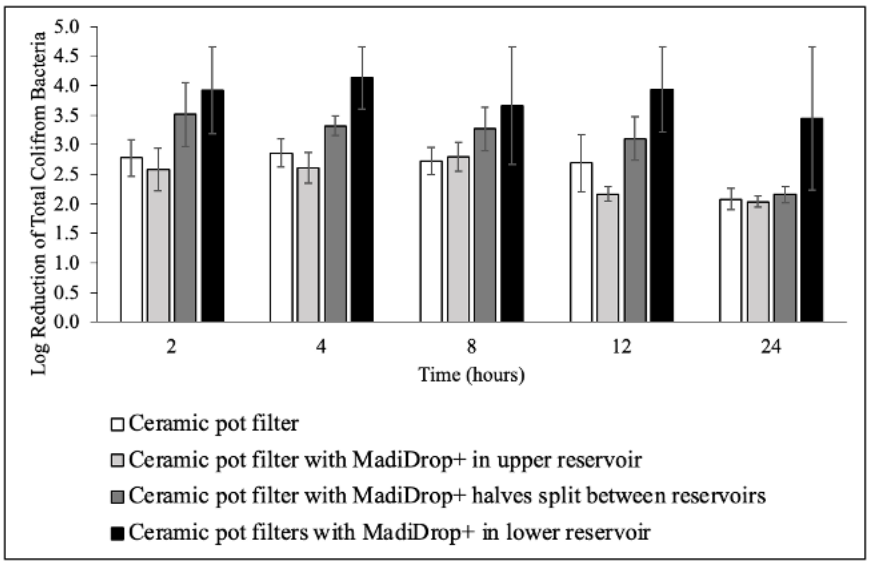


Fig. 3.7. Average log reduction of Total Coliform bacteria from ceramic pot filters with and without MadiDrop+ tablets in varying filter locations. Error bars represent standard error from duplicate trials.

3.4.4 Household study

A total of 36 households were enrolled and randomized to receive a Kohler Clarity filter (n = 12), a Kohler Clarity filter with a MadiDrop+ in the lower reservoir (n = 12), and a Kohler Clarity filter with MadiDrop+ halves split between upper and lower reservoirs (n = 12). Most households used surface water from a tap or pipe as their primary source of drinking water (n = 32, 88.9%). However, 83.3% (n = 30) of households described their primary source of drinking water as interrupted; their last interruption lasting an average of 8 days (± 2 days). This means that 41.7% and 36.1% of households in the study relied on stored water or directly collected from surface water, respectively, as a secondary source during interruptions. Only 30.6% of participants reported treating their drinking water. Participants who reported using bleach or chlorine agreed to cease doing so for the duration of the study to isolate the effects of the MadiDrop+ on residual disinfection.

Table 3.1 Baseline characteristics of 36 enrolled households by intervention group. `

Demographic/household characteristics	MadiDrop+ in lower reservoir (*n* = 12)	MadiDrop+ split between reservoirs (*n* = 12)	No MadiDrop+ (*n* = 12)	Overall (*n* = 36)
People in household, mean (±SD)	4 (1.7)	4 (1.8)	5 (3.6)	5 (2.5)
Primary drinking water source, *n* (%)				
Surface water from tap/pipe	10 (83.3)	10 (83.3)	12 (100.0)	32 (88.9)
Groundwater	2 (16.7)	2 (16.7)	0 (0.0)	4 (11.1)
Time to collect water from primary source (minutes), mean (±SD)	1 (0.3)	1 (0.0)	1 (0.0)	1 (0.2)

Main water supply, *n* (%)				
Interrupted	8 (66.7)	10 (83.3)	12 (100.0)	30 (83.3)
Continuous	4 (33.3)	2 (16.7)	0 (0.0)	6 (16.7)
Days of last interruption, mean (±SD)	7 (0.7)	8 (2.4)	8 (2.4)	8 (2.0)
Secondary water source, *n* (%)				
Storage tanks	4 (33.3)	6 (50.0)	6 (50.0)	15 (41.7)
Directly from surface water	3 (25.0)	4 (33.3)	5 (41.7)	13 (36.1)
Groundwater	0 (0.0)	0 (0.0)	1 (8.3)	1 (2.8)
Surface water from tap/pipe	1 (8.3)	0 (0.)	0 (0.0)	1 (2.8)
N/A	4 (33.3)	2 (16.7)	0 (0.0)	6 (16.7)
Time to collect water from secondary source (minutes), mean (±SD)	20 (25.8)	27 (33.5)	56 (92.4)	37 (63.3)
Frequency using secondary source, *n* (%)				
1-3 times per week	4 (33.3)	4 (33.3)	6 (50.0)	14 (38.9)
Always	4 (33.3)	5 (41.7)	6 (50.0)	15 (41.7)
N/A	4 (33.3)	3 (25.0)	0 (0.0)	7 (19.4)
Typical drinking water treatment, *n* (%)				
Use cloth as a filter	0 (0.0)	0 (0.0)	1 (8.3)	1 (2.8)
Add bleach/chlorine	2 (16.7)	3 (25.0)	1 (8.3)	6 (16.7)
Boil	1 (8.3)	0 (0.0)	3 (25.0)	4 (11.1)
None	9 (75.0)	9 (75.0)	7 (58.3)	25 (69.4)
Water storage vessels, *n* (%)				
Tanks	3 (25.0)	4 (33.3)	2 (16.7)	9 (25.0)
Plastic buckets	1 (8.3)	8 (66.7)	1 (8.3)	10 (27.8)
Plastic bottles	0 (0.0)	0 (0.0)	1 (8.3)	1 (2.8)

Combination of tanks, buckets, and bottles	8 (66.7)	0 (0.0)	8 (66.7)	16 (44.4)
Method of removing water from vessel, *n* (%)				
Spigot	3 (25.0)	7 (58.3)	3 (25.0)	13 (36.1)
Cup with a handle	8 (66.7)	4 (33.3)	6 (50.0)	18 (50.0)
Combination of spigot and cup with a handle	1 (8.3)	1 (8.3)	3 (25.0)	5 (13.9)
Self-reported description of drinking water quality, *n* (%)				
Poor	0 (0.0)	1 (8.3)	1 (8.3)	2 (5.6)
Average	4 (33.3)	5 (41.7)	3 (25.0)	12 (33.3)
Good	7 (58.3)	6 (50.0)	7 (58.3)	20 (55.6)
I don't know	1 (8.3)	0 (0.0)	1 (8.3)	2 (5.6)

3.4.5 Bacterial results

Influent TCB was similar between each intervention group, averaging between 1-2.5 log (Fig. 3.8A). The third week of the study showed around 1-log less influent TCB than weeks 1, 2, and 4. This could be due to variability in precipitation over the course of the study. In general, rainfall increases risk of microbial contamination in water sources (Tornevi, Bergstedt, and Forsberg 2014).

Over the four-week study, average effluent TCB was between 0 – 12, 1 – 36, and 509 – 5,916 CFU/100 mL when the MadiDrop+ was in the lower reservoir, split between reservoirs, and not present in filters, respectively. There was less TCB detected in the effluent of filters with a MadiDrop+, regardless of the tablet location in the filter (Fig. 3.8B).

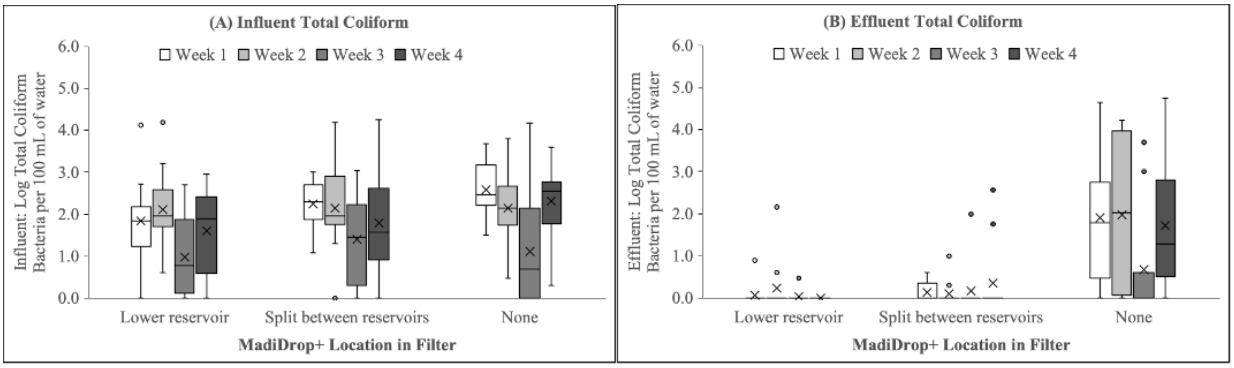


Fig. 3.8. Log scale of influent (A) and effluent (B) Total Coliform bacteria (CFU/100 mL) in each filter intervention over four weeks of use. Average values are represented by X's and outliers are represented by data points.

Influent *E. coli* was similar between intervention groups that received a MadiDrop+ in their filter. However, average influent *E. coli* was 0.2-0.5 log higher in the group that did not receive a MadiDrop+. It is worth noting that influent *E. coli* concentrations were relatively low among all intervention groups (Fig. 3.9A).

Effluent *E. coli* was below 1 CFU/100 mL in filters that received a MadiDrop+, except for an outlier in the "split between reservoirs" group (Fig. 3.9B). Filters without a MadiDrop+ had an average effluent log *E. coli* of 0.54, 0.58, 0.20, and 0.23 for weeks 1-4, respectively.

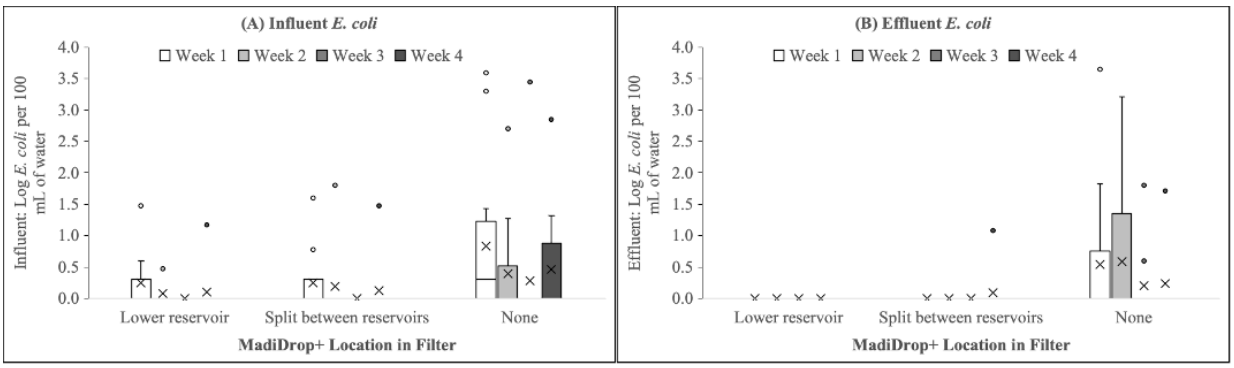


Fig. 3.9. Log scale of influent (A) and effluent (B) *E. coli* (CFU/100 mL) in each filter intervention over four weeks of use. Average values are represented by X's and outliers are represented by data points.

3.4.6 Turbidity

Turbidity was generally reduced by all filters, regardless of having a MadiDrop+. These results agree with literature that silver does not affect turbidity (Shepard et al., 2020). Effluent turbidity was less than 1 NTU for all intervention groups (Fig. 3.10B), meeting the World Health Organizations' ideal standard for household water treatment (World Health Organization, 2017b).

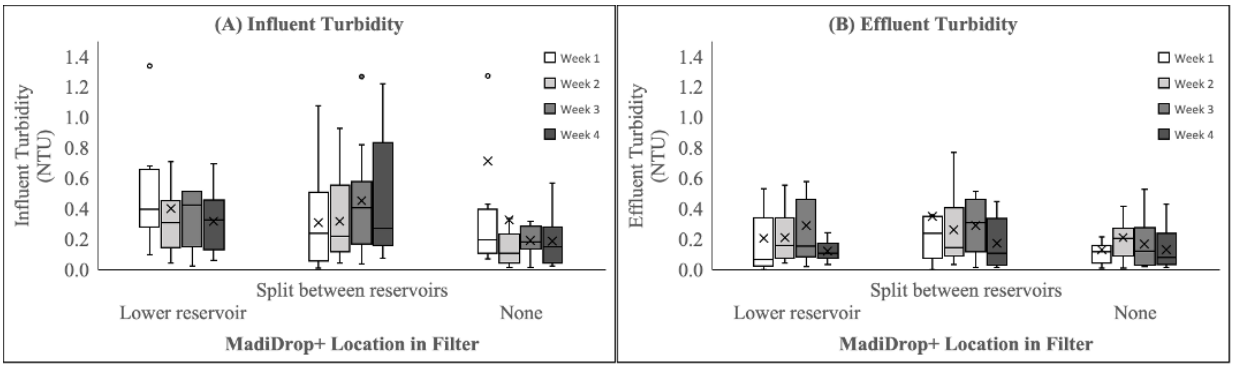


Fig. 3.10 Influent (A) and effluent (B) turbidity in each filter intervention group over four weeks of use. Average values are represented by X's and outliers are represented by data points.

3.4.7 Silver

Silver measured in the effluent of filters with a MadiDrop+ did not exceed 100 μg/L over the course of the study (Fig. 3.11). In week 4, one filter in the "lower reservoir" group measured at the upper limit of 100 μg/L. Researchers contacted the household immediately to notify them to empty their water container. Translators learned that the participant was ill and unable to change their water at least every 72 hours as directed, leading to the high silver concentration that week. Generally, filters with a whole MadiDrop+ in the lower reservoir released more silver into the effluent than filters with MadiDrop+ halves split between reservoirs. It is likely that silver ions released in the upper reservoir do not pass completely through the filter cartridge into the lower reservoir where silver was measured, possibly because of sorption to the filter matrix.

Values in Fig. 3.8-3.11 were considered as outliers if they were 1.5 times the interquartile range larger than the third quartile or 1.5 times the interquartile range smaller than the first quartile.

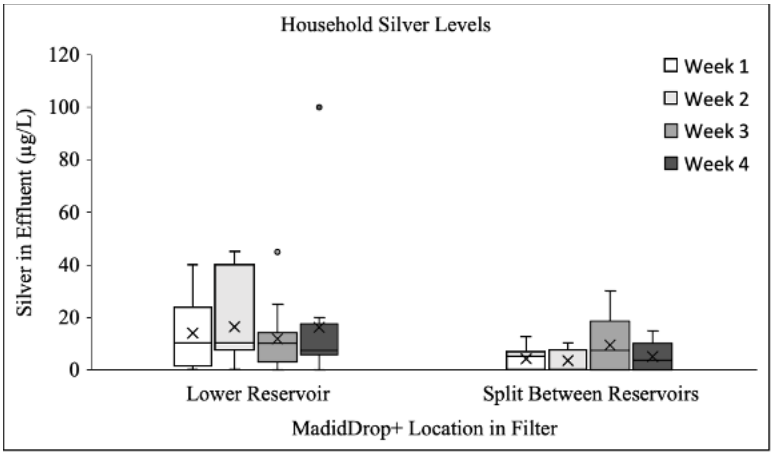


Fig. 3.11 Box and whisker plot of the effluent silver concentrations from filters that received a MadiDrop+ over four weeks of use. Xs represent the average concentration.

3.5 Discussion

In this study, the addition of chemical disinfection via the MadiDrop+ to several household water filters was evaluated for bacterial removal in 24-hour laboratory experiments and a four-

week household study. First, laboratory experiments evaluated differences in filter disinfection when varying the MadiDrop+ location. Results from the laboratory experiments informed the interventions chosen to be tested in households.

The MadiDrop+ improved filter performance in both laboratory and household environments for all filter types. In laboratory experiments, Kohler Clarity and Rama carbon filters removed the most *E. coli* when MadiDrop+ halves were split between reservoirs. PureMadi ceramic pot filters removed the most TCB when a whole MadiDrop+ was in the lower reservoir. The least beneficial MadiDrop+ location was in the upper reservoir for all three filters. The location of the MadiDrop+ within the filter affected disinfection because of the gradual release of silver from the tablet, contact time with contaminated water, and effect of turbidity.

The metallic silver patches in the ceramic are gradually oxidized to ionic silver by the dissolved oxygen in water. Water is not dosed with a set amount of silver at once. Therefore, the longer a volume of water is in contact with the MadiDrop+, the more ionic silver is available for disinfection. Water is only in the upper reservoirs of filters for a few hours before it passes into the lower reservoir for storage. The MadiDrop+ generally has less contact time with untreated water in the upper reservoir than the filtered water. Silver may become lodged in the pores of the filter medium and do not reach the lower reservoir for further disinfection. A MadiDrop+ in the lower reservoir has the most contact time with water.

In the laboratory environment, some Kohler Clarity filters demonstrated a higher log removal in the first few hours followed by a decrease in log removal over the 24-hour test (Fig. 3.5). The initial high log removal values could be explained by the slow rate of filtration, around 0.46 liters per hour, that seemed to produce a lag effect. The slow passage of water through the ceramic cartridges led to increased introduction of bacteria to the lower reservoir over the test period. Additionally, when MadiDrop+ was present in the lower reservoir, there was more ionic silver available per bacterium during the first few hours of low water levels. As the volume of water increased, there was less silver per bacterium available, possibly explaining the decrease in bacterial reduction over the first 12 hours.

Optimal MadiDrop+ location was different depending on the test water. Filters tested with SGW removed the most *E. coli* with MadiDrop+ halves split between reservoirs. Filters tested with surface water removed the most TCB with a whole MadiDrop+ in the lower reservoir. This is because the surface water had a turbidity of 21 NTU, much higher than the SGW that is composed of chemical reagents and deionized water. Having a half MadiDrop+ in the upper reservoir of turbid surface water may not provide as much disinfection as a half MadiDrop+ in the upper reservoir of less turbid SGW. Turbidity may consist of agglomerations of particles that can shield pathogens from chemical disinfection (Kahler et al., 2016).

Laboratory tests with different filters and test waters showed the optimal MadiDrop+ locations were in the lower reservoir and split between reservoirs. The household portion of this study sought to evaluate those best MadiDrop+ locations for performance with the end-user. Over four weeks of use, Kohler Clarity filters with a MadiDrop+ in the lower reservoir and split between reservoirs had average effluent TCB concentrations (CFU/100 mL) of 0 – 12 and 1 – 36, respectively. Whereas Kohler Clarity filters without the MadiDrop+ had average effluent TCB concentrations (CFU/100 mL) of 509 – 5,916 over four weeks. This suggests that during long-term use, Kohler Clarity filters without a disinfectant remove less bacteria or become recontaminated. Recontamination could occur if the user lifts the upper reservoir for any reason, exposing the treated water to contaminants.

There was not a significant difference in bacterial removal between the MadiDrop+ locations in filters used in households. The presence of any silver seemed to decrease bacteria regardless of being wholly in the lower reservoir or split between reservoirs.

A drawback of adding the MadiDrop+ to household water filters is the user's responsibility to fully empty their water container at least once every 72 hours to prevent silver exceeding 100 μg/L. Only one household, who had a whole MadiDrop+ in the lower reservoir, had exactly 100 μg/L silver in their effluent during the study. The participant reported they were ill that week and not fully emptying their filter at least once every 72 hours. Silver levels were generally lower with the MadiDrop+ halves split between reservoirs, never exceeding 40 μg/L.

Adding MadiDrop+ halves to upper and lower reservoirs of a Kohler Clarity filter increased bacterial removal compared to filters alone in laboratory and household settings. Silver did not exceed the drinking water limit. This combination of household filter and MadiDrop+ may be the most practical in communities without access to consistently treated drinking water.

3.6 Conclusion

The MadiDrop+ alone requires 8 hours of contact time to achieve a 3-4 log reduction of bacteria (Ehdaie, Krause, and Smith 2014). However, after only 2 hours, Kohler Clarity and Rama Water filters with MadiDrop+ halves split between upper and lower reservoirs removed 5.7 and 3.3 log *E. coli*, respectively (Fig. 3.5C, 3.6). The contact time for the MadiDrop+ is reduced when added to a household water filter.

As seen in the four-week household study, filters with a MadiDrop+ either in the lower reservoir or split between reservoirs removed more TCB and *E. coli* compared to filters without MadiDrop+ (Fig. 3.8, 3.9). The location of the MadiDrop+ in the filter did not significantly affect bacterial removal. However, silver levels were higher in the effluent of filters with a whole tablet in the lower reservoir (Fig. 3.11). Neither filter group with MadiDrops exceeded the drinking water limit of 100 μg/L.

This study evaluated the microbiological performance of combining two HWT interventions. A thorough financial analysis to compare the cost of the interventions and their corresponding microbiological benefits to users is a gap in this study. Future work is required in this area. In the short-term, aid organizations, government agencies, and other decision makers who seek to provide household water treatment to under-resourced communities may consider pairing common water filters with a MadiDrop+ to increase bacterial disinfection and prevent recontamination in treated water.

Chapter 4: Development of a copper-based water treatment technology

4.1 Abstract

The World Health Organization estimates that at least 2 billion people use a drinking water source contaminated with feces. Copper has been shown to have antibacterial, antiviral, and antifungal effects in drinking water to combat microbial contamination and thus decrease instances of waterborne disease. Studies have shown 2.5- and 1.8-log reductions of *E. coli* and MS2 bacteriophage, respectively, after 6 hours of contact time with 300 µg/L copper. This is well below the drinking water limit of 1,300 µg/L. The objective of this chapter was to develop a copper-based household water treatment product that could consistently release ~300 µg/L copper into 10 L of water daily for at least 1 week. Our first approach was to embed copper into ceramic tablets. We varied the copper salt, mass of copper, firing temperature, and firing environment. Copper-embedded ceramic tablets generally released high amounts of copper in the first few days of use followed by gradual or drastic declines over the following days. The second approach was immersing metallic (zero-valent) copper in water. We evaluated copper release from 3 copper metal products that vary in surface area: a sheet (lowest surface area), a coarse mesh (referred to as "mesh"), and a 200-mesh screen (highest surface area; referred to as "screen"). Unlike the copper-embedded tablets, none of the metallic copper interventions had initial spikes or drastic drops in copper release over 8 days of use. The copper sheet consistently released 70-129 µg/L copper over 8 days but was more than 3 times the cost of other interventions. The copper mesh and copper screen released 145-256 µg/L and 188-333 µg/L copper, respectively, over 8 days of use and cost $0.75 and $2.00 per 10 grams, respectively. Given consistency of copper release, target range of copper concentrations, and low cost, the copper mesh and copper screen are further evaluated in combination with the MadiDrop+ in Chapter 5.

4.2 Background

The World Health Organization (WHO) estimates that at least 2 billion people use a drinking water source contaminated with feces (World Health Organization, 2022). Household water treatment (HWT) can reduce the pathogen load in drinking water when centralized water treatment is unavailable or inconsistent. A successful HWT product must remove waterborne pathogens, and be low-cost, simple, and easy to distribute. The MadiDrop+ is a HWT product that uses silver as a disinfectant, which achieves a 4-log reduction of bacteria but only around a 1-log reduction of protozoa and viruses (Singh et al., 2019; World Health Organization, 2021). Copper, 1/10th of the cost of silver, may be an effective alternative or addition to silver-based disinfection for HWT.

Studies have shown 2.5- and 1.8-log reductions of *E. coli* and MS2 bacteriophage, respectively, after 6 hours of contact time with 300 µg/L copper (Armstrong et al., 2017, 2016). This is well below the drinking water limit of 1,300 µg/L (US EPA, 2015). Electrolytically generated copper has been used in hospital water systems to inactivate *Legionella pneumophila*

(Chen et al., 2008). Electricity is not consistent or available in some rural, and even urban, areas. Alternatively, passive copper release from copper pots and containers have been shown to have antibacterial, antiviral, and antifungal properties in water (Cervantes et al., 2013; Sudha et al., 2012, 2011, 2009). A copper pot or container may become difficult to transport or distribute due to their size. Smaller in size, and most likely cost, copper-embedded paper (Dankovich and Smith, 2014) and copper-embedded stones (Drelich et al., 2017) have inactivated bacteria in water.

This chapter aimed to develop and evaluate a practical, low-cost method for delivering 300 ug/L copper into 10 L of drinking water every day for at least one week. The two approaches for this research were: (1) embedding metallic copper into the pores of a ceramic tablet and (2) evaluating the oxidation of copper directly from pieces of copper metal in water. Copper interventions were placed in deionized water and sampled over several days of use to determine if copper release rates were consistently sufficient for disinfection.

To embed copper into ceramic tablets, we varied the copper salt, mass of copper, firing temperature, and firing environment. The steps for embedding copper into ceramic was as follows:

1. Apply copper compound to ceramic (Cu^{2+} or Cu^{1+})
2. Reduce copper to its metallic form in the pores and surface of the ceramic (Cu^0)
    a. Heat or
    b. Acid
3. Use the tablet in water
    a. Metallic copper is oxidized to ionic copper (Eq. 4.1)

$$Cu^0 + O_2^0 \rightarrow Cu^{2+} + OH^- \quad (4.1)$$

The second approach to achieving sustained release of copper in water was immersing copper metal in water. Since copper metal releases disinfecting ions upon oxidation of metal at the surface (Eq. 4.1), we evaluated copper release as a function of time from 3 copper metal products that vary in surface area: a copper sheet (lowest surface area), a coarse copper mesh (referred to as "mesh"), and a 200-mesh copper screen (highest surface area; referred to as "screen"). A product that can consistently provide copper into water will be further evaluated in combination with the MadiDrop+ to achieve greater disinfection of drinking water than the MadiDrop+ alone.

4.3 Methods and materials

4.3.1 Copper and silver quantification

Total residual copper and silver concentrations refer to the sum of zero-valent and ionic metal in solution. All samples were analyzed for total residual silver and copper concentrations by being prepared with trace metal grade nitric acid (Fisher Chemical, Fair Lawn, NJ, USA) and measured using graphite furnace atomic absorption spectrometry (GFAAS).

4.3.2 High temperature copper reduction – air

We attempted to reduce copper in the ceramic by firing at higher temperatures (600-750ºC). Ceramic was fired in air in a Lindberg/Blue M Muffle Furnace (Thermo Scientific). The firing temperature, mass of copper, and type of compound were tested for their effects on copper release from the tablet. The MadiDrop+ ceramic tablet is manufactured with a proprietary process by Du-Co Ceramics Company (Saxonburg, PA) using an aluminosilicate clay. Attempts to incorporate copper into ceramic used the same ceramic tablet as the MadiDrop+ without the silver.

To test the effect of firing temperature, four tablets were soaked in 1.75 M copper sulfate for two days. Two were fired at 600ºC and two were fired at 750ºC. Tablets were rinsed for 3 minutes with deionized water then placed in 400 mL of deionized water.

Three copper compounds were applied to the ceramic tablet: copper (II) sulfate, copper (II) nitrate, and copper (I) chloride (Thermo Fisher Scientific). The two former compounds are soluble in deionized water and the latter in hydrochloric acid. Ceramic tablets were soaked in the copper mixtures for 1-2 days then fired in air at 750ºC. Each tablet was placed in 400-mL of deionized water in a clean glass container. After 24 hours, 10 mL of water was sampled from the container. Water in the containers were refreshed and repeated to simulate daily use.

4.3.3 High temperature copper reduction – forming gas

Tablets were seeded with 1.5 grams of copper from copper sulfate and heated at 450ºC in forming gas, a mixture of hydrogen and nitrogen. Tablets were then rinsed for 3 minutes with deionized water to avoid a spike of copper release on the first day of use. They were placed in 10 liters of synthetic groundwater for 24 hours, after which a 14 mL sample of water was taken. Water was emptied then the process was repeated to simulate daily use of the tablets for 10 days.

4.3.3 Low temperature/acidic reduction

Methods were adapted from literature to attempt to form copper nanopatches on a porous ceramic tablet (Dankovich and Smith, 2014). Tablets were submerged in a mixture of 0.32 M copper sulfate and 1 M sodium hydroxide for 21 hours to allow copper uptake into the pores and surface of the ceramic. Following copper absorption, tablets were placed in a warm (85 ºC) ascorbic acid bath for 30 minutes to reduce the copper.

Glass beakers were washed with soap and water, rinsed with 10% v/v nitric acid, and thoroughly rinsed with deionized water. Each tablet was rinsed with deionized water and placed in 400 mL of deionized water in a clean glass beaker. After 24 hours, 10 mL of water was sampled from the container. Water in the containers were emptied and refilled and sampling was repeated to simulate daily use for ten days.

4.3.4 Placing copper metal directly into water

Since copper metal releases disinfecting ions upon oxidation of metal at the surface, we evaluated copper release as a function of time from 3 copper metal products with increasing surface

area: a copper sheet, a coarse copper mesh (mesh), and a 200-mesh copper screen (screen) (Fig. 4.1). To mimic expected use conditions, we placed the copper metal sources in 10 L of deionized (DI) water for 24 hours, mixed for 10 seconds, took a water sample and measured copper content by GFAAS, then emptied the container, refilled with 10 L DI water, and repeated for each time point.

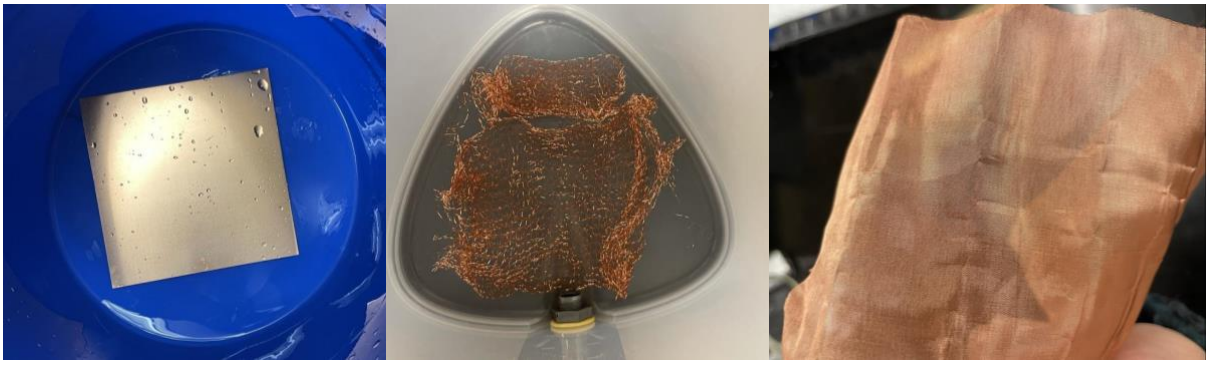


Figure 4.1 Photos of copper metal tested in 10 L of water for drinking water treatment. We used a full copper sheet that weighed 226 grams (left), 10 grams of copper mesh (middle), and 10 grams of copper screen (right).

4.4 Results

4.4.1 High temperature copper reduction in air

Ceramic tablets seeded with copper sulfate were fired at either 600 ºC or 750 ºC in air. We found that the tablets fired at or under 600 ºC formed a blue precipitate in the water after the first day of use. When fired at or above 750 ºC, tablets did not change the color of the water. We assume that copper sulfate does not reduce to metallic copper when fired below 600 ºC. Instead, tablets are most likely releasing the copper salt back into the water. Therefore, we pursued a firing temperature of 750 C to avoid the blue precipitate.

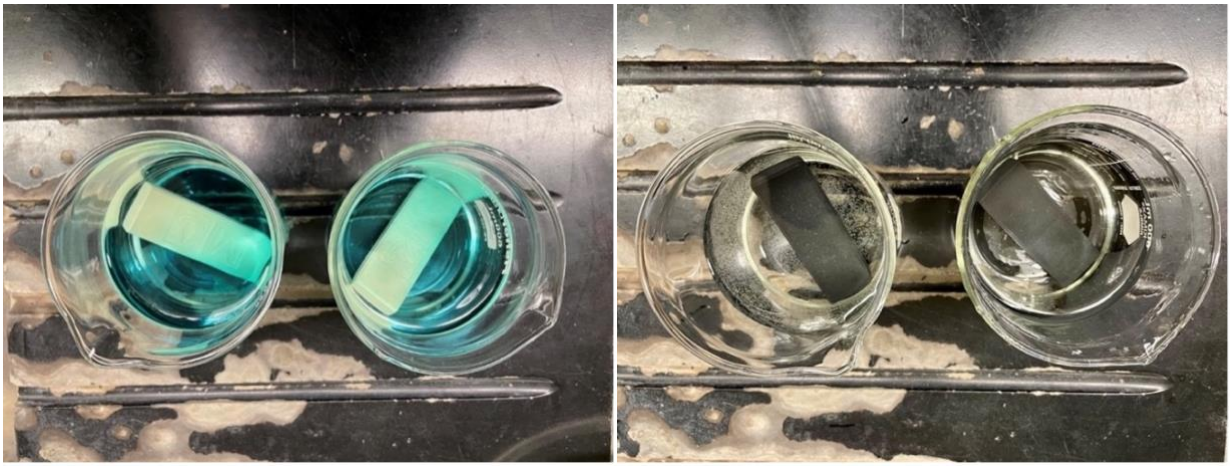


Figure 4.2 Photos of copper tablets after the first day of submersion in 400 mL deionized water. Tablets were seeded identically with copper sulfate then fired at 600 ºC (left) or 750 ºC (right).

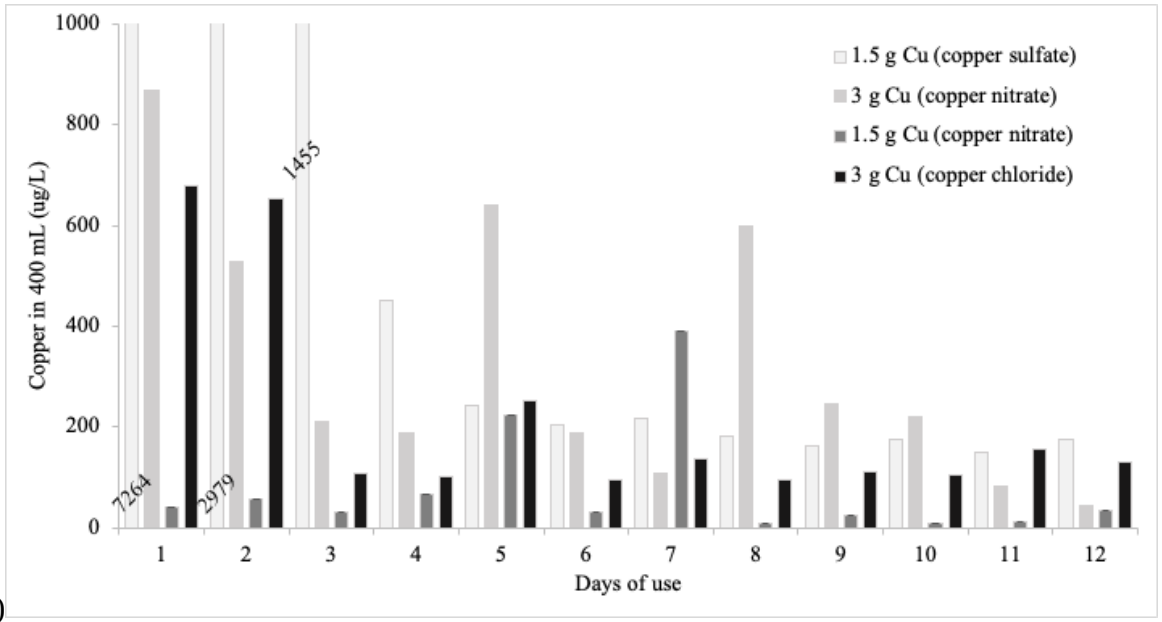
Tests comparing the copper release from identical tablets in either 400 mL or 10 L of DI provided a ratio of copper release based on volume of test water. The experimental ratio was used to predict an expected copper release in 10 L from the measured copper release in 400 mL.

Table 4.1 Copper release from identically seeded copper-ceramic tablets in either 400 mL or 10 L of DI water. The ratio of copper release was calculated by dividing the copper release from tablets used in 400 mL by the copper release from tablets used in 10 L. Each scenario was measured in duplicate.

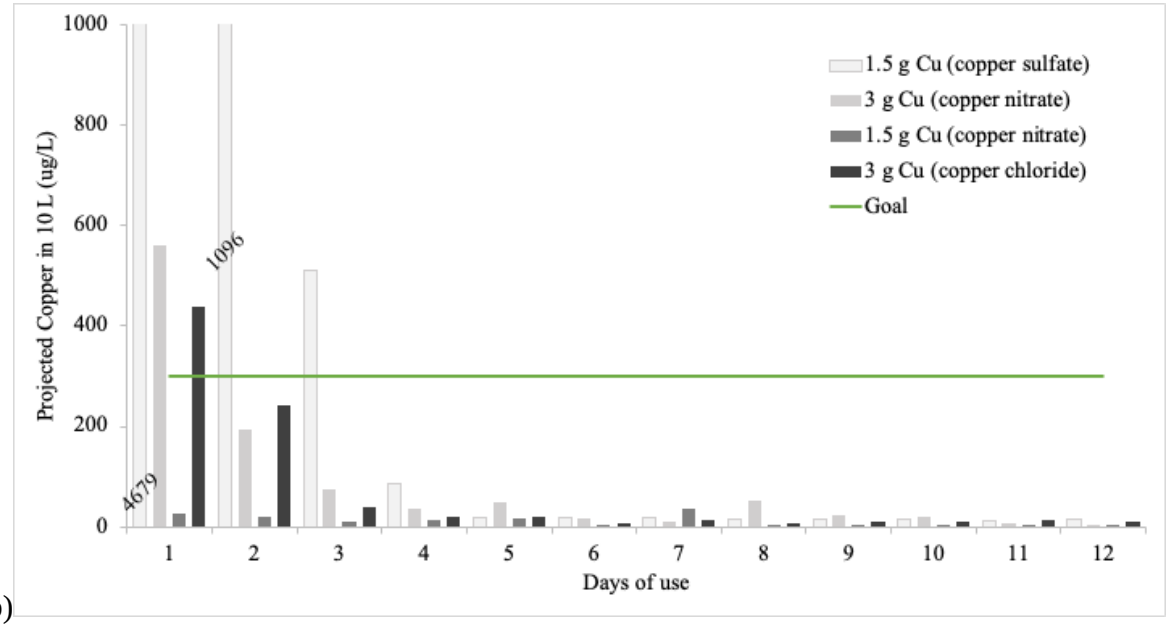
	Copper concentration (µg/L)		
Days of use	Tablets used in 400 mL	Tablets used in 10 L	Ratio of copper release
1	4389	2827	1.6
2	579	213	2.7
3	303	106	2.8
4	161	31	5.2
5	119	9	12.9
6	128	11	11.2

Ceramic tablets seeded with copper sulfate, copper nitrate, and copper chloride were tested for their copper release in 400 mL of DI water (Fig. 4.3a). Copper release was initially highest for the tablets seeded with copper sulfate. By day 5, copper sulfate tablets released copper comparably to the copper nitrate and copper chloride tablets. Copper nitrate tablets with 3 grams of copper released more than 2x copper compared to tablets with 1.5 grams of copper from copper nitrate, except on day 7. Most tablets released less copper with each progressing day, tapering off around day 9 of use. Tablets with 1.5 grams of copper from copper nitrate did not follow that trend, instead releasing lower levels most days except day 7.

If the tablets were intended to be used in 400 mL of drinking water, the tablets with copper sulfate may have been appropriate for use after day 3. However, the goal volume of treated water is 10 L/day to meet drinking water requirements for an average household. Therefore, predicted values of copper release in 10 L are shown in Fig. 4.3b using the predictive ratio calculated in Table 4.1. In 10 L, none of the copper-seeded tablets meet the goal of consistent release of 300 µg/L copper after day 3.



(a)



(b)

Figure 4.3 Concentration of copper released from ceramic tablets seeded with various copper compounds and fired at 750ºC when used in 400 mL of water (a) and the projected copper release if the same tablets were to be used in a larger volume of 10 liters (b).

4.4.2 High temperature copper reduction in forming gas

One study successfully incorporated copper sub-microparticles into ceramic stones by soaking them in a copper sulfate solution and firing them in hydrogen gas at 450 ℃ (Drelich et al., 2017). As hydrogen gas is highly flammable and unavailable for this research, we fired copper embedded tablets in forming gas, a mixture of hydrogen and nitrogen. It is possible that the hydrogen could replace oxygen in the reduction reaction of ionic to metallic copper:

$$Cu^{2+} + H_2 \rightarrow Cu^0 + 2H^+. \quad \text{(Eq. 4.2)}$$

The benefit of hydrogen replacing oxygen in the reduction reaction is to possibly prevent cupric oxide from forming, an insoluble copper state that is poor for disinfection and discussed later in this chapter. The hydrogen ions formed in Eq. 4.2 could attach to the aluminosilicate framework of the ceramic by electrostatic forces. Alternatively, the hydrogen ions could be consumed by the hydroxylation reaction at a site with local charge imbalance:

$$H^+ + A - O^- \rightarrow A - OH, \qquad \text{(Eq. 4.3)}$$

where O represents a lattice oxygen and A represents the aluminosilicate framework.

Copper-embedded tablets fired in forming gas released around 750 µg/L copper on the first day of use. That is slightly above our goal concentration of 300 µg/L, but still below the daily drinking water limit of 1,300 µg/L. By day 2, copper release fell to 200 µg/L, possibly sufficient for disinfection. However, by day 3, copper fell further to 45 µg/L, staying in that range for the remaining 7 days. One study found less than a 0.5-log reduction of MS2 Bacteriophage from 100 µg/L copper after 24 hours of contact time, meaning that concentrations below 100 µg/L will not provide sufficient viral disinfection (Armstrong et al., 2017).

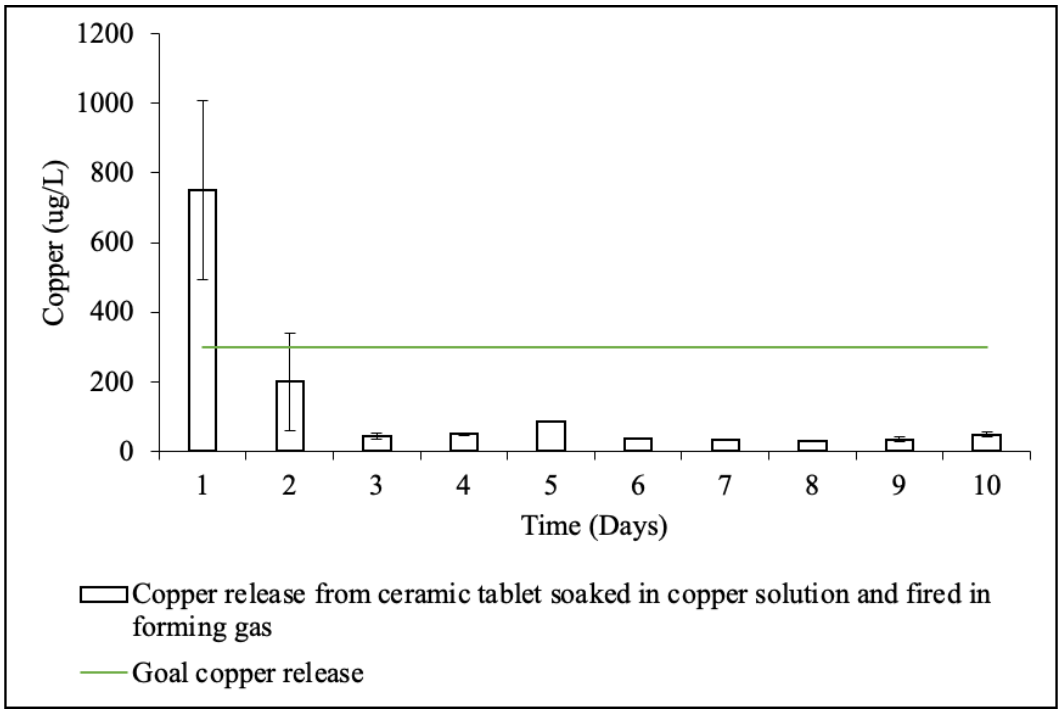


Figure 4.4 Copper concentrations from 10 L of synthetic groundwater treated with a ceramic tablet that was soaked in copper sulfate and fired at 450 °C in forming gas. Error bars represent standard error from duplicate tablets. The green line represents the goal concentration of copper for desired disinfection performance.

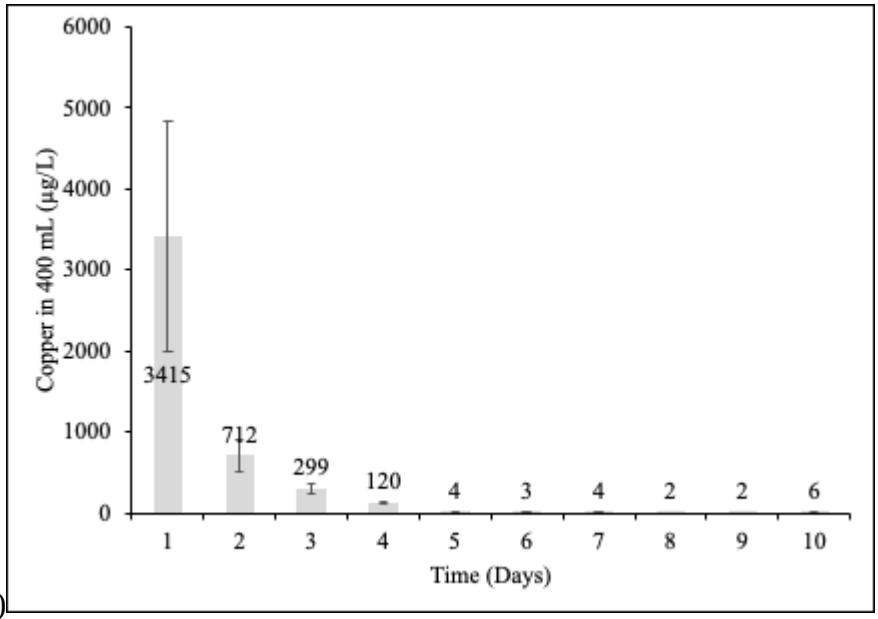
4.4.3 Low-temperature acidic reduction of copper

We attempted a low-temperature, acidic reduction of copper to form copper nanopatches on a porous ceramic tablet. We adapted methods from a study that successfully incorporated

copper nanoparticles into cellulosic paper (Dankovich and Smith, 2014). The copper-embedded papers were used as filters and released around 200 µg/L copper into effluent water.

The ceramic tablets embedded with copper using acidic reduction were tested in 400 mL of DI water for 10 days of use. Copper concentrations in the water began around 3,400 µg/L on the first day, then steadily declined to 4 µg/L by the fifth day (Fig. 4.5a). When we projected the equivalent copper concentration in 10 L of water, there is zero copper in the water from days 5 and onward (Fig. 4.5b). This method was designed for paper filters to be used once then replaced; sustained release of copper was not attained with a low temperature, acidic reduction approach.

(a)



(b)

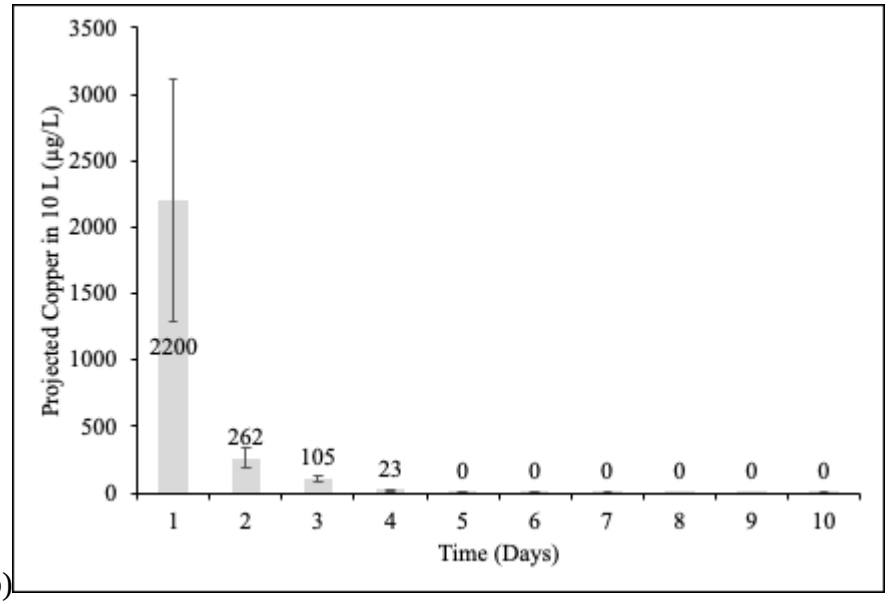


Figure 4.5 Copper release in 400 mL (a) and projected copper release in 10 liters (b) from ceramic tablets that were soaked in a copper sulfate and sodium hydroxide mixture then reduced in an ascorbic acid bath at 85 ^{o}C. Error bars represent standard error from triplicate tablets.

4.4.4 Placing copper metal directly into water

One copper sheet, 10 grams of copper mesh, and 10 grams of copper screen consistently released around 70-129 µg/L, 145-256 µg/L, and 188-333 µg/L, respectively, over 8 days (Fig. 4.6). The copper screen released the most copper over the test period, consistent with its relatively higher surface area than the other forms of copper metal we tested. The ranges of copper release from the copper metal interventions are much smaller than the ranges of copper release from the copper-embedded tablets (Figs. 4.3, 4.4, 4.5). Similarly, there was not a spike or sudden decrease in the copper levels from the copper metal interventions like there was with the copper-embedded tablets. The intended water treatment product would continue to release copper for longer than 8 days, requiring further testing for long-term performance.

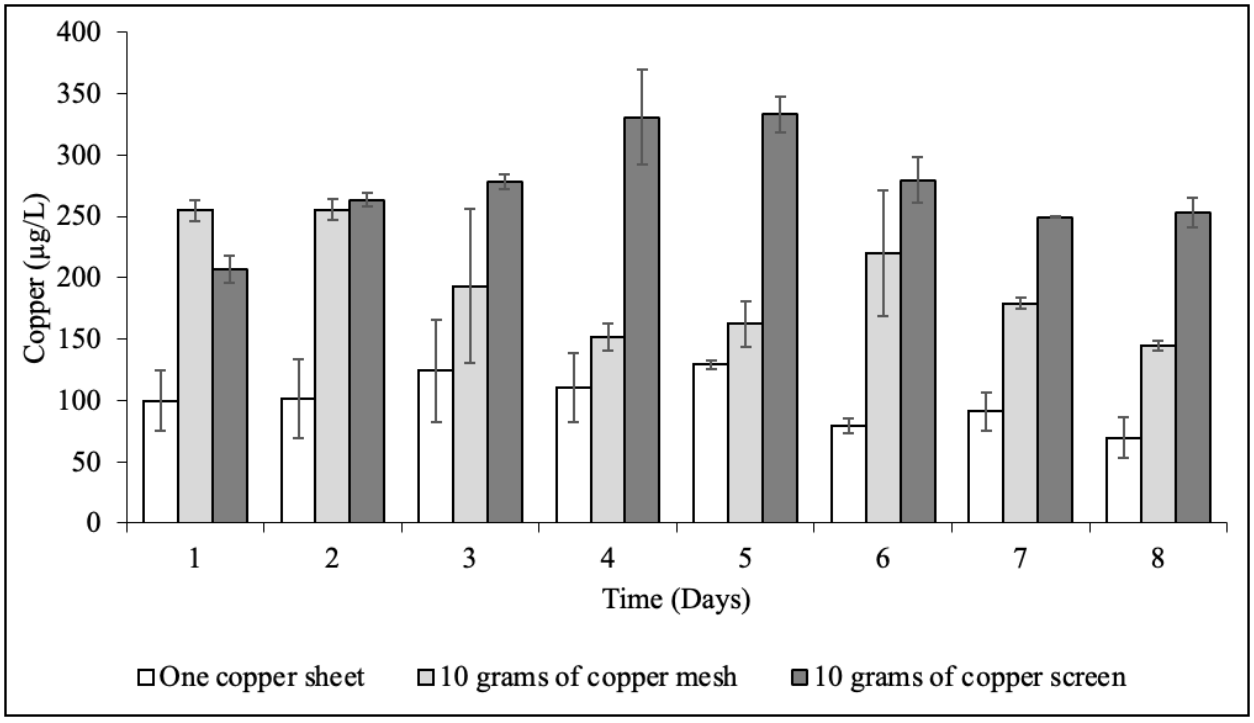


Figure 4.6 Copper concentrations in water containing either a copper sheet (226 grams), 10 grams of copper mesh, and 10 grams of copper screen. Error bars represent standard error from duplicate interventions.

4.5 Discussion

Copper-embedded ceramic tablets generally released high amounts of copper in the first few days of use followed by gradual or drastic declines over the following days (Figs. 4.3, 4.4, 4.5). Drelich et al. (2017) demonstrated that a cross section of a copper-embedded ceramic stone had a zone of saturation with copper sub-microparticles. The copper-embedded tablets presented in this dissertation may not have had a sufficient zone of saturation. It is possible that the porosity of the ceramic used in this study was too small for copper ions to travel further than the surface. If copper ions crowded on the surface of the ceramic, instead of saturating into the pores, the ions

may have been immediately released into water during the first use. This would explain high levels of copper during the first few days from copper-embedded tablets.

Another possibility is that insoluble cupric oxide formed in the pores or on the surface of ceramic after the first few days of use, blocking copper ions from being released from the tablet. When copper sulfate was heated at 750 ºC, the resulting black color indicates that copper(II) oxide, CuO, may have been formed (see Fig. 4.2). Minoshima et al. (2016) found no viral inactivation from solid-state cupric oxide. Another study found cupric oxide was less effective against a gram-positive bacterium, *E. hirae*, than cuprous oxide and metallic copper (Hans et al., 2013). Hosseini et al. (2021) observed that cupric oxide coating on door handles reduced SARS-CoV-2 infectivity by 99.9% in one hour compared to glass. However, they found that the virus needed direct contact with the solid CuO for disinfection. The leachate produced from CuO did not have antiviral properties (Hosseini et al., 2021). This indicates that a CuO-embedded tablet in drinking water would not provide sufficient disinfection as the pathogens would need direct contact with the tablet.

The benefit to the copper sheet, copper mesh, and copper screen is the consistency in release of copper into 10 L of water. Unlike the copper-embedded tablets, none of the metallic copper interventions had initial spikes or drastic drops in copper release over 8 days of use (Fig. 4.6). Large ranges in copper concentrations from a product could lead to users consuming copper over drinking water standards or insufficient copper for disinfection. Small, consistent ranges of copper release are desirable in a water treatment product to reliably disinfect similar amounts of pathogens every day.

The copper sheet consistently released 70-129 µg/L copper over 8 days but was more than 3 times the cost of other interventions (Table 4.2). The copper mesh and copper screen released 145-256 µg/L and 188-333 µg/L copper, respectively, over 8 days of use and cost $0.75 and $2.00 per 10 grams, respectively.

Given the consistency of copper release, targeted range of copper concentrations, and low cost, the copper mesh and copper screen were further evaluated in combination with the MadiDrop+ in Chapter 5.

Table 4.2 Summary of copper-based water treatment interventions that were developed and evaluated in Chapter 4.

Intervention	**Copper release range in 10 L (µg/L)**	**Cost of materials* (USD per one intervention)**
Tablets fired in air	1 - 4,679	2.27
Tablets with acidic reduction	0 - 137	???
Tablets fired in forming gas	29 - 751	1.90
Copper sheet	80 - 100	6.53

Copper mesh	150 - 250	0.75
Copper screen	180 - 330	2.00

*Excludes cost of labor and firing processes.

Chapter 5: Development and Evaluation of a Novel Water Treatment Product: MadiDrop+Cu

5.1 Abstract

The World Health Organization (WHO) estimates that microbiologically contaminated drinking water is estimated to cause 485,000 diarrheal deaths each year. Household water treatment is a low-cost method for reducing the pathogen load in drinking water to decrease instances of diarrhea and sometimes death. Chapter 5 developed and evaluated a new silver and copper water treatment product that meets WHO 1-star performance criteria for household water treatment, the MadiDrop+Cu. First, we tested different configurations of the copper mesh and copper screen with the MadiDrop+ to evaluate the effects of their proximity to one another on copper and silver concentrations in water. Wrapping the copper mesh around the MadiDrop+ decreased silver concentrations in water compared to the MadiDrop+ alone. Folding the copper screen into smaller dimensions decreased the copper concentrations in water compared to the copper screen unfolded. The MadiDrop+ and copper screen, coined MadiDrop+Cu, provided an average of 174 - 325 µg/L copper and 60 - 141 µg/L silver daily for the first 15 days of use. Copper concentrations remained between 149 - 365 µg/L for 92 days of use. When tested individually and in combination against *E. coli*, MadiDrop+Cu removed the most bacteria together rather than separately after 8 hours. A previous study found a prototypic chlorinated polymer gel removed more *E. coli* with the MadiDrop+ than either intervention alone after 8 hours (Estrella-You, 2023). Chapter 5 tested the viral (MS2 Bacteriophage) disinfection from the chlorinated polymer and MadiDrop+Cu by themselves and in combination with one another. The greatest viral disinfection occurred when all three interventions were used together. MadiDrop+Cu removed more MS2 Bacteriophage together than used separately after 24 hours. MadiDrop+Cu achieved >6-log reduction and >3-log reduction of bacteria and viruses after 8 and 24 hours, respectively. Further research is needed to elucidate the long-term disinfection performance of MadiDrop+Cu. The data collected in this chapter supports the feasibility and benefit of commercializing the new product, MadiDrop+Cu, which achieves World Health Organization 1-star performance criteria for household water treatment.

5.2 Background

The World Health Organization (WHO) estimates that microbiologically contaminated drinking water is estimated to cause 485,000 diarrheal deaths each year (World Health Organization, 2022). Household water treatment can reduce microbiological contamination and decrease instances of waterborne disease. The WHO has rigorous performance criteria for household water treatment technologies. Performance classifications are assigned to technologies that can reduce specified amounts of bacteria, viruses, and protozoa from drinking water (Table

1). Certification from the WHO is important for aid organizations and users to trust a water treatment product.

The MadiDrop+ is a commercial product developed at the University of Virginia and now sold by Silivhere Technologies that releases silver into 10-20 liter (L) containers of water and, since the tablets release silver ions over time, they provide disinfection for approximately one year. Silver ions are effective against bacteria, but only achieve around a 1-log reduction (i.e., 90% reduction) of protozoa and viruses (Ehdaie et al., 2020; World Health Organization, 2018). A 1-star WHO performance requires at least a 3-log reduction of viruses with a 2-log reduction of bacteria or a 2-log reduction of protozoa with a 2-log reduction of bacteria. The MadiDrop+ removes 3-4 log of bacteria after 8 hours (Singh et al., 2019), but does not meet the 1-star requirements for protozoa or viruses.

There is evidence that combining silver with copper disinfectants results in synergistic activity against bacteria and viruses (Arakawa et al., 2019; Chen et al., 2008; Soliman et al., 2020; Vaidya et al., 2017). This research sought to develop a product that could release both silver and copper into drinking water to increase disinfection properties from the MadiDrop+ alone. A household water treatment that utilizes silver and copper may have the potential to achieve WHO 1-star classification and, more importantly, reduce instances of waterborne disease.

In Chapter 4 of this dissertation, ten grams of a copper mesh and a copper screen were shown to produce average daily copper concentrations of 145-256 µg/L and 188-333 µg/L, respectively, in 10 L of water for 8 days. Therefore, we tested different configurations of the copper mesh and copper screen with the MadiDrop+ to evaluate the effects of their proximity to one another on copper and silver concentrations in water. The MadiDrop+ and copper screen, coined MadiDrop+Cu, was tested for 92 days in water for long-term copper and silver release. The MadiDrop+Cu was tested individually and in combination against *E. coli* after 8 and 24 hours of contact time.

The MadiDrop+ and free chlorine have demonstrated a synergistic relationship against bacteria in drinking water (Estrella-You and Smith, 2022). A prototypic chlorinated polymer gel was found to remove more bacteria with the MadiDrop+ than either alone (Estrella-You, 2023). The chlorinated polymer gel, MadiDrop+, and copper screen were tested individually and together against MS2 Bacteriophage after 8 and 24 hours of contact time. Given the data collected in this study, products were assessed for WHO performance criteria.

5.3 Methods and Materials

5.3.1 Copper and silver quantification

Total residual copper and silver concentrations refer to the sum of zero-valent and ionic metal in solution. All samples were analyzed for total residual silver and copper concentrations by being prepared with trace metal grade nitric acid (Fisher Chemical, Fair Lawn, NJ, USA) and

measured using inductively coupled plasma mass spectrometry (ICP-MS) and/or graphite furnace atomic absorption spectrometry (GFAAS).

5.3.2 Copper mesh and screen experimental setup

Ten grams of copper mesh (Amazon) was tested by itself, folded around a MadiDrop+ (Silivhere Technologies), or with a MadiDrop+ but not touching. Ten grams of copper screen (TWP, Inc.) was tested by itself, folded up into a 2-inch by 2-inch square with a MadiDrop+ but not touching, and unfolded with a MadiDrop+ but not touching. See Fig. 5.1 for photos and Table 5.1 for copper screen product specifications. A MadiDrop+ was also tested by itself to compare silver release to each scenario. Interventions were placed in 10 liters of deionized (DI) water for 24 hours, after which a water sample was taken, and acid digested to 2% nitric acid. Water was fully emptied from the containers and refilled with 10 L DI while keeping the interventions in the containers. We repeated this process every 24 hours to simulate daily use. In all tests, mixing occurred naturally at the time of filling and for 10 seconds prior to sampling at 24 hours.

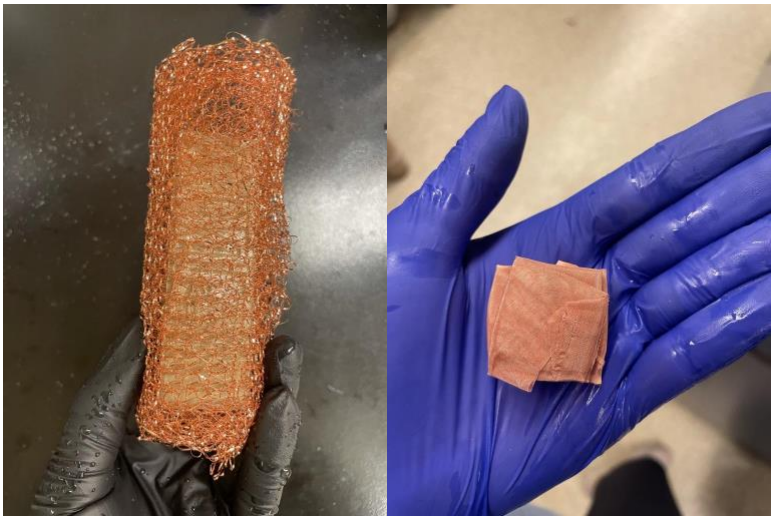


Figure 5.1 Photo of a MadiDrop+ wrapped in copper mesh (left) and a folded piece of copper screen (right). See Chapter 4.3.4 for more photos of the copper mesh and screen.

Table 5.1 Product specifications for the copper screen used in this study. All values were provided by the manufacturer, TWP Inc (twpinc.com/200-mesh-copper-002-wire-dia).

Specs	U.S.	Metric
Mesh size	200 per in	200 per 2.54 cm
Wire diameter	0.0020 in	0.0508 mm
Opening	0.0030 in	0.0762 mm
Opening (microns)	76	76

Opening (%)	35	35
Overall thickness	0.0044 in	0.1118 mm
Weight per square foot	0.0700 lb	0.0318 kg

5.3.3 Chlorinated polymer gels

Detailed methods for the development and bacterial testing of the chlorinated polymer gel are described in the thesis titled "Investigating the synergistic effect of free chlorine and silver ions on bacteria inactivation for point-of-use water treatment applications" (Estrella-You, 2023). Briefly, preparing the polymer gels consisted of two steps: (i) precursor (gel that does not contain chlorine yet) preparation, and (ii) precursor chlorination. The precursor was prepared using a polymerizable amine monomer called 3-(4'-vinylbenzyl)-5,5-dimethylhydantoin or VBDMH, which was synthesized from 4-vinylbenzyl chloride and 5,5-dimethylhydantoin in the presence of potassium hydroxide (Sun and Sun, 2001). After synthesizing the gels, they were soaked in methanol and deionized water to remove any unreacted chemicals to prevent them from being released in the water. The gels were then exposed to a sodium hypochlorite (NaOCl) solution and rinsed with deionized water to remove any unbound chlorine (Liang et al., 2005).

We used two chlorinated gels with 1 gram of monomer each in 5 L of SGW for MS2 Bacteriophage disinfection experiments because that amount was found to remove 1.1-log of *E. coli* after 8 hours (Estrella-You, 2023). SGW consisted of 1.2 g of $MgSO_4$, 1.92 g $NaHCO_3$, 0.08 g KCl, and 1.2 g of $CaSO_4$ per 20 L in a plastic container (US EPA, 2002).

5.3.4 MS2 Bacteriophage

ATCC medium #271 consisted of tryptone (10 g/L), yeast extract (1 g/L), NaCl (8 g/L), and agar (if required, either 0.45% w/v or 1% w/v for top or bottom agar, respectively) with DI water. Medium was autoclaved at 121℃ for 25 minutes and allowed to cool to 50 ℃ before adding supplements. Supplements consisted of glucose (1 g/L), $CaCl_2$ (0.294 g/L), and thiamine (10 mg/L). While the agar mediums were still liquid, 10 mL of bottom agar (1% w/v) was poured into sterile 100 mm petri dishes and 10 mL of top agar (0.45% w/v) was poured into sterile glass bottles with screw caps. Bottom agar plates and top agar bottles were stored at 4℃ until use.

Host *E. coli* (ATCC 15597) was rehydrated with 1 mL of medium without agar (referred to as broth). The rehydrated host was stored at 4°C for up to a week. Seventy-five µL of the rehydrated host was inoculated on a 90 mm tryptic soy agar plate (Millipore 1.46685) and spread with a sterile glass rod. The plate was incubated inverted at 37℃ overnight, after which it was stored at 4℃ for up to 5 days.

Log phase *E. coli* was cultured by scraping one colony from the plate into 10 mL of broth in a 250 mL flask with a foam stopper. The flask was incubated at 37°C while shaking at 180 RPM for 1.5-2.5 hours until the growth reached OD_{600} of 0.1 to 0.4 (HACH DR6000).

MS2 Bacteriophage (ATCC 15597-B1) was rehydrated with 1 mL of broth. Five mL of the log phase *E. coli* was infected with 100 µL of the rehydrated bacteriophage and incubated at 37°C while shaking at 180 RPM for 16-18 hours. After incubation, the phage culture was centrifuged at 4,000 g for 10 minutes. The lysate was filtered with a 0.45 µm PES sterile filter. The filtrate was stored at 4°C.

MS2 Bacteriophage (ATCC 15597-B1) was quantified by adapting methods from the manufacturer and double layer plaque assay (Cormier and Janes, 2014). Bottom agar plates were warmed in the incubator one hour prior to plating. Top agar was melted and poured into autoclaved glass bottles with screw caps (10 mL each). The top agar bottles were placed in a 50°C water bath for one hour prior to plating.

Once samples with bacteriophage were collected, they were neutralized with sodium thiosulfate (60 g/L; 26.4 µL per 1 mL sample). A previous study found that the addition of sodium thiosulfate did not affect viral activity (Ehdaie et al., 2020). Bacteriophage samples were diluted with #271 broth. One hundred µL of log phase *E. coli* and 30 µL of bacteriophage sample were added to a bottle of top agar, gently mixed, and immediately poured onto a bottom agar plate. The top agar was allowed to cool at room temperature until solidified, about 15-30 minutes. At each sampling interval, four plates were included as quality control: no top agar, top agar without host or bacteriophage, top agar with host only, and top agar with host and bacteriophage stock. All plates were incubated inverted overnight (37°C, 16-18 hours) after which visible plaques were counted (Fig. 5.2).

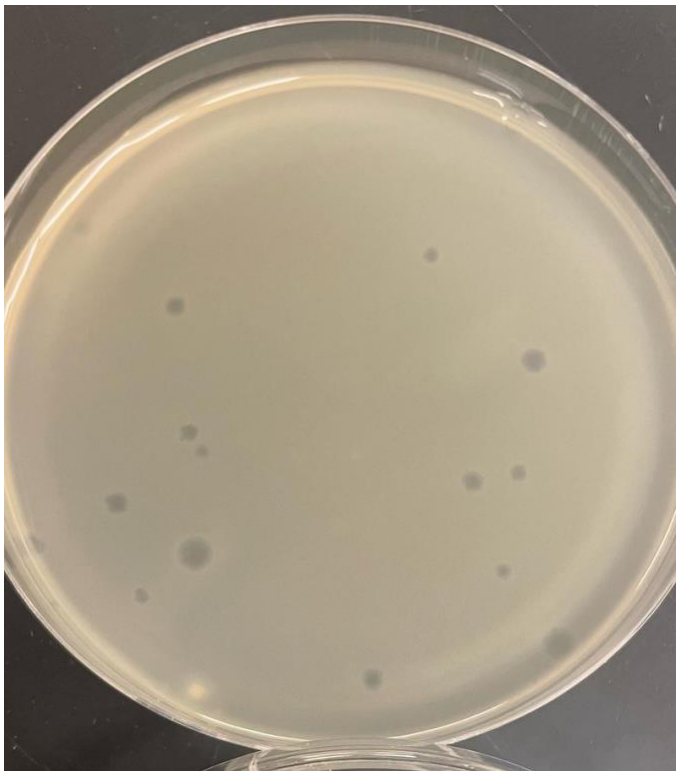


Figure 5.2 Photo of MS2 Bacteriophage plaques. Each dark circle was counted as one plaque-forming unit (PFU).

5.3.5 Escherichia coli

Escherichia coli (*E. coli* C300, American Type Culture Collection) was cultured using sterilized LB broth. LB broth was made with 0.5 g of yeast extract, 0.5 g of sodium chloride, 0.25 g of Bacto-Tryptone, and 50 mL of deionized water (DI). The *E. coli* culture was incubated at 37℃ overnight while shaking at 200 RPM. Overnight cultures were centrifuged for 20 minutes at 2,500 RPM. The supernatant was discarded, and *E. coli* was resuspended in a 10 mM phosphate buffer (0.056 g dipotassium phosphate, 0.024 g monopotassium phosphate, and 50 mL DI). This was stored at 4℃ and used within 5 days. For long-term storage at -20℃, cultures were diluted with 40% glycerol.

A sterilized pipette tip was used to collect *E. coli* samples from the test water. Samples were treated with 60 g/L sodium thiosulfate (26.4 μL per 1 mL sample) upon collection to stop disinfection. *E. coli* was quantified with the IDEXX Colilert Test and Quanti-Tray/2000, a method approved by the U.S. EPA (US EPA, 2017).

5.3.6 Disinfection experimental setup

Prior to testing, plastic containers were washed with soap and water, soaked in 10-20% nitric acid for 30 minutes, and thoroughly rinsed with DI water. SGW was added to each container and kept covered at room temperature overnight. Before each test, a new MadiDrop+ tablet was

unwrapped and rinsed for 3 minutes under running water, as per the manufacturer instructions. An unused piece of copper screen was confirmed to be its desired mass on a scale. Polymer gels were chlorinated with NaOCl and kept in a sealed glass bottle.

Containers of 10 L SGW were inoculated with ~10^6 PFU/mL bacteriophage or ~10^7 MPN/100 mL *E. coli* and received either a MadiDrop+, 10 grams of copper screen, a MadiDrop+ with 10 grams of copper screen, or nothing (control). Tests including the polymer gels were performed in 5 L SGW and thus required half the content of copper screen and MadiDrop+. Containers of 5 L SGW were inoculated with ~10^6 PFU/mL bacteriophage and received either 2 polymer gels, 2 polymer gels with 5 grams of copper screen, 2 polymer gels with half of a MadiDrop+, 2 polymer gels with 5 grams of copper screen and half of a MadiDrop+, or nothing (control).

In all disinfection experiments, the solutions were mixed for 10 seconds after inoculation at 0 hours and prior to sampling at 8 and 24 hours. Samples were collected and quantified as previously described in section 5.3.4 for bacteriophage and section 5.3.5 for *E. coli*.

Log reduction of *E. coli* or bacteriophage was calculated by subtracting the log of the *E. coli* or bacteriophage in the effluent at each time point from the log of the *E. coli* or bacteriophage of the control (water without treatment intervention) at the same time point. *E. coli* tests were conducted in triplicate trials. Bacteriophage tests without the polymer gels were conducted in triplicate trials. Bacteriophage tests with the polymer gels were conducted in duplicate trials.

5.4 Results

5.4.1 Optimizing copper and silver release from copper mesh/screen with MadiDrop+

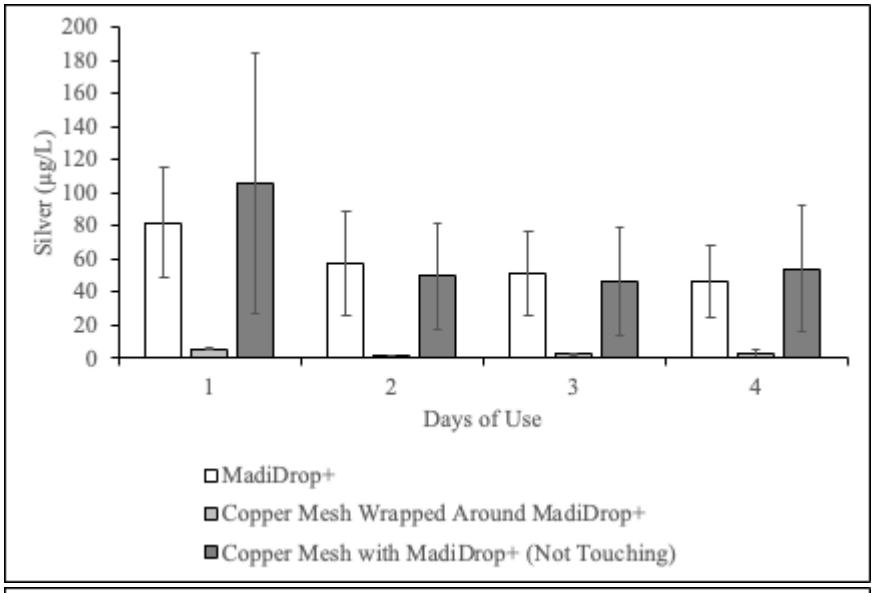
Ten grams of the copper mesh was either used by itself, wrapped around a MadiDrop+, or used with the MadiDrop+ but not touching. Silver release was comparable from the MadiDrop+ alone versus the MadiDrop+ with 10 grams of copper mesh in the same container but not touching (Fig. 5.3a). However, when 10 grams of copper mesh was wrapped around the MadiDrop+, silver release was below 6 µg/L over four days of use. In comparison, MadiDrop+ used without copper mesh released 47-82 µg/L silver over four days of use. Results indicate that wrapping the MadiDrop+ with copper mesh may provide a barrier for silver to diffuse out of the tablet and into the water.

Copper release from the copper mesh was highest when the copper mesh was used by itself for the first 3 days of use (Fig. 5.3b). By day 4, copper release was comparable between the copper mesh alone, wrapped around a MadiDrop+, or with a MadiDrop+ but not touching.

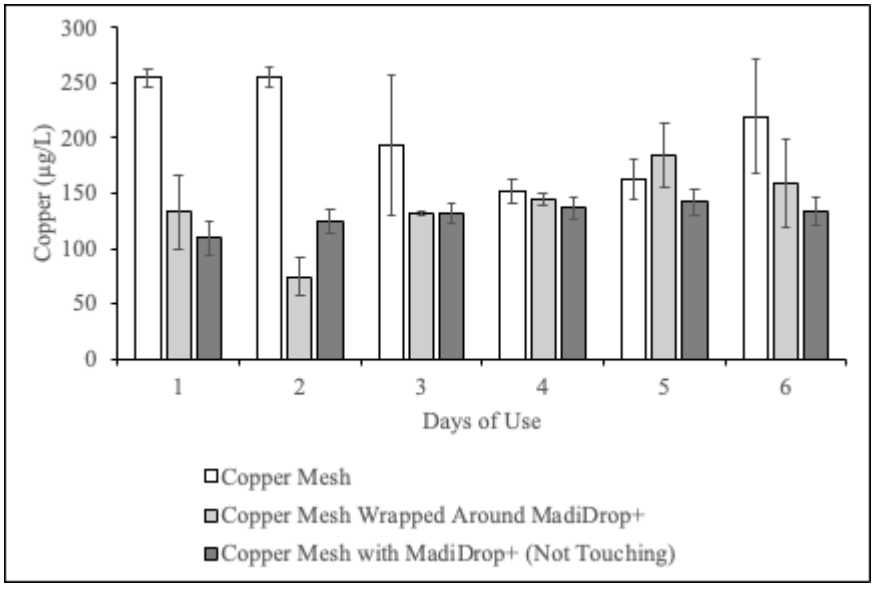
Ten grams of copper screen was tested unfolded by itself, folded up into a 2-inch by 2-inch square with a MadiDrop+, or unfolded with a MadiDrop+. Silver release was comparable between the MadiDrop+ alone and the MadiDrop+ with a copper screen, regardless of folding the screen (Fig. 5.4a). Copper release was similar between the unfolded copper screen with and without a MadiDrop+ over 4 days of use (Fig. 5.4b). However, copper levels were below 30 µg/L when the copper screen was folded. This leads us to believe that the entire surface area of the copper mesh

needs to be exposed to water for oxidation, and thus copper release, to occur. Folding the mesh may either (a) prevent oxidation by preventing diffusion of water or dissolved oxygen that allows oxidation, or (b) slow diffusion of copper ions. If kept separately, there is less of a chance that the two interventions will obstruct diffusion of copper and/or silver.

When comparing the copper mesh and copper screen, the highest copper release was achieved with 10 grams of unfolded copper screen not touching the MadiDrop+ (Fig. 5.4b). Additionally, we observed that the copper mesh would flake off into the water, posing a choking/health hazard if consumed. Therefore, we continued testing the copper screen completely unfolded with the MadiDrop+ while not obstructing each other (Fig. 5.5).



(a)



(b)

Figure 5.3 Silver (a) and copper (b) concentrations in water after 24 hours with a MadiDrop+ alone, 10 grams of copper mesh alone, 10 grams of copper mesh wrapped around a MadiDrop+, and 10 grams of

copper mesh and a MadiDrop+ in the same container but not touching each other. Error bars represent standard error of duplicate data, except for the MadiDrop+ only which was performed in triplicate.

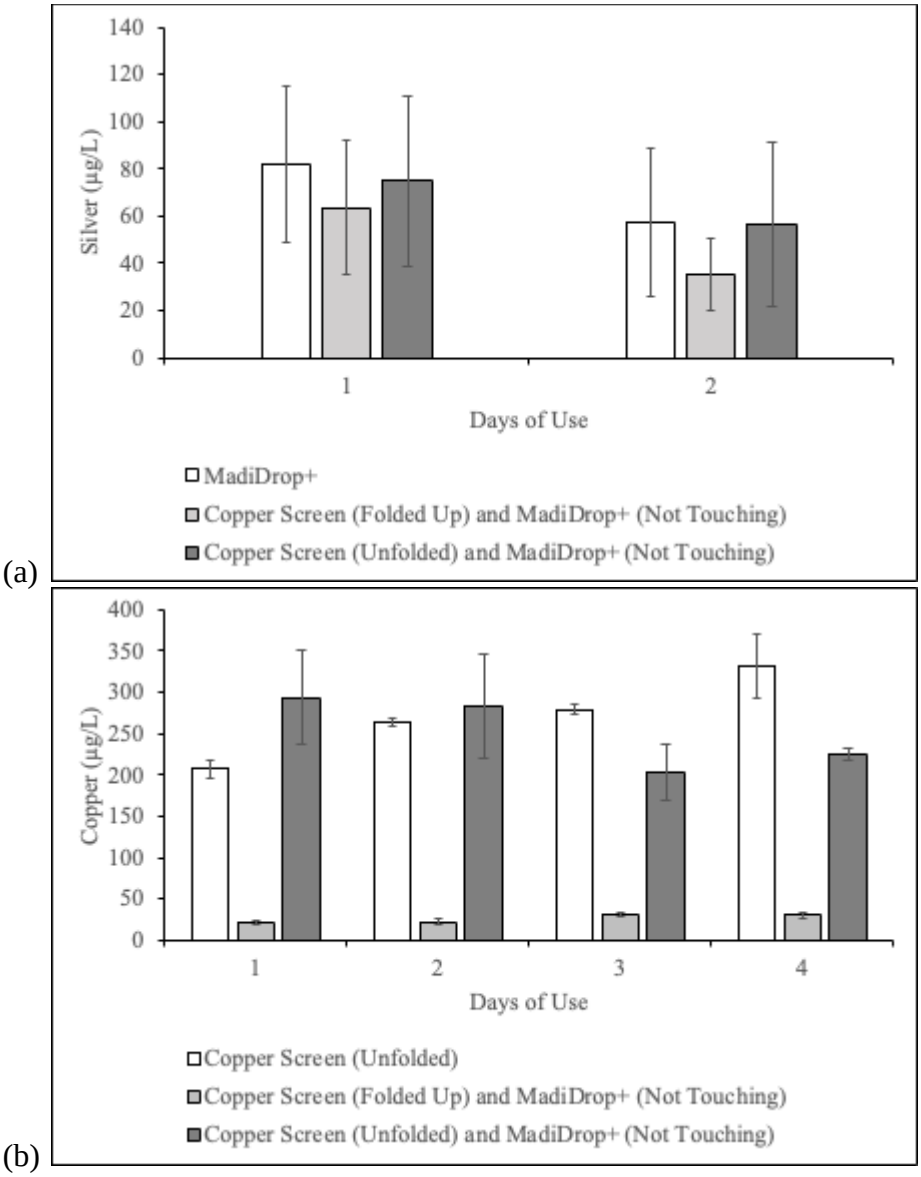


Figure 5.4 Silver (a) and copper (a) release from a MadiDrop+ alone, 10 grams of unfolded copper screen with and without a MadiDrop+, and 10 grams of folded copper screen with a MadiDrop+. Error bars represent standard error of triplicate data except for the copper release from the copper screen alone which was performed in duplicate.

Ten grams of unfolded copper screen and a MadiDrop+ were used in 10 L DI for 92 days. Average copper and silver concentrations were between 174 - 325 µg/L and 60 - 141 µg/L, respectively, for the first 15 days of use (Fig. 5.5). Average silver concentrations exceeded the drinking water limit of 100 µg/L on days 7 and 8 of use. The error bars for silver indicate that there was variability between silver release from the triplicate trials. Two of the trials showed silver

concentrations between 12 - 39 µg/L while one trial had consistently higher silver between 125 - 209 µg/L. It is possible that the latter MadiDrop+ tablet was dosed with excess silver during manufacturing. Further testing is required to evaluate the silver release from the MadiDrop+ when paired with the copper screen.

Copper was measured for days 20 through 92 of use, as well (Fig. 5.6). Copper concentrations remained between 193 - 365 µg/L from days 20 to 59 of use. Days 66 through 92 show lower levels of copper between 149 - 181 µg/L. Silver concentrations are still being quantified for days 20 through 92.

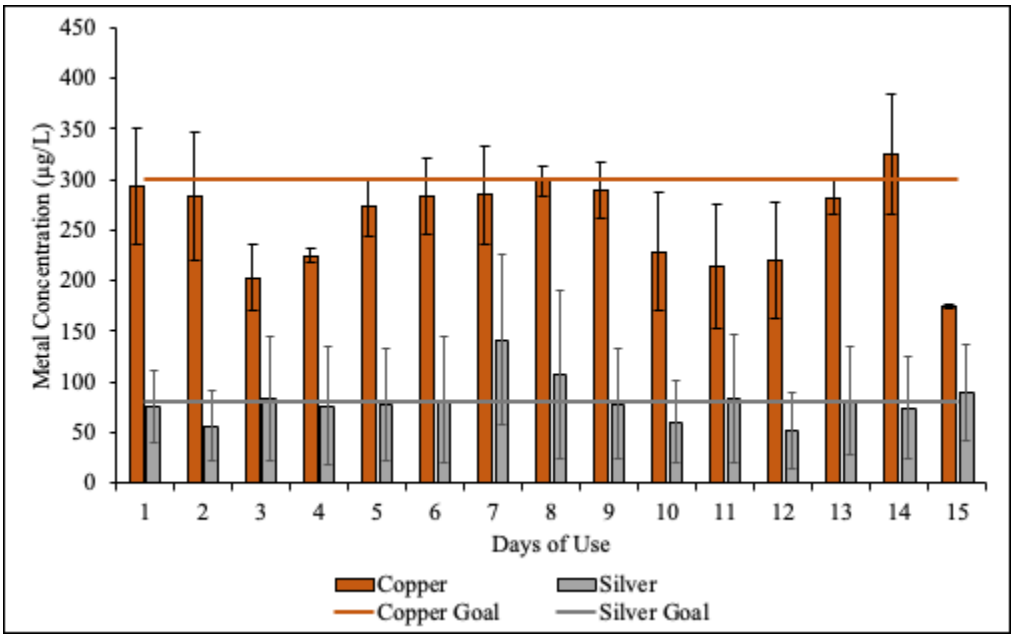


Figure 5.5 The first 15 days of copper and silver concentrations in 10 L of DI water with 10 grams of copper screen unfolded with a MadiDrop+ in the same container but not touching. Error bars represent standard error from triplicate trials.

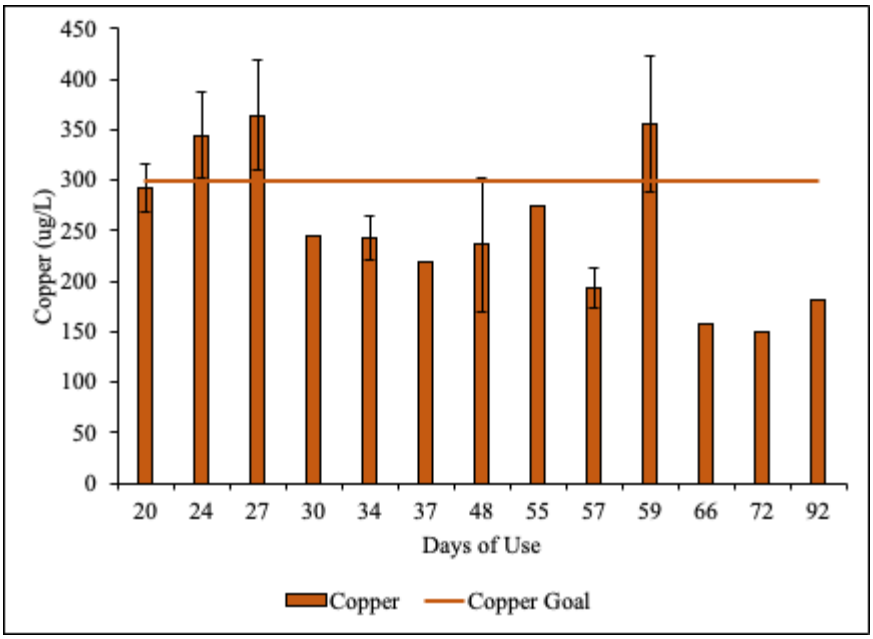


Figure 5.6 Days 20 to 92 of copper concentrations in 10 L DI with 10 grams of copper screen unfolded and a MadiDrop+ in the same container but not touching.

5.4.2 Inactivation of E. coli *and MS2 bacteriophage from MadiDrop+Cu*

We tested the copper screen and MadiDrop+ individually and in combination against both bacterial (*E. coli*) and viral (MS2 Bacteriophage) pathogens. After 8 hours, the combination of the MadiDrop+ with the copper screen (MadiDrop+Cu) achieved higher removal of *E. coli* compared to either intervention alone (Fig. 5.7a). By 24 hours, the MadiDrop+ alone achieved a similar log reduction of *E. coli* compared to the MadiDrop+Cu. This suggests that for shorter contact times, it would be beneficial to employ the MadiDrop+Cu to kill more bacteria. The least amount of contact time is preferable for ease of use for the consumer.

Viral disinfection required more contact time than bacterial disinfection. After 8 hours, the MadiDrop+Cu only removed 0.5-log of MS2 (Fig. 5.7b). However, that is still more than either intervention alone at 8 hours. The most disinfection occurred at 24 hours, where the MadiDrop+Cu removed 3.2-log of MS2 and the MadiDrop+ alone and the copper screen alone removed 0.8- and 2.1-log, respectively. The MadiDrop+Cu killed more viruses than either intervention alone at 24 hours of contact time.

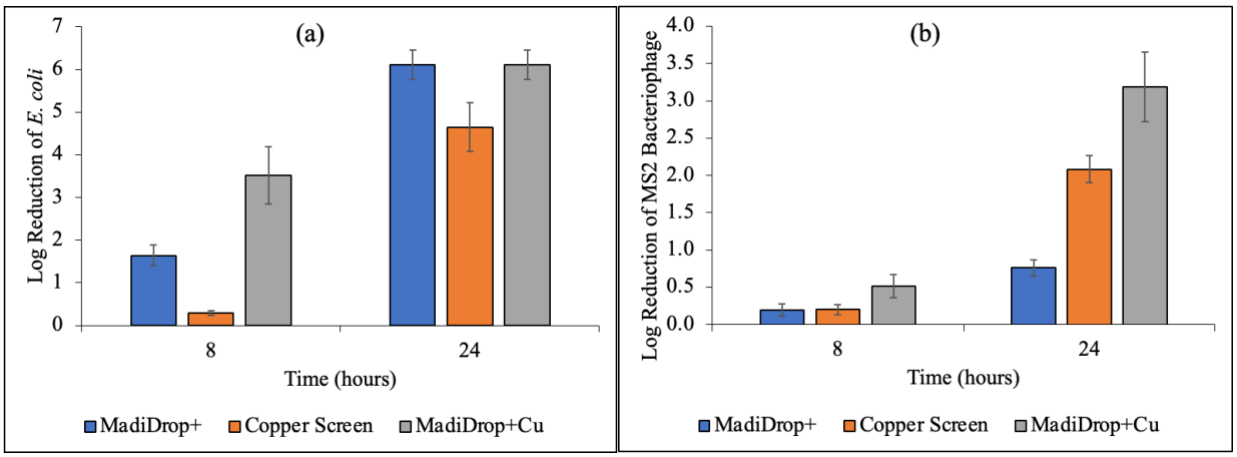


Figure 5.7 Disinfection of *E. coli* (a) and MS2 Bacteriophage (b) from the MadiDrop+, 10 grams of fine copper screen, and the combination of the two. We calculated the $\log_{10}$ of the concentration of the control at 8 and 24 hours divided by the concentration of the sample at the same time point. Error bars represent standard error of triplicate data.

The polymer alone did not remove MS2 Bacteriophage after 8 and 24 hours of contact time (Fig. 5.8). After 24 hours, the polymer and MadiDrop+ combination removed 0.6-log MS2 while the MadiDrop+ alone removed 0.8-log MS2. Combining the polymer with the MadiDrop+ did not increase disinfection of MS2 compared to the MadiDrop+ alone. After 24 hours, the copper screen removed 2.1-log by itself and 3.3-log with the polymer. Therefore, combining the polymer with the copper screen increased disinfection of MS2 compared to either the polymer or copper screen alone. The combination of the polymer, MadiDrop+, and copper screen removed 4.4-log MS2 after 24 hours. The greatest disinfection of MS2 occurred when combining all three technologies.

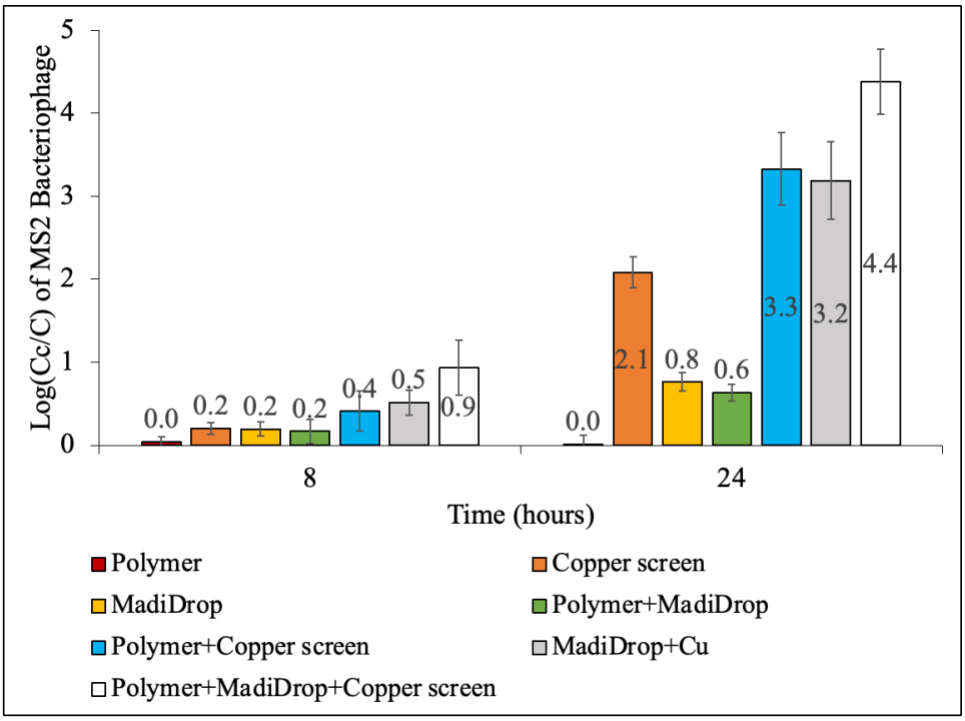


Figure 5.8 Disinfection of MS2 Bacteriophage with newly developed technologies individually and in combination. We calculated the $\log_{10}$ of the concentration of the control (C_C) at 8 and 24 hours divided by the concentration of the sample (C) at the same time point. Error bars represent standard error of triplicate data except for any treatments that include a polymer, which were collected in duplicate.

5.5 Discussion

Ten grams of copper mesh was tested by itself, wrapped around the MadiDrop+, and free-floating with the MadiDrop+. The MadiDrop+ alone released 47 - 82 µg/L per day over four days. Wrapping the copper mesh around the MadiDrop+ decreased silver concentrations to 1 - 6 µg/L per day (Fig. 5.3a). When the mesh and MadiDrop+ were in the same container but not touching, copper and silver concentrations were similar to when the interventions were used alone. Similarly, the copper screen did not appear to influence the silver release from the MadiDrop+ when they were not wrapped around each other (Fig. 5.4a). Copper concentrations did decrease 10-fold from 203 - 293 µg/L to 20 - 31 µg/L when the copper screen was folded into a 2x2 inch square (Fig. 5.4b). When the copper screen was unfolded with the MadiDrop+, the copper levels were similar to the copper screen unfolded by itself. This indicates that there is little interaction between the copper and silver products unless one is physically blocking the other from contact with water. Oxidation and surface area are keys to understanding this.

The dissolved oxygen in the water most likely reacts with the metallic copper or silver on the surface of the intervention to release copper or silver ions into the water. More surface area that is in contact with water leads to greater opportunity for the oxidation, and thus greater metal release rates. Chapter 4 of this dissertation reported that a piece of copper sheet with lower surface area than the copper screen released less copper into water, even though it weighed 22 times more

than the screen (Fig. 4.6). Sudha et al. (2009) reported that varying the surface area of a copper device in water affected the antibacterial performance of the device. However, further research is required to gather long-term effects of copper/silver release when interventions are used together versus separately.

The copper screen released an average of 149 - 365 µg/L per day for 92 days, well below the drinking water standard of 1,300 µg/L per day (Fig. 5.6). Copper concentrations were often below the goal of 300 µg/L per day, but there is evidence that copper can still disinfect below that. Sudha et al. (2012) inoculated *Vibrio cholerae* O1, *Shigella flexneri* 2a, enterotoxigenic *E. coli*, enteropathogenic *E. coli*, *Salmonella enterica* Typhi, and *Salmonella* Paratyphi in a copper pot for 16 hours. They could not recover any of the bacteria, indicating loss of culturability. The copper concentration in the copper pot water was only ~177 µg/L. However, their inoculation was lower than most studies at only 500 CFU/100 mL.

Additionally, copper concentrations below 300 µg/L may not be indicative of low disinfection since this intervention deploys both copper and silver. Silver concentrations are still being quantified for days 20 through 92. It is expected that disinfection will be higher than literature shows for copper alone. Regardless, future work is required to evaluate the long-term disinfection performance of the copper screen and MadiDrop+ combination, coined MadiDrop+Cu.

Although we do not know the long-term disinfection properties of the MadiDrop+Cu, upon first use, the MadiDrop+Cu eradicated more bacteria (*E. coli*) than the sum of each technology individually after 8 hours of contact time (Fig. 5.7a) and more viruses (MS2 Bacteriophage) than the sum of each technology individually after 24 hours of contact time (Fig. 5.7b). With > 6 log removal of bacteria and > 3 log removal of viral pathogen, this combination disinfectant-releasing product, MadiDrop+Cu, meets WHO 1-star performance criteria.

The chlorinated polymer alone did not remove MS2 Bacteriophage, even after 24 hours (Fig. 5.8). Estrella-You (2023) found that concentrations of the released chlorine were below the detection limit (<40 µg/L Cl_2) of the spectroscopic method Hach 10241. However, they confirmed the presence of Cl^+ in the polymer by staining it with iodide. It is possible that the chlorinated polymers did not release sufficient chlorine for disinfection of the virus. One study found 99% inactivation of 6 viral pathogens using chlorine concentrations over 400 µg/L (Engelbrecht et al., 1980). The concentration of chlorine from the polymers is at least 10 times lower than the concentrations found to inactivate viruses in literature.

The MadiDrop+ and polymer combination were shown to remove more bacteria than either intervention alone (Estrella-You, 2023). Additionally, free chlorine and silver have been shown to synergistically disinfect bacteria (Estrella-You and Smith, 2022). However, combining the MadiDrop+ and chlorine-charged polymer did not increase MS2 disinfection. It is possible that chlorine below the detection limit is sufficient for bacterial pathogens, but not viral pathogens. The study that found synergistic effects also used at least 50 µg/L, which is higher than what is detected from the polymer.

Alternatively, the addition of the polymer to the copper screen improved disinfection of MS2 compared to the copper screen alone. Free chlorine is known to cause corrosion of copper (Atlas et al., 1982). It is possible that the presence of the polymer increased oxidation of the copper screen to release more copper ions than without the polymer. A higher concentration of copper in the water would lead to greater disinfection of MS2 (Armstrong et al., 2017).

The polymer, copper screen, and MadiDrop+ together had the greatest effect on MS2. As discussed earlier, the polymer and MadiDrop+ combination did not increase disinfection. Therefore, it is possible that chlorine increased copper release and the greater copper/silver interaction allowed for the increased disinfection of MS2. It is believed that viral inactivation by copper and silver involves a modified site-specific Fenton mechanism, generating hydroxyl radicals near the target site (Choi and Hu, 2009; Samuni et al., 1984; Yahya et al., 1992). Since copper ions can repeat cyclic redox reactions, many OH radicals can be formed near the same site, multiplying damage for one target. The OH radicals may affect the peptide backbone of the capsid proteins of the virions (Abad et al., 1994).

Since log reduction is not additive, we cannot quantify synergy from the data collected in this study. Determining chemical synergy requires an understanding of the dose-response for each disinfectant in question. Due to limited resources and time, we did not test the effects of silver and copper salts on *E. coli* and MS2 Bacteriophage to quantify the doses of each chemical that result in synergy. Instead, we optimized the combination of a copper screen and MadiDrop+ tablet to release 174 - 325 µg/L copper and 51 - 141 µg/L silver per day for at least 15 days. The resulting product, MadiDrop+Cu, removed >6-log bacteria and >3-log virus, achieving WHO 1-star criteria for HWT. MadiDrop+Cu with chlorinated polymer gels removed >4-log MS2 Bacteriophage. Future studies should evaluate the bacterial and protozoan disinfection from the MadiDrop+Cu and polymer combination to assess for WHO HWT performance.

Chapter 6: Conclusions and Future Work

The research presented in this dissertation explored methods for increasing the disinfection performance of a silver-embedded ceramic tablet, the MadiDrop+, with the goal of reducing instances of waterborne disease. Chapter 3 evaluated the bacterial removal when combining the MadiDrop+ and household water filters. In both laboratory and field settings, placing a MadiDrop+ in the lower reservoir or split between reservoirs of filters increased bacterial disinfection compared to filters alone. A thorough financial analysis to compare the cost of the filters and MadiDrop+ and their corresponding microbiological benefits to users is a gap in this study. Future work is required in this area. In the short-term, aid organizations, government agencies, and other decision makers who seek to provide household water treatment to under-resourced communities may consider pairing common water filters with a MadiDrop+ to increase bacterial disinfection and prevent recontamination in treated water.

In Chapter 4, we developed and evaluated several copper-embedded ceramic tablets and metallic copper interventions for their ability to produce consistent, daily copper concentrations in 10 L of water. While copper-embedded ceramic tablets generally released high amounts of copper in the first few days of use, it was followed by gradual or drastic declines over the following days (Figs. 4.3, 4.4, 4.5). It is hypothesized that insoluble cupric oxide formed in the pores or on the surface of ceramic after the first few days of use, preventing copper ions from being released from the tablet. The mechanisms of metallic copper formation on ceramic needs to be studied in future research.

Unlike the copper-embedded tablets, none of the metallic copper interventions had initial spikes or drastic drops in copper release over 8 days of use. The copper sheet consistently released 70-129 µg/L copper over 8 days but was more than 3 times the cost of other interventions. The copper mesh and copper screen released 145-256 µg/L and 188-333 µg/L copper, respectively, over 8 days of use and cost $0.75 and $2.00 per 10 grams, respectively. It is hypothesized that the dissolved oxygen in the water makes contact with the surface of the copper metal and forms copper ions that are released into the water (Eq. 4.1). Given consistency of copper release, target range of copper concentrations, and low cost, the copper mesh and copper screen were further evaluated in combination with the MadiDrop+ in Chapter 5.

Chapter 5 tested different configurations of the copper mesh and copper screen with the MadiDrop+ to evaluate the effects of their proximity to one another on copper and silver concentrations in water. Wrapping the copper mesh around the MadiDrop+ decreased silver concentrations in water compared to the MadiDrop+ alone. Folding the copper screen into smaller dimensions decreased the copper concentrations in water compared to the copper screen unfolded. It is likely that the silver-ceramic surface and metallic copper surface need to have direct contact with water to promote oxidation of the metal.

The MadiDrop+ and copper screen, coined MadiDrop+Cu, provided an average of 174 - 325 µg/L copper and 60 - 141 µg/L silver daily for the first 15 days of use. Copper concentrations remained between 149 - 365 µg/L for 92 days of use. Future research is required to understand the

speciation of copper in water treated with the copper screen over months of use.

When tested individually and in combination against *E. coli*, MadiDrop+Cu removed the most bacteria together rather than separately after 8 hours. It is possible that silver disrupts the bacterial envelope, allowing more copper to enter the cell and bind to nucleic acids, catalyzing the formation of radicals (Kumar et al., 2005; Straub et al., 1995).

A previous study found a prototypic chlorinated polymer gel removed more *E. coli* with the MadiDrop+ than either intervention alone after 8 hours (Estrella-You, 2023). Chapter 5 tested the viral (MS2 Bacteriophage) disinfection from the chlorinated polymer and MadiDrop+Cu by themselves and in combination with one another. The greatest viral disinfection occurred when all three interventions were used together. Future studies should evaluate the bacterial and protozoan disinfection from using the three interventions together.

MadiDrop+Cu removed more MS2 Bacteriophage together than when used separately after 24 hours. MadiDrop+Cu achieved >6-log reduction and >3-log reduction of bacteria and viruses after 8 and 24 hours, respectively. Further research is needed to elucidate the long-term disinfection performance of MadiDrop+Cu. Given the data collected in this dissertation, the new product, MadiDrop+Cu, achieves World Health Organization (WHO) 1-star performance criteria for household water treatment.

While WHO approval increases product trust among organizations and users, the scheme has limitations. The WHO criteria do not include aspects like social acceptability, ease of use, and cost. If a product is culturally inappropriate, not used properly, or inaccessible, it has a higher likelihood of user rejection. In those cases, it does not matter how well it removes bacteria, viruses, and protozoa if the product is not designed with the needs of the user. Future work should focus on a low-cost and simple method for delivering the MadiDrop+Cu. Ideally, end-users should have input throughout the entire design process to ensure acceptance and ease of use. Additionally, thorough cost analyses of the MadiDrop+/filter and MadiDrop+Cu should be performed to determine if the additional cost is proportionate to the additional disinfection compared to either intervention alone.

The need for household water treatment varies from community to community, but generally it is prevalent in communities with greater underlying economic problems that prevent access to clean water. Decentralized, household water treatment should be continuously improved with the knowledge that simultaneously governments should be working towards long-term solutions for safe drinking water. Funding should be concurrently directed towards improving household water treatment technologies and strengthening governmental systems for water treatment and distribution.

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