Efficacy of Copper and Silver as Residual Disinfectants in Drinking water

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ABSTRACT

Contamination events and biofilms can decrease the amount of free chlorine available in drinking water systems. The efficacy of 100 µg/L silver and 400 µg/L copper, individually and combined, were evaluated as secondary, longer-lasting residual disinfectants against *Salmonella enterica* serovar Typhimurium*, Escherichia coli, Listeria monocytogenes,* and *Mycobacterium fortuitum* at 24°C and 4°C. A >5.0-$\log_{10}$ reduction was observed in *E. coli* and *L. monocytogenes* after three hours and *S.* Typhimurium following seven hours of exposure to silver. *M. fortuitum* was the most resistant species to silver (1.11-$\log_{10}$ after seven hours). Copper did not significantly reduce *S.* Typhimurium and *E. coli* at 24°C; ≥2.80-$\log_{10}$ reductions were observed in the Gram-positive *L. monocytogenes* and *M. fortuitum*. Longer exposure times were required at 4°C to achieve significant reductions in all species. A synergistic effect was observed when silver and copper were combined at 24°C. In addition, silver was not affected by the presence of organic matter at concentrations that completely inhibited 0.2 mg/L chlorine. The results of this study suggest that combinations of silver and copper show promise as secondary residual disinfectants. They may also be used in conjunction with low chlorine levels or other disinfectants to provide additional, long-lasting residuals in distribution systems.

Key words: Silver, copper, disinfection, disinfectant residual, water distribution system

INTRODUCTION

Chlorine based disinfectants are used in almost all surface water treatment plants in the United

States. [1] In addition to primary treatment with chlorine, a residual disinfectant is required because of both the potential for bacterial regrowth and the fact that water contamination can occur once the water enters the drinking water distribution system. [2-4] In the United States, the Environmental Protection Agency's (EPA's) Surface Water Treatment Rule requires the maintenance of at least a 0.2 mg/L residual of free chlorine [5-6]; however, it is sometimes difficult to maintain an adequate concentration of free chlorine throughout the distribution system. [7-8] Factors affecting the concentration of chlorine include the interaction of the chemical with organic matter in the water, the presence of biofilms, water age, pipe material, and tubercle formation on the pipes. [3,8-9] In addition, the intrusion of contaminated material during pipe repair, through illegal cross-connections, or through the loss of pressure in the distribution system may further promote disinfectant decay. [2,10]

The antimicrobial activity of silver (Ag) has been known since ancient times. Over the past several decades, silver has been used in water treatment, in dietary supplements, in medical applications, and to create antimicrobial coatings and products. [11-15] The antimicrobial properties of copper (Cu) have also been recognized throughout history. The Egyptians and Romans used this metal to treat wounds and to protect their drinking water quality. [16] The bactericidal, virucidal and algaecidal properties of copper are well documented. [13,17-22] As such, copper was recognized by the United States EPA as the first metallic antimicrobial agent in 2008. [23]

The objective of the current study was to evaluate the residual disinfectant efficacy of silver and copper, both individually and in combination, for the inactivation of *Escherichia coli,*

Salmonella enterica serovar Typhimurium*, Listeria monocytogenes* and *Mycobacterium fortuitum* in an aqueous model system. Although *L. monocytogenes* is not typically a waterborne pathogen, it was included to compare the disinfection efficacy against Gram-positive versus Gram-negative bacterial species.

MATERIALS AND METHODS

Glassware Preparation

All of the glassware were soaked overnight in a 10% nitric acid solution to reduce metal contamination and then rinsed with distilled water and autoclaved. Glassware used for the experiments involving chlorine were soaked for 24 hours in a 30% (v/v) chlorine solution then rinsed with distilled water and baked at 104°C for two hours to satisfy chlorine demand. Experiments were conducted in sterile 250 ml Erlenmeyer flasks.

Borosilicate glassware was used for the described studies. Although this material is not used in distribution systems, it was chosen due to the fact that these metals are known to have a tendency to adhere to many surfaces, forming a plate or layer on the surface. This “plating” phenomenon is more limited with borosilicate glassware. In addition, the glassware was treated with 10% nitric acid between experiments to remove any residual metals. It is important to limit the amount of metals bound to surfaces to ensure that the concentrations being evaluated are accurate and consistent.

Disinfectant Preparation

Stock solutions of silver and copper were prepared in distilled water using $AgNO_3$ (J.T. Baker, Phillipsburg, NY) and $CuCl_2 \cdot H_2O$ (J.T. Baker, Phillipsburg, NY) and then added to the test suspension at a final concentration of 100 µg/L silver and 400 µg/L copper. The silver and copper concentrations were measured at the beginning of each experiment by a colorimetric procedure using a Hach DR/2000 spectrophotometer. [24] These experimental concentrations were chosen for the following reasons: First of all, the EPA recommends a secondary non-enforceable standard of 100 µg/L for silver in drinking water [https://www.epa.gov/dwstandardsregulations/secondary-drinking-water-standards-guidance-nuisance-chemicals; accessed April 2018]. In addition, 400 µg/L copper has been used to treat water in numerous previously published studies. [25-29]

Similarly, a chlorine stock solution was prepared in distilled water using 5.0% sodium hypochlorite (NaOCl; J.T. Baker, Phillipsburg, NJ) and then added to the test suspension at a final concentration of 0.2 mg/L free chlorine. The initial free chlorine concentration was determined by the *N, N,*-dimethyl-*p*-phenylenediamine method adapted from Standard Methods for the Examination of Water and Wastewater. [30]

Humic acid (Aldrich Chemical Company, Inc., Milwaukee, WI) was used as a source of organic matter in order to achieve a concentration of 3 mg/L or 10 mg/L of total organic carbon (TOC). The final TOC concentrations were determined by combustion analysis using a TOC-VCSH instrument (Shimadzu, Columbia, MD).

Maintenance and Preparation of Bacteria

All test bacteria in this study were obtained from the American Type Culture Collection (ATCC; Manassas, VA). Strains included *E. coli* ATCC 25922, *L. monocytogenes* ATCC 19115, *S.* Typhimurium ATCC 23564*,* and *M. fortuitum* ATCC 6841. The bacteria were maintained on Tryptic Soy Agar (TSA; Difco, Sparks, MD). Erlenmeyer flasks containing 100 ml of Tryptic Soy Broth (TSB; Difco, Sparks, MD) were inoculated and incubated on an orbital shaker (250 rpm) at 37°C overnight prior to testing. In the case of *M. fortuitum,* Tween 80 (polyethylene glycol sorbitan monooleate; Sigma Aldrich, St. Louis, MO) was added to the broth to a final concentration of 0.1% (v/v) to inhibit the formation of bacterial aggregates. After incubation, the bacteria were pelleted via centrifugation (15,300 × *g*, 10 min, 10°C; JA-14 rotor, Beckman J2-21 centrifuge; Beckman Coulter, Inc., Fullerton, CA). The pelleted cells were washed by resuspension in 100 ml of 0.01 M phosphate buffer (3.8 mM Na_2HPO_4, 6.5 mM KH_2PO_4; pH 7.5) followed by centrifugation as described previously. This step was repeated one additional time. The final pellet was resuspended in 20 ml of 0.01 M phosphate buffer. The test suspensions were then prepared by adding small volumes of the bacterial suspension to 10 ml of 0.01 M phosphate buffer, resulting in an optical turbidity (measured using a BIOLOG turbidimeter, Hayward, CA) equivalent to a McFarland number 0.5 optical density standard [= 1.5×10^8 colony-forming units (CFU)/ml]. This solution was then diluted further in 0.01 M phosphate buffer to achieve the desired final test concentration (approximately 1.0×10^7 CFU/ml).

Experimental Procedure

All experiments were conducted in demand-free 0.01 M phosphate buffer (3.8 mM Na_2HPO_4, 6.5 mM KH_2PO_4; pH 7.5) so that the test solutions could be standardized. This is a standard method for evaluating the efficacy of various water treatments [e.g., calculating concentration-time (Ct) values for disinfectants such as chlorine, chloramines, chlorine dioxide, ozone, etc.] against specific microorganisms. [31-33] It has also been used in previous studies with metal ions. [11,34-35] In addition, the inoculation titers used in this study, though higher than those typically found in environmental samples, are necessary to demonstrate a ≥5-$\log_{10}$ reduction in the bacterial numbers and are also part of the standard methodology for disinfection studies. [31-33,36-37]

The disinfection treatments included the following: (*i*) 100 µg/L silver, (*ii*) 400 µg/L copper, (*iii*) 100 µg/L silver and 400 µg/L copper, *(iv)* 100 µg/L silver and 3 mg/L TOC (from humic acids), *(v)* 100 µg/L silver and 10 mg/L TOC (from humic acids), *(vi)* 0.2 mg/L chlorine, *(vii)* 0.2 mg/L chlorine and 3 mg/L TOC (from humic acids), and *(viii)* 0.2 mg/L chlorine and 10 mg/L TOC (from humic acids). A control with bacteria with no added disinfectant and another with disinfectant but no bacteria were also included. Experiments were performed in triplicate at room temperature (24°C) and at refrigeration temperature (4°C) for the silver, the copper, and the silver/copper treatments. Refrigeration temperature was included as a "worst case" scenario to mimic colder regions where water temperatures can be a few degrees above freezing. Such low temperatures can diminish the effectiveness of many antimicrobials. Experiments conducted with added TOC and/or chlorine were performed at 24°C only.

Purified stocks of the bacteria were added separately to the disinfection systems and the flasks were placed on an orbital shaker (200 rpm) to simulate flowing water in the water distribution system for the duration of the experiment. At predetermined time intervals, 1-ml samples were collected and neutralized with Dey Engley neutralizing broth (D/E; Difco, Sparks, MD) at a ratio of 1:1. Samples were assayed immediately. A neutralization validation test was successfully conducted prior to the use of D/E in the described experiments. [38] This validation test is necessary to perform to ensure that the neutralizing broth completely inhibits the action of the antimicrobials so that the duration of the time exposures are accurate. [39-40]

Assay for Surviving Bacteria

Bacterial samples were serially diluted in 0.01 M phosphate buffer and the surviving bacteria were enumerated in duplicate using the spread plate method on the following agar media: *E. coli,* mEndo agar (Difco, Sparks, MD); *L. monocytogenes, Listeria* selective agar with antibiotic supplements (LSA; Oxford, Columbia, MD); *S.* Typhimurium, hektoen agar (Difco, Sparks, MD); *M. fortuitum*, tryptic soy agar (TSA; Difco, Sparks, MD). The plates were incubated at 37°C for either 24 hours (*E. coli, S.* Typhimurium, and *L. monocytogenes*) or 72 hours (*M. fortuitum*).

Data Analyses

Data were reported as logarithmic reduction $-\log_{10} N_t/N_0$ where N_t was the concentration of surviving microorganisms at time *t* and N_0 was the concentration of microorganisms at time zero.

Synergy was evaluated by the following formula described by Koivunen and Heinonen-Tanski [41]: Synergy value = $\log_{10}$ reduction by a combination of treatments 1 and 2 – ($\log_{10}$ reduction by treatment 1 + $\log_{10}$ reduction by treatment 2). If the value of the equation is positive, a synergistic effect is present, whereas a negative value represents an antagonistic effect. A zero value indicates that the efficiency of the combined treatments is the same as the sum of the two individual treatments.

A Student's t-test was used to determine if there were significant differences between the control and the disinfection treatments. Differences were considered significant if the resultant *P* value was ≤ 0.05.

RESULTS AND DISCUSSION

It has been proposed that silver inactivates bacteria by binding to the sulfhydryl (-SH) groups of enzymes, affecting important metabolic processes such as respiration. [42-44] The formation of silver free-radicals inside the cell can also impair the electron transport chain and inactivate bacterial DNA and RNA, as well as damaging the cell membrane, leading to microorganism death. [13,45-47] Copper's antimicrobial action consists of inhibiting the respiratory chain on the bacterial membrane. This generates free oxidative radicals [48] that ultimately cause lipid peroxidation of cellular membranes, direct oxidation of proteins, and the inactivation of DNA and RNA. [49] Copper and silver may thus react with the outer and inner bacterial cell components, disrupting normal bacterial metabolism.

Concentration of Silver and Copper in Experimental Supernatants

One of the objections to using copper and silver in water distribution systems has been that these metals will concentrate or "plate" on surfaces and this will limit the availability of ions in the liquid phase. It is unclear as to whether these metals would then become unavailable and no longer provide an antimicrobial effect. Nevertheless, despite these concerns, the silver and copper ion concentrations (100 and 400 μg/L, respectively) remained constant in the control solutions with no added bacteria at both 24°C and 4°C throughout the course of all the experiments (Table 1). This demonstrated that the two metals did not bind to the surface of the test containers. These metals would be much more likely to adhere to surfaces and/or biofilms on the materials commonly used in water distribution systems. This could serve to concentrate the metals and provide an additional source of metal ions that could leach into the water, adding to the disinfectant residual available or alternatively the metals on the surfaces could help to prevent further biofilm formation. Silver and copper ions have been shown to have antimicrobial efficacy against bacterial pathogens in biofilms [50-51] and also to prevent the formation of biofilms. [52]

In the experiments with Gram-negative bacterial suspensions (i.e., *E. coli* and *Salmonella*) conducted at room temperature, no silver ions were detected in the supernatant. Silver may gain access to the inside of the cell by an active uptake mechanism of a microbial transport system. [53-54] In contrast, the concentration of copper did not change in the treatment suspensions containing these two species. This may suggest that silver was most likely bound to cellular components (e.g., proteins), whereas copper was either prevented from entering or interacting

with the microbial cells or was being actively removed from the cells by copper efflux pumps. [49,55] In the treatment suspensions containing *L. monocytogenes* at 24°C, both the silver and the copper levels decreased after seven hours.

At 4°C after seven hours, the concentration of both metals decreased, but was still detected in the supernatants for all three bacterial species. Thus, temperature had an effect on the amount of unbound silver. At low temperatures, bacterial growth, and therefore their metabolism, is perturbed. [56] Low temperatures can adversely reduce the antimicrobial properties of copper and silver by limiting the numbers of ions that can be successfully transported inside the cell. As with the 24°C *L. monocytogenes* experiment, the copper levels also decreased at 4°C, suggesting that both metals interacted with the cells.

Antibacterial Efficacy of Silver and Copper

There is considerable variation in the response of different microorganisms to antimicrobials. The underlying reasons are poorly understood, but the chemical composition of the outer cell layers is thought to be a key factor. [57-58] The impact of the metals on bacterial reductions of Gram-positive and Gram-negative species (with differing cell walls) is shown in Figures 1 and 2.

There was a significant reduction in *S.* Typhimurium after one hour of exposure with both the silver (100 µg/L) and the silver/copper (100/400 µg/L) treatments in the experiments conducted at 24°C (Figure 1A). The observed reductions were also significant for these treatments for every sampling time thereafter. After seven hours, a 5.42-$\log_{10}$ reduction was observed with silver and

a 3.51-$\log_{10}$ reduction was observed with silver/copper. No significant reductions of *S.* Typhimurium were found in any of the samples treated with copper (400 μg/L).

Significant reductions of 2.44-$\log_{10}$ and 3.12-$\log_{10}$ of *E. coli* were achieved after one hour of exposure at 24°C to silver (100 μg/L) and silver/copper (100/400 μg/L), respectively (Figure 1B); after ≥3 hours of exposure, a >5.62-$\log_{10}$ reduction was observed with both the silver and the silver/copper treatments. As with the *S.* Typhimurium experiments, no significant reductions in *E. coli* were observed in any of the samples with copper (400 μg/L).

L. monocytogenes was used as a model for the inactivation of Gram-positive microorganisms by silver and copper. Similar reductions for *L. monocytogenes* (Figure 1D) were achieved with both the silver and the silver/copper treatments at 24°C to those found for *E. coli*. Within three hours of exposure, reductions greater than 5.03-$\log_{10}$ were observed for both the silver and the silver/copper treatments; within five hours, no bacteria were recovered (>6.52-$\log_{10}$ reduction). Unlike the Gram-negative *E. coli* and *S.* Typhimurium, however, *L. monocytogenes* was also found to be sensitive to the 400 μg/L copper treatment, with a 2.43-$\log_{10}$ reduction after five hours. Gram-positive microorganisms are able to bind up to 30 times more Cu^{+2} than *E. coli*. [59] It has also been reported that the Gram-negative species *Pseudomonas* and *Ferrobacillus* can tolerate up to 300 mg/L copper before an inhibitory effect is observed. [60] This is 750 times the concentration used in this study.

M. fortuitum was the most resistant bacterial species to silver (Figure 1C) with only a 1.11-$\log_{10}$ reduction observed after seven hours of exposure at 24°C. In contrast, a 2.56-$\log_{10}$ reduction was

observed for the copper treatment after seven hours. *M. fortuitum* was therefore much more sensitive to copper than *S.* Typhimurium and *E. coli*. Mycobacteria, although they are Gram-positive, have an unusual cell wall that contains high levels of lipids, making these microorganisms intrinsically resistant to many disinfectants by reducing their uptake. [57,61] Furthermore, the ability of some strains of mycobacteria to clump together confers extra protection against chemical agents. Mycobacteria have been found to tolerate chlorine levels typically used in water supplies and swimming pools. Thus, the residual chlorine in distribution systems does not eradicate this pathogen. [62] *M. fortuitum* was included in this study as a model for *Mycobacterium avium* Complex (MAC) inactivation. *M. avium* is currently on the EPA's Contaminant Candidate List (CCL) for possible future regulation in drinking water [https://www.epa.gov/ccl/microbial-contaminants-ccl-4; accessed May 2018]. The results of the present study show a significant reduction of *M. fortuitum* after treatment with Ag/Cu (100/400 µg/L) and Cu (400 µg/L). It is interesting to note that the results for copper were quite similar for *M. fortuitum* and the other Gram-positive bacterium, *L. monocytogenes*, whereas *M. fortuitum* was much more resistant to silver. Although the overall reductions observed for *M. fortuitum* were lower than those observed for the other bacterial species in this study, they were still statistically significant in comparison to the control solutions.

Effect of Temperature on Antibacterial Efficacy

A lower temperature adversely affected the inactivation of all the bacterial species tested (Figure 2). Longer exposure times were needed at 4°C in order to achieve significant reductions and these reductions were generally lower than those observed during the tests conducted at 24°C. *S.*

Typhimurium was reduced by only 0.26-$\log_{10}$ after seven hours of exposure to silver at 4°C (Figure 2A) in comparison to the 5.42-$\log_{10}$ reduction achieved at 24°C with this treatment. As with the experiments conducted at room temperature, the copper treatment did not yield any significant reductions within seven hours. Interestingly though, a 2.39-$\log_{10}$ reduction in *S.* Typhimurium was observed after seven hours of exposure to silver/copper (100/400 μg/L). This was only about 10-fold lower than the reduction of 3.51-$\log_{10}$ achieved at room temperature. Thus, the lower temperature did not seem to have as great an effect when the two metals were used in combination. The reductions in *E. coli* observed in the tests conducted at 4°C did not appear to be as affected by the lower temperature as the reductions in the other bacterial species studied (Figure 2B), though copper was still ineffective against *E. coli* at this temperature.

Reductions of 0.30-$\log_{10}$ and 2.25-$\log_{10}$ were achieved for *M. fortuitum* after 24 hours of exposure to the copper and the silver/copper treatments, respectively at 4°C (Figure 2C). Likewise, only a 2.11-$\log_{10}$, a 0.15-$\log_{10}$, and a 1.37-$\log_{10}$ reduction were achieved for *L. monocytogenes* after seven hours of exposure to the silver, the copper, and the silver/copper treatments, respectively at 4°C (Figure 2D). These were significantly lower than the reductions achieved for these organisms with these treatments at room temperature (Figure 1C and D), particularly for the copper treatment.

Synergy between Silver and Copper

A synergistic effect with both metals present was observed for all of the bacterial species after one hour of exposure at 24°C (Figure 1). Nevertheless, at later exposure times, there at times

appeared to be a small antagonistic effect between the two metal ions. A similar early synergy (within one to two hours of exposure) was found for *S.* Typhimurium and *L. monocytogenes*, but not for the other bacterial species tested at 4°C. In addition, although synergy was not observed between the metals in the latter sampling times with the *S.* Typhimurium experiment conducted at room temperature, a significant synergistic effect was found throughout the course of the experiment at all exposure times at 4°C with *S.* Typhimurium (Figure 2A). The reasons for this enhanced effect are not known, but it could be due to different target sites for the two metals. The ability of the cell to recover is thus overwhelmed by the combined damage. Silver may also inactivate enzymes involved in the regulation of copper homeostasis, thus rendering the organisms more sensitive to copper.

Effect of Organics on Antibacterial Efficacy

The residual disinfectant (usually chlorine) in distribution systems can be severely affected by intrusions events (e.g., organic matter, microbial contamination) and by the presence of biofilms that might react with the chemical. [2] The effect of organic matter on the antimicrobial activities of both silver and chlorine are presented in Table 2. *E. coli* was chosen as a representative bacterial species because it is known to be sensitive to the effects of both disinfectants. A chlorine (Cl) concentration of 0.2 mg/L (the concentration generally used for chlorine residual in distribution systems) was almost completely neutralized by the addition of 3 mg/L and 10 mg/L TOC (from humic acids). Conversely, the inactivation of *E. coli* by 100 µg/L of silver (Ag) was not affected by the presence of either 3 mg/L or 10 mg/L of total organic carbon (TOC). This is despite humic acids being known to be strong chelators of metals. [63-64] Similarly, in an earlier

study, Butkus et al. [65] found silver to be unaffected by the presence of organic matter.

Nevertheless, the antimicrobial activity of metal ions such as silver can be hampered by ligands such as sulfide, chloride, phosphate, or organic acids whose presence reduce their bioavailability and thus their antimicrobial efficacy. [66] Other factors such as visible light can also promote the oxidation and subsequent degradation of silver which can limit its long-term effectiveness. [67] However, in the distribution system, such exposure to light would be minimal.

CONCLUSION

At moderate levels, copper is considered safe to humans, as demonstrated by the widespread and prolonged use of copper intrauterine devices [17] and copper pipes/plumbing fixtures which leach copper ions into potable water. [68-69] However, copper toxicity has been documented in specific populations with altered copper metabolism, such as individuals with Wilson's disease. [49,70] Because of this risk, the EPA has set a secondary non-enforceable standard of 1,000 µg/L for copper [https://www.epa.gov/dwstandardsregulations/secondary-drinking-water-standards-guidance-nuisance-chemicals; accessed April 2018]. In addition, the EPA's Lead and Copper Rule requires drinking water utilities to monitor water at customer taps. If greater than 10% of the taps sampled exceed 1,300 µg/L Cu, the system must take actions to control corrosion [https://www.epa.gov/dwreginfo/lead-and-copper-rule#rule-summary; accessed April 2018].

Both the EPA and the World Health Organization (WHO) regard silver as safe for human consumption. Only argyria, an irreversible but not dangerous skin discoloration, occurs with the

ingestion of large quantities of silver (e.g., grams) over several years or by the administration of higher concentrations to ill individuals. Based on epidemiological and pharmacokinetic data, the WHO has determined a No Observable Adverse Effect Level (NOAEL) for humans of a maxium of 10 grams of silver over the course of a lifetime. [71] In the United States, no primary drinking water standard exists for silver; however, the EPA recommends a secondary non-enforceable standard of 100 μg/L [https://www.epa.gov/dwstandardsregulations/secondary-drinking-water-standards-guidance-nuisance-chemicals; accessed April 2018]. The WHO has stated that this amount of silver for the treatment of water could easily be tolerated since the total absorbed dose would only be half of the NOAEL after 70 years. [71]

An early synergistic effect was observed within the first few hours of exposure when silver and copper were used in combination against all of the species tested. Although no such synergy was observed in the latter sampling times, a combination of silver and copper could still be preferable to the use of either metal individually to provide a disinfectant residual in water distribution systems that have diverse microbial populations that include resistant organisms such as the mycobacteria. For instance, silver and copper have been shown to be highly effective against the resistant organism *Legionella pneumophila* in tap water in numerous studies over long periods of time. [72-74]

There have also been a number of studies that suggest that silver and copper can be utilized along with low concentrations of chlorine to effectively treat water. In one study, the inactivation of *Legionella pneumophila* by a combination of copper and silver was shown to be relatively slow when compared to that of free chlorine; nevertheless, when silver and copper were

combined with low levels of free chlorine, the inactivation rates of bacterial indicator organisms were greater than those for free chlorine alone. [25,27] Beer et al. [75] found that electrolytically generated copper and silver ions used in swimming pool water along with lower levels of chlorine provided control of total coliforms and heterotrophic bacteria equivalent to the control provided by high levels of chlorine. Yahya et al. [27] demonstrated that adding 400 μg/L copper and 40 μg/L silver to water systems containing contaminants similar to those in swimming pools allowed the concentration of free chlorine to be reduced at least three-fold (from 0.3 to 0.1 mg/L). Enhanced inactivation rates for *E. coli, L. pneumophila*, *Staphylococcus aureus, Streptococcus faecalis* [25,27], and *Pseudomonas aeruginosa* [26] have also been obtained when water was treated with 400 μg/L copper, 40 μg/L silver, and 0.2 mg/L free chlorine.

As demonstrated in the current study, a combination of silver and copper should not be adversely affected by the presence of organic matter (at concentrations that would neutralize the chlorine residual levels usually found in drinking water) and is effective against diverse species of bacteria. Chlorine disinfection can also produce dangerous disinfection by-products (formed as the result of the reaction of the halogenated element with organic matter) such as trihalomethanes and haloacetic acids [76-78] and is known to be corrosive to most materials. [79-80] In contrast, silver and copper do not produce any harmful disinfectant by-products [28] or cause corrosion of pipes. [81] Silver and copper therefore show promise as secondary residual disinfectants for drinking water distribution systems, particularly in warmer regions or during warm seasons in colder regions. They may be used together or also in conjunction with a low level of chlorine or another disinfectant to provide an additional, long-lasting residual disinfectant in water distribution systems.

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List of Figure Captions

Figure 1. Reduction of (A) *Salmonella enterica* serovar Typhimurium, (B) *Escherichia coli*, (C) *Mycobacterium fortuitum*, and (D) *Listeria monocytogenes* after exposure to silver (100 μg/L), copper (400 μg/L), and silver/copper (100/400 μg/L) at 24°C.

Figure 2. Reduction of (A) *Salmonella enterica* serovar Typhimurium, (B) *Escherichia coli*, (C) *Mycobacterium fortuitum*, and (D) *Listeria monocytogenes* after exposure to silver (100 μg/L), copper (400 μg/L), and silver/copper (100/400 μg/L) at 4°C.

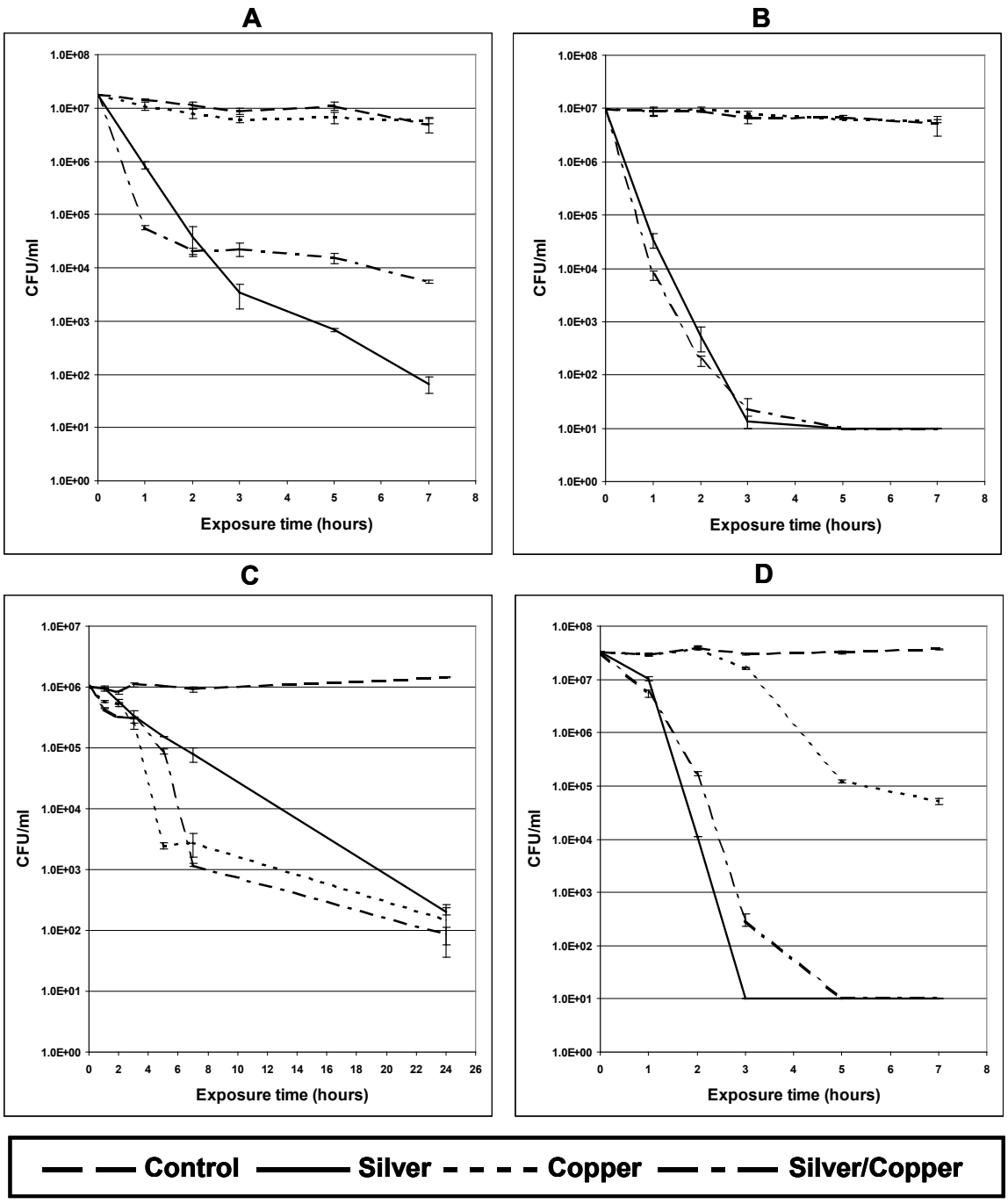
A
B
C
D
CFU/ml
Exposure time (hours)
Control
Silver
Copper
Silver/Copper

Figure 1

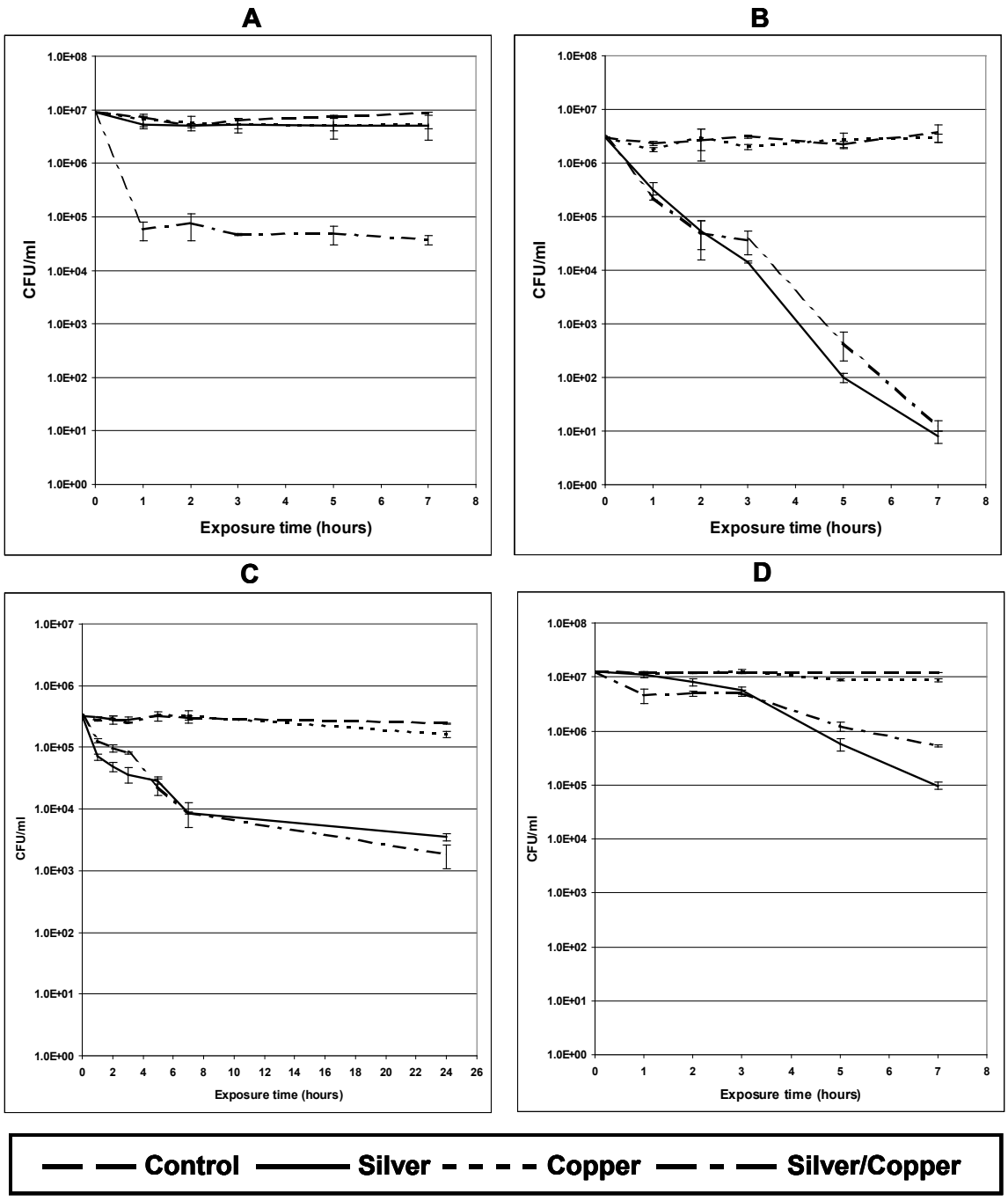
A
B
C
D
CFU/ml
Exposure time (hours)
Control
Silver
Copper
Silver/Copper

Figure 2

Table 1. Reduction in silver (Ag) and copper (Cu) concentrations after exposure to test solutions / containers with and without bacteria. Original amount of metal ions added to the solutions was 100 μg/L Ag and 400 μg/L Cu.

Experimental Temperature (°C)	**Bacterial Species Tested**	**Concentration in Supernatant (μg/L)**	
		Ag	**Cu**
4	NONE (Control)	100	400
	Escherichia coli	10	400
	Salmonella serovar Typhimurium	30	400
	Listeria monocytogenes	ND to 40	340
24	NONE (Control)	100	400
	Escherichia coli	ND	400
	Salmonella serovar Typhimurium	ND	400
	Listeria monocytogenes	10 to 20	290

ND = not detected

Table 2. Reduction[a] of *Escherichia coli* after exposure to chlorine (Cl) (0.2 mg/L) or silver (Ag) (100 µg/L) with or without organic carbon (from humic acids).

Time (h)	Control	Cl			Ag		
		Total organic carbon			Total organic carbon		
		0 mg/L	3 mg/L	10 mg/L	0 mg/L	3 mg/L	10 mg/L
1	0.00 ± 0.00	>5.70[b]	0.13 ± 0.09	0.14 ± 0.13	2.51 ± 0.21	2.51 ± 0.11	2.39 ± 0.22
2	0.02 ± 0.03	>5.70[b]	0.15 ± 0.15	0.16 ± 0.20	4.40 ± 0.11	4.40 ± 0.14	4.40 ± 0.19
3	0.04 ± 0.04	>5.70[b]	0.15 ± 0.12	0.17 ± 0.36	5.55 ± 0.38	5.55 ± 0.25	5.55 ± 0.17
5	0.06 ± 0.06	>5.70[b]	0.17 ± 0.31	0.17 ± 0.17	>5.70[b]	>5.70[b]	>5.70[b]
7	0.08 ± 0.07	>5.70[b]	0.19 ± 0.18	0.18 ± 0.25	>5.70[b]	>5.70[b]	>5.70[b]

[a] Average Log_{10} reduction of triplicate tests ± standard deviation. Original *E. coli* inoculum = $4.75x10^6$ CFU/ml.

[b] Detection limit of the assay