Inactivation of *E. coli* by copper and silver wire in the presence of synthetic sunlight for safe drinking water

Graham McLaughlin, Vikramjit Bajwa, Mukund Shukla, Kevin Hall and Praveen Saxena

ABSTRACT

Bacterially-contaminated water is a major health concern leading to infectious diseases in emerging nations. The use of copper (Cu) and silver (Ag) wire independently, and Cu wire in combination with synthetic sunlight (SySu), were investigated as a low-cost water treatment method. Water inoculated with 1,100 colony forming units/mL of *Escherichia coli* was treated with one of these four treatments: (1) 50 cm^2/L surface area (SA) pure Cu wire; (2) 37.8 W/m^2 SySu irradiation; (3) a 50 cm^2/L SA pure Cu wire combined with the 37.8 W/m^2 SySu irradiation; and (4) 5 cm^2/L pure Ag wire. An improved rate of bacterial inactivation was achieved using the combined Cu-SySu treatment method compared to either treatment applied independently. When given independently, the Ag wire treatment was more effective than the Cu wire treatment. Cu and Ag content in the water, tested at the end of the experiment, remained far below the safety limits suggested by the World Health Organization. The Cu-SySu combination method has a great potential to be used as a low-cost, re-useable, low-maintenance method of choice for purification of contaminated drinking water.

Key words | copper, drinking water, *E. coli*, silver, solar disinfection

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INTRODUCTION

Contaminated water is recognized as a direct and indirect source of several infectious diseases (Ashbolt 2004). Recurring infections such as diarrhoea, the second leading cause of death among children under five years of age in developing countries, can be prevented by improving water supply quantity and quality and promotion of community-wide sanitation systems (UNICEF/WHO 2015). However, the United Nations estimated that 663 million people were without access to an improved source of drinking water in 2014 despite a significant world-wide commitment of resources to achieve the Millennium Development Goals stated in 1990 (Hutton & Haller 2004; Way 2015).

The development of low-cost, maintainable and impactful solutions is imperative to assisting those who do not have the means of accessing their basic human rights of safe water and proper sanitation. Solar disinfection (SODIS) has been recognized as a viable low-cost solution for drinking water intervention in many regions with high levels of water contamination (UNICEF/WHO 2015). SODIS is a resilient point-of-use drinking water purification method because of its dependence on the sun as the source of disinfection. SODIS works under the principle of the UV-A and UV-B solar spectrums passing through transparent polyethylene terephthalate (PET) bottles (volumes typically between 1 and 2 L) and affecting an infectious agent's cellular repair capacity (Fisher *et al.* 2008). Several studies have shown that inactivation of *E. coli* by SODIS is caused by disrupting a sequence of normal cellular functions (Mcguigan *et al.* 1998; Berney *et al.* 2006). SODIS has been gaining momentum as a choice for household drinking water treatment since detailed field studies (Wegelin *et al.* 1994) and implementation projects began in the 1990s. Alternative

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methods for accelerating the water disinfection process are desirable because of the current limitations of SODIS.

In recent years, the use of copper (Cu) and silver (Ag) as a bactericide has re-emerged as a potential solution for inexpensive drinking water treatment. Cu coil disinfection is a very low-cost treatment method that has only recently been studied scientifically even though water storage in Cu pots has been practiced for centuries in India (Sudha *et al.* 2009). Fisher *et al.* (2008) has shown that the use of Cu wire in the presence of ascorbate (vitamin C powder) is an alternative means of disinfection (Fisher *et al.* 2008). The use of solid Cu metal as a disinfection method has also recently gained recognition as a potential alternative for inactivation of bacteria and viruses both for dry surface contact (Grass *et al.* 2011), as well as contact while submerged in water (Shrestha *et al.* 2009; Sudha *et al.* 2009, 2011, 2012). Research reports have shown a reduction in bacterial growth by storing contaminated drinking water in standard Cu pots and using Cu wire over a 16-hour period. Silver has also been used in combination with Cu for applications such as wastewater treatment and swimming pool disinfection (Landeen *et al.* 1989). Copper pots showed higher effectiveness as compared to Ag pots during the study of water storage containing *Salmonella paratyphi*, *Shigella* spp., and *E. coli* (Shrestha *et al.* 2009).

In this study, two independent drinking-water treatment techniques, Cu and synthetic sunlight (SySu) using sunlight-spectrum lamp, were tested to assess whether in combination the effectiveness of drinking water treatment was improved over the standard SODIS method.

MATERIALS AND METHODS

Experimental setup

All experiments were conducted using 330 mL Evian® PET water bottles containing deionized water. The deionized water was filtered through a *Milli-Q* Millipore ultraviolet disinfection and ultrafiltration system prior to adding bacterial culture. Before testing, each bottle was washed thoroughly with 95% alcohol for 2 minutes followed by 15% bleach solution for 10 minutes and later rinsed with deionized water three times for 4 minutes each. All bottles with lids were dried under a laminar flow bench for 3 hours.

Bacterial strain

Escherichia coli (*E. coli*) strain DH5α (subcloning efficiency DH5*α* Competent Cells, Invitrogen, Ontario, Canada) was used in all experiments. *E. coli* cultures were maintained on Luria-Bertani (LB) medium plates.

Inoculum preparation

E. coli were cultured by adding a single colony into a sterile 15 mL test tube containing liquid LB medium. After the colony was added, the test tube was placed in a shaking incubator at 37 °C for 16 hours. The resulting concentrated *E. coli* solution was diluted serially to 10^{-6} concentration in a 50 mL sterile test tube containing sterile deionized water. Each dilution was plated onto LB agar plates and incubated at 37 °C for 24 hours for growth and to estimate the concentration of the stock solution. A consistent volume was pipetted into each 330 mL bottle in an effort to achieve a target concentration of 1,100 colony forming unit/ml (CFU/mL).

Evaluation of bacterial growth

Three samples (0.2 mL) from each treated water bottle were plated on separate LB petri dishes at each time interval to evaluate the effect of treatments on bacterial growth. Visual plate counts for colonies were performed and recorded for all plated samples after 24 hours incubation time in a dark thermal chamber maintained at 37 °C.

Test material

Twenty-gauge Stinson® Cu wire (>99.9% purity) was used in all Cu treatment bottles. The Cu wire was cut into 64.8 cm strips, equating to 50 cm^2/L when submerged in the 330 mL water bottles. The strips were wrapped around a cylindrical object to create the coils seen in Figure 1. Sixteen-gauge Ag wire (>99.9% purity) was purchased from Working Ag (New Westminster, British Columbia, Canada). Silver was cut to a length of 3.84 cm, equating to 5 cm^2/L when submerged in the 330 mL water bottles.

Figure 1 | Copper (64.8 cm) and silver (3.84 cm) wires used for all treatments for water sanitation.

Initial trials with Cu and Ag wires showed that even surface area (SA)-volume ratio of 50 cm^2/L for Cu and 5 cm^2/L for Ag were effective in controlling bacterial growth (data not shown); therefore further study was carried out with these SA-volume ratios.

SySu lamp treatment

The Gravita Pro LEP 02 sunlight-spectrum lamp was used for the sunlight-portion of this experiment. The light intensity was measured at seven locations on the box-stand using an LI-COR LI-250A Light Meter directed at the light source. The resulting intensity was measured as 172.8 ± 1.3 μmol/m^2 s, equivalent to 37.8 W/m^2 in the sunlight spectrum. To confirm the proper functioning of the Gravita Pro LEP 02 sunlight-spectrum lamp, a spectral scan was also performed which shows the presence of UV A and UV B (Figure 2).

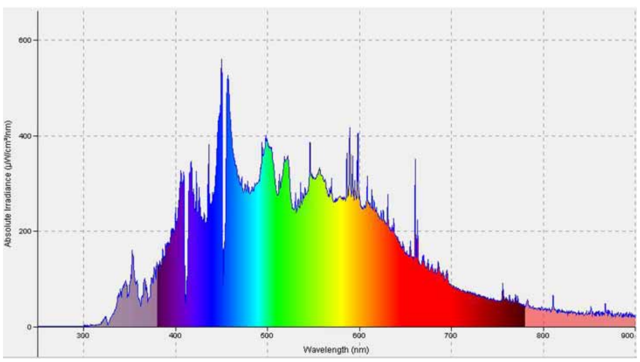

Figure 2 | Spectral scan results of the Gravita Pro sunlight-spectrum lamp used for the SySu treatment.

Three 0.2 mL samples were taken from each bottle at time intervals of 0, 60, 90, 120, 180, and 240 minutes for the bottles exposed to SySu. Bacterial enumeration was performed using the standard plate count method as described above in the inoculum preparation section. Turbidity was not induced in the bottles and remained below 0.5 Nephelometric Turbidity Units for all tested bottles.

SySu treatment chamber

The SySu chamber (Conviron, Winnipeg, Manitoba, Canada) used in this test had ambient temperature control that was set to 30 °C. Bottles were positioned on a modified cardboard box wrapped in aluminium foil. The modification was made to ensure that the irradiation was evenly distributed about the top surface of the box, while the aluminium foil's main purpose was to maintain a consistent backing surface for all bottles. Figure 3 shows a schematic of the box dimensions and distance from the light source.

Evaluation of effect of Cu coil, SySu and Ag on bacterial inactivation

All coils were autoclaved and placed into the treatment bottles immediately after the 0-min samples were taken. All bottles were placed in a thermal growth chamber set at 30 °C to mimic ambient air temperature and containing a sunlight-spectrum lamp. Bottles that were not subjected to the SySu treatment were wrapped in aluminium foil and placed underneath a modified cardboard box to ensure no light penetration (Figure 4(a) and 4(b)). A bottle containing a thermocouple was considered as negative control. An Omega HH374 (Omega®, Canada) thermometer was used with three thermocouples in order to monitor the ambient temperature as well as the water temperature. The ambient temperature of the chambers remained at 30 °C throughout the test, while the water temperatures rose from room temperature (23 °C) to the ambient air temperature before reaching steady state. Temperature was recorded throughout the experiment.

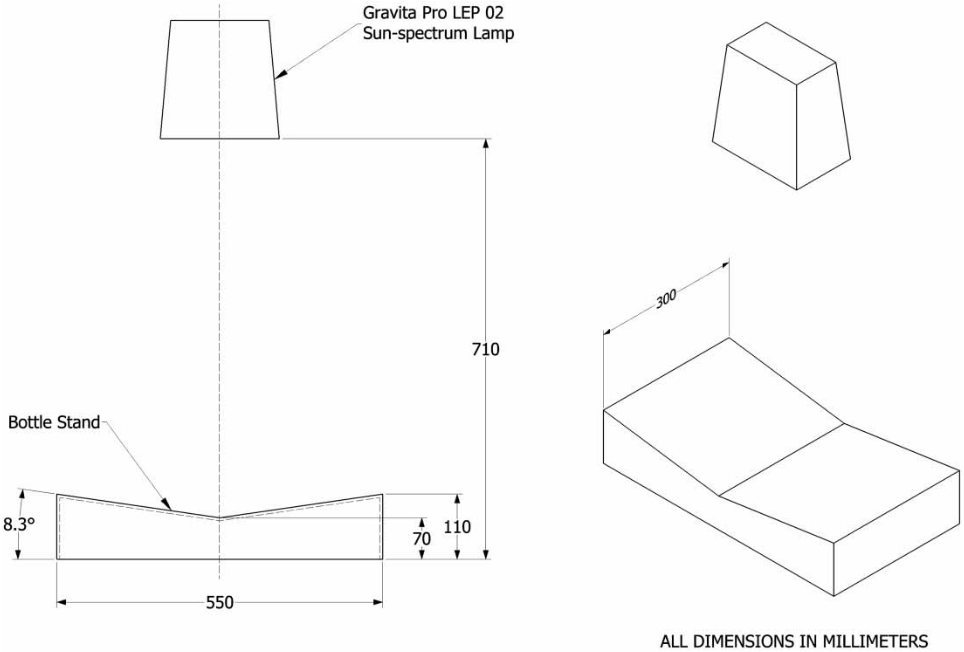

Figure 3 | Schematic outlines with dimensions of SySu stand used for the water bottle treatment.

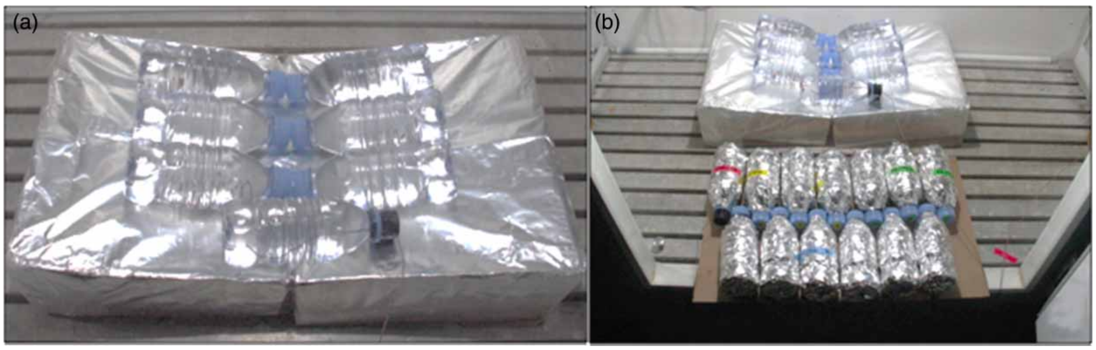

Figure 4 | SySu disinfection experiment arranged within the controlled chamber. The bottle in the front is the negative control containing a thermocouple (a). Bottles without SySu experiment in foreground were wrapped in tinfoil and placed beneath the bottle stand to ensure no light penetration (b).

Detection of Cu and Ag in test solution

After completion of the experiment (270 minutes), coils were removed from the bottles and water samples were collected to determine Cu level. After removing the coils using autoclaved tongs, each 330 mL bottle was sealed and sent to University of Guelph's Laboratory Services for analysis according to the Standard Methods for the Examination of Water and Wastewater (APHA 1999). Samples were prepared following procedure 3030: Preliminary Treatment of Samples followed by procedure 3120B: Metals by Plasma Emission Spectroscopy. Standard Methods 21st edn – Part 3000. Metals test setup is summarized in Supplementary Table S1 (available in the online version of this paper).

Statistical analysis

The experiment was conducted as a randomised complete block design with three replications. Data were analyzed using JMP 10.0.0 (SAS Institute, Cary, NC, USA), and ANOVA was conducted to determine significance of the model followed by means comparison using Tukey's test at

$\alpha = 0.05$ significance level. The CFU/mL values were compared for all treatments at each time and percentages for each time point was calculated based on the initial value for the same treatment at 0 h. Data were presented as the mean ± standard error of three biological replicates and each replicate analyzed in triplicate.

RESULTS

Effect of Cu, SySu and Cu-SySu on bacterial inactivation in drinking water

SySu treatment for 3 h or longer and the 50 cm^2/L SA Cu treatment alone for 90 minutes or longer was significantly effective in controlling the bacterial growth compared to the dark control treatment, with only 12 and 31% of the CFU/mL remaining (compared to 0 minute control for the same treatment) after 240 minutes of SySu and Cu treatment, respectively (Figure 5). However, when Cu treatment and SySu treatment were given in combination, their efficiency in inhibiting *E. coli* growth was increased significantly, with only 1% of the CFU/mL remaining (compared to the 0 minute control treatment) after 240 minutes of the combined treatment (Figure 5, Table S2). These results indicate that the combined treatment effect of Cu-SySu had greater efficiency in reducing bacterial number than either treatment method given independently.

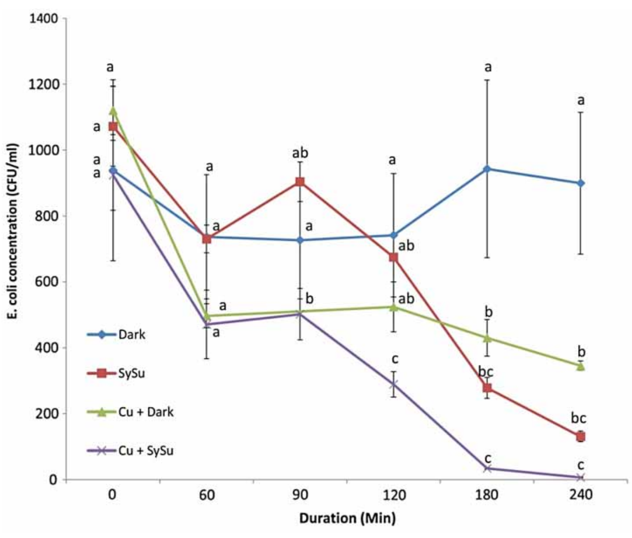

Figure 5 | Effect of Cu, SySu and Cu-SySu combination treatment given for five (0, 60, 90, 120, 180, 240 min) time intervals on reduction in CFU/mL observed after 24 h of plating. Data represent mean values ± SE of three replicates and for means in the same time interval, different letters indicate significant differences among treatments using Tukey's test at $P < 0.05$. Dark (positive control), SySu (Synthetic sunlight), Cu + Dark (copper and no light) and Cu + SySu (copper and SySu).

Effect of Ag and Ag-Cu combination on bacterial inactivation in drinking water

Two hours or longer treatment of contaminated water with 5 cm^2/L SA Ag wire was effective in controlling the bacterial growth, with only 20% of the CFU/mL remaining in 240 minutes treatment sample group compared to the 0 minute control (Figure 6, Table S3). The combination of Cu and Ag showed slightly higher efficiency in controlling growth compared to the Ag-alone treatment, with only 16% of the bacterial growth remaining after 240 minutes compared to the 0 minute control in the same group, however the combined effect of the Ag-Cu was not significantly better than the Ag-only treatment (Figure 6).

Residual amount of Cu and Ag in drinking water after the removal of the Cu and Ag coils

All pieces of metal wire were removed from the water bottles 270 minutes after the start of the test to measure the residual amount of Cu left in the water after treatment. Slightly higher

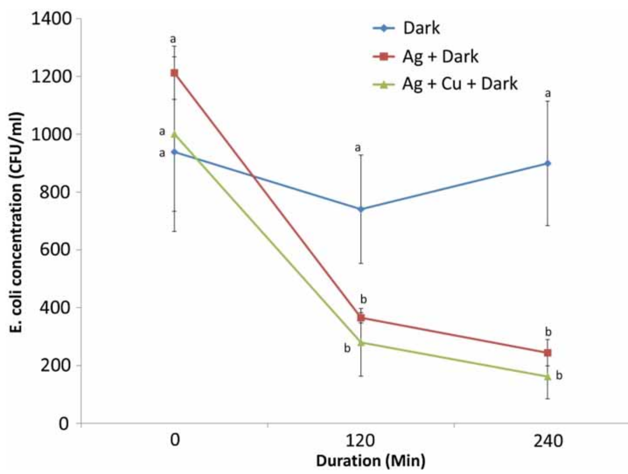

Figure 6 | Effect of Ag, and Ag-Cu combination treatment given for three (0, 120, 240 min) time intervals on reduction in CFU/mL observed after 24 h of plating. Data represent mean values ± SE of three replicates and for means in the same time interval, different letters indicate significant differences among treatments using Tukey's test at $P < 0.05$. Dark (positive control), Ag + Dark (Silver and no light) and Ag + Cu + Dark (silver, copper and no light).

Table 1 | Concentration of dissolved Cu and Ag in water after removal of the metal wires after 270 min. Data represent mean values ± SE of three replicates and for means in the same column, different letters indicate significant differences among treatments using Tukey's test at $P < 0.05$

Treatment	Cu concentration (mg/L)	Ag concentration (mg/L)
Dark	0.004 ± 0.002^{d}	0.001 ± 0.000^{a}
SySu	0.003 ± 0.0003^{d}	0.001 ± 0.000^{a}
Cu	0.075 ± 0.0003^{b}	0.001 ± 0.000^{a}
Cu + SySu	0.095 ± 0.0044^{a}	0.001 ± 0.000^{a}
Ag	0.003 ± 0.0007^{d}	0.001 ± 0.000^{a}
Cu + Ag	0.054 ± 0.0167^{c}	0.001 ± 0.000^{a}

amounts of Cu (0.075 ± 0.0003 mg/L) were detected in the water bottles treated with Cu compared to the bottles not treated with Cu (Table 1). Combined treatment of the Cu and SySu gave the highest level of Cu measured (0.095 ± 0.0044 mg/L) among all treatments (Table 1). The amount of Ag detected in all the treatment fluctuated around the detection limit of 0.001 mg/L (Table 1).

DISCUSSION

The results of our study demonstrate that a combined method incorporating both the solar and Cu coil disinfection provides improved bacterial inactivation compared to either method applied independently. Also, the combined effect SySu and Cu appears to exceed their additive effect (Figure 5). However, combining Cu (50 cm^2/L) with Ag (5 cm^2/L) did not improve the efficiency of bacterial inactivation compared to the application of these treatments individually. Very limited work has been carried out on either the effect of metals or the synergistic effects of solar irradiation with metals for safe drinking water. The effectiveness of the Cu pot and Cu device for killing enteric bacteria in drinking water has been shown earlier (Sudha *et al.* 2009). This Cu pot and Cu device study was limited to Cu treatment only and with a treatment time of 16 h but without accounting for the turbidity of treated water. Also, Sudha *et al.* (2009) used lower Cu SA (15.2 cm^2/L) as well as a lower ambient temperature (room), which is below the optimum area of 50 cm^2/L and a temperature of 40 °C as observed in the preliminary tests in our study (Sudha *et al.* 2009).

The SODIS in our experiments employed a synthetic setup to ensure that the time for inactivation through SODIS is similar to the suggested time of 6 hours in the field (Luzi *et al.* 2016) and also to give Cu wire a reasonable time to complete the disinfection process. The SySu intensity is much lower than in the field. Reduced bottle diameter and low turbidity were employed to counter-balance the low irradiation intensity in order to achieve a disinfection rate similar to that suggested for SODIS in the field.

The information on supplementation of SODIS with metals is rather limited. Fisher (2004) used four 2.5-cm lengths of 18-gauge wire in the presence of 200 μM ascorbate (vitamin C) in a 100 mL sample of river water, which equates to 32.17 cm^2/L SA to volume ratio. Inactivation rate in their study was 3 log CFU/hour in 20 minutes of treatment following a 15-minute shoulder period (Fisher 2004). Interestingly, Cu accumulation in the water was not observed in the dark control bottle containing the same amount of Cu. This was not replicated in the present study, as the Cu wire treatment in the darkness reached an average of 0.075 mg/L Cu after the 270 min test period (Table 1). Fisher (2004) induced a Fenton-type reaction by using ascorbate as an agent for induction. Ascorbate has not been used in this study to induce Fenton-type reaction, since ascorbate powder is not easily accessible to low-income people in developing countries. The other differences between this study and Fisher's experiments is the use of lemon juice as a natural supplement to ascorbate powder by Fisher (2004), however it was determined that the natural ligand (lemon) did not have the same effect on *E. coli* as the ascorbate powder. Fisher (2004) also did not perform any experiments with Cu wire independent of ascorbate.

Copper content was measured at the end of the trial in order to ensure that the concentration of dissolved Cu in the water remained well below the safe limit for drinking water of 1.3 mg/L (WHO 2004). As seen in Table 1, Cu content after 270 min was approximately 0.095 mg/L in the bottles containing 50 cm^2/L ratios of Cu wire to water volume, which is far below the safe limit of Cu in drinking water. Similarly, the residual amount of Ag was very low as it was not detected in the sample water. Total Ag must be maintained below 0.1 mg/L to ensure no adverse health effects from consumption (WHO 2003). All experiments in this series measured Ag far below the maximum limit, fluctuating around the detection limit of 0.001 mg/L (Table 1).

The use of Cu to enhance the practice of SODIS could provide several advantages that would make SODIS safer and more practical for households seeking a low-cost alternative to improved drinking water. As demonstrated in this report, the rate of bacterial inactivation can be increased through the addition of a small, reusable piece of Cu wire without addition of any chemical additives. The presence of Cu ions in water at a safe level may provide additional benefits beyond the inactivation of bacteria. One of the main disadvantages of the current SODIS method occurs on days with little to no sun due to cloud cover. The rate of inactivation achieved by using Cu wire independently is enough to improve lightly contaminated source water; therefore combining SODIS with Cu may be useful on cloudy days.

Another advantage to the proposed improved method is the residual effect of Cu ions on bacteria in water. Even after the piece of Cu is removed, Cu ions remaining in water will create a relatively long lasting residual effect (White *et al.* 1972). The ability for water to become re-contaminated after being removed from standard SODIS practices creates a hazard that is difficult for the user to identify. The use of Cu wire could provide a residual effect that would improve safety by preventing such re-contamination.

Field-testing of Cu-SODIS method is recommended, especially in drinking water contamination-prone areas to show the potential benefit of this method in preventing the water-related diseases. Based on the observation of this study the Cu-SySu combination method has a great potential to be used as a low-cost, re-useable, low-maintenance method of choice for purification of contaminated drinking water. Silver wire was also very effective in controlling bacterial growth in water. The rate of inactivation by Ag was more than 1.5 times as effective with a tenth of the SA of Cu. However, further investigation for the use of Ag wire combined with SODIS should be carried out. Also, the mechanisms of action for the antimicrobial effects of Cu or Ag are not understood. Mechanisms that have been proposed include the generation of hydroxyl radicals in a Fenton-type reaction that cause irreparable cellular damage such as oxidation of proteins, cleavage of DNA and RNA molecules, and possible membrane damage due to lipid peroxidation (Hambidge 2001; Grass *et al.* 2011; Longano *et al.* 2012) Reproducibility, ease of function and efficiency to determine contamination levels in the set up described in this study may be useful in investigating the synergy and mechanisms involved in the combined efficiency of metal and SODIS in the water purification process. Regardless of the mechanism of disinfection, the application of Cu-SODIS and Ag as shown in our results is recommended for improved SODIS in order to develop a safe, trusted, consistent, and maintainable method to improve water hygiene.

AUTHORS' CONTRIBUTIONS

G.M., V.B., M.S., K.H. and P.S. were involved in the development of the hypotheses and experimental design; G.M., V.S.B. and M.R.S. conducted the majority of the experiment; G.M., V.B. and M.S. participated in drafting the manuscript; and all authors were involved in subsequent revisions.

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