Effectiveness of silver and copper infused ceramic drinking water filters in reducing microbiological contaminants

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ABSTRACT

A series of bench-scale experiments were conducted on ceramic filters with various amounts of silver and/or copper nanoparticles fired-in during the manufacturing process. The experiments were designed to determine the efficacy of the filters on the removal of pathogens from drinking water. *Escherichia coli* and MS2 were employed as non-pathogenic surrogates for pathogenic organisms. Experiments were run on 23 ceramic filters – 10 replicates, one single filter, and two blanks – for approximately 10 days. Influent and effluent turbidity, *E. coli,* and MS2 concentrations were monitored regularly. Results showed that all ceramic filter configurations exceeded WHO standards for removal of bacteria under highly protective conditions, but few met these criteria for bacteriophage. The filters containing various concentrations of silver nanoparticles (25–100%) achieved an additional 2 log removal over the blank, copper, and mixed copper/silver filters for bacteria; however, it was difficult to determine the effect of nanoparticles on the removal of bacteriophage. A significant variation in effluent quality between filters of the same composition was observed, indicating the importance of variations in the manufacturing process on effluent water quality. Given the potential health risks of inadequate filtration, it is recommended that the quality control process be examined and upgraded.

Key words | ceramic filter, copper nanoparticles, household drinking water treatment, point-of-use filtration, silver nanoparticles

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INTRODUCTION

The United Nations Millennium Development Goals (MDGs) established water access targets to reduce global disease burden and support poverty eradication. Even though the global MDG target for water was achieved in 2010 using the surrogate measure of improved (i.e. protected, but not necessarily safe) water supplies, 663 million people still did not have access to improved water supplies in 2015 and there are serious inequities between those who have and those who do not (WHO & UNICEF 2015). During the tenure of the MDGs, the United Nations General Assembly passed a resolution declaring access to safe water and sanitation as a basic human right (Resolutions 64/292; 60/169). If water quality is taken into consideration, an estimated 1.8 billion people do not have access to safe drinking water (Onda *et al.* 2012). This means that over 1 billion of the estimated 6.2 billion people using improved water sources continue to consume unsafe water (Onda *et al.* 2012; Bartram *et al.* 2014) against the backdrop of a global agenda to ensure universal drinking water access by 2030 that is safe and affordable (SDG Goal 6) (UN 2015).

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Globally, insufficient drinking water, sanitation, and hygiene lead to 842,000 deaths per year, 48% of which occur in children under the age of five (Prüss-Ustün *et al.* 2014). Diseases caused by micro-organisms (parasites, bacteria, protozoa) and viruses could be prevented or significantly reduced if household-level point-of-use (POU) water treatment practices were properly implemented and used on a consistent basis (WHO/UNICEF JMP 2008). While numerous POU water treatment methods and processes are currently available, for example biosand filters, bucket filters, ceramic candle filters, ceramic (clay pot) filters, boiling, and chemical addition, ceramic filters are relatively low cost, and are easy to operate and maintain (Mwabi *et al.* 2012). They have also been shown to effectively remove micro-organisms and viruses from source water of varying quality (Clasen & Boisson 2006; Mwabi *et al.* 2012).

Ceramic water filters have been used in at least 17 countries to improve the quality of drinking water in an attempt to reduce the number of global deaths from waterborne disease (Clasen & Boisson 2006). Ceramic filters are manufactured by mixing clay and water with a combustible organic material such as sawdust or rice husks, molding it in a filter press, and then firing it in a kiln to approximately 870°C. The organic material burns off during firing, creating pores through which water can flow (Oyanedel-Craver & Smith 2008; Brown & Sobsey 2010). It has been proven that the efficacy of these filters is improved by the incorporation of silver into the filter's design (Oyanedel-Craver & Smith 2008; Brown & Sobsey 2010; Yakub *et al.* 2013). Several theories exist explaining how silver acts as a bactericide, including: silver promotes bacterial and viral oxidation, it affects bacterial cell viability, it inactivates DNA replication, or it inhibits enzyme function (Oyanedel-Craver & Smith 2008; Ravishankar Rai & Jamuna Bai 2011). Typically, a colloidal-silver solution is added to the filters after the firing process by painting it on or submerging the filters in an 800-mg/L colloidal-silver solution (Oyanedel-Craver & Smith 2008; Brown & Sobsey 2010; Yakub *et al.* 2013). Experiments conducted on filters with silver applied in these ways resulted in an average of 5.7–6.4 log reductions for *Escherichia coli* but only 1 log reduction for viruses (Brown & Sobsey 2010; Yakub *et al.* 2013). This may be due to the fact that pot filtration relies more on size exclusion and less on the bactericidal properties of silver to remove bacteria and viruses from water; if the pores are not small enough, viruses ranging from 10 to 100 nm in size may be able to pass through (Brown & Sobsey 2010; Yakub *et al.* 2013). However, differences have been noted in the number of log reductions achieved between filters manufactured in the same factory, and even the same batch, demonstrating a need for stricter production standards (Salsali *et al.* 2011).

Another, less common, method of incorporating silver and/or copper nanoparticles into ceramic filters is to mix the nanoparticles with the raw materials before the firing process; this technique is known as 'firing-in' (Ballantine & Hawkins 2009) and is the method utilized by Agua Pur in the Dominican Republic, which manufactured the filters in this study. To our knowledge, the effectiveness of firing-in has not been investigated in the literature to date. It is postulated that adding nanoparticles in this manner results in more effective removal of microorganisms than either the painting or submersion techniques because the silver will not wash off, oxidize, react with chlorine in chemically treated water, or be removed through regular filter cleaning once it becomes bound to the clay during firing (Ballantine & Hawkins 2009). Moreover, ion release is considerably lower than when silver is applied to the clay surface (Ren & Smith 2013).

Although silver nanoparticles improve filter performance, they are expensive. This research was designed to determine the potential for a modified ceramic filter manufacturing technique to improve the efficacy and affordability of these filters for the removal of bacteria and viruses from water by either reducing the amount of silver and/or replacing silver with less expensive copper in the manufacturing process. The ceramic filters employed in this work were manufactured especially for this study using the fired-in process; i.e. set quantities of clay, water, sawdust (which determines the pore size), and silver and/or copper are mechanically mixed, formed into individual filters using a pneumatic press, air dried, and fired in a wood-fueled kiln. The reference filter (100% silver) represents the filter that is currently being manufactured and distributed in the Dominican Republic; all nanoparticle contents reported herein are relative to the reference filter. Note that the specific amounts and sizes of sawdust and silver are proprietary. While the antimicrobial properties of silver and copper are well documented, the efficacy of ceramic filters modified with copper nanoparticles has not been proven in the literature to the same extent as silver. An additional objective of this work is to determine whether any of the copper and/or silver formulations meet the World Health Organization (WHO) General Testing Protocol for ceramic filters (WHO 2014a).

MATERIALS AND METHODS

Laboratory setup

Twenty-three ceramic pot filters, consisting of 11 replicates (of which one pair were blanks, i.e. no silver or copper) and one single filter (its pair broke in transit), were infused with varying representative concentrations of silver and/or copper nanoparticles and manufactured using the fired-in technique (Table 1). Each filter had a volume of approximately 6.5 L, and was set in a 20 L plastic bucket fitted with a spout to drain effluent water into a collection pail (Figure 1). Spouts were visually inspected for leaks to ensure the validity of flow rate measurements. Large, rectangular holes were cut into the side of each bucket to enable insertion of a sterile dish under the pot for sampling purposes.

Charging and sampling followed a 10-day cycle over the course of two weeks (WHO 2014a). Filters were charged with clean reverse osmosis (RO) water on the first and last day to assess changes in flow rate, and with challenge water on days 1–5 and 8–11 to assess microbiological reduction. Microbiological plating was done on days 8, 9, 10, and 11, and plates were counted on the following morning. The filters were not challenged on days 6 or 7, as it was the weekend and resources were not available.

To ensure that the *E. coli* RS2GFP was not affected by the addition of MS2 bacteriophage to contaminated water, challenge water was collected both before and after the phage were added. These samples were then plated at 0, −1, −2, and −3 dilutions following conventional protocol (see Microbiological methods section) and counted the following morning. The two original sample vials were then left at room temperature for approximately 24 hours before being re-plated at the same dilutions, and counted the following morning. Counts from both days, with and without phage, were compared to assess bacterial death potentially caused by MS2. It was found that there was no significant difference between *E. coli* RS2GFP mixed with MS2 and *E. coli* by itself, verifying that the filters were the sole factor in *E. coli* reduction.

Table 1 | Filter composition, mean effluent flow, and mean effluent turbidity

Filter composition[a]	Mean flow (L/h)	Standard deviation of mean flow (*n*)	Mean effluent turbidity (FTU)	Standard deviation of mean turbidity (*n*)
Blank	0.858	0.177 (8)	14.0	9.4 (8)
Blank	0.819	0.127 (8)	12.9	10.1 (8)
25% Ag	0.982	0.140 (8)	18.3	13.8 (7)
25% Ag	1.046	0.078 (8)	27.6	15.1 (7)
30% Ag	1.534	0.251 (9)	7.6	1.3 (5)
30% Ag	1.572	0.078 (9)	7.0	1.2 (5)
40% Ag	1.048	0.141 (9)	12.2	8.6 (5)
40% Ag	0.828	0.145 (9)	8.8	8.0 (5)
50% Ag	1.138	0.118 (9)	1.0	0.0 (5)
50% Ag	1.524	0.054 (9)	10.2	2.0 (5)
75% Ag	0.492	0.094 (9)	3.4	3.1 (5)
75% Ag	0.444	0.081 (9)	6.2	5.4 (5)
100% Ag	1.203	0.073 (8)	18.9	13.5 (8)
100% Ag	1.055	0.089 (8)	10.1	7.4 (8)
100% Cu	1.823	0.124 (8)	18.9	14.3 (7)
100% Cu	1.588	0.221 (8)	21.3	14.9 (8)
300% Cu	1.580	0.086 (8)	24.0	14.9 (7)
300% Cu	1.513	0.076 (8)	21.9	13.8 (7)
100% Cu + 25% Ag	1.039	0.146 (9)	3.0	0.7 (5)
100% Cu + 37.5% Ag	1.131	0.119 (8)	17.0	13.5 (7)
100% Cu + 37.5% Ag	1.412	0.127 (8)	25.9	13.3 (7)
100% Cu + 50% Ag	1.089	0.109 (9)	11.2	8.2 (5)
100% Cu + 50% Ag	0.828	0.036 (9)	3.4	2.6 (5)

[a] Percentages are referenced against the nanosilver amounts used in current production (i.e. 100%). Copper percentages are referenced against the equivalent weight for the same antimicrobial properties of silver.

Challenge water characteristics and flow measurements

Synthetic challenge water was prepared as per the WHO (2014a) protocol. First, RO water was added to a large, clean bin, and an air stone was inserted for mixing purposes; humic acid and $NaHCO_3$ were added to the

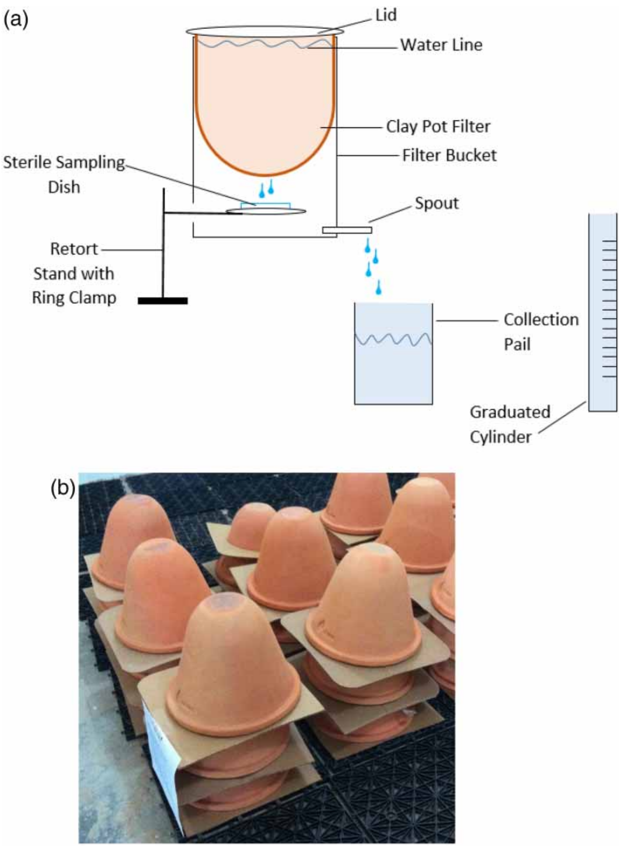

Figure 1 | (a) Schematic illustration of filter and effluent sample collection setup; (b) image of actual filters in factory (courtesy of Schuster-Wallace).

water to achieve the required total organic carbon (TOC) (15 ± 5 mg/L) and alkalinity (100 ± 20 mg/L) respectively; synthetic sea salts were then gradually added until the total dissolved solids fell within the desired range (1,500 ± 150 mg/L); NaOH was added drop-wise to achieve the required pH (9.0 ± 0.2); the turbidity was measured to ensure that it fell within the required range (40 ± 10 Nepholometric Turbidity Units); and, finally, the required number of non-pathogenic *E. coli* RS2GFP ($\geq 10^5$/100 mL) and MS-2 bacteriophage ($\geq 10^8$/L) were added. A sample of the challenge water was then analysed for chemical oxygen demand, which was converted to TOC to ensure that it remained within the desired range.

Filters were challenged once daily for 10 days with 6 L of synthetic challenge water. The flow rate was measured by collecting the effluent water from the spigot of the filter bucket in a collection pail for 1 hour, after discarding the first two to three drops from the spigot that would have been left there from the previous day. This was repeated for each filter until all were charged with 6 L of challenge water. After 1 hour, a graduated cylinder was used to measure the volume of water that had collected in the pail; these data were used to calculate the flow rate. It should be noted that this flow rate represents the fastest rate (i.e. when the driving force is greatest).

Escherichia coli RS2GFP and MS2 bacteriophage characteristics

E. coli RS2GFP was supplied by Dr Larry Halverson from the Department of Agriculture and Biosystems Engineering at Iowa State University via the laboratory of Dr Monica Emelko in the Department of Civil Engineering at the University of Waterloo. *E. coli* RS2GFP is resistant to the antibiotics kanamycin and rifampicin, minimizing contamination in the laboratory. *E. coli* was suspended in sterile PBS and added to the challenge water prior to charging the filters. The MS2 bacteriophage was supplied by the American Type Culture Collection (ATCC 13706) and purchased through Cedarlane Labs, as was the host *E. coli*.

Turbidity and microbiological methods

Once daily flow rate measurements were complete, water samples were collected for turbidity and microbiological analyses from the subsequent effluent flow. Approximately 35 mL of effluent was collected in a sterile plate, which was placed directly under the filter using a ring clamp. Serial dilutions (1:10 and 1:100) were prepared from a 10 mL sample using sterile Hach tubes containing 9 or 9.9 mL of PBS and plated for *E. coli* RS2GFP and MS2. *E. coli* RS2GF was plated and enumerated at 10^0, 10^{-1}, and 10^{-2} dilutions using a standard plating method. MS2 was plated at varying dilutions between 10^{-4} and 10^{-18} as our knowledge about the stock MS2 concentration developed. Enumeration of MS2 bacteriophage was accomplished through a standard double agar overlay method as described by Adams (1959) and optimized by Mesquita (2011). The remaining sample was used to measure turbidity.

RESULTS AND DISCUSSION

The highest median log reductions of *E. coli*, without significant deviation, were achieved by the 25, 30, 40, 50, and 75% Ag filters and ranged from 6.25 to 6.8 log reductions (Figure 2). The median log removal for all filters met the WHO highly protective 4 log removal criteria (WHO 2014a). The minimum median log reduction was 4.4 (blank) and the maximum median log reduction was 6.8 (25 and 50% Ag). Even the minimum log reductions for the 25, 30, 40, 50, and 75% Ag filters exceeded the WHO highly protective removal criteria with the exception of one 30% Ag filter. The term 'highly protective' means that the filters, if used correctly and consistently over a full year, will limit the burden of disease from drinking-water to 10^{-6} disability-adjusted life year (DALY) per person. A DALY represents the years of life lost due to mortality coupled with the years of life lived with a disability due to illness, in this case a water-borne illness (WHO 2011a). All except one minimum log removal (100% Cu at 1.3 log removal) also met the WHO protective or limited protection requirement of 2 log removal (WHO 2014a). 'Limited protective' implies that the filters, if used correctly and consistently over one full year, will limit the burden of disease due to drinking water to 10^{-4} DALYs per person. None of the copper filters performed as well as the silver ones, even those that had silver mixed with copper. The two blank filters, with median log reductions of 4.4 and 5.6, performed at about the same level as the filters fired with copper nanoparticles. Overall, the silver filters clearly outperformed the blank, copper, and mixed metal filters in the reduction of *E. coli* RS2GFP. These results indicate that nanoparticle inclusion is not necessary for achieving the WHO minimum reduction requirements (limited protection status). This is not surprising, as Brown & Sobsey (2010) and Yakub *et al.* (2013) suggest that ceramic filters likely rely more on size exclusion for reducing bacteria than on the antimicrobial properties of silver and copper. This suggests that larger pore sizes, as indicated by faster flow rates, will result in higher effluent turbidities and lower removal of *E. coli*. Although nanoparticle inclusion is not required to achieve acceptable effluent quality, it is clear that an infusion of 25–75% silver

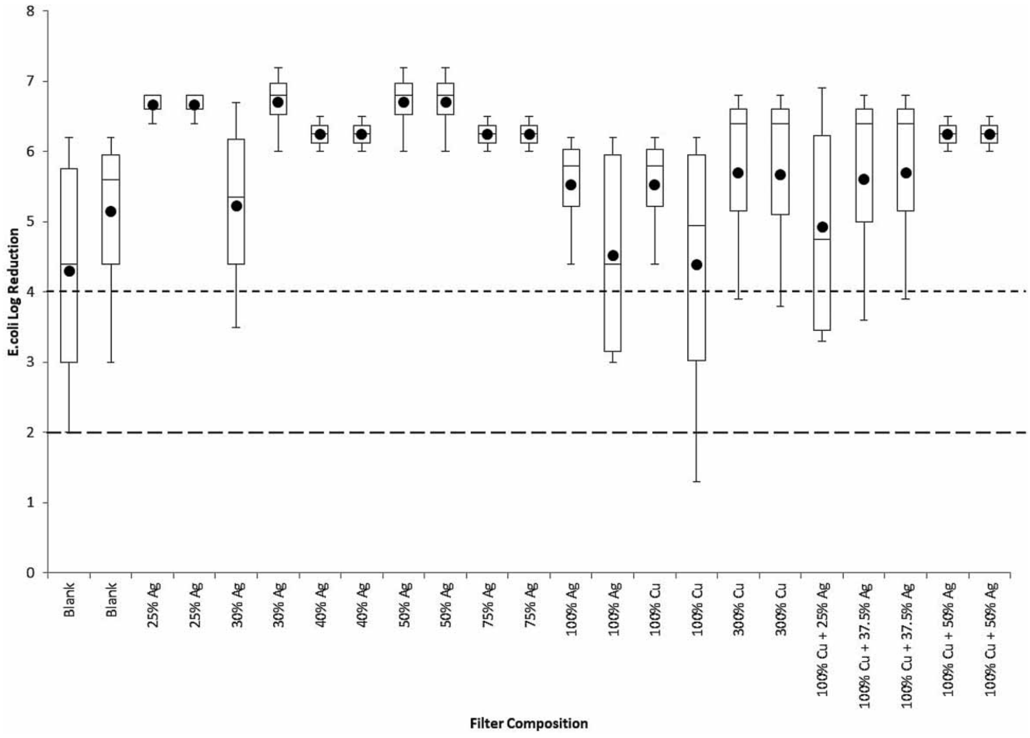

Figure 2 | Log reduction of *E. coli* in filter effluent water samples. The short dashed line indicates the WHO standard for minimum log reduction under highly protective conditions, while the long dashed line indicates the WHO standard for minimum log reduction under protective or limited protection conditions.

does increase *E. coli* reduction by up to 2 log, sufficient to consistently meet the WHO highly protective conditions. It is also noteworthy that the current silver content (100% Ag) performed at a lower level than the lower silver contents, suggesting that the amount of nanosilver currently used to manufacture the filters can be reduced without loss of efficacy.

Enumerating phage was problematic in the early plates, as the MS2 bacteriophage had an extremely high titre, which made growing and enumerating it difficult to do without overwhelming the host *E. coli*. Therefore, the phage data reported are limited to the later plates, which represent removals by the blank, 100% Ag, and 100% Cu filters for a total of six filters. It should be noted that based on individual plates counted, the highest median log reduction of MS2 bacteriophage measured (7.9) was achieved by a 75% Ag filter, and the lowest median log reduction (2.1) was measured in a 25% Ag filter. The median log removals for all six filters (blank, 100% Ag, and 100% Cu in duplicate) (Figure 3) exceeded the WHO protective or limited protection required reduction (3 log). However, none of the filters met this threshold for the minimum observed reduction. While maximum log reductions measured met the WHO highly protective criteria for minimum required reduction (5 log), they all failed at the third quartile. While the data collected suggest that neither silver nor copper nanoparticles provided additional removal benefits over those of the blank, additional experiments are required to confirm this finding due to the limited data collected. However, the literature suggests that significant contact time with silver is required for bacteriophage inactivation (De Gusseme *et al.* 2010), which would not be realized in these filters.

It is notable that the same filter configuration achieved significantly different median removals ($\alpha < 0.1$) of *E. coli and* MS2 (Table 2). A likely explanation is that the filtration mechanisms responsible for removal of *E. coli* and MS2 are not the same. Factors affecting filtration mechanisms

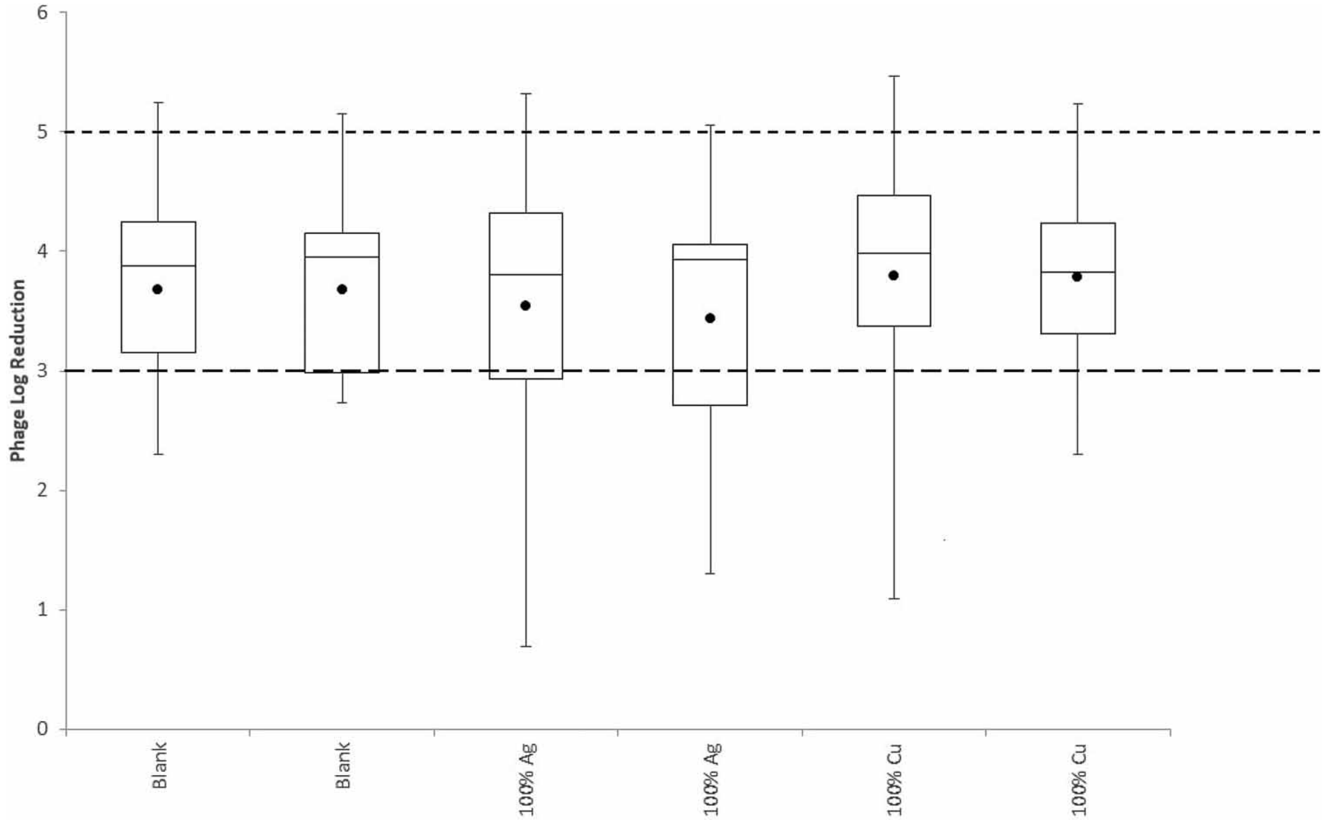

Figure 3 | Log reduction of phage in filter effluent water samples. The short dashed line indicates the WHO standard for minimum log reduction under highly protective conditions, while the long dashed line indicates the WHO standard for minimum log reduction under protective or limited protection conditions.

Table 2 | Comparison of *E. coli* and MS2 log removals in blank, 100% Ag, and 100% Cu filters

	***E. coli* mean log removal**	**MS2 mean log removal**	**t^a**
Blank	4.30	3.68	2.39
Blank	5.15	3.68	7.50
100% Ag	5.52	3.54	7.78
100% Ag	4.52	3.44	3.93
100% Cu	5.52	3.79	7.32
100% Cu	4.39	3.79	1.98

[a] $t_{\alpha/2=0.1,\ \mathrm{dof}=10} = 1.81$.

include pore size, morphology, and surface properties, which will vary with different filter configurations and microorganisms. As such, if the *E. coli* and MS2 are capitalizing on different removal mechanisms, it is expected that the same filter would perform differently for both.

Production inconsistences (i.e. pore size variation and nanoparticle distribution), and/or development of microscopic cracks, are likely to explain the differences in removals of the same microorganism between duplicate filters (e.g. Figure 2, 30% Ag). While the purpose of this experiment was not to follow the WHO (2014a, 2014b) protocol, it is interesting to note that based on their criteria of less than or equal to one log variance between duplicates, the 30% Ag and 100% Cu filters would have failed the consistency test for *E. coli* removal even though their mean removals exceeded the highly protective minimum removal criteria. All three duplicate pairs reported for MS2 met consistency requirements for protective or limited protection. However, whisker plots (Figure 3) clearly indicate that each filter fails the protective or limited protection criteria at least some of the time.

Prior to testing, all filters were examined for microscopic cracks by gently tapping on the side of the pot and checking its resonance (perfect filters will have a bell sound). One of the 300% Cu filters was found to have a microscopic crack prior to testing, which could have developed during the firing process or resulted from aggressive handling. Regardless, the crack does not appear to have impacted the filter's ability to remove *E. coli* consistently, as its performance cannot be differentiated from that of its duplicate (Figure 2).

In keeping with the WHO general testing protocol for ceramic filters, an influent turbidity of 30–50 Formazin Turbidity Units (FTU) was maintained over the course of testing, with an average of 43.4 FTU. Effluent turbidity and flow rate are positively correlated (Figure 4), indicating

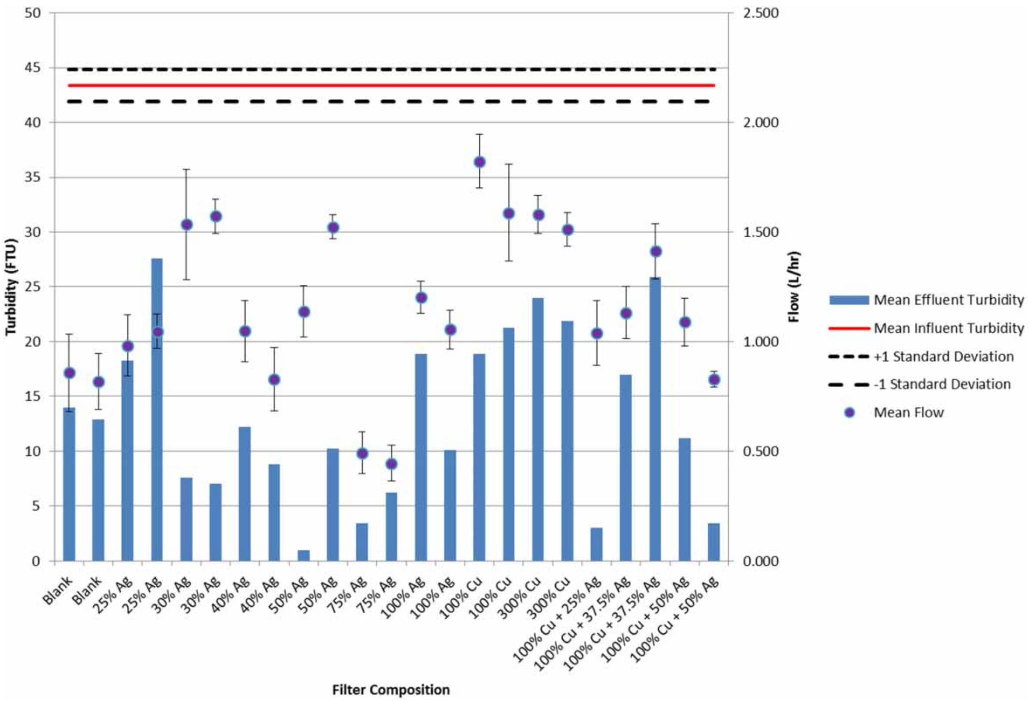

Figure 4 | Mean filter flow rates and turbidity measurements.

that larger pore sizes facilitate the passage of both water and particulates. According to the WHO (WHO 2011b), in communities where drinking-water resources and treatment options are very limited, filtered water should have a turbidity consistently less than 5 FTUs, though ideally it should be less than 1 FTU. Only one filter (50% Ag) met the WHO criteria, with an average effluent turbidity of 1 FTU. An additional three filters had average effluent turbidities of less than 5 FTUs (75% Ag, 100% Cu + 25% Ag, and 100% Cu + 50% Ag, with turbidities of 3.4, 3, and 3.4 FTU respectively), although none of these were duplicate pairs. The best performing duplicate pair was 75% Ag with mean effluent turbidities of 3.4 and 6.2 FTUs. Given that a key criterion for assessing whether or not to drink water is how clear it looks (Levison *et al.* 2011), this will have implications for whether filters are used, even though scientifically they are removing microorganisms and therefore improving potability. Similarly, from an ease of use perspective, all except one filter had mean flow rates of less than 1.75 L/h. Given that WHO standards require a minimum of 7.5 L/capita/day of potable water, increasing to 20 L/capita/day to ensure basic and food hygiene needs are met (Hutton & Bartram 2003), it would take over 17 hours to produce sufficient water for a family of four to meet the minimum requirement of 7.5 L/capita/day and 45 hours to meet the requirement of 20 L/capita/day.

CONCLUSION

These results indicate that copper and silver nanoparticles are not necessary to reduce *E. coli* colonies in contaminated water to a level that is acceptable for ceramic filter performance. However, the addition of 25–75% Ag during the firing process gives the pot an additional 2 log reduction capability for *E. coli* over the blank ceramic filters, conferring WHO highly protective status. Despite being a cheaper alternative, it does not appear that addition of copper nanoparticles provides enhanced benefits over the blanks, and it therefore should not be considered in filter production. Unfortunately, replacing some of the silver with copper in order to reduce costs does not appear to confer any benefits in terms of log removal for *E. coli*. Reported results were insufficient to comment on enhanced phage reduction efficacy associated with nanoparticles, although it is clear that these filters were unable to meet WHO highly protective removal standards, regardless of nanoparticle inclusion.

It is noteworthy that significant variations in effluent quality were observed in different filters with the same composition. This demonstrates the clear need for quality control practices in order to reduce inconsistencies in pore size between filters. Without greater consistency in the production practices and standards, the potential range of bacterial, viral, and turbidity reduction of each pot will be wide, and the lower end of the range may not only not meet the WHO reduction criteria, but instill a false sense of security in filter users. An additional factor in this is the fragility of the filters. They can crack easily, without any visible signs. If the consumer is unaware of a fault in their pot, it may continue to be used with a compromised degree of effectiveness. Care instructions become critical, and recommendations should include that filters be handled as little as possible and with extreme care. A method of testing, such as checking for a bell sound, should also be implemented at regular intervals, instead of just once before use, in order for users to ensure that they are not using compromised filters.

A final observation reflects the utility of the filters, given the overall low turbidity removal and slow flow rates. While it is clear that microbial removal is sufficient to protect health to WHO standards, ease of use is a critical element for widespread, sustained uptake. While filters are affordable, requiring and managing multiple filters to provide sufficient water for family use, or aesthetically displeasing water quality, may be enough of a disincentive to stop using the filters, leaving families unprotected.

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