Virus removal efficiency of Cambodian ceramic pot water purifiers

Hamidreza Salsali, Edward McBean and Joseph Brunsting

ABSTRACT

Virus removal efficiency is described for three types of silver-impregnated, ceramic water filters (CWFs) produced in Cambodia. The tests were completed using freshly scrubbed filters and de-ionized (DI) water as an evaluation of the removal efficiency of the virus in isolation with no other interacting water quality variables. Removal efficiencies between 0.21 and 0.45 log are evidenced, which is significantly lower than results obtained in testing of similar filters by other investigators utilizing surface or rain water and a less frequent cleaning regime. Other experiments generally found virus removal efficiencies greater than 1.0 log. This difference may be because of the association of viruses with suspended solids, and subsequent removal of these solids during filtration. Variability in virus removal efficiencies between pots of the same manufacturer, and observed flow rates outside the manufacturer's specifications, suggest tighter quality control and consistency may be needed during production.

Key words | Cambodia, ceramic water purifiers, enteric virus, MS2 virus, virus filtering

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ABBREVIATIONS

CRC	Cambodian Red Cross
CWF	Ceramic water filter
DAL	Double agar layer
DI	De-ionized
IDE	International Development Enterprises
NTU	Nephelometric turbidity units
PFU	Plaque forming units
POU	Point of use
RDIC	Resource Development International Cambodia

INTRODUCTION

Today, 1.1 billion people, or approximately 15% of the world's population, do not have access to a source of safe drinking water. In response, increasing reliance is being placed upon point-of-use (POU) technologies as interventions to provide safe water to rural households as a key element to improve the situation. Simple, low-cost water treatment technologies are capable of dramatically improving the microbial water quality at the household level (Murphy *et al*. 2010a) and are being proposed as part of the solution for meeting the Millennium Development Goal for safe water. However, while investigators have assessed the effectiveness of removal of *Escherichia coli* and an array of metals and nitrates (e.g. Brown 2007; Murphy *et al*. 2010b), fewer studies have done a focussed evaluation of the effectiveness of POU technologies on viruses.

One POU technology currently employed is the ceramic water filter (CWF). CWFs are devices produced and used in developing countries around the world, including Honduras, Kenya, Cambodia, Ghana and Nicaragua (van Halem *et al*. 2009). Three organizations in Cambodia produce these filters: Resource Development International Cambodia (RDIC), International Development Enterprises (IDE) and the Cambodian Red Cross (CRC). Clay (sourced either from open pits or from unfired clay bricks) is put through a hammer mill. This is mixed with fine rice husks (<1 mm diameter) to increase porosity and flow rate post-firing, and also (in the case of RDIC) laterite, which is an iron oxide mineral shown in some tests by RDIC to bind viruses (Hagan *et al*. 2008). The

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dry ingredients are thoroughly mixed, followed by mixing with an appropriate proportion of water. Of these three pot types, the fractions by weight of initial ingredients range from 56–63% clay, 15–18% rice husks and 21–23% water, with 4% laterite in the case of RDI. The mixture is separated into 8.0–10.0 kg balls and pressed into a pot form approximately 24 cm high by 34 cm wide at the top by 2.0 cm thick.

After pressing, the surface is finished by smoothing and the pots are set to dry outside for 7–18 days depending on season, and then fired to at least 830 °C. After firing, each is inspected for damage and flow testing is done, with an acceptable rate generally in the range of 1.5–3.0 L/hr. Figure 1 presents a picture of one of the finished products from RDIC with the receptacle.

The final production step involves painting each filter with a colloidal silver solution as a biocide. Brown (2007) investigated the effects of additives such as laterite and colloidal silver and found them to be negligible, although Bloem *et al.* (2009) did find a significant difference with silver treatment enhancing *E. coli* removal.

Brown (2007) and Lantagne (2001a; 2001b) reported microbial removal efficiencies of 2 to 6 log, 0.5 to 5 log and 4 to 6 log were achieved for *E. coli*, virus and protozoa, respectively. However, source water characteristics such as turbidity and organic matter influence removal effectiveness, particularly for viruses. As a result, the purpose of this investigation was to examine the filtration/adsorption effect on virus removal efficiency, under conditions of clean filters and clear water, of several types of CWFs manufactured in Cambodia. Due to the significant differences in size and physiological structure between viruses and bacteria, mechanisms of removal are quite different, with smaller viruses likely being removed primarily through adsorption on filter surfaces (van Halem 2006). To test these aspects of the CWFs, they were tested with de-ionized (DI) water spiked only with viruses.

Figure 1 | RDIC clay pot filter and receptacle (picture used with permission from RDIC).

The virus used in this investigation was the MS2 virus (ATCC 15597 B1), a male-specific (F+), F-RNA, single-stranded, non-enveloped coliphage of diameter 23–25 nm with icosahedral shape. Bacteriophages share many fundamental properties with human viruses, allowing testing as surrogates to assess removal effectiveness of enteric viruses. F-RNA male-specific bacteriophages are especially useful as surrogates for enteric viruses because of similarities in origin (faeces of warm-blooded animals), failure to multiply in the environment and similarity in composition, structure and size (Grabow 2001). This is also useful as a comparison to similar investigations by Brown (2007) and van Halem (2006) who employed the same virus. Virus stock and influent and effluent samples were assayed for coliphage concentration by the double agar layer (DAL) procedure derived from a standard procedure by USEPA (2001).

In terms of removal mechanisms of indicator organisms, van Halem (2006) found that the effective pore diameter of similar filters from Cambodia, Ghana and Nicaragua was in the range of 40 μm. In comparison to the size of *E. coli* (0.5 μm × 1.0–3.0 μm) and MS2 virus (23–25 nm), this suggests removal by mechanisms other than absolute screening, namely sedimentation, diffusion, inertia, turbulence and adsorption (van Halem 2006).

MATERIALS

The CWFs were sourced from local suppliers in Cambodia and shipped by air to Guelph for testing. The plastic receptacles used were converted polyethylene rain barrels. All laboratory materials were selected based on USEPA (2001) or appropriate substitutes for recommended equipment.

METHODS

Production of *E. Coli* and MS2 stock

The *E. coli* Famp (ATCC 700891) stock used to produce the detection bacteria for each experiment run was a log-phase

culture grown in tryptic soy broth (TSB), generated from a single colony, and frozen with glycerol in a 1 : 4 ratio. The MS2 stock was generated in a procedure similar to that of Brown (2007).

Spiking and filtration procedure

The laboratory had four of each of these types of CWFs. Two of each type were randomly selected for testing with one of all types randomly selected to use as a blank during testing. Before a testing day, an overnight stock of *E. coli* host was prepared and used to produce a log-phase culture for the following morning. The filters were prepared by light scrubbing and rinsing with DI water. The receptacles were prepared by washing with soapy water and rinsing, disinfection with 70% ethanol and letting dry overnight. The same was done for a 10 L mixing bucket. On the testing day, the virus solution was prepared by mixing 10 L of DI water with 80 g NaCl (common table salt) and the required volume of virus stock. An influent sample was taken using a sterile bottle and fresh laboratory glove, the solution was poured into the filter and the lid was replaced. The volume of filtrate was recorded hourly, with an effluent sample taken after three hours. All samples were refrigerated before processing on the same day.

DAL procedure for sample analysis

The following is a short summary of the DAL testing procedure, as derived from USEPA Method 1602 (USEPA 2001). Influent and effluent samples were serially diluted in TSB between 10^{-1} and 10^{-4}. Tubes of 0.5% tryptic soy agar (TSA) with antibiotics (i.e. soft agar) were prepared in 5 mL aliquots in sterile test tubes with caps and kept in a water bath at 45 °C until used. Plates of hard bottom agar (1.5% TSA with antibiotics) were pre-prepared and kept refrigerated until use. For plating, each tube of top agar was inoculated with 0.1 mL of log-phase *E. coli* host, then 0.5 mL of the sample dilution being tested was added. This was gently swirled in the palm of the hand for three seconds and then carefully poured onto a bottom agar plate, with gentle tilting to achieve even distribution. The plate cover was carefully replaced and the agar was allowed to harden for at least five minutes before placing the plate in the incubator. The plates were allowed to incubate for 18–24 hours before counting.

QUALITY CONTROL

Each dilution of each sample was completed in duplicate, along with a method blank for each sample (soft agar tube with no sample dilution added). If any contamination was detected in the blank, the entire set of plate counts for that sample was discarded. In addition to this, for each run of filters, a seventh filter was run with clean water and tested as a control for contamination. If any plaques were found, then the results for that day were considered suspect.

Plate counts and concentration calculations

Colonies of virus were observed as clean plaques (in the range of approximately 1–5 mm) on the opaque bacterial lawn, as the virus infected the surrounding bacterial host. These were counted manually, with counts between 3 and 400 used for calculations of sample concentrations in pfu/mL. USEPA (2001) recommends using plate counts between 0 and 300; however, it was observed that colonies were distinct and countable up to 400.

RESULTS AND DISCUSSION

The results for log removal efficiency of each pot are shown in Table 1. Six experiments on each pot were conducted; however, due to some laboratory errors some tests had to be discarded. Particularly, any results showing negative or otherwise severely outlying results in terms of removal efficiency were discarded when calculating the arithmetic average. '# of tests used in calculation' refers to those successful tests used to compute results.

As shown, there is some variability between the individual CWFs and their performance with respect to virus removal efficiency. Of particular note are the differences between RDI-1 and RDI-2 at 0.21 log and 0.42 log, respectively, and the differences between IDE-1 and IDE-2 at 0.45 log and 0.26 log, respectively. This is suggestive of a need for tighter quality control during production to ensure consistent performance. One aspect that was noted during testing which also lends credence to the need for a tighter regulation on quality control and consistency is the

Table 1 | Virus removal results for different CFs

CF type	Avg. influent concentration in pfu/mL (std. dev.)	Avg. effluent concentration in pfu/mL (std. dev.)	Avg. log removal efficiency (std. dev)	# of tests used in calculation
RDI-1	7.0×10^4 (3.2×10^4)	4.2×10^4 (1.5×10^4)	0.21 (0.08)	5
RDI-2	5.7×10^4 (2.5×10^4)	2.3×10^4 (1.3×10^4)	0.42 (0.09)	3
IDE-1	6.1×10^4 (2.8×10^4)	2.2×10^4 (9.5×10^3)	0.45 (0.12)	4
IDE-2	9.5×10^4 (3.2×10^4)	5.1×10^4 (1.3×10^4)	0.25 (0.09)	4
CRC-1	8.4×10^4 (5.2×10^4)	4.8×10^4 (2.4×10^4)	0.21 (0.10)	4
CRC-2	7.7×10^4 (5.9×10^4)	4.0×10^4 (1.8×10^4)	0.22 (0.16)	4

outlying flow rates from manufacturers' specifications. RDI considers 1.5–3.0 L/hr to be acceptable (Hagan *et al.* 2008), and IDE and CRC consider a range of 2.0–3.0 L/hr to be acceptable (IDE 2008; Potters for Peace [no date]). RDI-1 and RDI-2 both fell slightly below the acceptable specified range at 1.3 and 1.4 L/hr, respectively, whereas pots IDE-3 (the control) and IDE-2 were both above the acceptable range at 3.8 and 3.9 L/hr, respectively. Only the CRC CWFs fell completely within the given acceptable range in average results for the measured flow rate per hour.

In regard to the relationship between flow rates and removal efficiency, it was observed that the higher flow IDE filter (IDE-2) had lower removal efficiency than that of the lower flow IDE filter (IDE-1). It is possible that the higher flow rates could be due to greater concentration of flow paths such as micro-fractures, which could contribute to lower removal efficiency. However, with a broad comparison across filter types and comparing flow rates and removal efficiencies, no clear trend was observed. Van Halem (2006) suggests that CWFs with initial flow rates greater than 1–2 L/hr perform equally as well as those with somewhat higher flow rates, and Bloem *et al.* (2009) found that filters with initial flow rates of up to 6 L/hr performed equally as well in microbial treatment as those with initial flow rates of 2 L/hr.

In comparison to each other, the IDE and RDI CWFs had average removal efficiencies higher than those of the CRC CWFs. However, the pots tested from RDI and IDE both showed higher variability in performance within manufacturer type, with the two CRC pots being more consistent. In comparison to investigations on CWFs of similar construction, the removal efficiencies in these tests were generally lower. Results from various other investigations on CWFs are shown below in Table 2. As shown, tests by van Halem (2006) with MS2 virus on similar filters from Ghana, Nicaragua and Cambodia show a broader range (from 0.5 log to 3.0 log) of removal. However, important differences exist in comparison between the experimental results from this research and those for van Halen (2006),

Table 2 | Results from various investigations

Investigation	Pot sources	Water type	Cleaning procedures	Virus log removal	Comments
This experiment	Cambodia (RDI, IDE, CRC)	DI	Before every test	Range 0.21–0.46	
Brown (2007)	Cambodia (RDI, several recipe variations)	Surface water and collected rain water	Once per week during frequent testing	Mean 1–2 log	
van Halem (2006)	Ghana, Nicaragua, Cambodia	Canal surface water	Very infrequent (3–6 weeks between cleanings during frequent testing)	Range 0.5–3.0 log	Apparent filter clogging enhancement effects producing higher removals
Lantagne (2001a)	Nicaragua (Potters for Peace)	Filtered, disinfected well water	Unknown	<1	Tests only done on 1 filter, very preliminary results

as van Halem used surface water in trials for 13 weeks, and the filters were apparently only scrubbed in weeks 6 and 9. The lower removal values were observed for the tests in the 5th week and the higher values in week 13, after filters had started to become clogged. The clogging and build-up of biofilm is hypothesized to enhance the virus removal efficiency (van Halem 2006).

In laboratory testing of Cambodian filters made by RDI, using Cambodian surface and rain waters as spike waters with pot scrubbing once per week, Brown (2007) reported virus reductions averaging 1–2 log. He found a mean log reduction of 1.3 for surface water and 1.4 for rain water tested on a similar filter as used in this study (laterite and silver nitrate added). The rain water used by Brown (2007) was fairly high quality with an average NTU (nephelometric turbidity units) of 1.1, and the surface water source had higher NTU with an average turbidity of 8.4 NTU. Given the fact that these other investigations used surface waters or rain water rather than very high-quality DI water (as in this investigation), and given the significant difference in results, it is considered likely that performance with regard to virus removal is enhanced by binding with small particulates in the water which enhances virus removal.

Table 3 and Figure 2 show a comparison of virus removal results based on turbidity, with an assumed turbidity of 0.02 NTU for de-ionized water (Global Water Instrumentation Inc. 2010). Brown (2007) used two source waters, namely rain water and surface water, which had average turbidities over his testing period of 1.1 and 8.4, respectively. His average MS2 removal results for both surface waters and for different filter types are shown as data points B, C, F and G below. These are combined with results from our experiments and separated into two categories for comparison: clay pots treated with both silver and laterite (all by RDI) and clay pots treated with only silver (from RDI, CRC and IDE). These are shown on separate trend lines in Figure 2.

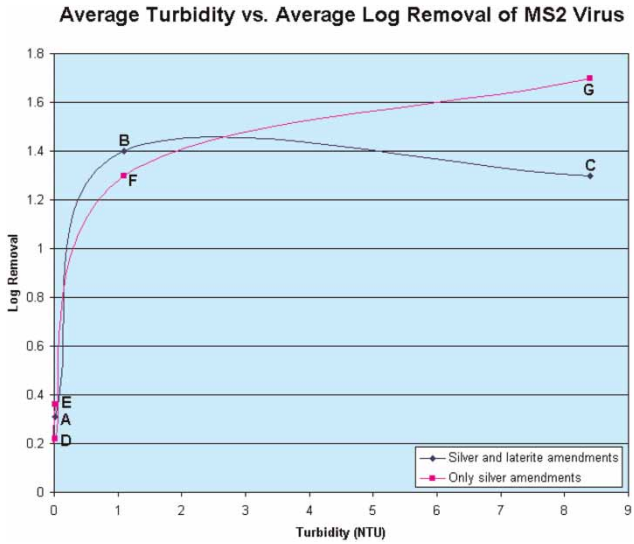

Figure 2 | Graphical comparison of the results in Table 3.

Due to the exceptionally small size of viruses (even relative to that of bacteria), the binding of viruses to other larger particulates very likely enhances the removal effectiveness as these larger particulates are strained out or are subject to alternative removal mechanisms suggested by van Halem (2006), namely sedimentation, and adsorption contributed to by diffusion, inertia and turbulence. The adsorption of viruses to water particulates at higher turbidities and the greater removal of this larger particulate due to straining or the same alternative mechanisms mentioned above may account for the differences between results at varying turbidities. Further research is needed to confirm

Table 3 | Comparison between this experiment and results from Brown (2007)

Point	Source	Description	Water	Average turbidity (NTU)	Average log removal
A	This experiment	RDI – Silver nitrate and laterite	De-ionized	0.02	0.31
B	Brown (2007)	RDI – Silver nitrate and laterite	Rain water	1.1	1.4
C	Brown (2007)	RDI – Silver nitrate and laterite	Surface water	8.4	1.3
D	This experiment	IDE – Colloidal silver applied	De-ionized	0.02	0.36
E	This experiment	CRC – Colloidal silver applied	De-ionized	0.02	0.22
F	Brown (2007)	RDI – Silver nitrate applied	Rain water	1.1	1.3
G	Brown (2007)	RDI – Silver nitrate applied	Surface water	8.4	1.7

and investigate this possible mechanism and its characteristics.

CONCLUSIONS

In testing the virus removal efficiency of three types of ceramic silver-impregnated pot water filters, removal efficiencies of between 0.21 and 0.45 log were observed. This test represents the removal efficiency of a clean filter with clear water (i.e. removal efficiency of viruses by sorption to the pot and filtration by the pot with negligible particulates). Although this is not entirely representative of the actual point-of-use conditions, it does give an indication of the adsorption and filtration potential of the filter materials for the viruses by themselves. The removal efficiency is substantially lower than that observed in other tests of similar filters which utilized representative surface waters and less frequent cleaning, suggesting that removal may be enhanced under these latter conditions. More work is needed in order to compare performance between manufacturers and also to investigate the relationship between turbidity and virus removal efficiency.

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