Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2020.26395

205 (2020) 286–295
November

Mechanism of silver incorporated in biosand zeolite clay granular filters for the removal of *Cryptosporidium parvum* and *Giardia lamblia* from surface water at point of use

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Received 29 January 2020; Accepted 27 July 2020

ABSTRACT

This study was designed to assess the performance of the biosand zeolite silver-impregnated clay granular (BSZ-SICG) in removing *Cryptosporidium* and *Giardia* from surface water and to establish the mechanism of action involving Ag^+ embedded in granular-clay in the deactivation of these parasites. Untreated water revealed a higher concentration of *Cryptosporidium* oocysts and *Giardia* cysts which ranged from 16–32 oocysts/1 mL and 32–128 cysts/1 mL, respectively. To ascertain the mechanism of Ag^+ in removing target protozoans, granular-clay-impregnated with $AgNO_3$ was soaked in synthetic water prepared with normal saline (0.9% w/v). The oocysts isolated from surface water samples were spiked in synthetic water containing 0.1 mg/L of Ag^+ for 30 min. Scanning electron microscopy revealed the interaction of Ag^+ with the wall surface of both oocysts and cysts. The transmission electron microscopy also showed the presence of Ag^+ within the cytoplasm of the oocysts and cysts. The mechanism of action of the silver-incorporated in the granular-clay is still not well understood. However, the silver ions were fully able to enter the cytoplasm of the oocyst and cyst. This study therefore strongly recommended the use of BSZ-SICG in rural households for the provision of safe potable water and to counteract diarrhoeal diseases within the communities.

Keywords: Cryptosporidium parvum; *Giardia lamblia*; Removal; Biosand zeolite silver-impregnated clay granular (BSZ-SICG)

Importance section

It is well documented that *Cryptosporidium* and *Giardia* sp. are commonly found in surface water sources because surface water is more vulnerable to direct contamination from sewage discharges and runoff. Moreover, it is also well known that these two species are resistant to most of the disinfectants including chlorine, which is widely used in water treatment plants. The underserved rural communities are at risk of diseases associated with the consumption of water contaminated with *Cryptosporidium* and *Giardia* sp. The results of this study showed that biosand zeolite silver-impregnated clay granular (BSZ-SICG) filters are effective in removing the *Cryptosporidium* oocysts and *Giardia* cysts from surface water. These filters produce drinking water that is safe for human consumption. The incorporation of silver in granular clay is crucial to increase the mode of action of the silver in the destruction of the oocysts and cysts of *Cryptosporidium* and *Giardia*.

1. Introduction

Untreated drinking water has been reported to be one of the most important contributors to the human disease burden, being responsible for an estimated 1.9 million deaths each year [1]. For the treatment of water that is meant

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for human consumption, the concept of multiple barriers is a vital principle for the production of safe drinking water. For this purpose, the conventional water treatment involves a series of processes such as coagulation, flocculation, and clarification through sedimentation, filtration and disinfection in addition to the protection of water sources. These processes are applied to raw water sources for the reduction of microorganisms of public health concern [2]. Filters within a conventional water treatment process are known as the last barrier to prevent the release of particles and protozoan cysts/oocysts into the distribution system [3]. Chlorine is known to be the common drinking water disinfectant used worldwide. However, waterborne pathogens such as *Giardia lamblia* and *Cryptosporidium parvum*, which contribute to a significant proportion of human deaths [4,5] represent a major challenge to public health and drinking water suppliers. This is due to their tenacity and their resistance to some drinking water treatment methods such as chlorination [6–8].

The past two decades have led in an intense awareness of the potential public health risks posed by *Giardia lamblia* and *Cryptosporidium parvum* because of their resilience to chlorination. Furthermore, *Cryptosporidium* and *Giardia* have been confirmed to be the causative agents of enteritis, worldwide [9]. In addition, it has been reported by the Centers for Disease Control and Prevention (CDC) that during the past three decades, *Cryptosporidium* has been recognized as one of the most common causes of waterborne diseases in humans. Therefore, due to the obstinate nature of *Cryptosporidium* and *Giardia*, their treatment is typically addressed through filtration and pre-treatment with coagulation-flocculation to optimize their physical removal from water sources [10]. The optimal mechanism for the removal of protozoan parasites by coagulation in conventional water treatment systems is precipitate enmeshment [11,12]. According to Fox and Reasoner [13], removal using biological mechanisms such as slow sand filtration is accomplished by the filtering action of the *Schmutzdecke* (biological layer), which is the top layer of sand and particulate materials (fine soil particles, plant debris, algae, free-living or non-pathogenic protozoa). Moreover, these are removed from the water, as there is percolation downward through the sand filter bed. The *Schmutzdecke* is therefore the most important process for the removal of microorganisms in biosand filters.

In developing countries, where centralized water treatment facilities are almost non-existent in rural settings, a number of authors have repeatedly demonstrated that point-of-use (PoU) household treatment systems are the prime alternative solution for access to safe drinking waters [14–19]. Numerous studies have highlighted the importance of improved water quality and the reduction of diarrhoeal diseases in both children and adults by using PoU water treatment technologies [20–25]. Other studies have recognized silver coated/impregnated ceramic water filters as a promising technology based on performance and social acceptability, which results in higher long-term use [22,25,26]. Studies have demonstrated that silver nanoparticles have antimicrobial properties, and do not pose adverse health risks if the concentration is within the drinking water quality guideline value, which is 0.1 mg/L according to World Health Organization (WHO) [27,28].

Silver has been known to have antibacterial properties since Roman times. Moreover, the increased use of nanosilver in a range of experimental drinking water treatment systems, its use in combination with ceramic filters and its perceived potential to be a water disinfectant that does not result in disinfection by-products (DBPs) in the treated water have raised the profile of this metal [29]. Several studies have been conducted on the disinfection efficacy of silver and AgNP applications against a range of microorganisms found in water [30–34]. However, the majority of these studies have focused on the impact of silver on bacteria, bacteriophages and viruses.

Little is known about the mechanism of action of silver nanoparticles on protozoan parasites such as *Cryptosporidium* and *Giardia*. To date, most studies have been focusing on the removal of bacteria and protozoans by silver-coated/impregnated ceramic or porous pot filters [19,35–37]. Currently, no documented data are available on the removal of protozoans by BSZ-SICG filters. The BSZ-SICG filter also involves the concept of multiple barriers, which include both physical and chemical (disinfection) technologies. However, this study was designed to ascertain the performance of these filters, and especially to determine the mechanism of action of silver-impregnated clay granular filter on the removal/deactivation of protozoans parasites, specifically *Cryptosporidium parvum* and *Giardia lamblia* isolated from surface water when these components (biosand, zeolite and silver) are included in the BSZ-SICG filter.

2. Methodology

2.1. Construction and deployment of the BSZ-SICG filter

The biosand with zeolite (BSZ) was constructed by Tshwane University of Technology Water Research Group (TUT-WRG) as previously done by Mwabi et al. [19] with some modifications. In brief, a layer of silver-impregnated granular clay, which was prepared by mixing ball clay, sawdust, paper fiber, and silver nitrate powder ($AgNO_3$), and molded into small granulates prior to firing was added to the biosand filters with zeolite to form a BSZ-SICG filter. The silver-impregnated granular clay and the schematic representation of BSZ-SICG (Patent No. 2017/02594) are shown in Fig. 1. The BSZ-SICG water treatment devices were deployed in Makwane Village of the Limpopo Province in South Africa, subsequent to construction.

2.2. Performance of BSZ-SICG filters in removing Cryptosporidium oocysts and Giardia cysts from the surface water samples

2.2.1. Study area and water sample collection for assessment of the performance of BSZ-SICG filters in removing the target protozoan parasites

Water samples were collected on a monthly basis for a period of twelve months from the time of the deployment of the BSZ-SICG filters in the Makwane Village located in the Limpopo Province of South Africa (SA). For untreated surface water, 96 samples were collected using 50 L sterile plastic containers. For the treated water, 96 samples were collected using 50 L sterile plastic containers after filtration

through the BSZ-SICG filters. All samples were transported to the Tshwane University of Technology Water Research Laboratory (TUT-WRL) for analysis within 24 h. Furthermore, the water treatment devices (BSZ-SICG) were assessed for their performance in removing *Cryptosporidium* oocysts and *Giardia* cysts from surface water while in use in homes of Makwane Village.

2.2.2. Recovery of Cryptosporidium oocysts and Giardia cysts

The recovery of *Cryptosporidium* oocysts and *Giardia* cysts from untreated surface water and treated water samples was done using the Envirochek™ filters as described by the US Environmental Protection Agency [38] Briefly, about 50 L of untreated water samples or 50 L of treated water were separately filtered at a flow rate of 2 L/min through an Envirochek™ cartridge (1 μm pore size). The retained material was eluted from the Envirochek™ cartridge using phosphate-buffered saline and concentrated by centrifugation in order to collect the oo/cysts. For the detection of oocysts and cysts, the light microscope (100× magnification) was used, whereby 250 μL of the concentrated sample was placed on the light microscope slide for a wet mount to detect *Giardia* cysts. In addition, Ziehl–Neelsen staining technique was performed to detect *Cryptosporidium* oocysts, thus 250 μL of the concentrate was fixed on the microscope slide, upon which Ziehl–Neelsen stains were applied and observed under the light microscope (100× magnification). Furthermore, the remaining pellet was re-suspended in 30 mL of distilled water and kept at 8°C for further analysis. The concentrations of oocysts and cysts in the initial (untreated water samples) and final (treated water samples) were calculated using the Eq. (1), and the results were expressed as oocysts and cysts/1 mL.

$$\text{Initial / final concentration} = \frac{N}{V} \quad \text{(1) (in this study)}$$

where N = number of oocysts/cysts detected on the surface of the light microscope slide and V = volume of water sample (concentrate) pipetted on the slide.

2.2.3. DNA extraction of Cryptosporidium oocysts and Giardia cysts

The DNA was extracted using the freeze and thaw method described by Xiao et al. [39], with some modifications. Briefly, 500 μL of lysis buffer was added to 500 μL of the concentrated samples (Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa) in a 2 mL Eppendorf tube. The samples were then subjected to 5 cycles of freeze and thaw (30 min at –70°C and 2 min in a water bath set at 60°C). Upon completion of the cycles, 200 μL of proteinase K (200 μg/mL) was added to the samples and they were frozen overnight at –70°C. Thereafter, these frozen samples were placed in a water bath set at 90°C for 20 min to denature the proteinase K. The DNA was further extracted by phenol-chloroform, whereby 200 μL of phenol-chloroform-isoamyl alcohol was added to the samples and centrifuged for 5 min at 12,000 rpm at 4°C. The aqueous phase, on the surface, was transferred to a new Eppendorf tube, to which 500 μL of ice-cold ethanol 99.9% was added to precipitate the DNA. The samples were then frozen overnight at –20°C upon which they were centrifuged for 3 min at 12,000 rpm. The pellet was further washed with 500 μL of 70% ethanol and finally suspended in 200 μL of elution buffer and stored at –20°C until further analysis.

2.2.4. Molecular characterization of Cryptosporidium and Giardia

2.2.4.1. Nested-PCR (polymerase chain reaction) for Cryptosporidium

The ssUr DNA for *Cryptosporidium* (850 bp) was amplified by nested-PCR as previously described by [39] using

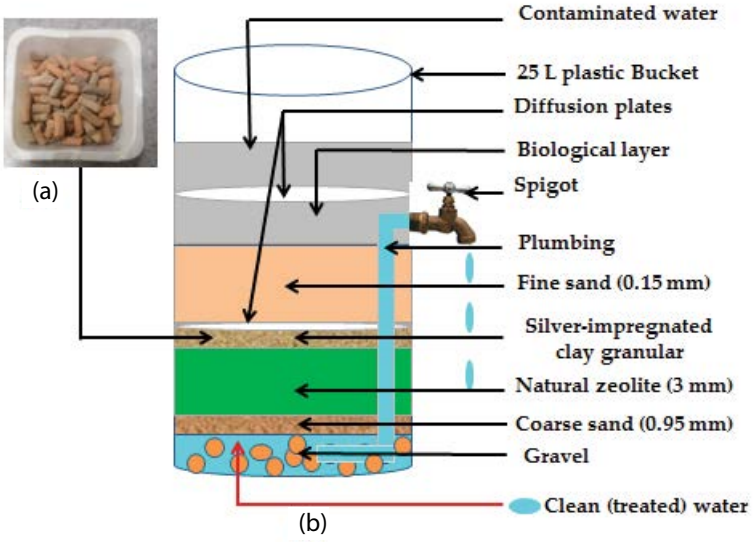

Fig. 1. (a) Silver-impregnated clay granules and (b) schematic representation of BSZ-SICG.

primers listed in Table 1. The PCR reaction contained 0.2 μL of each primer, 0.2 mM dNTP, 1.5 mM $MgCl_2$, 2.5 μL of DNA polymerase, 3 μL of 10× PCR buffer (Inqaba Biotechnical Industries (Pty) Ltd., Pretoria, South Africa), 1 μL of bovine serum albumin (BSA, 10 mg/mL), and nuclease-free water to make a final volume of 30 μL. Nested PCR involves the use of two primer sets and two successive PCR reactions. The following thermal protocol was used for the first set of primers: a pre-denaturation cycle at 94°C for 3 min; 35 cycles at 94°C for 45 s, at 60°C for 50 s and at 72°C for 1 min and a final extension at 72°C for 7 min. The thermal protocol followed for the second set of primers was the same as the first, with the exception of the annealing step which was done at 58°C for 50 s. The PCR products were electrophoresed on a 1.5% agarose gel containing ethidium bromide and visualized with the UV Transilluminator device (Lasec, Johannesburg, SA).

2.2.4.2. Semi-nested PCR for Giardia

A semi-nested PCR for *Giardia* was performed using the primers listed in Table 1 to amplify a 432-bp fragment of *Giardia* glutamate dehydrogenase (GDH) gene as previously described by [40]. The PCR was performed in standard mixtures of 30 μL containing 200 nmol of each primer, 0.2 mM dNTP, 1.5 mM $MgCl_2$, 2.5 U/μL Taq DNA polymerase, 3 μL of 10× PCR buffer (Inqaba Biotechnical Industries (Pty) Ltd., Pretoria, South Africa), and 1 μL of bovine serum albumin (BSA, 10 mg/mL). The template was subjected to initial denaturation at 94°C for 2 min, 35 cycles at 94°C for 2 min, at 55°C for 10 s, at 72°C for 30 s and a final extension at 72°C for 5 min.

2.3. Mechanism of silver-impregnated granular clay in removing/deactivating Cryptosporidium oocysts and Giardia cysts from surface water

To evaluate the mechanism involving the silver-impregnated granular clay in removing/deactivating *Cryptosporidium* oocysts and *Giardia* cysts from surface water, the following procedure was followed: firstly, the silver-impregnated granular clay was spiked in 1 L beaker × 2 containing sterile saline water. One beaker contained the silver nitrate ($AgNO_3$) at a concentration of 0.1 mg/L and another one a concentration of 0.05 mg/L, which were confirmed by SPECTRO ARCOS ICP spectrometer (SPECTRO Analytical Instruments (Pty) Ltd., Kempton Park, RSA). Secondly, each of these beakers was then spiked with the cyst-seeded solution (69 cysts/1 mL) and oocyst-seeded solution (24 oocysts/1 mL) obtained from the surface water samples. These beakers were then allowed to stand at room temperature for 30 min prior to the analysis of the samples. For analysis purposes, scanning electron microscopy (SEM) (JEOL JSM-5800LV, JEOL Ltd, Tokyo, Japan) and transmission electron microscopy (TEM) (JEOL 2100F, JEOL Ltd, Tokyo, Japan) was used. The samples were sent to the Council for Scientific and Industrial Research (CSIR) Nanotechnology Innovation Centre (Pretoria, South Africa) for analyses of Ag^+ on the wall surface of *Cryptosporidium* oocysts and *Giardia* cysts under SEM. Briefly, the oocyst/cyst-positive water concentrates were fixed with 2.5% glutaraldehyde for 2 h. Thereafter, they were washed five times with phosphate buffer and further fixed with 0.5% osmium tetroxide. Subsequently, they have washed 5 times again with sterile deionized water and dehydrated with different concentrations of ethanol (25%, 50%, 75% and 100%), after which they were analyzed under SEM for detection of Ag^+ on the wall surface of oocysts and cysts. Furthermore, all the samples with SEM images showing Ag^+ were further analyzed under TEM to confirm if Ag^+ had passed through the thick walls of oocysts and cysts of *C. Parvum* and *G. Lamblia*, respectively. In brief, the dehydrated samples were infiltrated with equal parts of epoxy resin and placed overnight at room temperature, upon which they were polymerized for 8 h at 70°C prior to TEM analysis. Fig. 2 depicts a schematic representation of the preparation for the deactivation of *Cryptosporidium* oocysts and *Giardia* cysts. Moreover, one sample without the addition of silver nitrate was included in the experimental study for control purposes.

3. Results

3.1. Performance of BSZ-SICG in removing Cryptosporidium oocysts and Giardia cysts from surface water samples

Table 2 provides a summary of the results for the detection of *Cryptosporidium* oocysts and *Giardia* cysts in

Table 1
Primers used in this study for amplification of *Cryptosporidium* and *Giardia* sp.

Primer name	Sequence 5′-3′	Target genes	Gene size (bp)	References
	Cryptosporidium primers for primary and secondary PCR			
Excry1	GCCAGTAGTCATATGCTTGTCTC	ssUr DNA	850	[39]
Excry2	ACTGTTAAATAGAAATGCCCCC			
NesCry3	GCGAAAAAACTCGACTTTATGGAAGGG			
NesCry4	GGAGTATTCAAGGCATATGCCTGC			
	Giardia primers for primary and secondary PCR			
CpbDiag-F1	AAGCTCGTAGTTGGATTTCTG	GDH gene	432	[40]
CpbDiag-R	TAAGGTGCTGAAGGAGTAAGG			
N-Diag-F2	CAATTGGAGGGCAAGTCTGGTGCCAGC			
N-Diag-R2	CCTTCCTATGTCTGGACCTGGTGAGT			

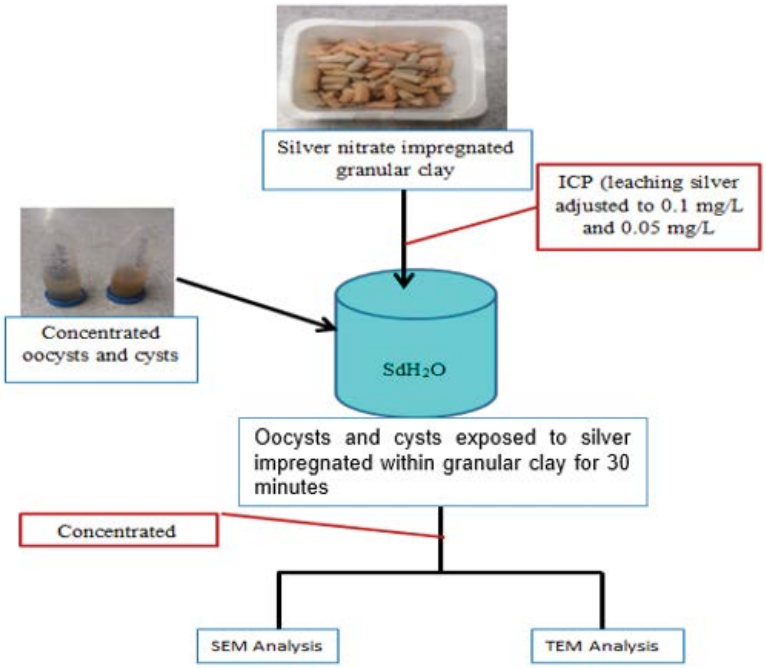

Fig. 2. Schematic representation of the preparation for deactivation of *Cryptosporidium* oocysts and *Giardia* cysts.

Table 2
Concentration of *Cryptosporidium* oocysts and *Giardia* cysts in treated and untreated water samples

Study period	Untreated surface water		Treated water samples (BSZ-SICG)	
	C. Parvum (oocysts/1 mL)	*G. Lamblia* (cysts/1 mL)	*C. Parvum* (oocysts/1 mL)	*G. Lamblia* (cysts/1 mL)
April 2015	32	128	ND	ND
May 2015	32	128	ND	ND
June 2015	16	48	ND	ND
July 2015	16	32	ND	ND
August 2015	16	32	ND	ND
September 2015	32	48	ND	ND
October 2015	32	78	ND	ND
November 2015	32	130	ND	ND
December 2015	34	128	ND	ND
January 2016	34	98	ND	ND
February 2016	32	126	ND	ND
March 2016	30	124	ND	ND

untreated and treated water samples. These results revealed that the untreated water samples tested positive for both *Cryptosporidium* oocysts and *Giardia* cysts; however, the treated water samples tested negative for both of these target protozoan parasites over the study period.

The concentration of *Cryptosporidium* oocysts and *Giardia* cysts ranged from 16 to 32 oocysts/1 mL and 32 to 128 cysts/1 mL. The results for *Cryptosporidium* oocysts and *Giardia* cysts were further confirmed by a light microscope following Ziehl–Neelsen staining and wet mount, respectively. Figs. 3a and d depict the results of *Giardia* cysts and *Cryptosporidium* oocysts from untreated surface water samples as observed under the light microscope, while Figs. 3b and d exhibit the results obtained from the treated water samples, respectively.

3.2. Molecular characterization of Cryptosporidium and Giardia

Results obtained from molecular studies (nested and semi-nested PCR) confirmed that the untreated surface water samples were positive for both *C. Parvum* and *G. Lamblia* during the study period. Nonetheless, water samples treated

by filtering through BSZ-SICG were found to be negative for both target protozoans. The results are summarised in Table 3.

3.3. Confirmation of isolates of Cryptosporidium oocysts and Giardia cysts by SEM

SEM images confirmed the presence of both *Cryptosporidium* oocysts and *Giardia* cysts in untreated surface water samples from the Makwane community (Figs. 5a and b).

3.4. Identification of Ag^+ on the surface of the cell wall of Cryptosporidium oocysts and Giardia cysts under SEM and energy-dispersive X-ray analysis

SEM was further used to identify Ag^+ on the wall surface of *Giardia* cysts and *Cryptosporidium* oocysts. The results are revealed in Fig. 6. The SEM micrograph revealed damaged walls of *Giardia* cyst and *Cryptosporidium* oocyst (Figs. 6a and b) after being exposed to 0.1 mg/L $AgNO_3$.

3.5. Identification of Ag^+ within the oocysts walls of Cryptosporidium and cyst walls of Giardia under TEM

The TEM micrograph shows the wall surface of native cysts and oocysts being smooth and intact (Fig. 7a). In contrast, after treatment, the oocyst and cyst walls were found to be severely damaged. In addition, Ag^+ was also observed within the cytoplasm of the cysts and oocysts. The results are presented in Figs. 7b and c.

4. Discussion

The use of nanomaterials in PoU water treatment systems has been investigated over the past three decades. Ceramic filters coated/impregnated with $AgNO_3$ are an example of PoU water treatment systems that have been trialed in several developing countries [41–43]. Most researchers have shown that certain nanoparticles exhibit antibacterial activity; however, only a few studies have investigated the mode of action of silver on protozoa [23,44]. The approach for this study was first to assess the performance of the BSZ-SICG filter in removing *Cryptosporidium* and *Giardia* from surface water and secondly to establish the mechanism involving Ag^+ embedded in granular clay.

To our knowledge, no previous study has systematically examined the removal of *C. Parvum* oocysts and *G. Lamblia* cysts by BSZ-SICG water filters. Most studies have focused on biosand filters with zeolite, ceramic water filters and silver-impregnated porous pot (SIPP) filters for the removal of *Cryptosporidium* and *Giardia* by size exclusion or adsorption method [45–48]. Adeyemo and co-workers [37]compared the performance of the household water treatment systems in removing the *Cryptosporidium* and *Giardia* from surface water and groundwater. Results revealed that none of these devices could produce drinking water that complied with the limits set by the South African National Standards (SANS 241) and international (WHO) standards for drinking water, which is zero (oo)cysts per 10 L of water. In the present study, it was observed that the BSZ-SICG filter produced drinking water that met the standards set by WHO [49] for household water treatment systems (Table 2). Thus, according to WHO, *Cryptosporidium* and *Giardia* must not be detected in treated drinking water; hence, the water treated by BSZ-SICG in this study was found not to contain any of the target protozoans (Fig. 3).

Silver has been shown to have strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities for bacteria, fungi, and viruses [50–52]. Moreover, it is also well known that $AgNO_3$ can be oxidized in aqueous solutions, leading to the release of silver ions, which can in turn interact with the cell surface of the bacteria. However, the information on how silver removes/

Table 3
Targeted protozoans confirmed by nested and semi-nested PCR from surface water samples over the study period

Study period	Untreated surface water		Treated water samples (BSZ-SICG)	
	C. Parvum	*G. Lamblia*	*C. Parvum*	*G. Lamblia*
April 2015	+	+	–	–
May 2015	+	+	–	–
June 2015	+	+	–	–
July 2015	+	+	–	–
August 2015	+	+	–	–
September 2015	+	+	–	–
October 2015	+	+	–	–
November 2015	+	+	–	–
December 2015	+	+	–	–
January 2016	+	+	–	–
February 2016	+	+	–	–
March 2016	+	+	–	–

+: detected (positive);
–: not detected (negative).

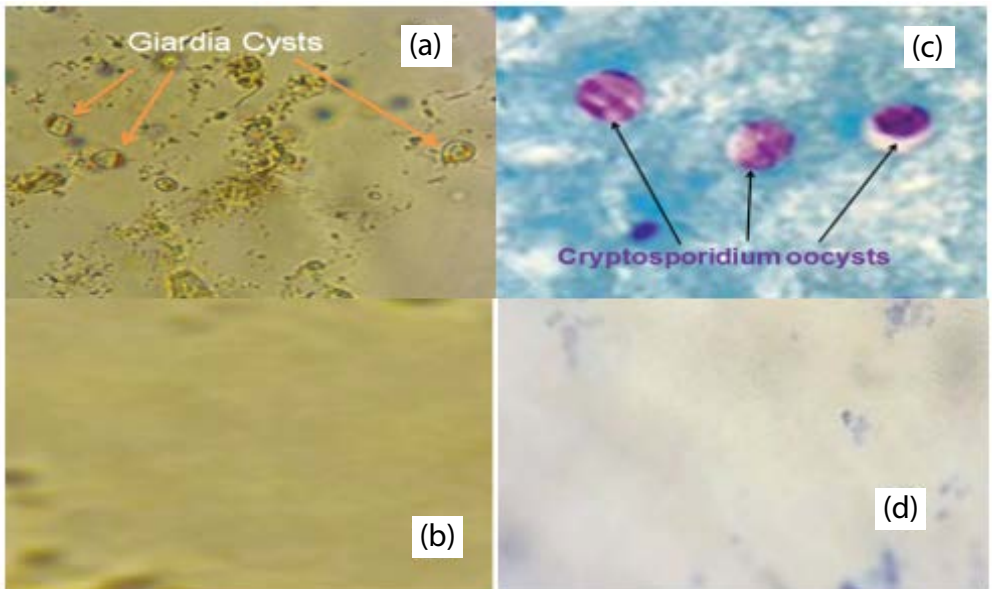

Fig. 3. *Giardia* cyst (a) and *Cryptosporidium* oocysts (c) under 100× oil magnification of a light microscope and control samples (b and d).

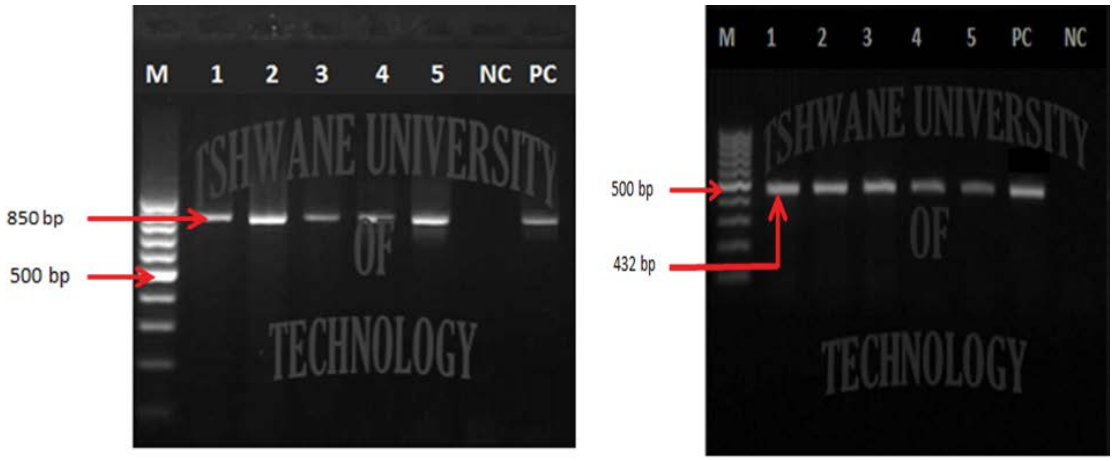

Fig. 4. Agarose gel electrophoresis of PCR products of *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts.

deactivates protozoans is still not well understood. A study by Abebe et al. [48], which investigated the effect of silver ions on *Cryptosporidium* using the mouse model and filtration technique by filtering through the ceramic water filters impregnated with $AgNO_3$, demonstrated that the physical removal mechanism of *C. Parvum* was achieved by size exclusion wherein oocysts are attached or retained in pores. Furthermore, these authors demonstrated that the presence of silver has disinfection effects when there was a decrease in the severity of infection in mice as well as when they identified modifications in the morphology of oocysts as a result of direct exposure to silver. These findings are in agreement with those of the present study whereby contaminated surface water was filtered through the BSZ-SICG filter after which the treated water was found to be free from *Cryptosporidium* oocysts and *Giardia* cysts. The observations were further confirmed with the molecular technique and neither *Cryptosporidium* oocysts nor *Giardia* cysts were detected in treated water (Table 3 and Figs. 4 and 5). However, in this current study, the removal of *Cryptosporidium* and *Giardia* by the BSZ-SICG filter could not be attributed to size exclusion, as the pore size of BSZ-SICG was unknown.

In an attempt to further investigate the role of silver ions (Ag^+) in the removal/deactivation of protozoan parasites, surface water samples containing *Cryptosporidium* oocysts and *Giardia* cysts were exposed to $AgNO_3$ impregnated within the granular clay for 1 h before SEM analysis. The SEM results revealed the interaction of Ag^+ with the wall surfaces of both the oocysts and the cysts (Fig. 6). Furthermore, the samples were assessed with TEM to determine the presence of silver ions within the oocysts and cysts. The TEM results revealed a transparent wall of the *Cryptosporidium* oocyst treated with $AgNO_3$ and the silver ion was observed scattered throughout the cytoplasm of the oocyst (Fig. 7). Based on the observations in this

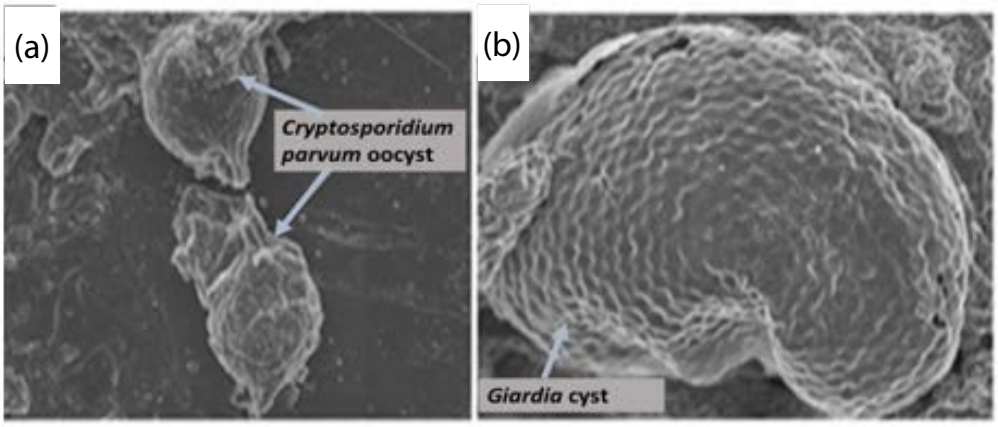

Fig. 5. SEM images of (a) *Cryptosporidium* oocysts and (b) *Giardia* cysts.

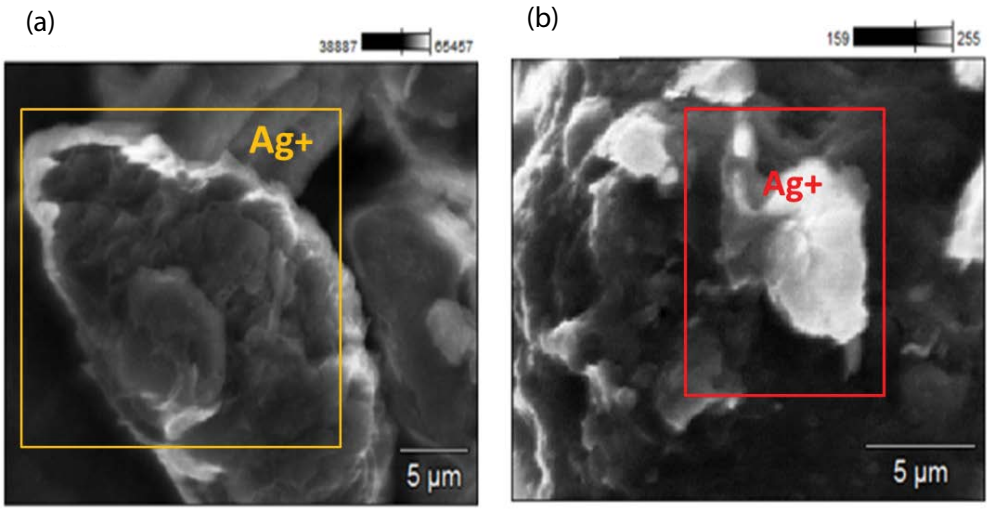

Fig. 6. The action of Ag^+ on *Giardia* cyst walls and *Cryptosporidium* oocyst walls as observed under the SEM (a) and (b) *Giardia* cyst walls and *Cryptosporidium* oocyst walls, respectively, treated with 0.1 mg/L $AgNO_3$.

current study, the action of silver on *Cryptosporidium* and *Giardia* may be described as the ability of the silver ions to attach to the walls of oocysts and cysts, after which they pass through the thick walls and once inside the cytoplasm, they cause damage to the oo(cyst) contents and also destroy the sporozoites within the oocysts. This mode of action was observed with *E. coli* whereby silver nanoparticles were able to pass through the outer membrane resulting in the leakage of cellular contents [28]. Although the cell wall of *E. coli* differs from that of the oocysts and cysts in that the cytoplasm of the bacteria hs aqueous solution that easily leaks out, the contents of both cells are flimsy. It could be that the cysts and oocysts cytoplasm leaks out to resulting in cell death, this means that silver ions can also destroy the oocysts and cysts after passing through their walls and entering the cytoplasm due to cell lysis.

5. Conclusion

It is well documented that *Cryptosporidium* and *Giardia* sp. are commonly found in surface water sources because surface water is more vulnerable to direct contamination from sewage discharges and runoff. Moreover, it is also well known that these two species are resistant to most of the disinfectants including chlorine, which is widely used in water treatment plants. The underserved rural communities are at risk of diseases associated with the consumption of water contaminated with *Cryptosporidium* and *Giardia* sp. In this study, it was found that *Cryptosporidium* and *Giardia* sp. in untreated surface water by far exceeded the recommended limits set by SANS 241, WHO and the United States Environmental Protection Agency for drinking water. The results further showed that BSZ-SICG filters are effective in removing the *Cryptosporidium* oocysts and *Giardia* cysts from surface water. These filters produce drinking water that is safe for human consumption. The incorporation of silver in granular clay is crucial to increase the mode of action of the silver in the destruction of the oocysts and cysts of *Cryptosporidium* and *Giardia*. Therefore, the present study recommends the implementation of the BSZ-SICG filters in the underserved communities of developing countries where access to safe drinking water remains a dream.

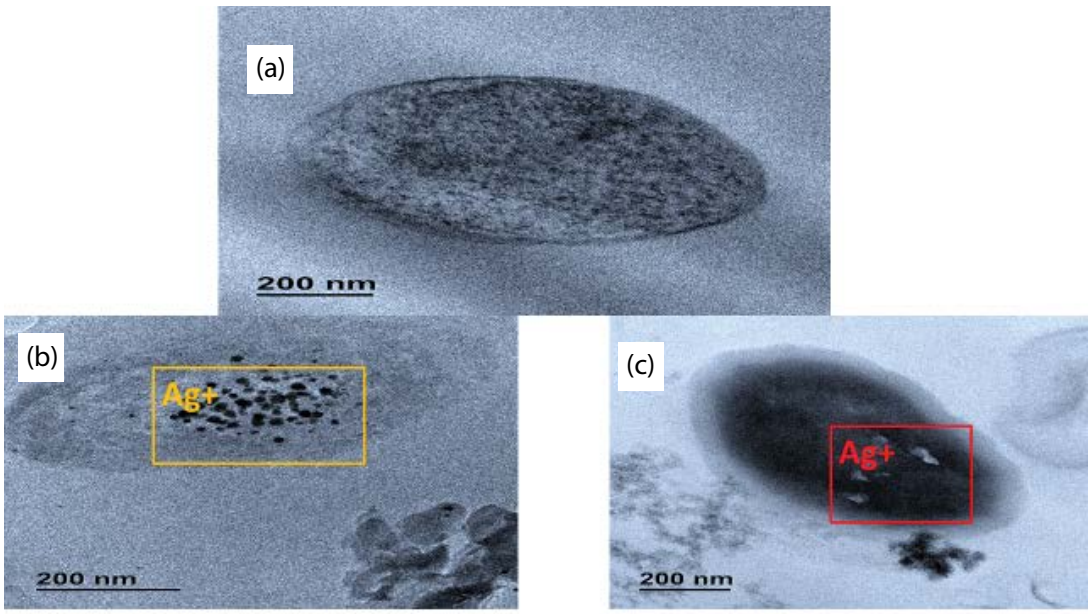

Fig. 7. The action of Ag^+ on *Cryptosporidium parvum* oocyst walls as observed under TEM (a) structure of native oocyst wall (control without $AgNO_3$), (b) and (c) structure of *Cryptosporidium* oocyst wall treated with 0.1 mg/L of $AgNO_3$ showing silver ions within the cytoplasm.

Author contributions

Resoketswe Charlotte Moropeng and Maggie NB Momba conceived and designed the experiments; Resoketswe Charlotte Moropeng performed the experiments; Resoketswe Charlotte Moropeng and Maggie NB Momba analyzed the data; Resoketswe Charlotte Moropeng wrote the paper.

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