

Parameters for effective sand filtration of *Schistosoma mansoni* cercariae from water

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ABSTRACT

Schistosomiasis is a water-based neglected tropical disease that is prevalent in over 78 countries. It affects communities that are reliant on freshwater bodies contaminated with Schistosome cercariae for their daily water activities. Whilst treatment with the drug praziquantel is relatively effective, it does not prevent reinfection. One option for reducing schistosomiasis infection is providing at-risk communities with treated water, thereby reducing contact with cercaria-infested water for activities such as bathing or doing laundry. This study aims to establish design guidance for sand filtration to remove schistosome cercariae from water. Four sand filters were tested, varying from 300 to 2000 μm in sand grain size. Each filter was tested with a sand depth of 20 cm, which was increased until no cercariae were detected in the effluent. The required filter depth to remove 100% of cercariae ranged between 40 and 70 cm depending on sand grain size. Cercaria removal was more effective in filters with smaller sand grain size and larger filter depth. These results are valid for intermittent flow, for up to six cycle flushes. While more rigorous testing is needed, these initial results suggest that sand filters can be an effective way to treat cercaria-contaminated water in low-income settings.

Key words: cercariae, filtration, NTD, Schistosomiasis, WASH, water treatment

HIGHLIGHTS

- These experiments have determined what sand grain sizes and bed depths are required to remove *Schistosoma mansoni* cercariae from water.
- Results suggest that sand filters can be an effective way to treat cercaria-contaminated water, such as water collected from lakes or rivers, in low-income settings.

INTRODUCTION

Cercariae are the infectious larvae of the *Schistosoma* parasite. They are released into the water by freshwater intermediate host snails and will penetrate a human host's skin to develop into adult schistosome worms, resulting in schistosomiasis infection (Colley *et al.* 2014). Schistosomiasis is primarily found in sub-Saharan African, with *Schistosoma mansoni*, *S. haematobium* and *S. japonicum* being the three main species infecting humans (Gryseels *et al.* 2006). One way of reducing disease transmission is through water treatment, which removes or inactivates cercariae to provide safe water for people to use as an alternative to infested lakes or rivers (Jordan & Unrau 1978; Kosinski *et al.* 2012; Evan Secor 2014; Braun *et al.* 2018; Mogeni *et al.* 2020). Water treatment should remove or inactivate all cercariae, as only two cercariae are required for an active schistosomiasis infection. A recent review about water treatment and schistosomiasis highlighted that filtration may be an effective process for removing cercariae from water, but showed some variability between studies (Braun *et al.* 2018).

Filtration is a water treatment method that works by physically retaining particles while allowing water to pass. Filters can retain solid matter much smaller than the pores of the filter material due to physical and biological processes (Kristian Stevik *et al.* 2004). Granular filters, which use sand or other granular material, are suitable for use in less developed areas because the filter media are often locally available, and no external power input is required to pass the water through the filter. Sand

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filters are often installed as part of a chain of water treatment steps either as pre- or post-treatment but can also be used as a standalone treatment system, especially at household scale (Verma *et al.* 2017).

Filters can be categorised into slow sand and rapid sand filters, depending on the filtration rate (approximately 0.1 m/h vs 5 m/h, respectively) (World Health Organization 2003; Verma *et al.* 2017). In slow sand filters, a biologically active *Schmutzdecke* (a biological layer on the surface of the filter) develops over time which uses organic matter as a food source and also contributes to pathogen reduction, thereby further treating the water (Campos *et al.* 2002). Rapid sand filtration uses coarser sand and requires a disinfection step (e.g. chlorination or UV disinfection) if water is intended for drinking. Intermittently operated slow sand filters are known as bio-sand filters, and are recommended as a point-of-use technology by the World Health Organization (World Health Organization 2012).

Sand filters primarily remove pathogens through physical straining and adsorption, as well as biological removal in the case of slow sand filters (Adin 2003; Kristian Stevik *et al.* 2004). Straining physically blocks pathogens from moving through the filter and is therefore influenced by sand grain size, pathogen size, water saturation (in case the filter is not fully saturated before adding water) and clogging of the filter (Kristian Stevik *et al.* 2004). Sand grain size and particle removal are inversely proportional (Gerba & Bitton 1994) as filters with smaller sand grains have smaller pores, making it more difficult for particles to pass (Muhammad *et al.* 1996). It has been suggested that effective removal occurs when pathogens are greater than 0.2 times the sand grain size (Bouwer 1984), i.e. sand grain size approximately <300–1800 µm for Schistosome cercariae, based on cercaria dimensions (60 µm wide × 360 µm long (Manson-Bahr 1920). Non-uniform grain size is favourable, as this can create smaller pores (Kristian Stevik *et al.* 2004; Keraita *et al.* 2008). Similarly, irregular or rod-like shaped pathogens have been found to be more easily removed (Weiss *et al.* 1995), suggesting that the forked-tail Schistosome cercariae may have enhanced removal, assuming the tail stays attached. High flow rates have been shown to reduce filter efficiency, especially in unsaturated filter medium (where the pores are occupied by air after the water has passed through), as this increases transport through larger filter pores. For this reason, water should be applied uniformly to filters. Clogging is caused by biomass growth on the filter and can significantly increase the filter effectiveness by reducing pore size. However, clogging can reduce the filtration rate and lead to anoxic conditions (McDowell-Boyer *et al.* 1986; Kristian Stevik *et al.* 2004). Adsorption, in the context of filtration, is described as the adhesion of pathogens or particulate matter to the sand grain surface. It is affected by pore size, organic matter content, biofilm, and filtration rate, amongst many other factors that influence the adhesion potential (Kristian Stevik *et al.* 2004).

A recent review about water treatment and schistosomiasis summarized the results of existing filtration experiments using Schistosome cercariae (Braun *et al.* 2018). Overall, no reliable conclusions could be drawn regarding the filter design (depth and grain size). However, studies were in agreement that slow filtration (0.04–0.19 m/h) retained more cercariae than rapid filtration (0.27–0.4 m/h), as cercariae were not washed through the filter (Kawata 1982; Fadel 1993). These studies also showed that ripening of filters to develop a *Schmutzdecke* can improve the overall efficacy of sand filters.

This research aims to determine what sand grain sizes and bed depths are required to achieve consistent *Schistosoma mansoni* cercaria removal, and what the corresponding filtration rates are. It also determines how far cercariae travel through filters of different grain sizes.

MATERIALS AND METHODS

Four filters with varying sand grain size and filter depth (Figure 1) were tested in this study for their effectiveness in removing *S. mansoni* cercariae.

Sand preparation

Sand was collected from the shore of Lake Victoria in Mwanza, Tanzania and allowed to dry on large plastic sheets. It was manually sieved using soil sieves (300, 425, 500 and 710 µm). This produced three grades of sand size: 300–425, 425–500 and 500–710 µm, making up the three graded filter types in this study. A fourth filter with 'mixed' sand was also tested, which consisted of sand collected from the field that was only passed through a 2000 µm sieve. Therefore, this sand had sand grain size of <2000 µm. Soil analysis of 100 g (repeated twice) of mixed sand is shown in Table 1. Additionally, fine and coarse gravel was purchased from building supply providers and sieved with a 2, 6 and 12 mm sieve. Gravel between 2 and 6 mm was used as fine gravel, and between 6 and 12 mm as coarse gravel.

Table 1 | Soil analysis of 100 g of sand collected from the shore of Lake Victoria

Sand grain size (μm)	<300	300–425	425–500	500–710	710–2,000
Content (%)	27.4%	28.2%	5.4%	9.0%	30.0%

The content is the percentage of sand (by mass) in the given sand grain size category. Results are the average of two soil analyses.

Filter preparation

Filters were constructed using locally available materials. Four 110 mm diameter PVC pipes were cut to 120 cm and capped at one end, sealed with silicone. A 6 mm hole was drilled into the pipe, 3 cm from the sealed end, into which a 6 mm plastic hose was slotted and sealed with silicone (a rubber *suba*-seal was used in some cases). The filters were filled with enough water to cover the hose and checked for leaks, which were sealed with additional silicone. Once the filters were fully sealed, the drainage layer was added to the filter, consisting of 7 cm of coarse gravel (5 cm above the hose outlet) followed by 5 cm of fine gravel (Figure 1). These layers are crucial for preventing the sand from clogging the hose. The filter was gently shaken to pack the gravel more closely. The depth of the filter from the surface of the gravel was subtracted from the height of the pipe to indicate the depth of the gravel drainage layers. The filter was then filled with 20 cm of sand, which was the lowest sand depth tested. Again, the filter was gently shaken to pack the sand and reduce pore size. The filters were then rinsed with 10 L of bottled water (Jibu brand), after which the effluent water ran clear. The depth was re-measured and the filter was topped up to 20 cm sand depth, and rinsed again with 10 L of bottled water. Finally, a 2 mm sieve was placed on top of the filter to act as a diffuser to ensure even distribution of the influent water onto the filter surface.

Cercaria preparation

Biomphalaria sudanica snails (intermediate hosts in this area) were collected from a transmission site at Kigongo Ferry, Lake Victoria, Tanzania (approximately 2°42'47.6" S 32°53'37.0" E). Snails were placed in 12-well multi-well plates (Corning) filled with 5 mL bottled water and exposed to light for 30 minutes, to induce cercarial shedding. Unless otherwise stated, all water used in experiments was Jibu bottled water. The plates were examined under a stereomicroscope and infected snails shedding only *S. mansoni* cercariae were used for experiments. Infected and uninfected snails were kept separately at ambient temperature (average 27.2 °C) and fed with dried lettuce every other day. Uninfected snails were checked every week for infection. 24 hours prior to the experiment and 10 actively shedding snails were placed in the dark to allow for heavier cercaria production. Snails were rinsed with bottled water, transferred to a 50 mL glass beaker containing 30 mL of bottled water, and placed under a 5 W LED lamp. After 60 minutes the snails were removed and three 200 μL aliquots were taken by pipette. Fifteen μL of Lugol's iodine solution was added to each aliquot. This instantly stained and killed cercariae, which could then be easily counted under a stereo microscope. The average cercaria concentration was calculated and the cercariae were used immediately for experiments. If there were <70 cercariae/mL the snails were exposed to light for a further 15 minutes to increase the number of cercariae. The average cercaria concentration was 95 ± 10 cercariae/mL. The 30 mL cercaria sample was added to a glass beaker containing 970 mL of non-chlorinated bottled water and stirred with a glass rod, thereby bringing the total volume to 1 L.

Filtration

While the snails were being exposed to light, the filter was primed by allowing 1 L of bottled water to wet the sand. This ensured that the filter had the same condition at the start of each experiment. The timer was started when the 1 L of cercaria sample was carefully added to the filter (via the 2000 μm sieve previously used to grade sand, to enable equal distribution of water). The effluent was collected in a large glass beaker, via the 6 mm hose fitted to the filter assembly. Once all the effluent had been collected, the time was recorded. The second cycle was started by adding 1 L of bottled water to the filter and restarting the timer. In the meantime, the effluent of the first cycle was carefully concentrated by passing it through a 20 μm Pitchford funnel which allowed water to pass through but retained the cercariae. The home-made funnel follows the design of Pitchford & Visser (1975), consisting of a cylindrical bag made from 20 μm mesh nylon (PlastOk Ltd, UK) attached to a solid funnel with a tap at the base. Once all water was passed through the Pitchford funnel's nylon mesh, a wash bottle was applied from the outside and inside of the funnel to wash down any cercariae on the mesh into the basal funnel. The Pitchford-funnelled effluent, now reduced to approximately 20 mL, was poured into a 25 mL glass beaker via the funnel

tap. With the tap open, the funnel was rinsed with the wash bottle once more and water was allowed to flow into the beaker. Finally, the cercariae in the effluent were counted under a stereo microscope.

The sand filter was filled six times in total, once with cercaria water (time 0) and five times with 1 L of bottled water. The cercariae in the effluent were counted after each cycle, thereby giving a profile of cercaria numbers in the effluent over time. Each condition (sand depth and grain size) was repeated three times. After each experiment, the filter was stored dry for 48 hours to ensure any cercariae left in the filter were dead. The filter was then flushed through with 5 L of bottled water. Once a filter condition had been tested three times, 10 cm of sand was added to the filter depth, unless more than 100 cercariae were carried through to the effluent, in which case 20 cm of sand were added. The filter depth was increased until no cercariae were present in the effluent of any of the three replicates. The depths for 100% cercaria removal were replicated using water collected from Lake Victoria. Cercaria age (time from shedding) was on average 2.0 ± 0.5 hours, and ambient temperature was on average 27.2 ± 2.9 °C. Filtration rates are calculated by dividing the flow rate (m^3/h) by the surface area of the filter.

Quality control

Initially, the 1 L effluent volume was split into five 200 mL volumes and analysed under the microscope for cercariae. However, the volumes were too large to accurately count cercariae. Therefore, the effluent was reduced to 20 mL using a Pitchford funnel which significantly facilitated sample analysis. To confirm that cercariae could not pass through the 20 μm Pitchford funnel and did not remain on the filter mesh, ten 1 L water samples containing between 50 and 200 cercariae were passed through the Pitchford funnel. The funnel was rinsed as described above, and the number of cercariae were counted in the reduced 20 mL effluent. Results indicate that approximately $96 \pm 3\%$ of cercariae were retained in the effluent.

Macropores are large pockets of air in the filter media which allow movement of pathogens through the filter. To reduce macropores, the filters were tapped on the ground to compress the sand. They were rinsed with 10 L of water which further compressed the sand. The sand was topped up to the correct amount of filter depth and re-rinsed. Pre-tests (data not included) conducted at the Natural History Museum in London used clear PVC pipes and showed no visible macropores on the outside of the filter.

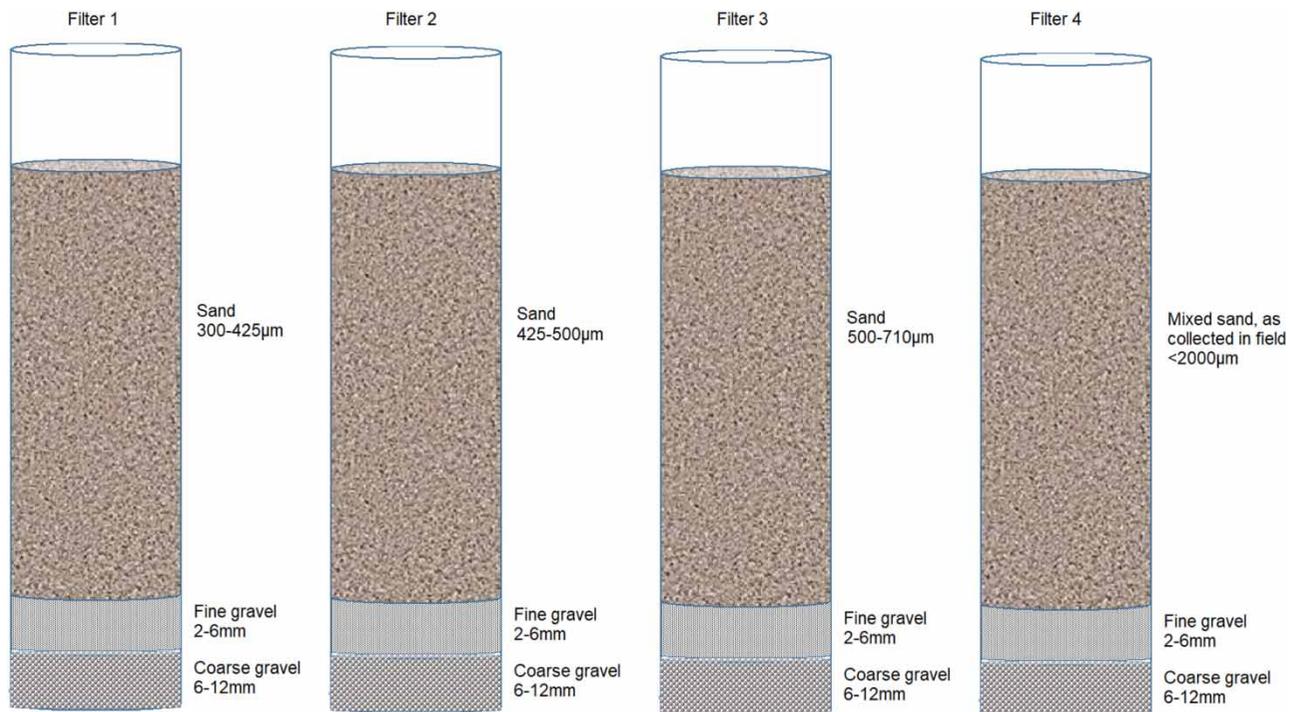


Figure 1 | Filter set-up of the four filters tested. Each filter had a drainage layer consisting of fine and coarse gravel. Filters 1–3 contain graded sand, and filter 4 contains mixed sand.

RESULTS AND DISCUSSION

These results are from experiments were conducted at the National Institute for Medical Research in Mwanza, Tanzania. Although experiments were also replicated at Addis Ababa University in Ethiopia, the data are not included as they include tests involving non-human schistosome cercaria species.

The results in Figure 3 indicate that all filters can effectively remove *S. mansoni* cercariae. The lowest depth, 40 cm, was required for the most finely graded sand (300–425 μm), whereas 70 cm were required for the coarsest sand (500–710 μm), as anticipated. Mixed sand, which was ungraded sand collected from the shore of Lake Victoria, performed well, with 50 cm of sand being enough to remove cercariae in all three replicates. This is even though mixed sand contained 30% sand grains exceeding 710 μm . The effectiveness of the mixed sand highlights the improved efficiency associated with ungraded sand. This is an important result, as it indicates that sand from schistosome endemic regions such as Lake Victoria can be directly used to build filters for treating water for cercaria removal.

The filter profiles in Figure 2 show how cercariae travel through the filters. The shallower the filter and coarser the sand, the sooner the cercaria numbers peak in the effluent (e.g. for 425–500 μm filter at 20 cm depth, cercaria numbers peak at the fourth cycle). The fact that the effluent does not show peak cercaria numbers in mixed sand or 300–425 μm sand at 20 cm depth suggests that the filters were potentially not operated for long enough. If additional water would be flushed through

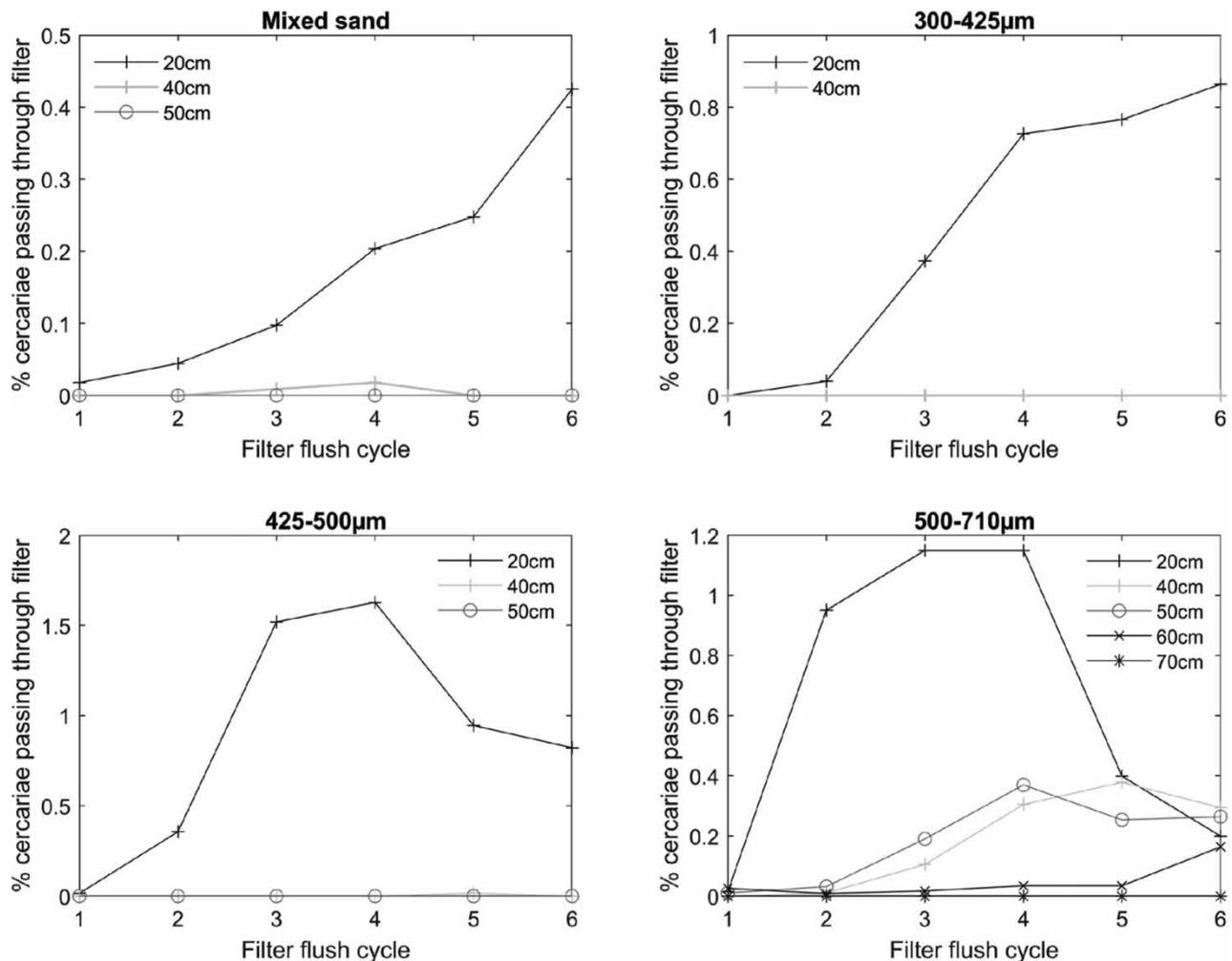


Figure 2 | Percentage of cercariae passing through the filter. Four sand filters were tested with varying sand grain size: mixed, 300–425 μm , 425–500 and 500–710 μm . Mixed sand consisted of sand grains as shown in Table 1. Each filter was tested for a sand depth of 20 cm. The depth was increased until no cercariae were detected in the effluent in any of the three replicates. The sand depths are shown in the legends. Percentage of cercariae passing through the filter is calculated as the number of cercariae in the effluent over cercariae in the influent. Influent in cycle 1 contained on average $2,857 \pm 313$ cercariae, all other cycles contained bottled water only.

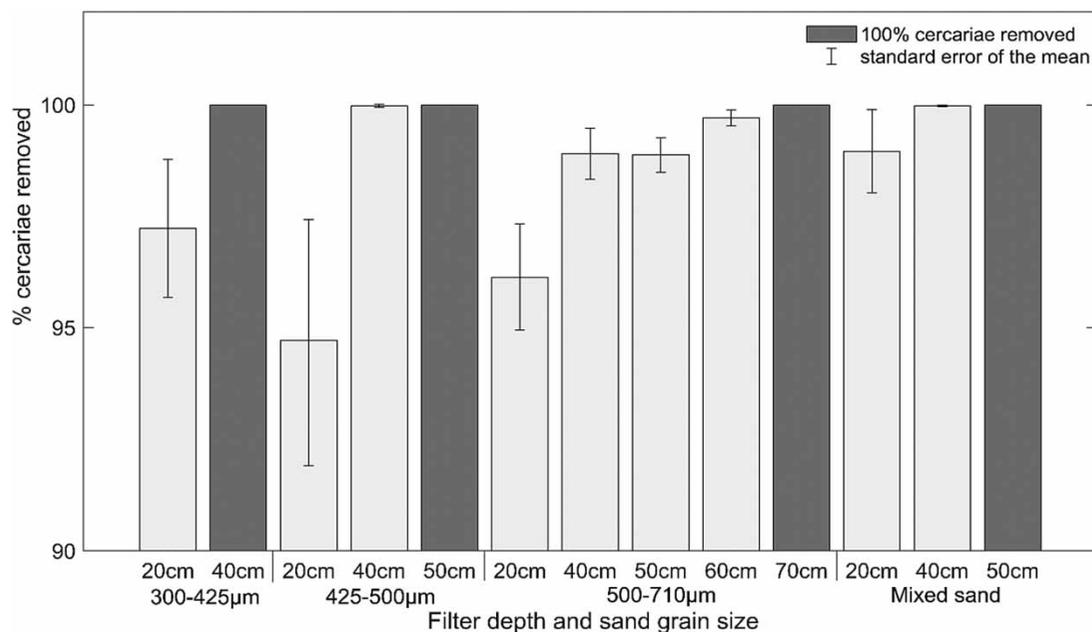


Figure 3 | Cercaria removal in the different filters. Four sand filters (varying sand grain size) were tested for filter depths between 20 and 70 cm using bottled water. Results are average cercaria removals based on three replicates, and error bars are standard errors of the mean. Columns highlighted in dark grey are the filter depths required for 100% cercaria removal for each of the four filters. These 100% removals were confirmed using natural water collected from Lake Victoria.

the filter, then cercariae could possibly still be present in the effluent. However, the aim of this research was to determine which filter conditions removed all cercariae, and therefore there was less focus on exploring ineffective filter depths. Results for 100% removal are only valid up to six cycles, and therefore the filter conditions should be tested over longer periods of time (i.e. exceeding six filter flush cycles). Filters should also be tested for both continuous and intermittent flow.

Given their size, cercariae are likely removed through straining and adsorption. Cercariae produce sticky secretions when damaged or under stress (Howells *et al.* 1974). This secretion may increase the effectiveness of filters, especially if cercariae become damaged by the sand. Future experiments could examine the cercariae in the effluent or backwash for visible damage (e.g. membrane rupture, deformation, tail loss).

Filters were primarily tested using bottled water to control the water matrix. Although this is an unrealistic water condition in the field, it presents the worst case as low-turbid water leads to less clogging and therefore reduced effectiveness. Given that the sand grain sizes and filter depths in Table 2 could remove all cercariae in bottled water suggests that filters would also achieve these removals using natural, more turbid water. Experiments using natural water from Lake Victoria indicate that results for 100% removal are valid under both water conditions as no cercariae were detected in the effluents.

The aim of these filtration experiments was to determine which filter conditions remove all cercariae, as only two cercariae are required for an active schistosomiasis infection. Therefore, results are shown in percent removal to indicate which filters removed all cercariae, as opposed to \log_{10} removal as is commonly used in water treatment. One-litre influents contained on average 2857 ± 313 cercariae, equating to \log_{10} removals of at least 3.5-log. Although the filters showed 100% removal, it must be noted that the cercaria enumeration using the Pitchford funnel is only $96 \pm 3\%$ accurate, and that these results are based on three replicates only.

Table 2 | Filtration rates of the four tested filters

Sand grain size (µm)	300–425	425–500	500–710	Mixed (300–2,000)
Filtration rate (m/h)	0.46 ± 0.02	0.54 ± 0.05	0.68 ± 0.08	0.54 ± 0.04
Sand depth for 100% cercaria removal (cm)	40	50	70	50

Rates are calculated by dividing the flow rate (m^3/h) by the surface area of the filter.

The sand grain size and filtration rates of the four tested filters match the typical characteristics of a slow sand filter more closely as opposed to a rapid sand filter. However, the crucial difference is that the tested filters were not ripened, i.e. the filters were not operated for several weeks before the experiments to develop a *Schmutzdecke*. It is likely that the filters would operate more effectively if they were conditioned with a biological layer, as highlighted in the systematic review (Braun *et al.* 2018). The effect of a *Schmutzdecke* could be examined in future experiments that test natural water. This study focused on parameters that can be easily controlled (sand grain size, filter depth) to determine suitable filter designs for cercaria removal in unconditioned filters. It is assumed that this is the worst-case condition, as the development of a biological layer over time would likely decrease pore size and thereby increase cercaria removal (whilst keeping in mind the negative effects of clogging on filter operation).

There is a risk that cercariae passed through sand filters but were not captured in the enumeration process. This may be due to cercariae attaching to the plastic hose, and therefore the hose pipe length was reduced to a minimum. It may also be due to cercariae attaching to the Pitchford funnel. An alternative to reducing the filter volume using a Pitchford funnel would be to kill the cercaria in the one-litre effluent, for example with chlorine or iodine. Inactivated cercariae sink to the bottom, allowing the top layer of cercaria-free water to be poured or pipetted off (Braun *et al.* 2020). The remaining effluent can then be examined under the microscope. This method would reduce the effluent volume without the use of a Pitchford funnel, and therefore reduce the potential error in cercaria numbers.

The filters remain to be extensively tested under real world conditions. It should be noted also that these experiments only considered filters operated in intermittent mode, as might be the case in household-scale filters, rather than continuous flow. Future experiments should test different filtration rates and different natural mixed sand (e.g. from riverbeds or lakeshores in endemic communities). Operating times of several weeks could test the impact of the *Schmutzdecke* and if cercariae are washed through after longer periods of operation. In addition, the results should be confirmed for different *Schistosoma* species as the respective cercariae vary in size and may hence influence filtration effectiveness.

CONCLUSION

All four filters could remove 100% of cercariae which demonstrates that sand filters can effectively remove *S. mansoni* cercariae in water. The required filter depth for 100% removal ranged between 40 and 70 cm depending on the sand grain size. Further experiments, focusing on longer operating times and continuous versus intermittent flow, are required to develop these initial results into robust design recommendations.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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