Household slow sand filter to treat groundwater with microbiological risks in rural communities

F. C. Andreoli [a], L. P. Sabogal-Paz [a, b]

[a] Department of Hydraulics and Sanitation, São Carlos School of Engineering, University of São Paulo, 400 Trabalhador São-carlense Avenue. Zip code: 13566-590. São Carlos, São Paulo. Brazil.

[b]e-mail: lysaboga@sc.usp.br

Abstract

Household slow sand filters (HSSFs) improve the quality of life in rural communities as they provide safe water. However, HSSFs require time for the growth of the biological layer (*schmutzdecke*) to achieve maximum performance, especially when groundwater is used as it normally has few nutrients. In this ripening period, pathogenic microorganisms can pass through the filter. In this context, this study reports the performance of two HSSF settings, intermittent (I-HSSF) and continuous (C-HSSF) flows followed by disinfection with sodium hypochlorite to treat groundwater with *Escherichia coli*, *Giardia muris* cysts and *Cryptosporidium parvum* oocysts. The weekly introduction of river water was tested as a filter-ripening agent and this procedure reduced the ripening time in approximately 80 days. Filtered water disinfection improved the water quality and inactivated protozoa. The costs and operational challenges addressed in this study can provide support to HSSF technology transfer in rural communities worldwide.

Keywords: social technology, pathogens, schmutzdecke, disinfection, drinking water, slow sand filtration.

1. Introduction

Almost a fifth of the world's population does not have access to water supply services, mainly in rural areas and suburban regions (Pinderhughes, 2004). Untreated groundwater catchment for consumption is common in these areas. Precarious conditions of use and conservation of wells can contaminate drinking water, causing health problems to the supplied community. Water treatments in a household level are essential to ensure human rights to safe water established by the United Nations (UN) (UNGA, 2015).

Consumption of contaminated water usually leads to diarrhoea that can cause approximately 485,000 deaths per year in low- to medium- income countries (WHO, 2019). Diarrhoea and pneumonia are the two main causes of infant mortality (WHO, 2013). The presence of pathogens (i.e. protozoa, bacteria and viruses) in drinking water is a health risk, especially regarding the adverse events caused by climate changes, which represent a threat from outbreaks of infectious diseases transmitted by water (Efstratiou et al., 2017). Protozoa, more specifically *Giardia* spp. and *Cryptosporidium* spp., are responsible for gastrointestinal diseases with outbreaks recorded in several locations worldwide. Efstratiou et al. (2017) observed 82% of the documented outbreaks between 2011 and 2016, reporting water supply system problems as the transmission route.

According to Jadhav et al. (2015), a household water treatment system can reduce the risk of waterborne disease transmission, as well as best practices, such as water source protection and safe storage. Household water treatment should be

accessible to anyone, easy to operate and maintain, as well as culturally accepted. Das et al. (2015) reported the difficulty of breaking habits and convincing communities to accept interventions for groundwater treatment when untreated water appears visually clear. Among the efforts for developing technologies adapted to local contexts is the so-called Point-of-Use (POU) (Sobsey et al., 2008) or Household Water Treatment and Safe Storage (HWTS) (Eawag and Sandec, 2008).

In the 1990s, David Manz adapted the conventional slow sand filter for household level and intermittent flow, which resulted in the Household Slow Sand Filter (HSSF). This technology has been spread by several organizations and over 300,000 HSSFs have been installed in more than 69 countries (CAWST, 2012).

HSSF consists of combining physical and biological processes. According to Jadhav et al. (2015), physical mechanisms include the retention of larger particles in the pores of the filter medium and adsorption, which enable organic removal, whereas the biological mechanisms include predation, elimination, natural death/inactivation, and partial reduction of organic carbon due to the microorganism metabolism. Therefore, HSSF treats water by combining physical processes with biological activity present on the sand filter surface (*schmutzdecke*). Nevertheless, achieving optimum technology performance requires a ripening period for the biological layer growth, especially when groundwater is used. In general, groundwater has few nutrients when compared to surface water (Maciel and Sabogal-Paz, 2020). During the ripening time, the treatment efficiency is reduced, thus increasing the risk of pathogens passing through the pores of the filter media. Speeding up of the ripening period in HSSF to treat groundwater is essential for safe water supply. However, ripening poses as a challenge, especially considering simple solutions adapted to isolated communities in developing countries (Calixto et al., 2020). According to Bradley et al. (2011), HSSF can significantly

increase the ripening time (i.e. up to 200 days), when groundwater is treated. It should be noted that the HSSF performance changes due to filter cleaning as biological layer growth is required. Thus, filtered water must be disinfected for both the ripening period and complete maturing.

Studies have aimed at optimizing HSSF performance, introducing biomass in the filter layer (Baig et al., 2011; Zhang et al., 2016; Calixto et al., 2020) or iron oxide (Tellen et al., 2010; Bradley et al., 2011; Ahammed and Davra, 2011). Comparisons are also made between different flows e.g. continuous and intermittent (Young-Rojanschi and Madramootoo, 2014; Maciel and Sabogal-Paz, 2020; Sabogal-Paz et al 2020) and the structure of the filter body in different materials, such as concrete and plastic (Stauber et al., 2012; Arnold, 2015).

It is important to highlight that stored filtered water can be contaminated due to hygiene habits and the type of storage used by the user, as observed by Duke et al. (2006), therefore, disinfection of drinking water is required. Specifically in household disinfection with chlorine, up to 6 log of bacteria or viruses and up to 5 log of some protozoa can be removed, as long as the water turbidity remains below 1.0 uT (WHO 2017). WHO (2019) highlights the importance of specific chlorine dosage in water for each location based on the chlorine demand in the water to be treated and also monitoring to ensure the maintenance of free chlorine concentrations between 0.2 and 0.5 $mg.L^{-1}$.

Chlorination with sodium hypochlorite is commonly used for water disinfection; however, this technology generates by-products and is not always accepted by isolated communities due to changes in both taste and odour of water (Schmidt and Cairncross, 2009). This is often aggravated when chlorine is incorrectly applied (i.e. excessive dosage) by the population.

Boiling water is a common technique in rural communities to obtain safe water (Clasen et al., 2008); however, the volume of treated water is limited (< 5.0 $L.day^{-1}$) and energy consumption (gas, electricity and wood) can be considerable and even economically unfeasible. In this context, HSSF followed by chlorination may be more practical than boiling filtered water. Moreover, chlorinated water remains safe for a longer time.

Considering this background, this study evaluated the performance of two models of HSSFs in full scale and in duplicate (two filters in continuous flow and two filters in intermittent flow) for the treatment of groundwater containing high concentrations of *E. coli*, *G. muris* cysts and *C. parvum* oocysts (situation that can happen, for example, by leaking septic tanks). Two operation phases were evaluated with and without ripening agents (river water) to speed up the HSSF ripening. Ripening consisted of a controlled introduction of river water to the filters for providing nutrients and improved conditions for developing the biological layer in a short time. The ripening agent was selected because it can be easily accessible in rural areas; thus, river water emerged as a possible solution for an exploratory study. When groundwater is used for supply and HSSF as a treatment, rapid biological layer ripening of the filter is essential in order to reduce the microbiological risk of the effluent. In our study, filtered water was disinfected with sodium hypochlorite daily and the microbiological quality was evaluated before and after chlorination. The costs and operational challenges of assembling, using and maintaining the HSSFs were analysed in terms of facilitating technology transfer in rural communities in developing countries.

2. Materials and methods

Four HSSFs were tested (two in continuous flow and two in intermittent flow, in duplicate) as an improved version of the HSSFs investigated by Terin and Sabogal-Paz (2019), including the disinfection system of filtered water using sodium hypochlorite. HSSF had a daily production of 48 L each. HSSFs were tested with and without a ripening agent (river water) for biological layer maturation. Research steps are described in Table 1.

Table 1. Research stages

Stage	Description
1	HSSF construction
2	Characterization of HSSFs with tracer tests
3	HSSF operation: - First phase: without ripening agent - Cleaning - Second phase: with ripening agent (river water)
4	Analysis of results - Statistical analysis - Analysis of operational challenges of the proposed treatment

2.1 Stage 1 - Construction

Modified Polyvinylchloride (MPVC) pipe used in water supply networks, with a nominal diameter of 250 mm, was used to form the HSSF body. PVC pipes and fittings were used to complete the filter structure. The filtering surface was covered with a non-woven synthetic blanket (100% polyester of 2.8 mm thickness). A drawing of the prototypes in continuous (C-HSSF1 and C-HSSF2) and intermittent (I-HSSF3 and I-HSSF4) flows is shown in Figure 1.

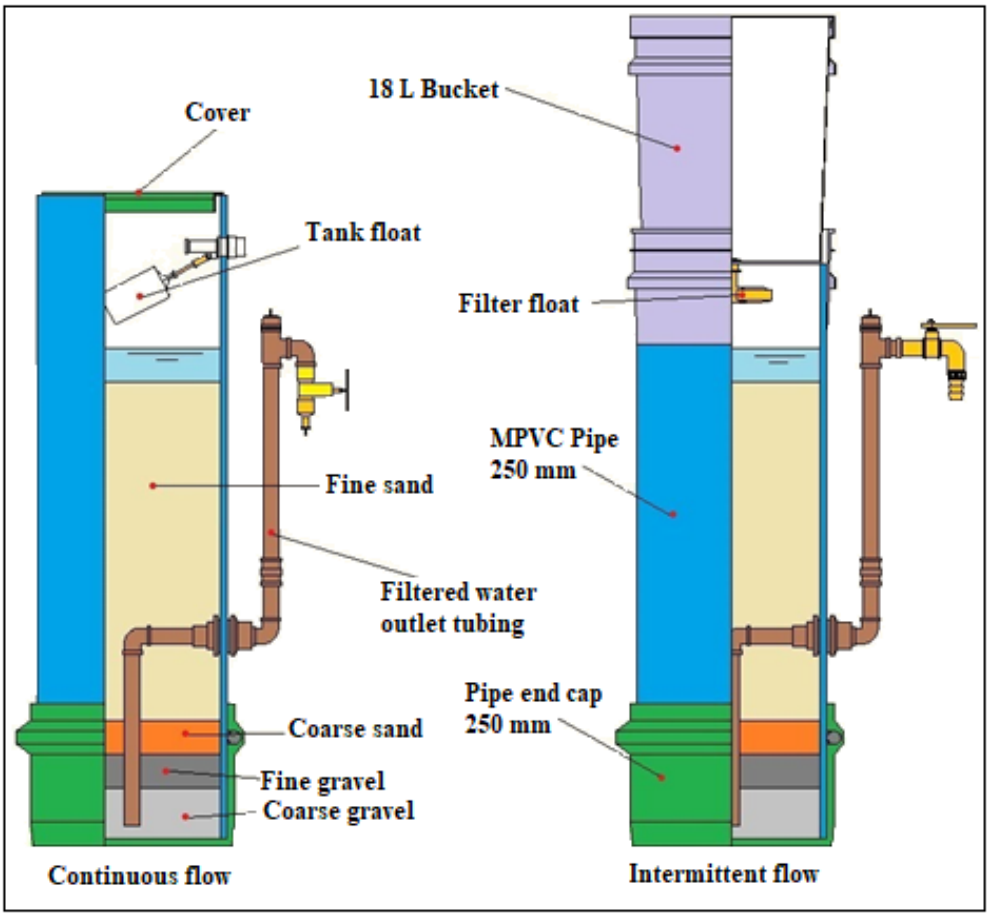


Figure 1. HSSFs in continuous and intermittent flows

Filtering material was obtained from construction local stores, washed with well water, dried in the sun and sieved, as recommended by CAWST (2012). Granulometric tests were applied according to Brazilian standard NBR-11799 (ABNT, 2016). A support layer consisted of 7.5 cm of coarse gravel (12 - 15 mm; 2.6 $g.cm^{-3}$ density and 41 % porosity), 5 cm of fine gravel (5 - 8 mm; 2.7 $g.cm^{-3}$ density and 44 % porosity) and 5 cm of coarse sand (1.5 - 3 mm; 2.6 $g.cm^{-3}$ density and 45 % porosity). The upper layer consisted of 50 cm of fine sand (0.075 - 1.0 mm; D_{10} = 0.19 mm; UC = 2.08; 0.16% < 0.1 mm; 2.7 $g.cm^{-3}$ density and 37 % porosity).

C-HSSFs and I-HSSFs were operated at filtration rates of 0.91 $m^3.m^{-2}.day^{-1}$ and between 0 to 8.64 $m^3.m^{-2}.day^{-1}$, respectively. In order to estimate the total volume of

water stored in the filter, the sum of the stationary water volume (in the upper layer of the filter medium) was added with the void volume of the filter layers and the internal volume of the filtered water outlet pipe. The volume of the layers multiplied by the porosity of the respective materials was considered when calculating the void volume.

After assembling, each empty prototype was weighed. The estimated weight of the filled HSSF was obtained considering the specific masses, volumes and porosities of the filtering material. Material costs used in the filters were calculated according to three different budgets in São Paulo State (Brazil) in January 2020.

2.2 Stage 2 – Characterization

After assembling the filters, tracer tests were conducted to analyse the flow of each prototype. A 100 $mg.L^{-1}$ sodium chloride (NaCl) solution was used as a tracer and the conductivity of the filtered water was recorded by the *Logger Lite* program (*Vernier Sofware & Technology*, USA). The tests were performed in triplicate for each filter.

In the I-HSSF tests, two 16 L feeds of the salt solution were added, followed by two batches of the same volume with water from the well to remove the whole tracer. The tracer *versus* filtrate volume curves were obtained as in Elliott et al. (2008) and Bradley et al. (2011). Moreover, the Morrill dispersion index (MDI) and modified Morrill dispersion index (MMDI) proposed by Lynn et al. (2013) were calculated.

In the C-HSSFs, NaCl solution fed the filter and the recording of the tracer concentration in the filtered water over time enabled the analysis of the degree model, according to Levenspiel (1999). Data were normalized by curve F and derived (curve E) by *Origin 8.6* software (*OriginLab*, EUA). Likewise, the residence time, the correlation of the results, the N-continuous stirred tank reactors (N-CSTRs) and the analysis of the

178 experimental curves for the distribution of hydraulic retention time with the
179 uniparametric theoretical models (small and large dispersion) were also calculated.
180
181 **2.3 Stage 3 – Operation**
182 The 4 HSSFs were fed over 374 days, which were divided into two phases. The
183 first operation phase comprehended the first 246 days and the filters were fed only with
184 studied water. The HSSFs were cleaned and the operation was restarted. The second
185 phase lasted 128 days and included accelerating the growth of the biological layer by
186 replacing the studied water weekly with river water. River water was used as a ripening
187 agent and the equivalent of one void volume was introduced into the filter.
188 Studied water was a mixture of well water and *Escherichia coli* (ATCC 11229).
189 A lyophilized *E. coli* strain was activated, replicated and cultivated periodically in
190 nutrient medium (70122 Nutrient Broth N ° 1 *Sigma-Aldrich*®) for an approximately
191 10^3 UFC.100mL^{-1} daily inoculation in the studied water.
192 A mixture of 200 L of studied water (well water and *E. coli*) was prepared daily.
193 100 L studied water was pumped into an elevated reservoir to supply the two C-HSSFs
194 and the remaining water was reserved for manual feeds of the two I-HSSFs. A needle
195 valve corrected the filtered water flow in the I-HSSFs and these filters were fed 16 L
196 three times a day (i.e. a 14-hour break and two 5-hour breaks), filling the bucket
197 installed on top of each filter (Figure 1).
198 Temperature, filtration rate, turbidity, dissolved oxygen (DO), pH and total
199 organic carbon (TOC) were analysed according to the methodologies of APHA et al.
200 (2012). Microbiological analyses of *E. coli* were performed by the membrane filtration
201 technique with growth in *Chromocult® Coliform Agar* (Merck KGaA).

A disinfection system was joined to the filtered water outlet by a 50 L reservoir and a 2 % sodium hypochlorite solution was dosed towards maintaining 0.5 $mg.L^{-1}$ of residual chlorine daily. The free chlorine concentration was measured using the chlorine reagent kit (HACH-TM, 2105569-BR).

After the first phase, the 4 HSSFs were cleaned to match the conditions for the beginning of the new operation (i.e. without *schmutzdecke*) and the procedure was: i) the non-woven blanket was scraped and the top layer of the sand bed was removed, washed and relocated; ii) the HSSFs were filled with sodium hypochlorite (4.0 $mg.L^{-1}$) and kept in 2 days of contact time; and iii) chlorine was removed by introducing well water for 7 consecutive days.

In the second phase, river water (Espraiado River, São Carlos/SP, Brazil) was introduced to the filters weekly to facilitate the formation of the *schmutzdecke*. Due to possible peaks of turbidity in the river water, a pre-treatment, including sedimentation for 24 h and a passage of river water through a non-woven blanket (100% polyester and 2.8 mm thickness) were applied. This pre-treated river water replaced 16 L of the studied water in each HSSF weekly (i.e. volume corresponding to approximately once the void volume).

Ten days before the end of the first phase, *Giardia muris* cysts and *Cryptosporidium parvum* oocysts were inoculated daily directly into the stationary zone of the HSSFs (i.e. liquid over the fine sand top). Protozoa suspensions were purchased from Waterborne™ (USA). Prior inoculation, a 5 µL aliquot of each suspension was evaluated under a microscope (BX51, Olympus®) in triplicate to know the number of (oo)cysts to inoculate approximately 500 (oo)cysts per each HSSF.

A sample concentration was performed by membrane filtration for protozoa detection in filtered water, according to a protocol described in Franco et al. (2016). In

this case, samples passed through a membrane of mixed cellulose esters (3 μm porosity and diameter 47 mm), which were scraped with Tween 80 (0.1 % heated to 45º C). Collected content from the membrane was centrifuged at 1500x*g* for 15 min. Next, the supernatant was discarded, leaving 0.5 mL; the pellet was resuspended, 50 μL aliquot of the resuspended pellet (0.5 mL) was prepared on microscopic slides, and consequently a multiplication factor of 10 for each (oo)cyst detected was applied. Slides were evaluated using a commercial kit (Merifluor®, Meridian Bioscience, USA) with an addition of 4',6-diamino-2-phenyl-indole (DAPI) (Fluoroshield™ *Sigma-Aldrich*®) and propidium iodide (PI) (*Sigma-Aldrich*®). The (oo)cysts were counted by fluorescence microscopy (BX51, O*lympus*® microscope) combined with confirmatory tests in DAPI and differential interferential contrast (DIC).

An analytical quality assay validated the protozoa detection method when evaluating the target organisms' recoveries, according to USEPA (2012). Four 1 L filtered water samples were inoculated with a known number of (oo)cysts using an *AccuSpyke® kit* (WaterborneTM, USA), and a blank test. Recovery percentages were calculated by the proportion between the quantified organisms and those present in the sample.

Protozoa inoculation and reduction efficiency in HSSF started on the last days of the first phase (ripe filter and in better operating conditions) and during the whole second phase. Protozoa inactivation by chlorine disinfection was evaluated in the last two months of HSSF operation in the second phase.

The (oo)cyst viability was estimated according to the permeability of the parasite membrane using PI as vital dye. In each test, the filtered water produced in one day by each filter (48L) was divided into two equal parts (24 L) and one of them received chlorine dosage to assess the disinfection performance in relation to parasite

252 inactivation. The 24 L without chlorine evaluated the HSSF performance in
253 removing/inactivating (oo)cysts. After that, the protozoa detection method was applied
254 to both 24 L samples above and positive (oo)cysts for PI (stained) and negative
255 (oo)cysts for PI (unstained) were counted.
256
257 **2.4 Applied statistics**
258 HSSF performance was statistically analysed by the PAST program
259 (Palaeontological Statistics, manufactured by Hammer et al. 2001) and the normality of
260 the data checked by Shapiro-Wilk test with 95 % confidence interval. When the data
261 were non-parametric, the C-HSSF and I-HSSF efficiencies and the comparison between
262 the disinfected filtered water were compared using the Mann-Whitney non-parametric
263 test. Bivariate analysis checked the correlation between *E. coli* reduction efficiency in
264 filtered water for different operation phases with temperature. To do this, the Spearman
265 correlation coefficient (for non-parametric analyses) was used with 95% confidence
266 interval.
267
268 **3. Results and Discussion**
269
270 **3.1 HSSF construction**
271 The construction of the filter included cutting, chamfering, drilling and sealing
272 the parts. A plumber or a bricklayer normally uses tools and skills needed to build a
273 filter. It should be noted that the HSSF version reported by Smith (2013) required skills
274 in cutting and folding the material used, therefore communities must have professionals
275 who are experts in making the proposed prototype.

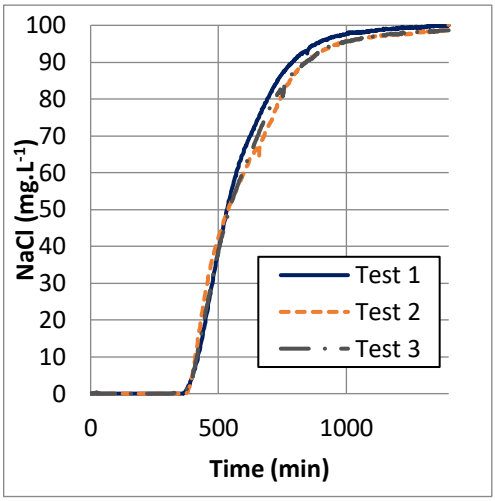
The HSSF body weight was 13 kg and it reached 92 kg when filter media was introduced. According to the results, the tested prototypes were lighter than other existing HSSFs (CAWST, 2010; Smith, 2013), facilitating the movement of the prototype for field tests. Nevertheless, HSSF became 7 times heavier after the granular media had been introduced. In this context, the empty HSSF must be transported to the final place, inside the residence, before being filled by the filtering materials. Napotnik and Jellison (2014) stated that both transport and movement of the finished HSSF (with body and filter media) can reduce the filtration rate by the filter bed compaction.

HSSF estimated costs (filter media, filter body, pipes, valves and fittings) were US$ 76 for I-HSSF and US$ 98 for C-HSSF in January 2020. For a family with a US$ 250 monthly income (equivalent to a minimum wage in Brazil in 2020), our HSSF prototypes correspond to at least 2.5 % of the annual income. In Nepal, for example, the CAWST HSSF model costs were 15 % of the annual income (Yung, 2003). According to Maciel and Sabogal-Paz (2020), their prototypes had a cost of US$ 97 per unit, which was a similar value in our study.

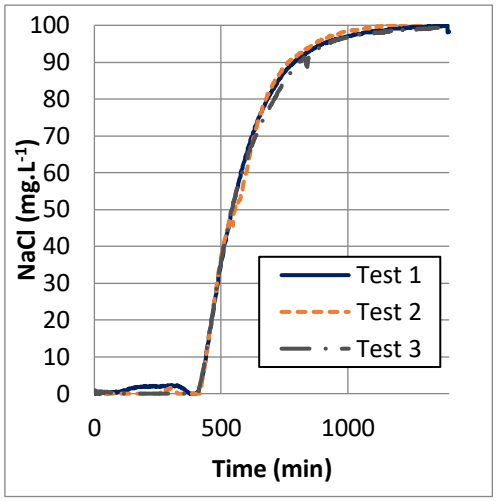
Water stored in the I-HSSFs was 16.24 L (sum of stationary water volume, void volume and internal volume in the HSSF outlet pipe). Introducing a feeding volume smaller than the void volume is recommended for a microbiological reduction (Elliott et al., 2008); therefore, each filter was fed three times per day with 16 L. On the other hand, water stored in the C-HSSFs was 18.9 L. This value divided by the daily flow, resulted in a theoretical hydraulic retention time of 567 ± 34 min that was later compared with the tracer tests.

3.2 Characterization of HSSFs

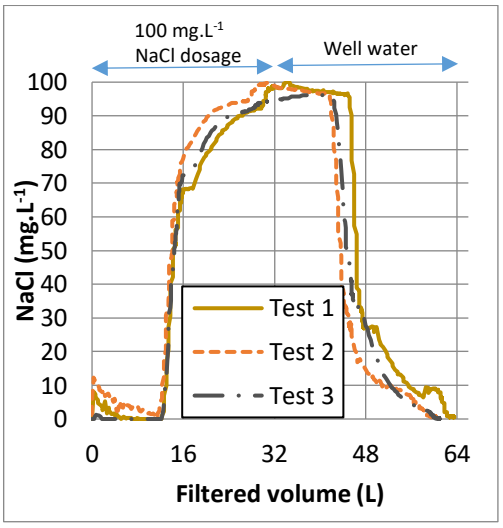
The results of the tracer assays on C-HSSFs and I-HSSFs are shown in Figure 2.



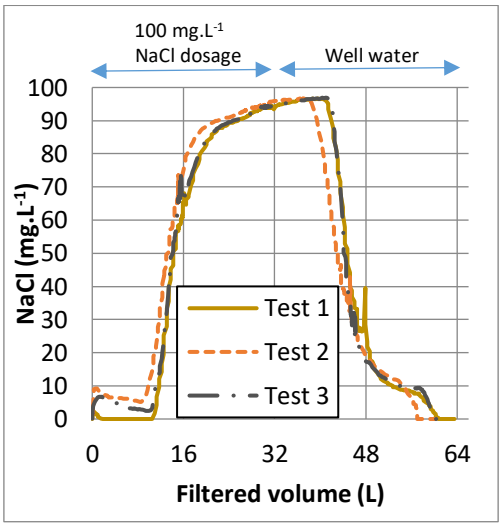
a) Tracer concentration in filtered water for C-HSSF1



b) Tracer concentration in filtered water for C-HSSF2



c) Accumulated tracer concentration for I-HSSF3



d) Accumulated tracer concentration for I-HSSF4

301 Figure 2. Tracer tests results in C-HSSF and I-HSSF

302

303 The graphs obtained in I-HSSFs are similar to those found by Elliott et al. (2008)
304 with an average vertical line close to the accumulated volume of 32 L (two feeds). A
305 change in the tracer concentration can also be observed introducing well water that aims
306 to remove it.

307 Regarding MDI, the values were 2.19 ± 0.01 for I-HSSF3 and 2.21 ± 0.07 for I-
308 HSSF4. This index considers the passage time of 10% and 90 % of the tracer mass in
309 the filter, therefore when closer to 1.0, it is close to the ideal plug flow reactor. USEPA
310 (1986) and Tchobanoglous et al. (2003) consider MDI ≤ 2 a plug flow reactor. The
311 HSSF model studied by Elliot et al. (2008), Bradley et al. (2011) and Yong-Rojanschi
312 and Madramootoo (2015) obtained MDI values of 1.3, 1.4 and 1.8, respectively.
313 According to the flow rate variation in the I-HSSF model studied by Lynn et al. (2013),
314 they adapted the MDI to the mMDI (i.e. index that took into account the accumulated
315 volume of filtered water) and found values around 2.9. In our study, the mMDI was
316 2.47 ± 0.03 for I-HSSF3 and 2.56 ± 0.13 for I-HSSF4, values close to those found by
317 Lynn et al. (2013).
318 The residence time distribution was 578 ± 4.4 min for C-HSSF1 and 578 ± 9.3
319 min for C-HSSF2. These values are close to the theoretical hydraulic retention time
320 (567 ± 34 min), previously shown. Data adjustment for the N-continuous stirred tank
321 reactors model and for the low and high dispersion intensity models are shown in Table
322 2. The N-CSTR model provided the best results in both prototypes, therefore 8 perfect
323 complete mix reactors in series are required for C-HSSF1 and 13 are necessary for C-
324 HSSF2. The C-HSSF model tested by Terin and Sabogal-Paz (2019) obtained N = 6
325 (filter cross sectional area = 0.049 m^2) and the one tested by Sabogal-Paz et al. (2020)
326 yielded N = 17 (filter cross sectional area = 0.0075 m^2), therefore the difference in
327 results may be associated with the filters' geometry. The largest number of reactors in
328 series indicates a reactor close to the plug flow (Levenspiel, 1999).
329
330
331

Table 2. Tracer test results for the C-HSSF

HSSF	Statistic	N-CSTR		Small dispersion model		High dispersion model	
		N	r^2	$D.\mu L^{-1}$	r^2	$D.\mu L^{-1}$	r^2
C-HSSF1	Mean	13	0.8	0.04	0.72	0.04	0.72
	Standard deviation	4	0.05	0.01	0.05	0.01	0.05
C-HSSF2	Mean	8	0.74	0.07	0.68	0.06	0.67
	Standard deviation	4	0.18	0.04	0.11	0.03	0.08

Notes: HSSF: household slow sand filter; N-CSTR: N-continuous stirred tank reactors model; N: number of stirred tank reactors; $D.\mu L^{-1}$: dimensionless group characterising the spread in the whole reactor; r^2: coefficient of determination.

When evaluating the development of the biological layer in the HSSF, Sabogal-Paz et al. (2020) suggested that the plug flow reactor may allow the same treatment time to be available for all water parcels that enter the filter, improving the performance of the HSSF.

3.3 HSSF operation

E. coli reductions are shown in Figure 3. The data showed that C-HSSFs displayed stability (ripening) after 100 days with around 3 log reductions in the first operation phase. In the second phase, the same efficiency was achieved in approximately 20 days. I-HSSF had almost 1.5 log reduction in the first operation phase, however the weekly inclusion of river water (i.e. ripening agent) in the second phase increased the efficiency of I-HSSF in reducing microorganisms (i.e. close to 2 log).

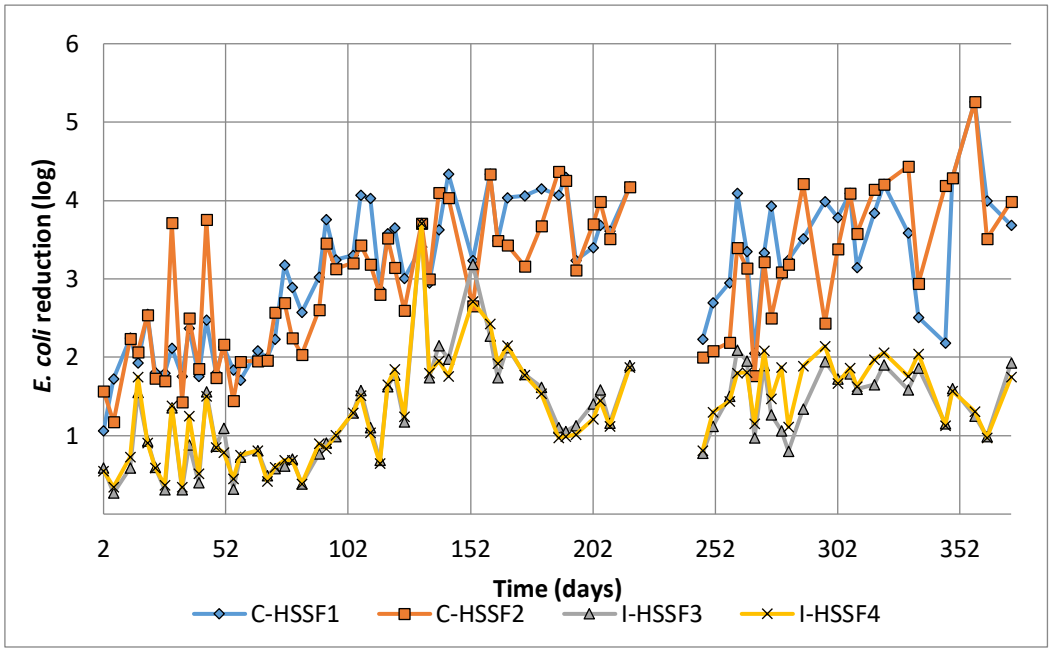


Figure 3. HSSF efficiency for *E. coli* reduction

The application of the Wilcoxon statistical test, to compare the *E. coli* data from filtered water, resulted in p-values of 0.018 between continuous filters and 0.264 between intermittent filters. The same test between the different HSSF configurations resulted in significantly lower values, indicating the similarity between the duplicates. Bivariate statistical analyses between the operation time and *E. coli* reduction showed a correlation ($p < 0.05$) in the first 20 days of operation in all HSSFs in the second phase and only in C-HSSF1 in the first phase. This indicates that there was a speed-up of filter performance in the second phase. Maciel and Sabogal-Paz (2020) in their bivariate analyses also observed that the time after HSSF maintenance correlated positively with microbiological removal.

Based on their data, Young-Rojanschi and Madramootoo (2014) proposed modelling *E. coli* reduction as a function of time for HSSFs in continuous and intermittent flows. For each HSSF, the aforementioned authors considered the initial reduction in bacteria (a), the reduction after ripening (b) and the ripening factor

364 characteristic (c). The results obtained from our data are shown in Table 3 after
365 applying the aforementioned modelling.
366
367 Table 3. Parameters for ripening models

Phase	HSSF	a	b	c	E
First phase: without ripening agent to speed up the biological layer growth	C-HSSF1	1.06	4.26	99	0.838
	C-HSSF2	1.37	4.27	169	0.753
	I-HSSF3	0.43	2.8	238	0.669
	I-HSSF4	0.45	2.79	285	0.612
Second phase: with ripening agent (river water) to speed up the biological layer growth	C-HSSF1	2.14	4.47	97	0.428
	C-HSSF2	1.89	4.62	96	0.725
	I-HSSF3	0.78	2.01	93	0.137
	I-HSSF4	0.81	1.95	52	0.078

Notes: a: initial reduction (log); b: final reduction (log); c: ripening factor; E: correlation between modelled and real values of *E. coli* reduction (Spearman); modelling: a + b. t / (t + c) (Young-Rojanschi and Madramootoo, 2014).

368
369 C-HSSFs showed greater *E. coli* reductions than I-HSSFs at the beginning of the
370 first and second phases. At the end of the same phases, bacterial reductions increased in
371 all filters, however showing a better performance for C-HSSFs. C-HSSFs presented a
372 filtration rate about 10 times lower than I-HSSFs and this lower filtration rate may have
373 helped the adsorption, filtration and microbiological treatment processes. In our study, it
374 is possible that the second phase, the microbiological mechanisms (i.e. faster
375 *schmutzdecke* formation) helped to reduce *E. coli*.

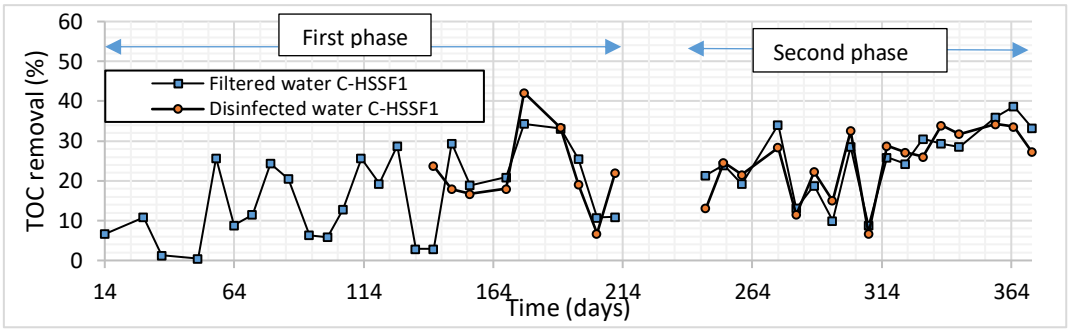
The ripening factor of all HSSFs was significantly higher in the first phase than in the second, therefore the river water use as a ripening agent changed the *E. coli* reduction curve, decreasing the ripening factor. In the modelling performed by Young-Rojanschi and Madramootoo (2014) with lake water as influent water, ripening factors of 2 to 4 were obtained in intermittent and continuous HSSFs, respectively.

Disinfection with sodium hypochlorite led to a reduction in the remaining *E. coli* in the filtered water in all HSSFs analysed (they were no longer detected). Hussain et al. (2015) also achieved a total coliform reduction, evaluating the HSSF followed by solar disinfection.

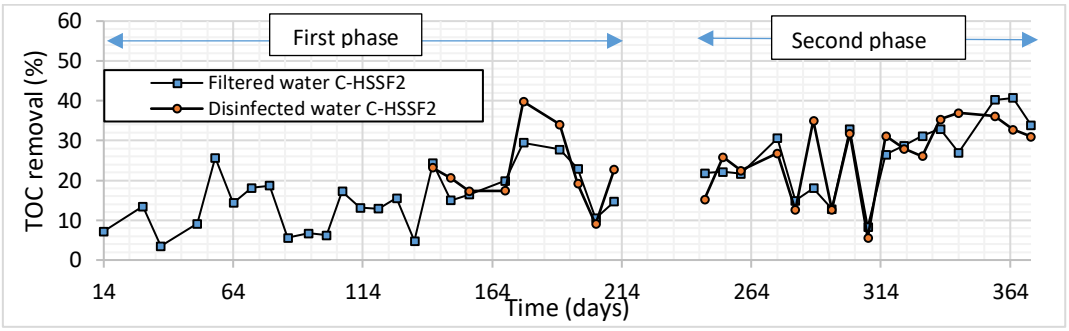
E. coli data of filtered water in the first week of HSSF operation were compared between the two phases. The Mann-Whitney test resulted in values of $p=0.698$ for all HSSFs. Therefore, it is emphasised that cleaning the HSSF leaves the filter in conditions close to the beginning of the operation in both studied phases.

TOC removal for each HSSF over the two phases is shown in Figure 4. The TOC of influent water was 6.8 ± 0.96 mg.L^{-1} in the first phase and 7.96 ± 1.21 mg.L^{-1} in the second. In this case, the first phase showed 30 % TOC removal after 140 operation days in the C-HSSF1 and only after 176 days in the C-HSSF2. On the other hand, the second phase achieved the same performance after 29 operation days in C-HSSFs and 99 days in I-HSSFs.

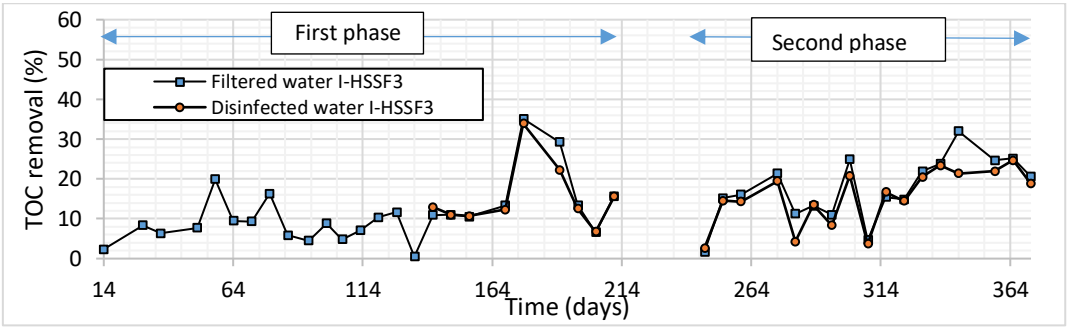
Campos et al. (2002) affirmed that the unstable organic carbon in slow sand filtration could be used by microorganisms for microbial biomass grow, thus providing its removal. In our study, the second phase, which included the ripening agent (river water) to speed up the biological layer growth, reduced the time to obtain 30% TOC removal (i.e. 111 operation days for C-HSSF1, 147 days for C-HSSF2 and 77 days for both I-HSSF3 and I-HSSF4) when compared to the first phase.



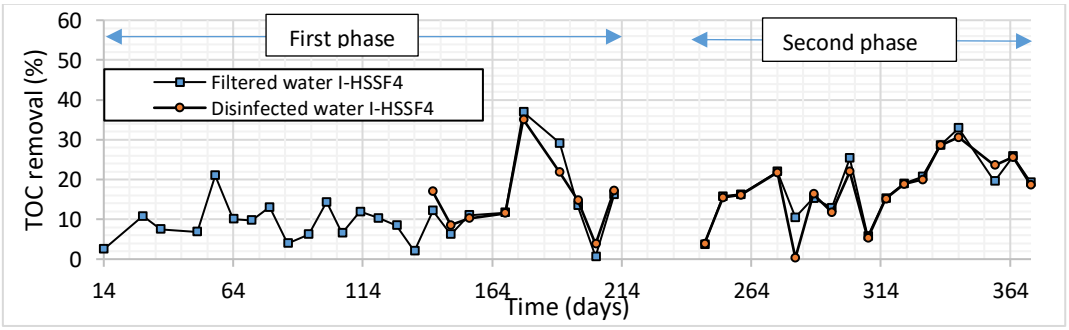
a) C-HSSF1



b) C-HSSF2



c) I-HSSF3



d) I-HSSF4

401 Figure 4. TOC removal for each HSSF

402

403 Disinfected water showed TOC removal similar to filtered water for all the
404 HSSFs and the Mann Whitney statistical test revealed no significant differences (p-
405 values: 0.67 for C-HSSF1, 0.63 for C-HSSF2, 0.51 for I-HSSF3 and 0.96 for I-HSSF4).
406 Monitored parameters throughout the operating time (turbidity, pH, temperature,
407 head loss and filtration rate) are summarized in Table 4. In the first phase, the turbidity
408 was 0.53 ± 0.13 NTU for the studied water. Despite low values, filtered water samples
409 presented removal with values of 9.5 ± 41 % in C- HSSF1, 8.7 ± 36 % in C- HSSF2,
410 14.8 ± 36% in I- HSSF3 and 16.5 ± 42% in I- HSSF4. In general, when influent water
411 turbidity is low, the removal is also low (Sabogal Paz et al., 2020). The Mann-Whitney
412 statistical test detected significant differences between filtered and chlorinated water
413 only in C-HSSF1.
414
415 Table 4. Analysed parameters during HSSF operation

First phase: without ripening agent				
Parameters	C-HSSF1	C-HSSF2	I-HSSF3	I-HSSF4
	(M ± SD)	(M ± SD)	(M ± SD)	(M ± SD)
Turbidity for filtered water (NTU)	0.44 ± 0.15	0.45 ± 0.13	0.42 ± 0.11	0.41 ± 0.16
Turbidity for disinfected water (NTU)	0.35 ± 0.09	0.4 ± 0.1	0.4 ± 0.15	0.34 ± 0.1
pH for filtered water	7.48 ± 0.07	7.51 ± 0.07	7.11 ± 0.24	7.09 ± 0.25
pH for disinfected water	7.51 ± 0.07	7.52 ± 0.05	7.02 ± 0.18	6.97 ± 0.18
Temperature at 8:00 am (ºC)	21 ± 2.9	21.2 ± 3	20.4 ± 2.8	20.4 ± 2.9
$dH.L^{-1}$	0.01 ± 0.003	0.02 ± 0.007	0.14 ± 0.009	0.14 ± 0.005
Flow rate ($m^3.m^{-2}.day^{-1}$)	0.88 ± 0.07	0.87 ± 0.11	6.93 ± 0.55	6.59 ± 0.51
Particle size for filtered water (nm)	651 ± 346	775 ± 485	922 ± 472	892 ± 675
Second phase: with ripening agent (river water)				

Parameters	C-HSSF1 (M ± SD)	C-HSSF2 (M ± SD)	I-HSSF3 (M ± SD)	I-HSSF4 (M ± SD)
Turbidity for filtered water (NTU)	0.65 ± 0.38	0.74 ± 0.58	1.22 ± 2.02	1.17 ± 1.98
Turbidity for disinfected water (NTU)	1.12 ± 2.38	1.09 ± 2.41	1 ± 1.96	1 ± 1.97
pH for filtered water	7.49 ± 0.16	7.49 ± 0.12	6.96 ± 0.15	6.95 ± 0.15
pH for disinfected water	7.53 ± 0.15	7.54 ± 0.13	6.9 ± 0.14	6.92 ± 0.13
Temperature at 8:00 am (ºC)	24 ± 2	23.8 ± 2	23 ± 2.1	22.8 ± 2.1
$dH.L^{-1}$	0.01 ± 0.002	0.02 ± 0.002	0.14 ± 0.003	0.14 ± 0.005
Flow rate ($m^3.m^{-2}.day^{-1}$)	0.88 ± 0.06	0.87 ± 0.09	6.87 ± 0.46	6.86 ± 0.5
Particle size for filtered water (nm)	496 ± 112	350 ± 165	544 ± 176	399 ± 104

Notes: M: mean; SD: standard deviation; $dH.L^{-1}$: head loss divided by thickness bed

In the second phase, the studied water showed 0.62 ± 0.41 NTU turbidity and the filtered and chlorinated water presented higher values. This increase was due to the ripening agent (river water) introduced weekly with 21.07 ± 25.32 NTU. However, only one day per week were there turbidity peaks of a maximum of 4.28 NTU in C-HSSFs and 12.3 NTU in I-HSSFs and the others days the filtered and chlorinated water remained below 1.0 NTU.

Regarding pH, the filtered water of the C-HSSFs showed slightly higher values than those of I-HSSFs. Filter media leaching, as pointed out by Sabogal-Paz et al. (2020), can explain the pH increase. Disinfection altered the pH values, however the treated water was within the recommended range, between 6.5 and 8.5 (WHO, 2017).

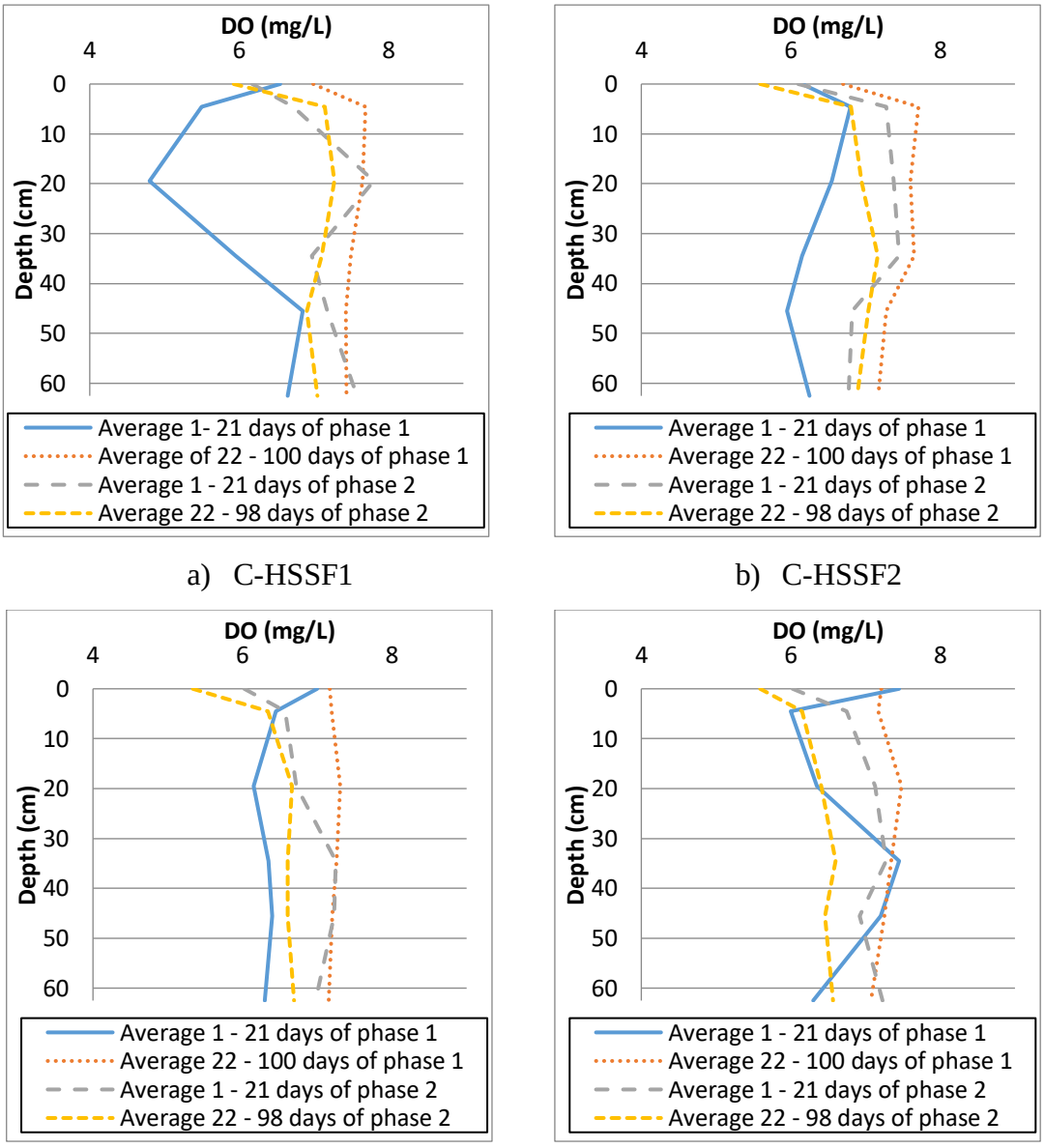
HSSFs internal temperature was higher in the second phase than the first as the room temperature was 24 ± 3.7 ºC in the first phase and 26.1 ± 2.2 ºC in the second. Filter ripening is known to improve at higher temperatures (Unger and Collins, 2006;

Arnold et al., 2016), therefore the temperature between phases may have influenced our results with favourable conditions to speed up the biological layer in the second phase. Nevertheless, this difference was very low (± 2ºC) and bivariate statistical analyses between the values of *E. coli* reduction and temperature showed no correlation ($p<0.05$)

Head losses and filtration rates varied slightly over time as low values of standard deviations were found. The variation head loss in relation to the filter media thickness ($dH.L^{-1}$) was higher for I-HSSFs than C-HSSFs. It should be noted that the low flow rate of C-HSSFs (33 $mL.min^{-1}$) resulted in a small head loss variation between the piezometers (i.e. in order of millimetres) while the instant high flow rate of I-HSSF (300 $mL.min^{-1}$) caused high head loss variation among piezometers (i.e. in order of centimetres). Young-Rojanschi and Madramootoo (2014) also observed this difference in HSSFs and they point out that the piezometers of the filters in continuous flow were not sensitive enough to detect the head loss.

The particle size of the studied water was 909 ± 298 nm in the first phase and 354 ± 140 nm in the second phase; however, filtered water showed a slight variation of this parameter (Table 4).

DO analyses in the filter depth are shown in Figure 5. Lower DO concentrations were found close to the surface, indicating consumption by the *schmutzdecke*. DO values were reduced with the depth and Young-Rojanschi and Madramootoo (2015) observed this phenomenon. DO profiles are similar between duplicates (C-HSSF1 versus C-HSSF2 and I-HSSF3 versus I-HSSF4) and different between continuous and intermittent flow configurations, with higher values for C-HSSFs. The pause periods may explain the higher DO consumption in I-HSSFs.



a) C-HSSF1 b) C-HSSF2

c) I-HSSF3 d) I-HSSF4

453 Figure 5. Dissolved oxygen (DO) variation for each HSSF according to the filter depth

454

455 DO profile in the initial 21 days of the first phase showed a different layout from
456 the other days in all HSSFs. On the other hand, in the second phase, the DO layout for
457 the same period was similar. In this case, there were differences in the DO profile
458 between the phases, in which the second phase had a DO profile closer to the days when
459 the HSSF was mature in the first days of operation. According to Adin (2003), DO
460 concentration can be an excellent indicator for the biological layer ripening.

The analytical quality test for the protozoan detection method is shown in Table 5. When the results are compared with the criteria established by USEPA (2012), the data obtained met the minimum recovery of 22 % for *Giardia*, but it did not meet the variation coefficient of 39 %.

Table 5. Analytical quality test for the protozoa detection method

G. lamblia	Sample	1	2	3	4
	Number of cysts	3	12	5	3
	Total average cysts ± Standard deviation	57.5 ± 42.7			
	Cyst recovery (%) ± CV(%)	57.6 ± 42.5			
C. parvum	Sample	1	2	3	4
	Number of oocysts	4	3	3	2
	Total average oocysts ± Standard deviation	30 ± 8.2			
	Oocyst recovery (%) ± CV(%)	30 ± 8.1			

Notes: CV: Coefficient of variation; number of inoculated organisms in *AccuSpyke*® kit (Waterborne™, USA): 99.9 cysts and 100.6 oocysts; final volume of each sample: 0.5 mL; aliquot assessed under microscope: 50 µL; multiplication factor = 10.

For *Cryptosporidium*, the method did not meet the minimum of 38 % recovery and met the variation coefficient of 37 %. When performing analytical quality tests of a similar protocol to the one used in our research, Maciel and Sabogal-Paz (2016) found recoveries of 80 ± 20.4 % for *Giardia* spp. and 5 ± 200 % for *C. parvum*, indicating a variability of the protozoa detection, according to the tested environmental matrix.

473 HSSF efficiency to protozoa reduction was performed when the filters were
474 mature (near the end of the operation). The results are shown in Table 6.
475
476 Table 6. HSSF efficiency for protozoa reduction

Protozoa	Day	Phase	Number of inoculated organisms (M ± SD)	Reduction (log)			
				C-HSSF1	C-HSSF2	I-HSSF3	I-HSSF4
G. muris	191	1	932 ± 177	TR	TR	TR	TR
	244	2	842 ± 424	TR	TR	TR	1.93
	275		2028 ± 453	2.32	2.32	2.02	2.02
	310		283 ± 138	0.98	1.15	TR	TR
	335		491 ± 137	1.21	TR	TR	1.69
	358		2735 ± 247	TR	TR	TR	1.55
C. parvum	191	1	942 ± 191	1.67	1.97	TR	1.67
	244	2	1086 ± 675	1.43	1.34	2.04	1.19
	275		690 ± 311	0.84	1.54	1.36	1.54
	310		175 ± 90	0.94	0.64	0.94	1.24
	335		233 ± 6	0.41	1.37	0.46	0.67
	358		595 ± 346	0.93	1.3	1.77	1.47

Notes: M: mean; SD: standard deviation; TR: total reduction (i.e. not detected in the sample).

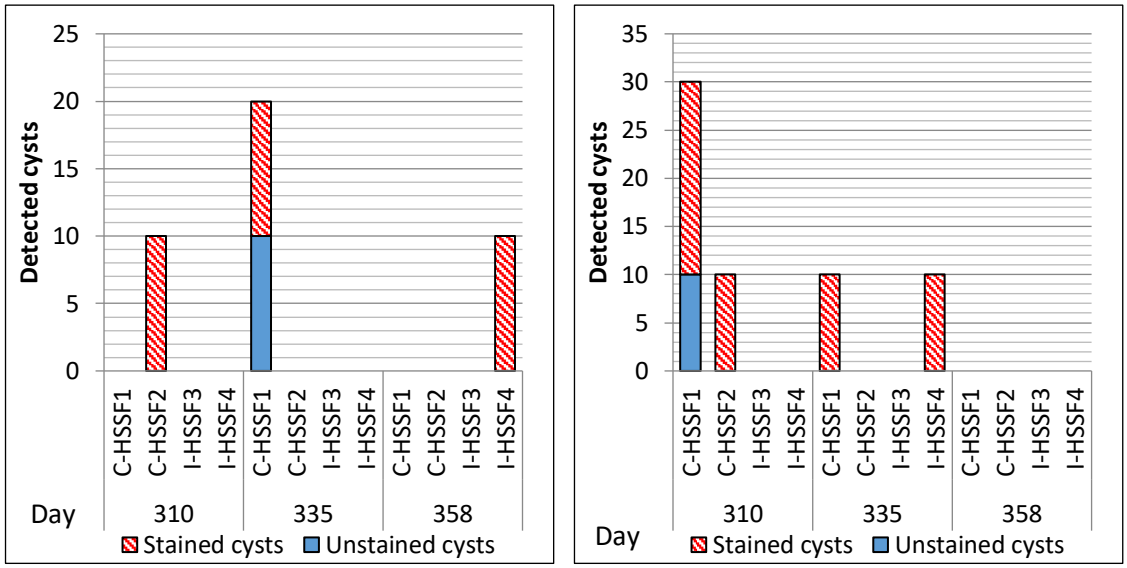
477
478 The maximum protozoa reductions occurred at the end of the first season,
479 obtaining a total removal (> 5 log) of *G. muris* in all HSSFs and 1.67 log to total
480 removal (> 5 log) of *C. parvum*. The end of the second phase also showed a total

481 reduction of cysts in C-HSSF1, C-HSSF2 and I-HSSF3, but not for I-HSSF4 (i.e. 1.55
482 log). The results suggest that a filter mature has a higher performance in protozoa
483 reduction due to the microbiological treatment promoted by the *schmutzdecke*.
484 Palmateer et al. (1999) achieved a reduction of 99.98 % (> 3 log) of *Cryptosporidium*
485 and 100 % (> 5log) of *Giardia* after 22 days of massive inoculation in I-HSSF model.
486 Although the HSSFs significantly reduced the amount of pathogens in the water, they
487 did not guarantee a complete reduction, mainly for *C. parvum* due to its small size and
488 ability to compress when it passes through the filter bed (Giglio and Sabogal-Paz,
489 2018), thus suitable disinfection is required.

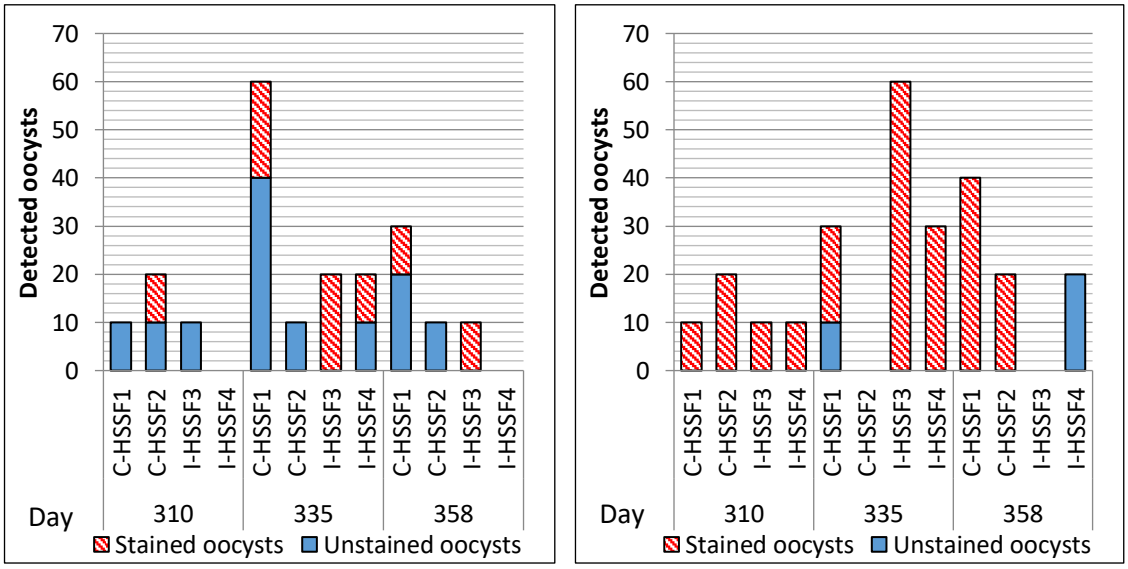
490 Higher bacterial reduction compared with protozoa may be explained by the fact
491 that *Giardia* and *Cryptosporidium* are present in their resistance form, cysts and oocysts
492 respectively, when in the environment. Usually, these forms show a double-layered
493 membrane protecting the parasites from adverse external conditions. Consequently, they
494 can tolerate the filtration process stress (i.e. predation, competition and lack of
495 nutrients) with less impact than bacteria (Ramo et al., 2017; Berglund et al., 2017). In
496 addition, the initial concentration of microorganisms inoculated in the study water may
497 have influenced the efficiency of the filter, since a high reduction of (oo)cysts was
498 observed when a high concentration of them was inoculated in the water, as reported by
499 Palmateer et al. (1999) and Adeyemo et al. (2015). Thus, low concentration in raw
500 water may underestimate the real protozoa removal rates.

501 The protozoan membrane permeability was evaluated using PI as an indicator of
502 the viability of the protozoa and the results are shown in Figure 6, at the end of the
503 second phase.

504



a) *G. muris* cysts in filtered water b) *G. muris* cysts after disinfection



c) *C. parvum* oocysts in filtered water d) *C. parvum* oocysts after disinfection

505 Figure 6. Potentially viable cysts and oocysts (unstained by propidium iodide) in filtered
506 and disinfected water

507

508 Potentially viable (unstained) *G. muris* cysts were detected in 25% of the filtered
509 water samples and in 20% of the disinfected water samples in the HSSFs. Potentially
510 viable *C. parvum* oocysts were detected in 60 % of filtered water and 12 % of
511 disinfected water samples. The findings indicated that the disinfection reduced the

512 amount of protozoa viable (i.e. 5 % for cysts and 48 % for oocysts), therefore, exposure
513 to chlorine may have altered the membrane permeability, contributing to the water
514 treatment. However, the existence of uncolored (oo)cysts in disinfected water shows
515 that chlorination was not fully efficient in removing the microbiological risk. Chlorine
516 doses even higher than the one used in our study (i.e. 0.5 $mg.L^{-1}$) do not inactivate
517 oocysts completely, according to Keegan et al., (2008). On the other hand, PI can also
518 underestimate the treatment efficiency as non-viable cells may be negative for PI
519 (Roussean et al., 2018). Regarding protozoa reduction, more research is needed to
520 provide safe water to the target population.

521 The main challenges observed in our study during the routine HSSF operation,
522 aiming at technology transfer in rural areas were: i) the change in the quality of filtered
523 water on the day of the addition of when the ripening agent (i.e. river water) was added;
524 ii) risks of chlorine splashing on clothes during disinfection; and iii) maintaining the
525 three daily feeding commitments for the I-HSSFs.

526 An alternative to motivate user acceptance for weekly river water feeds (ripening
527 agent introduction) would be to reserve the water from that day for less noble purposes
528 (e.g. general cleaning), since on that day the water quality is poor. When disinfecting,
529 using gloves and aprons and training the user can minimize any problems. The daily
530 HSSF operation routine requires discipline and commitment from the user; evidently,
531 these behaviour changes require training, monitoring and follow-up, as observed by
532 Vanderzwaag et al. (2009) and Sisson et al. (2013).

533

534 **4. Conclusions**

535 C-HSSFs efficiency was better than I-HSSFs in reducing the microbiological risk
536 found in groundwater.

The weekly feeding of HSSFs with river water accelerated the ripening process in approximately 80 days, thus improving the treatment efficiency compared to the operation without the ripening agent.

Disinfection with sodium hypochlorite improved the quality of the filtered water and generated an increase in the protozoa membrane permeability.

Despite the costs and operational challenges encountered, the HSSF can improve the quality of life in rural communities providing safe water worldwide.

Statement: The authors hereby declare previous originality check, no conflict of interest and open access to the repository of data used in scientific research purposes.

Acknowledgements: This research was supported by the Global Challenges Research Fund (GCRF) UK Research and Innovation (SAFEWATER; EPSRC Grant Reference EP/P032427/1) and by the Coordination for the Improvement of Higher Education Personnel (CAPES-Brazil, Financial Code: 001) for the PhD scholarship awarded to Fernando Cesar Andreoli. The authors are grateful to the Mexichem (Amanco-Brazil) company for donating the PVC pipes and fittings used to construct the HSSF.

Supplementary Material

Images from the microscopic examinations of the non-woven fabric installed on the top sand layer, and sand samples are provided as supplementary material. Statistical analysis used in the study is also provided.

5. References

562 Adeyemo, E., Kamika, I., Momba, M.N.B., 2015. Comparing the effectiveness of five
563 low-cost home water treatment devices for *Cryptosporidium*, *Giardia* and somatic
564 coliphages removal from water sources. Desalination and Water Treatment, 56:9, 2351-
565 2367. https://doi.org/10.1080/19443994.2014.960457
566
567 Adin, A., 2003. Slow granular filtration for water reuse. Water Science and Technology:
568 Water Supply, 3(4), 123-130. https://doi.org/10.2166/ws.2003.0053
569
570 Ahammed, M. M., Davra, K., 2011. Performance evaluation of biosand filter modified
571 with iron oxide-coated sand for household treatment of drinking water. Desalination,
572 276(1), 287-293. https://doi.org/10.1016/j.desal.2011.03.065
573
574 APHA, AWWA, WEF, 2012. Standard Methods for Examination of Water and
575 Wastewater, twenty-second ed. American Public Health Association, Washington,
576 ISBN 978-087553-013-0, 1360.
577
578 Arnold, N. B., 2015. Household Biosand Water Filters: Materials, Capacity, and
579 Temperature Effects on Performance. https://digitalcommons.mtu.edu/etds/976/
580
581 Arnold, N., Archer, A., Barkdoll, B., 2016. Bacterial adaptation and performance of
582 household biosand water filters in differing temperatures. Water Sci Tech Water
583 Supply, 16(3), 794-801. https://doi.org/10.2166/ws.2015.192
584

ABNT. NBR 11799: Material filtrante – Areia, antracito e pedregulho – Especificação [Filter material - Sand, anthracite and gravel - Specification]. 2016. Associação Brasileira de Normas Técnicas – ABNT.

Baig, S. A., Mahmood, Q., Nawab, B., Shafqat, M. N., Pervez, A., 2011. Improvement of drinking water quality by using plant biomass through household biosand filter–A decentralized approach. Ecological Engineering, 37(11), 1842-1848. https://doi.org/10.1016/j.ecoleng.2011.06.011

Berglund, B., Dienus, O., Sokolova, E., Berglind, E., Matussek, A., Pettersson, T., Lindgren, P. E., 2017. Occurrence and removal efficiency of parasitic protozoa in Swedish wastewater treatment plants. Science of the Total Environment, *598*, 821-827. https://doi.org/10.1016/j.scitotenv.2017.04.015

Bradley, I., Straub, A., Maraccini, P., Markazi, S., Nguyen, T.H., 2011. Iron oxide amended biosand filters for virus removal. Water Res. 45, 4501–4510. https://doi.org/10.1016/j.watres.2011.05.045

Calixto, K. G., Sabogal-Paz, L. P., Pozzi, E., Campos, L. C. 2020. Ripening of household slow sand filter by adding fish food. J Water Sanit Hyg De. 143. https://doi.org/10.2166/washdev.2020.143

Campos, L. C., Su, M. F. J., Graham, N. J. D., Smith, S. R., 2002. Biomass development in slow sand filters. Water Res. 36(18), 4543-4551. https://doi.org/10.1016/S0043-1354(02)00167-7

Cawst, 2012. Biosand Filter Construction Manual, Centre for Affordable Water and Sanitation Technology. Canada, Calgary.

Cawst, 2010. Biosand Filter Manual. Design, Construction, Installation, Operation and Maintenance. Training Manual. Centre of Affordable water and sanitation technology. Canada.

Clasen, T., McLaughlin, C., Nayaar, N., Boisson, S., Gupta, R., Desai, D., & Shah, N. 2008. Microbiological effectiveness and cost of disinfecting water by boiling in semi-urban India. Am J Trop Med Hyg, 79(3), 407-413. https://doi.org/10.4269/ajtmh.2008.79.407

Das, K., Roy, D., Mostafa, M. G., 2015. Sustainable Management for Arsenic (As) Free Safe Drinking Water in Bangladesh: A Review. J Pet Environ Biotechnol, 6(228), 2. https://doi.org/10.4172/2157-7463.100022

Duke, W. F., Nordin, R. N., Baker, D., Mazumder, A., 2006. The use and performance of BioSand filters in the Artibonite Valley of Haiti: a field study of 107 households. Rural Remote Health, 6(3), 570. www.rrh.org.au/journal/article/570

Eawag, Sandec – Department of Water and Sanitation in Developing Countries, 2008. Sandec Training Tool 1.0 – Module 3, Household Water Treatment and Safe Storage (HWTS).

635 Efstratiou A., Ongerth J. E., Karanis P., 2017. Waterborne transmission of protozoan
636 parasites: review of worldwide outbreaks-an update 2011–2016. Water Res., 114, 14-
637 22. https://doi.org/10.1016/j.watres.2017.01.036
638
639 Elliott, M.A., Stauber, C.E., Koksal, F., DiGiano, F.A., Sobsey, M.D., 2008. Reductions
640 of *E. coli*, echovirus type 12 and bacteriophages in an intermittently operated
641 household-scale slow sand filter. Water Res. 42, 2662–2670.
642 https://doi.org/10.1016/j.watres.2008.01.016
643
644 Franco R.M.B, Branco N., Amaro B.C.T, Neto, R.C.; Fiuza V.R.S. 2016.
645 *Cryptosporidium* species and *Giardia* genotypes detected in surface water supply of
646 Campinas, Southeast Brazil, by Molecular Methods. J. Vet. Med. Res.3:1–7.
647 http://agris.fao.org/agris-search/search.do?recordID=US2019V00122
648
649 Giglio, G. L., Sabogal-Paz, L. P. 2018. Performance comparison of three methods for
650 detection of Giardia spp. cysts and Cryptosporidium spp. oocysts in drinking-water
651 treatment sludge. Environmental monitoring and assessment, 190(11), 686.
652 https://doi.org/10.1007/s10661-018-7057-9
653
654 Hammer O, Harper DA, Ryan PD. 2001. PAST-palaeontological statistics, ver. 1.89.
655 Paleontological Museum, University of Oslo, Norway.
656
657 Hussain, G., Haydar, S., Bari, A. J., Anis, M., Asif, Z., Aziz, J. A., 2015. Evaluation of
658 Plastic Household Biosand Filter (BSF) In Combination with Solar Disinfection

659 (SODIS) For Water Treatment. Journal of the Chemical Society of Pakistan, 37(2).
660 https://doi.org/10.3390/ijerph9113806
661
662 Jadhav, D., Husain, M., Chavan, F. I., 2015. Biosand Water Filter: A Boon.
663 International Research Journal of Engineering and Technology, 2(3).
664 https://www.irjet.net/archives/V2/i3/Irjet-v2i3262.pdf
665
666 Keegan, A., Daminato, D., Saint, C. P., Monis, P. T., 2008. Effect of water treatment
667 processes on *Cryptosporidium* infectivity. Water research, 42, 1805-1811.
668 https://doi.org/10.1016/j.watres.2007.11.008
669
670 Levenspiel, O., 1999. Chemical Reaction Engineering, Industrial & Engineering
671 Chemistry Research. https://doi.org/10.1021/ie990488g
672
673 Lynn, T. J., Wanjugi, P., Harwood, V. J., Ergas, S. J., 2013. Dynamic performance of
674 biosand filters. J Am Water Works Assoc, 105(10), E587-E595.
675 https://doi.org/10.5942/jawwa.2013.105.0116
676
677 Maciel, P. M. F., Sabogal-Paz, L. P., 2020. Household slow sand filters with and
678 without water level control: continuous and intermittent flow
679 efficiencies. Environmental technology, *41*(8), 944-958.
680 https://doi.org/10.1080/09593330.2018.1515988
681
682 Maciel, P. M. F.; Sabogal-Paz, L. P., 2016. Removal of Giardia spp. and
683 Cryptosporidium spp. from water supply with high turbidity: analytical challenges and

684 perspectives. Journal of water and health, 14(3), 369-378.
685 https://doi.org/10.2166/wh.2015.227
686
687 Napotnik, J., Jellison, K., 2014. Transport effects on hydraulic loading rate and
688 microbial removal performance in biosand filters. Journal of water and health, 12, 686-
689 691. https://doi.org/10.2166/wh.2014.167
690
691 Palmateer, G., Manz, D., Jurkovic, A., McInnis, R., Unger, S., Kwan, K. K., Dutka, B.
692 J., 1999. Toxicant and parasite challenge of Manz intermittent slow sand
693 filter. Environmental toxicology, 14(2), 217-225. https://doi.org/10.1002/(SICI)1522-
694 7278(199905)14:2<217::AID-TOX2>3.0.CO;2-L
695
696 Pinderhughes, R., 2004. Alternative urban futures: Planning for sustainable
697 development in cities throughout the world. Rowman & Littlefield.
698
699 Ramo, A., Del Cacho, E., Sánchez-Acedo, C., Quílez, J., 2017. Occurrence and genetic
700 diversity of Cryptosporidium and Giardia in urban wastewater treatment plants in north-
701 eastern Spain. Science of the Total Environment, *598*, 628-638.
702 https://doi.org/10.1016/j.scitotenv.2017.04.097
703
704 Rousseau, A., La Carbona, S., Dumètre, A., Robertson, L. J., Gargala, G., Escotte-
705 Binet, S., Aubert, D., 2018. Assessing viability and infectivity of foodborne and
706 waterborne stages (cysts/oocysts) of *Giardia duodenalis*, *Cryptosporidium* spp., and
707 *Toxoplasma gondii*: a review of methods. Parasite, 25.
708 https://doi.org/10.1051/parasite/2018009

Sabogal-Paz, L. P., Campos, L. C., Bogush, A., Canales, M., 2020. Household slow sand filters in intermittent and continuous flows to treat water containing low mineral ion concentrations and Bisphenol A. Science of the Total Environment, 135078. https://doi.org/10.1016/j.scitotenv.2019.135078

Schmidt, W. P., Cairncross, S., 2009. Household water treatment in poor populations: is there enough evidence for scaling up now? Environ Sci Technol., 43(4), 986-992. https://doi.org/10.1021/es802232w

Sisson, A. J., Wampler, P. J., Rediske, R. R., Mcnair, J. N., Frobish, D. J., 2013. Long-term field performance of biosand filters in the Artibonite Valley, Haiti. The American journal of tropical medicine and hygiene, 88, 862-867. https://doi.org/10.4269/ajtmh.12-0345

Smith, A., 2013. Sandstorm: A biosand filter designed for small-scale enterprises. 36th WEDC International Conference, Nakuru, Kenya.

Sobsey, M.D., Stauber, C.E., Casanova, L.M., Brown, J.M., Elliott, M. a., Elliot, M.A., 2008. Point of Use Household Drinking Water Filtration: A Practical, Effective Solution for Providing Sustained Access to Safe Drinking Water in the Developing World. Environ. Sci. Technol. 42, 4261-4267. https://doi.org/10.1021/es702746n

Stauber, C. E., Printy, E. R., McCarty, F. A., Liang, K. R., Sobsey, M. D., 2012. Cluster randomized controlled trial of the plastic biosand water filter in

734 Cambodia. Environmental science & technology, 46(2), 722-728.
735 https://doi.org/10.1021/es203114q
736
737 Tellen, V., Nkeng, G., Dentel, S., 2010. Improved filtration technology for pathogen
738 reduction in rural water supplies. Water, 2(2), 285-306.
739 https://doi.org/10.3390/w2020285
740
741 Terin, U. C., Sabogal-Paz, L. P., 2019. Microcystis aeruginosa and microcystin-LR
742 removal by household slow sand filters operating in continuous and intermittent flows.
743 Water Res. 150, 29-39. https://doi.org/10.1016/j.watres.2018.11.055
744
745 Tchobanoglous, G., Burton, F.L., Stensel, H.D., 2003. Wastewater Engineering:
746 Treatment and Reuse. McGraw-Hill Higher Education, New York.
747
748 Unger M., Collins, M.R., 2006. "Assessing the role of the schmutzdecke in slow sand
749 and riverbank filtration." Proceedings, AWWA Annual Conference, San Antonio, TX.
750
751 United Nations General Assembly – UNGA, 2015. Transforming Our World: The 2030
752 Agenda for Sustainable Development. Draft resolution referred to the United Nations
753 summit for the adoption of the post-2015 development agenda by the General Assembly
754 at its sixty-ninth session. UN Doc. A/70/L.1 of 18 September 2015.
755
756 US Environmental Protection Agency – USEPA, 1986. Design manual, municipal
757 wastewater disinfection, Cincinnati, OH, EPA/625/1-86/021
758

US Environmental Protection Agency – USEPA, 2012. Method 1623.1: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA. United States Environmental Protection Agency. Office of Water. US.

Vanderzwaag, J. C., Atwater, J. W., Bartlett, K. H., Baker, D., 2009. Field evaluation of long-term performance and use of biosand filters in Posoltega, Nicaragua. Water Quality Research Journal, 44(2), 111-121. https://doi.org/10.2166/wqrj.2009.012

World Health Organization – WHO, 2019. Results of round II of the WHO international scheme to evaluate household water treatment technologies.

World Health Organization – WHO, 2017. Guidelines for Drinking-water Quality: fourth edition incorporating the first addendum. World Health Organization. Geneva, Switzerland.

World Health Organization – WHO, 2013. Ending preventable child deaths from pneumonia and diarrhoea by 2025: the integrated Global Action Plan for Pneumonia and Diarrhoea (GAPPD). https://apps.who.int/iris/bitstream/handle/10665/79200/9789241505239_eng.pdf

Young-Rojanschi, C., Madramootoo, C., 2015. Comparing the performance of biosand filters operated with multiday residence periods. J. Water Supply Res. Technol. 64, 157. https://doi.org/10.2166/aqua.2014.027

783 Young-Rojanschi, C., Madramootoo, C., 2014. Intermittent versus continuous operation
784 of biosand filters. Water Res. 49, 1–10. https://doi.org/10.1016/j.watres.2013.11.011
785
786 Yung, K., 2003. Biosand filtration: Application in the developing world. In: University
787 of Waterloo. Available online at:
788 https://sswm.info/sites/default/files/reference_attachments/YUNG%202003%20Biosand
789 %20Filtration.pdf
790
791 Zhang, B., Gao, L., Fazal, S., Mahmood, Q., Laghari, M., Sayal, A., 2016. Biosand
792 filter containing melia biomass treating heavy metals and pathogens. Polish Journal of
793 Environmental Studies, 25. https://doi.org/10.15244/pjoes/60433
794

795 **Supplementary Material**

796 **Statistical analysis**

797 Table S1 – Shapiro-Wilk test to verify the normality of the data

Data	p-value First phase of operation	p-value Second phase of operation
E. coli filtered water C-HSSF1	5.39E-12	8.29E-7
E. coli filtered water C-HSSF2	2.33E-9	3.62E-7
E. coli filtered water I-HSSF3	0.002	1119E-4
E. coli filtered water I-HSSF4	0.006	3.46E-6

798 Note: $P < 0.05$ was considered statistically significant.

799

800 Table S2 – Wilcoxon test to verify the difference in *E.coli* reduction in filtered water

801 between HSSFs

Comparison	p-value
C-HSSF1 = C-HSSF2	0.018
C-HSSF1 = I-HSSF3	1.2E-13
C-HSSF1 = I-HSSF4	1.18E-13
C-HSSF2 = I-HSSF3	1.73E-13
C-HSSF2 = I-HSSF4	1.73E-13
I-HSSF3 = I-HSSF4	0.264

802 Note: $P < 0.05$ was considered statistically significant.

803

804

805

806

807

Table S3 – Mann-Whitney test to check differences between the turbidity of filtered water before and after disinfection

HSSF	Comparison	p-value
C-HSSF1	Filtered water = Disinfected water	0.026
C-HSSF2	Filtered water = Disinfected water	0.365
I-HSSF3	Filtered water = Disinfected water	0.559
I-HSSF4	Filtered water = Disinfected water	0.804

Note: $P < 0.05$ was considered statistically significant.

Table S4 – Analysis of Spearman's correlation between *E. coli* reduction and variables of interest in the first phase of operation

Operating interval	HSSF		Variables of interest	
			Time of operation	Temperature
First 20 days of operation	C-HSSF1	r_s	**0.9**	-0.359
		p	**0.017**	0.633
	C-HSSF2	r_s	0.8	0.051
		p	0.083	1
	I-HSSF3	r_s	0.8	0.205
		p	0.0833	0.767
	I-HSSF4	r_s	0.631	0.358
		p	0.254	0.554
First 100 days of operation	C-HSSF1	r_s	**0.628**	0.016
		p	**0.001**	0.94
	C-HSSF2	r_s	**0.457**	0.169
		p	**0.022**	0.419
	I-HSSF3	r_s	0.122	0.21
		p	0.56	0.314
	I-HSSF4	r_s	0.091	0.139
		p	0.666	0.508

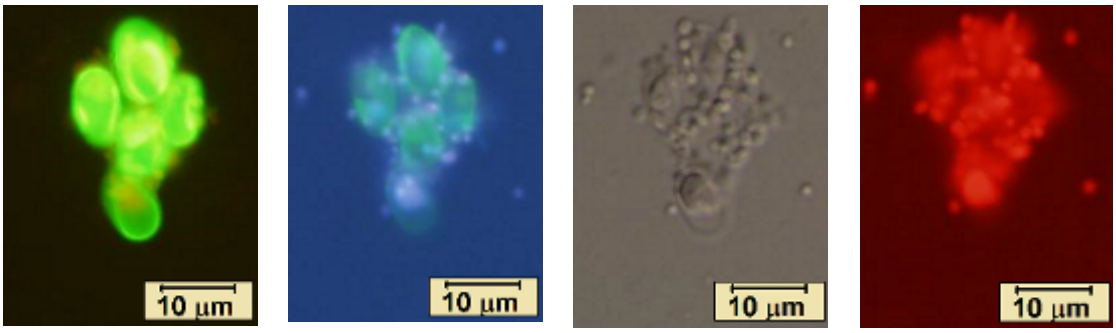
Note: r_s: Spearman's correlation coefficient; in bold: significant correlation ($p < 0.05$).

819 Table S5 – Analysis of Spearman's correlation between *E. coli* reduction and variables
820 of interest in the second phase of operation

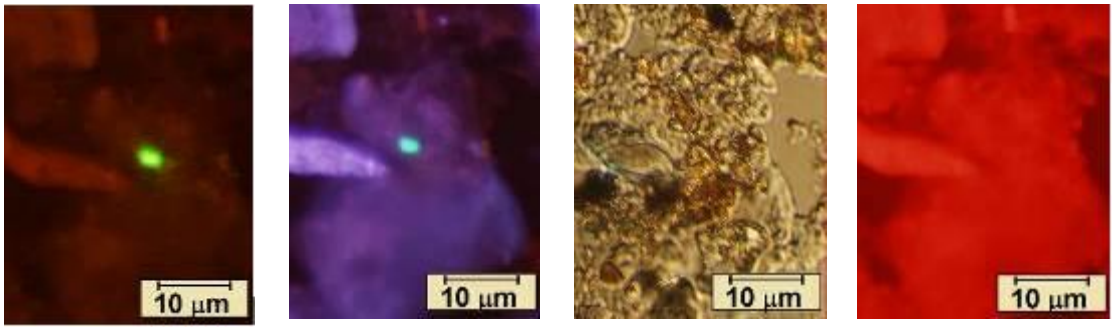
Operating interval	HSSF		Variables of interest	
			Time of operation	Temperature
First 20 days of operation	C-HSSF1	r_s	**0.9**	0.359
		p	**0.017**	0.633
	C-HSSF2	r_s	**0.9**	0.359
		p	**0.017**	0.633
	I-HSSF3	r_s	**0.9**	0.359
		p	**0.017**	0.633
	I-HSSF4	r_s	**1**	0.103
		p	**0.017**	0.9
First 100 days of operation	C-HSSF1	r_s	0.202	0.241
		p	0.394	0.306
	C-HSSF2	r_s	**0.684**	0.022
		p	**0.001**	0.927
	I-HSSF3	r_s	0.226	0.16
		p	0.339	0.501
	I-HSSF4	r_s	0.355	0.06
		p	0.125	0.802

821 Note: r_s: Spearman's correlation coefficient; in bold: significant correlation ($p < 0.05$).

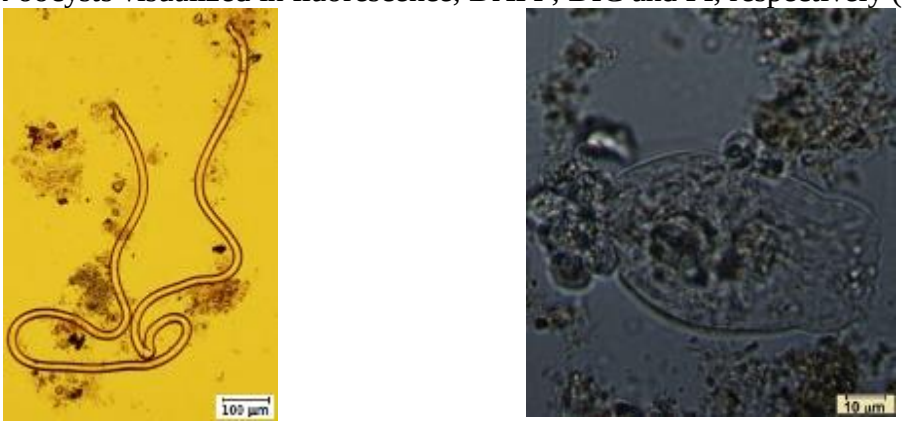
822



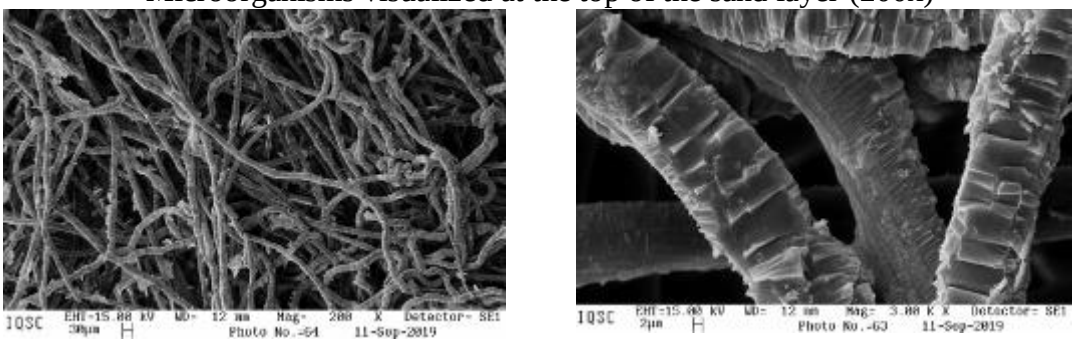
G.muris cysts visualized in fluorescence, DAPI , DIC and PI, respectively (400x)



C. parvum oocysts visualized in fluorescence, DAPI , DIC and PI, respectively (400x)



Microorganisms visualized at the top of the sand layer (200x)



Scanning electron microscopy images for blanket after filters operation (second season)

823 Figure A1 – Some microscopic images used in the research

824

825

826