Drinking Water Treatment by Multistage Filtration on a Household Scale: Efficiency and Challenges

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Universalising actions aimed at water supply in rural communities and indigenous populations must focus on simple and low-cost technologies adapted to the local context. In this setting, this research studied the dynamic gravel filter (DGF) as a pre-treatment to household slow-sand filters (HSSFs), which is the first description of a household multistage filtration scale to treat drinking water. DGFs (with and without a non-woven blanket on top of the gravel layer) followed by HSSFs were tested. DGFs operated with a filtration rate of 3.21 $m^3.m^{-2}.d^{-1}$ and

HSSFs with 1.52 $m^3.m^{-2}.d^{-1}$. Influent water contained kaolinite, humic acid and suspension of coliforms and protozoa. Physical-chemical parameters were evaluated, as well as *Escherichia coli*, *Giardia* spp. cysts and *Cryptosporidium* spp. oocyst reductions. Removal was low (up to 6.6%) concerning true colour, total organic carbon and absorbance (λ=254nm). Nevertheless, HMSFs showed turbidity decrease above 60%, *E. coli* reduction up to 1.78 log, *Giardia* cysts and *Cryptosporidium* oocysts reductions up to 3.15 log and 2.24 log, respectively. The non-woven blanket was shown as an important physical barrier to remove solids, *E. coli* and protozoa.

Keywords: drinking water; low-cost technology; slow sand filtration; protozoa; *Escherichia coli.*

Abbreviations:

DGF: dynamic gravel filter

HMSF: household multistage filter

HSSF: household slow-sand filter

SSF: slow sand filtration

MSF: multistage filtration

VSS: volatile suspended solids

1. Introduction

According to Sustainable Development Goal 6, the aim is to achieve universal and equitable access to safe drinking water, sanitation and hygiene, particularly for the poorest and most vulnerable communities by 2030 (WHO and UNICEF, 2017). Inadequate sanitation produces millions of waterborne diseases (Perez et al., 2012) and the higher risks are for children living in low- and middle-income countries (Speich et al., 2016). Clearly, there are large gaps between urban and rural coverage of drinking water and sanitation services in these areas (WHO and UNICEF, 2017). In this context, Efstratiou et al. (2017) emphasised that *Giardia* cysts and *Cryptosporidium* oocysts were the main causes of waterborne outbreaks worldwide.

Decentralised water treatment is crucial in improving the drinking water consumed by the poorest population (Baig et al., 2011). The WHO recommended household water treatment as a way to increase access to safe water for people, who live in rural areas in developing countries (WHO, 2011).

Household slow sand filters (HSSFs) are highlighted as a technology for drinking water treatment in rural communities. HSSFs can promote effective removal of pathogens and particulate matter. Its simple design, easy and cheap construction, operation and maintenance may contribute to improving life quality in rural communities (Manz, 2007).

The main HSSF mechanisms to remove microbiological and physicochemical parameters are filtration, adsorption and microbiological activity (Jenkins et al., 2011). Helminths and particulate matter removal are due to trapping in the pores between sand grains and attachment to the surfaces of the sand grains (Jenkins et al., 2011; Manz, 2007). There are studies that have reported bacteria, viruses and protozoa reductions, as

well as cyanobacteria, cyanotoxins and turbidity removals (Elliott et al., 2011; Terin and Sabogal-Paz, 2019; Wang et al., 2014). Clasen et al. (2015) reported that HSSF reduced 50% of diarrhoea cases in children.

Recently, HSSFs have been optimised by using new materials, sand bed depth reduction, different sand sizes and filter ripening ways, adding non-woven blankets to the top layer and operation in continuous and intermittent flows (Calixto et al., 2020; Elliott et al., 2008; Faria Maciel and Sabogal-Paz, 2018; Napotnik et al., 2017; Souza Freitas and Sabogal-Paz, 2019; Young-Rojanschi and Madramootoo, 2014).

HSSFs have limitations that are analogous to conventional slow filters when removing solids and organic compounds. The excess of suspended material in the influent water obstructs the intergranular voids causing a reduction in the filter run and an increase in cleaning activities (Souza Freitas and Sabogal-Paz, 2019). Therefore, coarse media filtration could be used as a pre-treatment, creating the multistage filtration (Galvis et al. 2002). There should be more than one treatment stage, within the multi-barrier concept, which would act in the gradual removal of fine particles and microorganisms in order to produce safe water (Visscher, 2006). Consequently, pre-filtration with coarse gravel (when included) would make the HSSF more efficient when turbid water is treated.

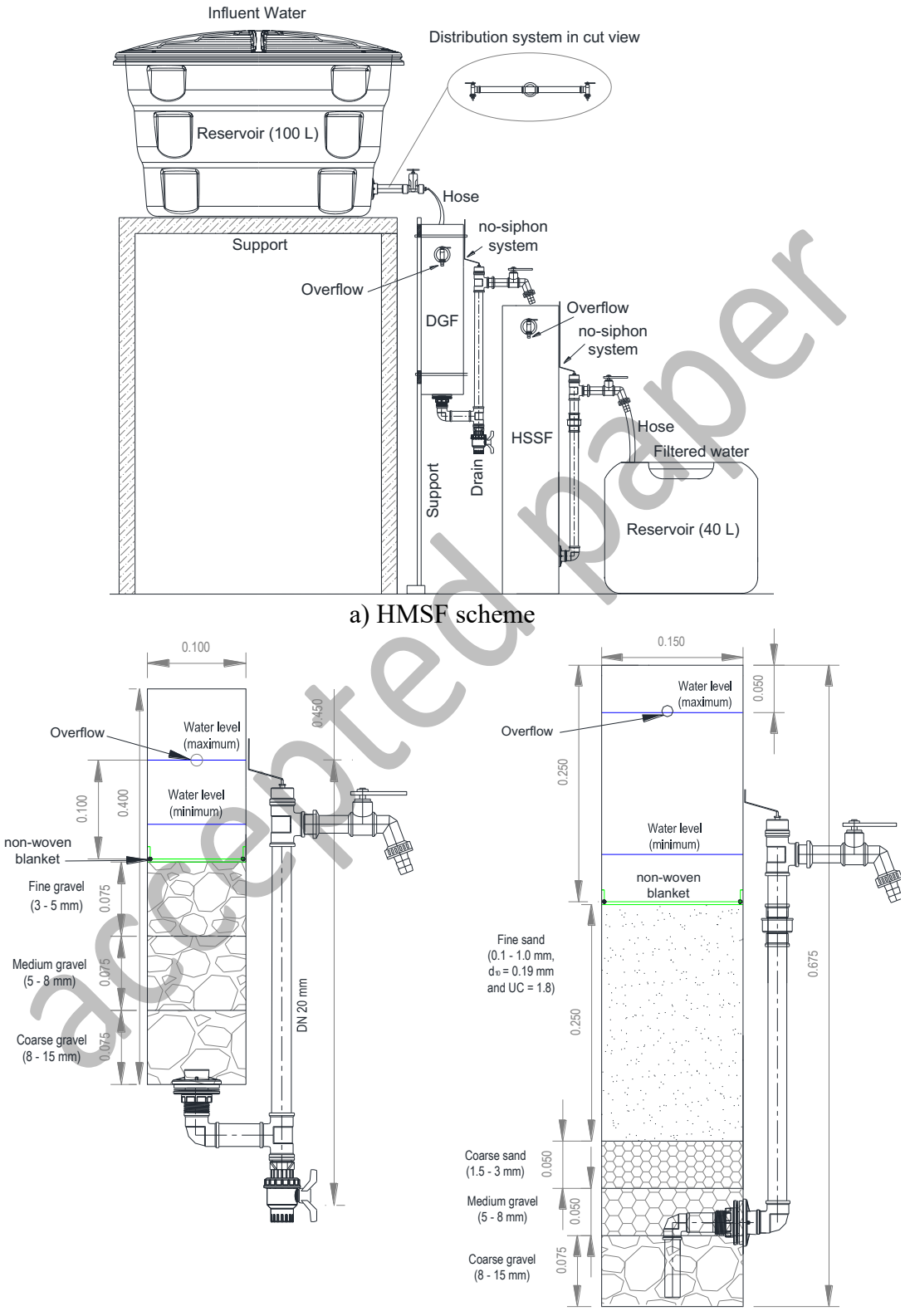
In this context, the aims of this study were to evaluate the HMSF performance to remove physicochemical and microbiological parameters from influent water with high levels of colour and turbidity.

2. Materials and Methods

2.1. HMSF Construction

96 HMSF had a dynamic gravel filter (DGF) as a pre-treatment of HSSFs (Figure

97 1).



a) HMSF scheme

b) DGF scheme (units in meters) c) HSSF scheme (units in meters)

98 Figure 1. HMSF with a dynamic gravel filter (DGF) as a pre-treatment of an HSSF

Two HMSFs were evaluated wherein DGF (with and without a non-woven blanket on top of the gravel layer) was followed by HSSFs. DGFs were constructed in PVC pipes with a 99.8 mm inside diameter (cross-sectional area = 0.0078 m²). DGF was filled with three gravel layers of 7.5 cm thickness each (coarse gravel with 8.0 to 15 mm, medium gravel with 5.0 to 8.0 mm and fine gravel with 3.0 to 5.0 mm). HSSFs were equally built out of PVC with 145 mm inside diameter (cross-sectional area = 0.0164 m²) and they were filled with two gravel layers which worked as support media (sizes: 5 to 8 mm and 8 to 15 mm) followed by a coarse sand layer (1.5 to 3.0 mm) and fine sand (0.1 to 1.0 mm) with an effective size (D_{10}) of 0.19 mm and uniformity coefficient (D_{60}/D_{10}) of 1.8, as recommended by CAWST (2012).

The filters were called DGF1 (with a non-woven blanket in the top layer), DGF2 (without non-woven blanket), HSSF1 and HSSF2 (household sand filters with a non-woven blanket in the top layer with identical characteristics between them). A non-woven blanket (100% polyester, specific mass of 0.2 g cm^{-3} and thickness of 2 mm) was positioned and fixed by a PVC ring slightly smaller than the inside filter diameter.

2.2. HMSF Operation

HMSFs were operated in continuous flow with a daily production of 25 L, more than the 20 L per day established as a minimum volume for basic health protection (WHO, 2003), thus DGFs and HSSFs operated with filtration rates of 3.21 ± 0.09 $m^3.m^2.d^{-1}$ and 1.52 ± 0.04 $m^3.m^2.d^{-1}$, respectively. HMSFs were monitored over 140 days and during this period, two stops in the filter operation took place, one lasting 19 days and the other 14 days. The stops were purposeful in order to assess what would happen in a home when the filters stop feeding, for example, during family holidays.

HMSFs worked closely to what would happen in a rural residence, that is, the reservoir of 100 L was filled and 25 $L.d^{-1}$ were forwarded to each HMSF; therefore, there was a declining filtration rate and valves were calibrated daily for each HMSF. Filter head loss was evaluated every other day and the HMSF stopped for maintenance when the flow rate was less than 25 $L.d^{-1}$.

2.3. HMSF maintenance

Blankets were removed from each filter and cleaned with deionised water and the cleaning liquid was stored for physicochemical and microbiological analysis. The same procedure was followed with the fluid drained from each DGF. Blankets were removed from each HSSF and the biological layer (*schumutzdecke*) was removed by splashing deionised water. The sand top was agitated manually three times and after was left steady for 1.0 min for sedimentation, then the supernatant was removed and stored for analysis as well.

2.4. Tracer tests

Tracer tests were performed three times prior to HMSF operation. A solution of 100 $mg.L^{-1}$ of NaCl was used as the tracer. A 100-L reservoir was filled with saline solution and a submersible water pump HM-5063 (Jeneca®, China) was placed for homogenisation to take place. A conductivity probe (Vernier® Software & Technologies, USA) with a Go!link® interface was positioned at an outlet pipe and the data was collected by Logger Lite® software (Vernier Software & Technologies, USA). The tracer test was carried out until the salt solution was close to 100 $mg.L^{-1}$ in the filter

output. Microsoft Excel® was used to develop the normalisation curve of tracer concentration over time and Origin 8.6® (Originlab, EUA) was used for data analysis resulting in the residence time distribution curve. Mean residence times in each filter were determined and the flow pattern was adjusted according to three hydrodynamic mathematical models (low dispersion, high dispersion and N-continuous stirred tank reactors) as recommended by Levenspiel (1999).

2.5. Influent Water

Influent water was a mixture of well water, 60 mg.L^{-1} of kaolinite (Sigma Aldrich®), 20 mg.L^{-1} of humic acid (Sigma Aldrich®) and *Escherichia coli* (ATCC 11229) which were agitated for 30 min by a mechanical mixer. Influent water was prepared to reach similar characteristics of challenge test water used for validating drinking water technologies, as described in WHO (2014). Well water and influent water characteristics are shown in Table 1.

Table 1 - Well water and influent water characteristics for the study

Parameter	Mean ± Standard deviation	
	Well water	Influent water
pH	6.24 ± 0.33	7.65 ± 0.15
Temperature (ºC)	22.7 ± 1.7	22.7 ± 0.8
Total Alkalinity (mg $CaCO_3$ L^{-1})	26.4 ± 3.8	34.03 ± 8.31
Conductivity (µS cm^{-1})	59.7 ± 6.7	68.1 ± 6.7
True Colour (HU)	3.2 ± 3.6	246 ± 22
Apparent Colour (HU)	1.8 ± 2.8	338 ± 36
Turbidity (NTU)	0.177 ± 0.091	42 ± 16.7
Absorbance (λ = 254 nm)	0.015 ± 0.031	0.554 ± 0.101
Total organic carbon -TOC (mg L^{-1})	3.13 ± 3.95	7.63 ± 0.71
Particle size (nm)	Not analysed	1116 ± 317
Escherichia coli (CFU 100 mL^{-1})	0	1.03 x 10^5

Total coliforms (CFU 100 mL^{-1})	0.2 ± 0.4	0

166

167 After 53, 64 and 88 days of continuous operation, approximately 10^3 cysts of
168 *Giardia lamblia* and 10^2 oocysts of *Cryptosporidium parvum* from purified suspensions
169 (Waterborne® Inc, USA) were added to the DGFs and HSSF inlets. In these three
170 assays, cysts and oocysts were added over four consecutive days prior to protozoa
171 analysis. Between the 101st and 140th days of continuous operation, cysts and oocysts
172 were added daily and four protozoa analyses were performed.

173

174 **2.6. Sampling and analysis**

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176 Temperature, pH, turbidity, apparent colour, true colour, absorbance (λ=254
177 nm), total alkalinity, conductivity, particle size, total organic carbon (TOC), *E. coli* and
178 total coliforms were analysed according to APHA et al. (2012).

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180 **2.6.1. Protozoa analysis**

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182 Protozoa protocols included membrane filtration and triple centrifugation.
183 Filtration with cellulose mixed ester membranes (47 mm diameter and 3 μm nominal
184 porosity, Millipore®) was performed according to Franco et al. (2016) without
185 immunomagnetic separation (IMS). Samples from DGFs and HSSFs were filtered until
186 reaching the number of five ester membranes used. Cysts and oocysts were eluted by
187 scraping the membrane three times using Tween 80 (0.1%, 45 °C). Samples were kept
188 in 50 mL Falcon tubes for centrifugation at 1,500 *x g* for 15 min. Supernatant was
189 discarded until the pellet was 5 mL, and then it was mixed for homogenisation. After

another centrifugation (1,500 *x g*; 15 min), the supernatant of each sample was discarded until 1 mL pellet was left for analysis.

Samples from the non-woven blanket cleaning water, the DGF drain and the HSSF biological layer were concentrated by triple centrifugation at 1,500 *x g* for 15 min, following the Medeiros and Daniel (2018) protocol. Samples were kept in 50 mL Falcon tubes for centrifugation at 1,500 *x g* for 15 min. Afterwards, the supernatant was removed until 5 mL. 10 mL of elution solution (Tween 80, 0.1% v/v) was added and mixed by 30s. Centrifugation was performed again and the supernatant was removed, 10 mL of deionised water were added and, after mixing, a third and last centrifugation was done. The remaining 5 mL were stored overnight in a refrigerator. The final pellet was vortexed and the Dynabeads™ GC-Combo (TermoFisher Scientific®) manufacturer's protocol was followed to perform immunomagnetic separation (IMS). Two acid dissociations were carried out to increase cyst and oocyst recoveries, according to Method 1623.1 (USEPA, 2012).

Protozoa detection for both methods (membrane filtration and triple centrifugation) was performed by immunofluorescence assay (IFA) using the Merifluor® kit (Meridian Bioscience Diagnostics, USA), following the manufacturer's protocol and Method 1623.1 (USEPA, 2012). Sample observations were made using an epifluorescence microscope (Olympus® BX51). Cysts and oocysts were identified by their size, morphology, shape and fluorescence and their concentration per litre was calculated according to Method 1623.1 (USEPA, 2012) in filtered water. Protozoa concentration per gram of total solids (referring to 50 mL of sample) was calculated for samples obtained from non-woven blanket cleaning, DGF drain and the HSSF biological layer.

Analytical quality assays were performed for each protozoan concentration method to verify how the matrix would influence protozoan recovery. The assays were performed four times plus the blank test, under equal conditions, inoculating approximately 3,000 *Giardia* cysts and 300 *Cryptosporidium* oocysts extracted from purified suspensions purchased from Waterborne® Inc, USA. Moreover, 15 µL of purified *Cryptosporidium* oocyst suspension and 5 µL of *Giardia* cysts were evaluated in triplicate to estimate the mean number of inoculated organisms in the matrix.

For membrane filtration protocol, four beakers containing 1.0 L of filtered water were spiked with cysts and oocysts and mixed with magnetic stirring for 2 min. After this period, the method explained above was followed.

For the triple centrifugation method with IMS, a sample of the drainage liquid from DGF was utilised since it showed turbidity and colour similar to the HSSF biological layer and non-woven blanket cleaning samples. In this case, a 25 mL sample was disposed into 50 mL Falcon tubes and cysts and oocysts were inoculated. Falcon tubes were mixed for 30s and they were filled again with the sample upon reaching 50 mL. A final mixture lasting 30s was performed on the sample before starting the method described above. Recovery (R%) for each protocol was calculated by Equation 1.

$$R(\%) = \frac{\text{cysts and oocysts recovered}}{\text{cysts and oocysts spiked} + \text{number of indigenous (oo)cysts of the sample}} X\ 100 \quad (1)$$

2.7. Microorganisms present in the non-woven blanket

Bright field microscopy was performed with 20 µL of samples from DGF1 and HSFF blankets, in Agar 2%, after the last maintenance. Microorganism visualisation was carried out under a microscope (Olympus® BX60) at 100x to 2000x magnification.

239 Samples of each used blanket (DGF1 and HSSFs) and new blanket (blank test) were
240 analysed by a Scanning Electron Microscope (SEM), (Zeiss® LEO 440) to capture
241 photomicrographs at 300 to 10,000 x magnification.
242
243 **2.8. Statistical analysis**
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245 Statistica® 7.0 (StatSoft, Inc, 2004) was used for statistical analysis. The
246 Shapiro-Wilk test was applied in order to verify data normality. Comparisons between
247 DGFs, HSSFs and HMSFs were made by the Student's t-test and Tukey test for
248 multiple comparisons. When data, even after transformation, did not present normality,
249 we resorted to the Mann-Whitney U test. There was a study of Pearson's correlation
250 (parametric data) and Spearman's (non-parametric data) correlation between physical
251 and operating variables and *E. coli* and protozoa reductions. P-values less than 0.05
252 were considered significant.
253
254 **3. Results and Discussion**
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256 **3.1. Tracer Tests**
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258 Tracer test results for the four filters are shown in Figure 2. The N-CSTR model
259 offered the best fit to all of the filter data, considering Pearson's correlation coefficient
260 (r^2): DGF1 (0.93); DGF2 (0.91); HSSF1 (0.99) and HSSF2 (0.99). Therefore, the numbers
261 of reactors in series were 9 ± 2 for DGF1, 8 ± 2 for DGF2, 8 ± 2 for HSSF1 and 7 ± 0.1
262 for HSSF2, closer to the plug flow reactor, according to Levenspiel (1999). A similar
263 performance was described by Faria Maciel and Sabogal-Paz (2018), Terin and Sabogal-

Paz (2019) and Sabogal-Paz et al. (2020), characterising a plug flow reactor for the HSSF as well.

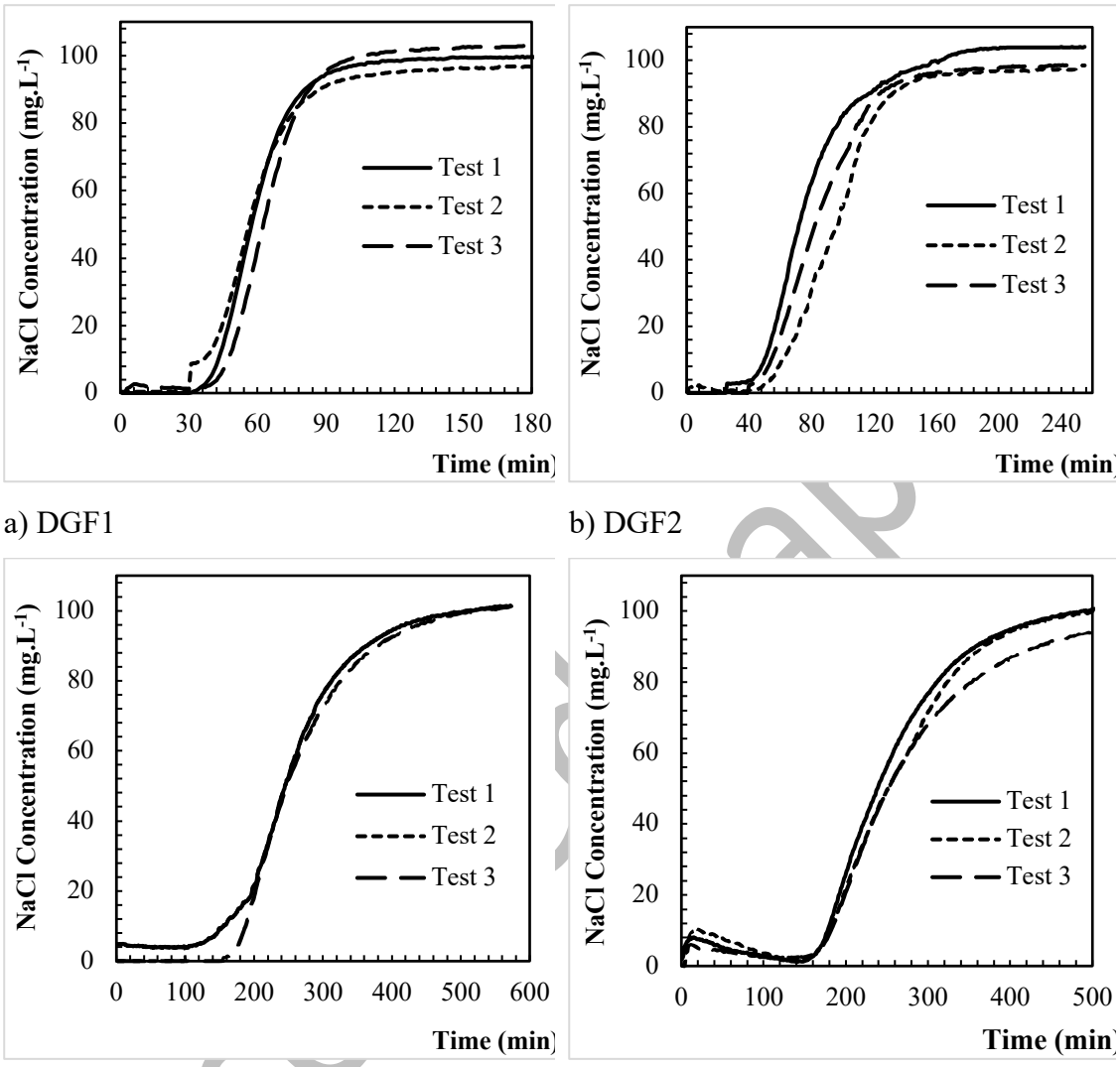


Figure 2 - Tracer tests results in triplicate

Mean residence times used for estimating the sampling times were 61 ± 4 min for DGF1, 86 ± 7 min for DGF2, 258 ± 8 min for HSSF1 and 261 ± 3 min for HSSF2. HSSF flow characterisation is an important operational parameter (e.g. it can define the water sampling time) and few studies have considered this aspect (Sabogal-Paz et al. 2020).

274 **3.2. HMSF Operation**

275 Filtered water features and HMSF efficiencies (DGF+HSSF) are shown in Table 2.

276 Table 2. Filtered water characteristics for each filter and HMSF efficiencies

Parameter	Mean ± Standard deviation (SD)			
	DGF1	HSSF1	DGF2	HSSF2
pH	7.59 ± 0.11	7.61 ± 0.09	7.58 ± 0.12	7.62 ± 0.08
Temperature (ºC)	22.4 ± 0.6	22.4 ± 0.7	22.4 ± 0.6	22.3 ± 0.6
Conductivity ($\mu S.cm^{-1}$)	68.2 ± 6.8	68 ± 6.4	68.1 ± 6.5	68 ± 7
True Colour (Hu)				
Mean ± SD	244 ± 24	236 ± 35	244 ± 25	232 ± 45
Removal (%)	1.3 ± 2	3.4 ± 8	0.9 ± 1.9	5.9 ± 14
DGF + HSSF removal (%)	4.6 ± 8.3		6.6 ± 14.4	
Apparent Colour (Hu)				
Mean ± SD	306 ± 32	286 ± 35	311 ± 34	285 ± 42
Removal (%)	10.3 ± 4.1	6.5 ± 6.4	8.6 ± 3.8	8.7 ± 8.5
DGF + HSSF removal (%)	16.2 ± 5.7		16.6 ± 8.4	
Turbidity (NTU)				
Mean ± SD	18.1 ± 3.5	13.8 ± 3	19.2 ± 4	14.1 ± 3.3
Removal (%)	53.6 ± 11.7	23.2 ± 9.8	50.7 ± 12.2	26 ± 11.3
DGF + HSSF removal (%)	64.6 ± 8.9		64 ± 9.1	
Absorbance (λ254 nm)				
Mean ± SD	0.550 ±	0.537 ±	0.551 ±	0.541 ±
Reduction (%)	0 ± 2.1	1.3± 2.9	0.1 ± 1.9	0.5 ± 2.6
DGF + HSSF removal (%)	1.2 ± 2.9		0.5 ± 2.2	
TOC ($mg.L^{-1}$)				
Mean ± SD	7.76 ± 0.76	7.40 ± 1.03	7.76 ± 0.82	7.36 ± 1.37
Removal (%)	-0,3 ± 4.6	5.8 ± 7.5	0.7 ± 3.2	5.4 ± 12.5
DGF + HSSF removal (%)	5.6 ± 7.5		6.0 ± 13.6	
Particle size (nm)				
Mean ± SD	583.1 ± 81	453.4 ± 32.5	595.8 ±	453.4 ± 40.9
Removal (%)	43.9 ± 16.3	21.1 ± 10.7	42.6 ± 16.8	23 ± 10.2
DGF + HSSF removal (%)	56 ± 13.2		55.9 ± 14	
E. coli (CFU 100 ml^{-1})				
Geometric Mean	1.8×10^4	1.7×10^3	2.6×10^4	3.0×10^3
Maximum value	8.8×10^4	3.5×10^4	1.1×10^5	6.9×10^3
Minimum value	5.0×10^2	5.6×10^1	1.0×10^3	1.0×10^2
Reduction (log)	0.76 ± 0.36	1.02 ± 0.49	0.55 ± 0.32	0.98 ± 0.71
DGF + HSSF reduction	1.78 ± 0.65		1.53 ± 0.77	

Note: HMSF = DGF + HSSF

277 DGF and HSSF were not efficient in true colour removal, as also reported by
278 Sánchez et al. (2006). This might be related to the difficulty in slow sand filtration (SSF)
279 in removing humic substances (Ellis and Wood, 1985). As apparent colour is influenced
280 by turbidity and particle size, its removal was superior to the true colour (Table 2). There
281 were no statistical differences among the filters in the removal of true and apparent colour.
282 Turbidity removal mainly happened in DGF (about 50%) and this confirms the
283 role of this filter in protecting the HSSF against high turbidity, smoothed turbidity peaks
284 and avoiding filter clogging (Galvis et al., 2002; Sánchez et al., 2006; Visscher, 2006).
285 DGF1 and DGF2 provided higher turbidity removal than the findings obtained by Franco
286 et al. (2012). Nevertheless, these authors found higher apparent colour removal.
287 HMSF turbidity removals were higher than those found by Galvis et al. (2002)
288 and Sánchez et al. (2012). However, when HSSF1 and HSSF2 were evaluated, their
289 efficiencies (around 64%) were lower than that reported by Elliott et al. (2008), Faria
290 Maciel and Sabogal-Paz (2018), Frank et al. (2014), Lynn et al. (2013), Murphy et al.
291 (2010) and Young-Rojanschi and Madramootoo (2014), with turbidity removals in the
292 range from 74 to 96%. This divergence is associated to influent water characteristics
293 between studies. There were no statistical differences between DGF, HSSF and HMSF in
294 the study.
295 Influent water turbidity and filtered water during the operating time are shown in
296 Figure 3. Turbidity peaks for influent water happened when the parameter measurement
297 occurred on the same day as the water preparation. HMSFs were able to maintain final
298 turbidity around 20 NTU. However, filtered water did not meet the World Health
299 Organisation (WHO) guidelines for drinking water, that is, 5.0 NTU, as also reported by
300 Baig et al. (2011). It should be noted that turbidity below 1.0 NTU is associated with 1-
301 2 log and 2.5-3 log reduction of viruses and protozoa, respectively (WHO, 2017). Some

studies used influent water with low turbidity (3.90-12.6 NTU), such as Ahmmed and Davra (2011), Elliot et al. (2008) and Stauber et al. (2006), achieving better HSSFs performances. Influent water prepared with kaolinite and low nutrient concentration may have influenced the filter efficiency in our study, as reported by Faria Maciel and Sabogal-Paz (2018) and Sabogal-Paz et al (2020).

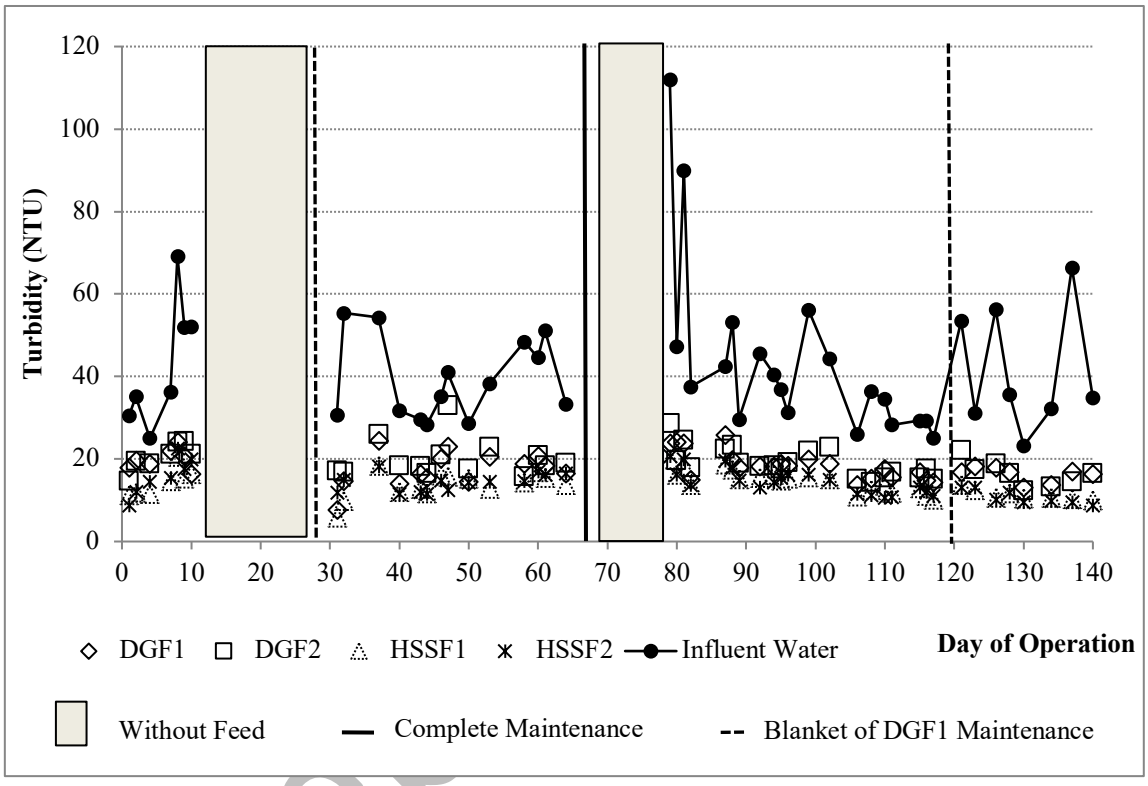


Figure 3 - Performance of DGFs and HSSFs in turbidity removal.

There was significant correlation between the influent water turbidity with both DGF efficiencies (r = 0.724 and 0.783, for DGF1 and DGF2, respectively). Similar findings were found by Franco et al. (2012) and Galvis et al. (2002), who reported that turbidity removal increased in the occurrence of peaks in raw water for DGF.

For all of the filters under study, turbidity removal did not correlate to the HMSFs' running time, when analysing the total period (140 days). However, there was significant correlation between the running time and turbidity removal during the period after

317 maintenance of the non-woven blankets on the 64th operation day, for DGF2 (r = 0.61)
318 and HSSF1 (r = 0.57).

319 Particle size evaluation was important to understand how each filter in HMSF
320 works. After the 53rd day, after adding cysts and oocysts, the particle size of the influent
321 water increased (1205.8 ± 296.3 nm) and showed a statistical difference in relation to
322 prior protozoan inoculum (768 ± 131.2 nm) (p = 0.0043). Higher particle size removal
323 can be seen in the DGFs (Table 2), analogous to the turbidity results obtained. There were
324 no statistic differences between the DGFs, HSSFs and HMSFs.

325 Filter ripening for the operation days was significantly correlated to a reduction in
326 particle size for DGF2 (r = 0.41), HSSF1 (r = 0.50), HMSF1 (r = 0.55) and HMSF2 (r =
327 0.53). This find may indicate that DGF removed the larger particles when compared with
328 HSSFs and this might be due to the lower media depth present in the latter (Elliott et al.,
329 2008).

330 There was no statistical difference between DGFs, HSSFs and the HMSFs (Mann-
331 Whitney U test) when TOC was evaluated. HSSF efficiency in organic compound
332 removal was lower (around 5%) than the results found by Lynn et al. (2013) and Souza
333 Freitas and Sabogal-Paz (2019). Nevertheless, the discrepancy in organic carbon removal
334 may be related to compound composition (high or low biodegradability) and influent
335 water characteristics (Campos et al., 2002; Modal et al., 2007). Low nutrient
336 concentrations in the influent water can impair the biological activity in HSSFs (Lynn et
337 al., 2013) and this situation may explain the lowest absorbance (λ=254 nm) and colour
338 removals in our study, since only humic acid, kaolinite and *E. coli* were added to the
339 influent water.

340 *E. coli* reduction during filter operation is shown for HMSF1 (Figure 4a) and for
341 HMSF2 (Figure 4b). Among HSSFs there were no significant statistical differences;

however, DGF1 showed a better performance than DGF2, according to the statistical test (p = 0.018). HSSFs had greater efficiency than DGFs, among HSFF1 and DGF1 (p = 0.014), and HSSF2 and DGF2 (p = 0.023).

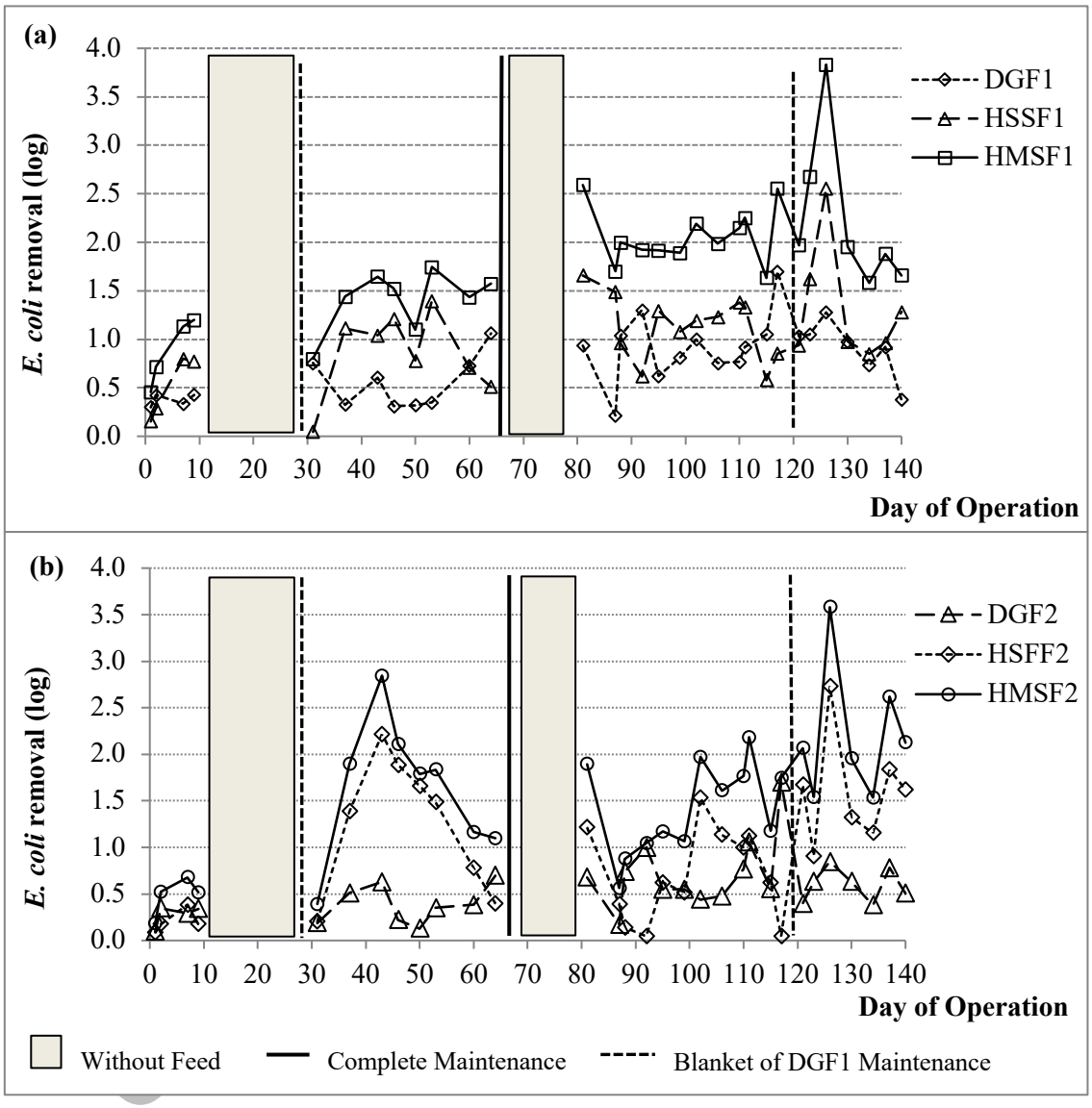


Figure 4 - *E. coli* reduction for DGFs and HSSFs

Young-Rojanschi and Madramootoo (2014) achieved removals up to 3.7 log and Souza Freitas and Sabogal-Paz (2019) obtained reductions close to 3.0 log in HSSFs, values higher than those obtained in our study (around 1.0 log, according to Table 2). On

352 the other hand, HMSFs showed mean reductions close to that obtained by Galvis et al.
353 (2002), between 1.9 to 4.0 log for full-scale MSF systems composed by DGF followed
354 by SSF.

355 *E. coli* reductions provided by DGF1, DGF2 and HSSF1 had a correlation with
356 the operation days, due to filter ripening, and this finding matches the results obtained by
357 Faria Maciel and Sabogal-Paz (2018) and Stauber et al. (2006). In addition, DGF ripening
358 occurred through the progressive accumulation of particles and microorganisms as it
359 happens in SSFs (Galvis et al., 2002).

360 Natural die-off can contribute to *E. coli* reductions due to stress, lack of nutrients,
361 lack of oxygen, entrapment in sand pores and predation in the biological layer, as well as
362 adsorption in the filter media (CAWST, 2012; Elliott et al., 2015).

363 Blanket cleaning in DGF1 negatively affected the HSSF1 performance (after the
364 31st day) and in *E. coli* reduction DGF1 (after the 121st), with r = -0.77 and r = -0.82,
365 respectively, according to the statistical study.

366 Complete HMSF maintenance, with blanket cleaning, DGFs drained and HSSF
367 surface layer cleaning was done aiming to assess system resilience. Prior to that, there
368 was no significant statistical difference between HMSFs for *E. coli* reduction, which did
369 not happen after complete maintenance, with HMSF1 providing a better performance than
370 that compared to HMSF2, according to the statistical test (p = 0.0015). HMSF1 showed
371 nearly constant *E. coli* reduction of 2.0 log, after 10 days of complete maintenance, while
372 HMSF2 presented greater instability (Figure 4). HMSFs obtained higher *E. coli* reduction
373 at 126 days of operation, with 3.83 log and 3.53 log for HMSF1 and HMSF2, respectively.
374 Faria Maciel and Sabogal-Paz (2018) reported a need for 140 days to reach maximum
375 HSSF efficiency due to a low concentration of nutrients in the influent water that affected
376 filter ripening. After complete HMSF maintenance both HMSFs required around 14 days

377 to achieve progressive *E. coli* reduction and this fact was caused by their biofilm change,
378 affecting HSSF efficiency.

379 A filter ripening period after cleaning must be carefully evaluated since the
380 development of the biological layer is essential to improve microorganisms and turbidity
381 removals in HSSFs (Ahammed and Davra, 2011; Bellamy et al., 1985; Napotnik et al.,
382 2017).

383 Significant statistical results (Pearson test) were found by correlating physical
384 variables with *E. coli* reduction in the following cases: i) HSSF2, with turbidity removal
385 ($r = 0.41$) and a reduction in particle size ($r = 0.46$); and ii) after complete maintenance,
386 in HSSF2 ($r = 0.57$) and HMSF2 ($r = 0.55$) with a decrease in particle size. However,
387 turbidity and particle size in DGF output did not influence the *E coli* reductions in HSSFs,
388 according to the statistical test.

389 HMSFs were not fed for 19 days at the beginning of the operation and 14 days
390 near the end of the operation to evaluate the HMSF performance after normal stops such
391 as family holidays. Evidently, the HSSFs were affected and they took days to reach their
392 efficiency and this phenomenon was also reported by Souza Freitas and Sabogal-Paz
393 (2019). Filter ripening depends on the influent water quality, including nutrients and
394 biodegradable carbon such as D-glucose (Modal et al., 2007) and natural coagulant
395 (Souza Freitas and Sabogal-Paz, 2019). However, biological layer formation can reach
396 days or even months to get completely formed. Therefore, the rapid ripening of the filter
397 should be better studied to avoid abandoning technology in rural areas when it presents
398 poor performance in some periods.

399

400 **3.3. Protozoan tests**

401

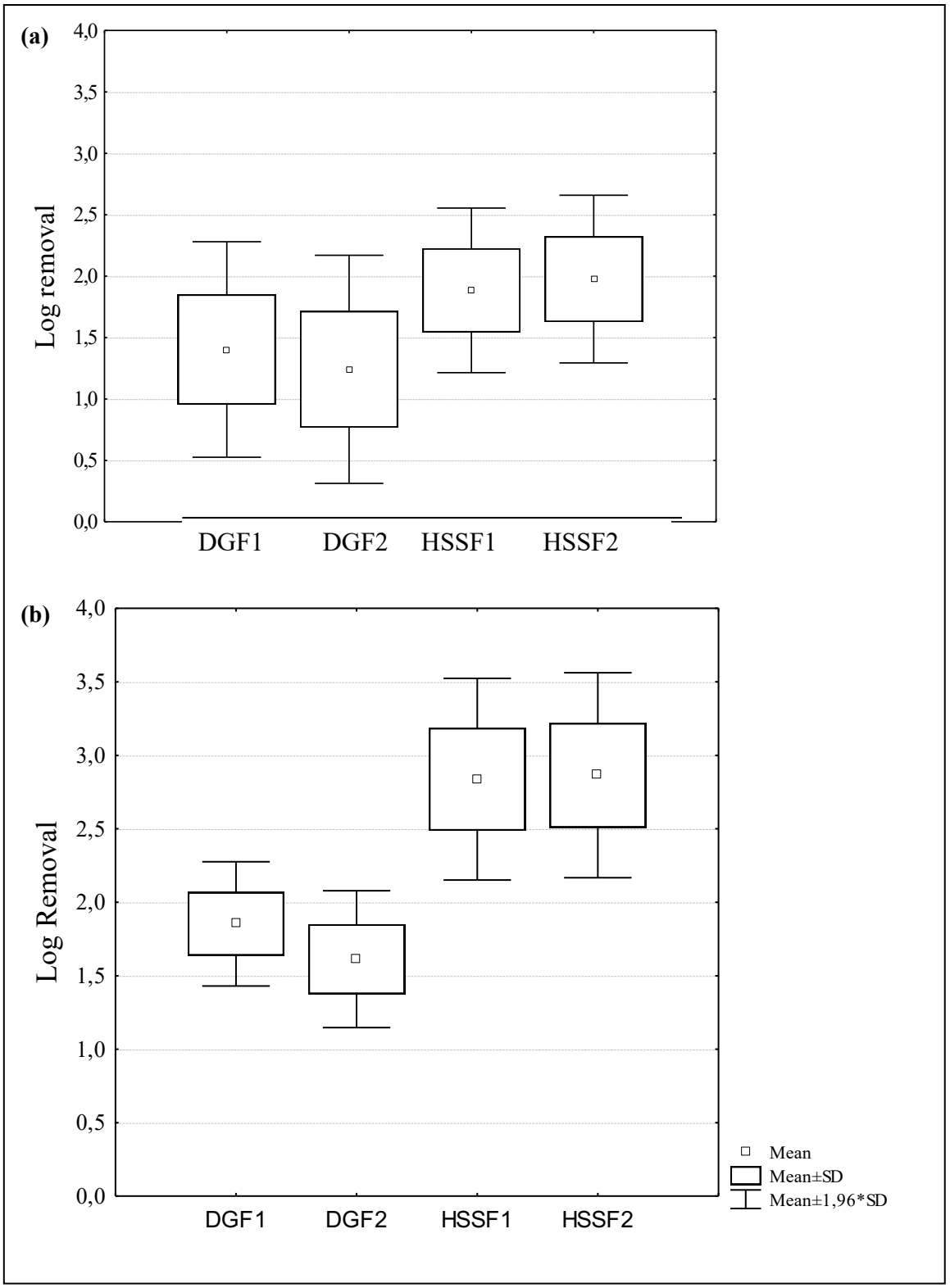
Analytical quality assays results are shown in Table 3. *Giardia* spp. cyst recovery was statistically higher that *Cryptosporidium* spp. oocysts for both methods. The relative standard deviation and mean met the Method 1623.1 (USEPA, 2012) and blank tests did not present protozoa for both protocols.

Table 3 - Analytical quality assays results for *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts

Methods	Membrane Filtration + IFA		Triple Centrifugation + IMS +	
Protozoa	*Cysts*	*Oocysts*	*Cysts*	*Oocysts*
Cysts and oocysts inoculated	3329 ± 149	314 ± 8	3387 ± 155	307 ± 12
	Recovery (%)			
Tests	*Cysts*	*Oocysts*	*Cysts*	*Oocysts*
Test 1	106	45	79	58
Test 2	90	29	79	36
Test 3	81	51	73	45
Test 4	95	45	87	47
Mean ± RSD	93 ± 11.4	42.2 ± 22.5	79.3 ± 7.2	46.7 ± 19.2

Note: RSD: relative standard deviation; IFA immunofluorescence assay; and IMS: immunomagnetic separation.

Giardia spp. cysts were detected in DGF and HSSF filtered water samples (93% and 21%, respectively). *Cryptosporidium* spp. oocysts were also found in filtered water (71% of DGFs and 43% of HSSFs). Standard deviation and the average protozoa removal are shown in Figure 5.



414

415 Figure 5 – DGF and HSSF efficiencies in *Cryptosporidium* spp. oocyst removal (a) and

416 *Giardia* spp. cyst removal (b).

417

418 Filters removed *Giardi*a spp. cysts more than *Cryptosporidium* spp. oocysts,
419 except for DGF2, that did not show a statistical difference. HSSFs were more efficient in
420 removing both protozoa than DGFs, due to their low filtration rate and sand grain size.
421 DGFs showed no difference in protozoa removal, according to statistical tests,
422 with 1.40 log ± 0.45 (DGF1) and 1.24 log ± 0.47 (DGF2) for oocysts (p = 0.490) and 1.85
423 log ± 0.22 (DGF1) and 1.61 log ± 0.24 (DGF2) for cysts (p = 0.096). There were also no
424 statistical differences between HSSFs for protozoa removal as well, reaching 1.88 log ±
425 0.34 (HSSF1) and 1.98 log ± 0.35 (HSSF2) for oocysts (p = 0.789). *Giardia* spp. cyst
426 removal efficiency was also equal between the HSSFs with 2.84 log ± 0.35 (HSSF1) and
427 2.86 log ± 0.36 (HSSF2) (p = 0.966). Our results are similar to those obtained by Bellamy
428 et al. (1985) and Palmateer et al. (1999) and these authors emphasized the role of the
429 biological layer on the filter performance. Sand grain size and sand bed depth are also
430 important in protozoa removal (Hijnen et al., 2007). Our findings were better than those
431 obtained by Fogel et al. (1993). Higher uniformity coefficient of the sand bed helps
432 protozoan removal, especially oocysts, due to the inequality of the grain size of the sand,
433 which generates winding water paths inside the filter.
434 *Giardia* cyst removals had a correlation with the filter operation time for DGF2 (r
435 = 0.82) and HSSF2 (r = 0.77). Consequently, filter ripening as well as adherence and
436 transport mechanisms are important for cyst and oocyst removals (Fogel et al., 1993;
437 Tufenkji et al., 2006; Verma et al., 2017).
438 HMSFs showed no statistical differences for cyst and oocyst removals. HMSF1
439 obtained 3.13 log ± 0.35 and 2.16 log ± 0.35 and HMSF2 obtained 3.15 log ± 0.36 and
440 2.24 log ± 0.39 for cysts (p = 0.898) and oocysts (p = 0.928), respectively. HMSF2
441 operation time had a relation with *Giardia* (r = 0.78) and *Cryptosporidium* (r = 0.84)
442 removals, according to the statistical test.

Protozoan removal had no correlation with particle size decrease and with influent water particle size, according to the statistical test. The analogous result happened when *E. coli* reduction, turbidity removal and influent water turbidity were associated in the statistical test.

3.4. Sludge characteristics generated in HMSFs

Sludge characteristics generated in HMSFs are shown in Tables 4 and 5. Complete filter maintenance occurred on the 64th and 140th days and DGF1 blanket cleaning occurred on the 121st day (Figure 4).

Table 4 – DGF sludge characteristics

Parameter	Non-woven blanket (DGF1)			Drainage water DGF1		Drainage water DGF2	
	I	II	III	I	III	I	III
Apparent colour (HU)	2820	4020	3340	820	1510	655	568
Turbidity (NTU)	10200	4130	3340	640	1140	421	468
TS (mg L^{-1})	10898	27280	27900	1084	1912	1214	842
TDS (mg L^{-1})	1248	22670	23273	172	372	570	134
TSS (mg L^{-1})	9650	4610	4627	912	1540	644	708
FSS (mg L^{-1})	8038	3900	3909	786	1273	540	558
VSS (mg L^{-1})	1613	710	718	126	267	104	150
VSS/TSS (%)	17	15	16	14	17	16	21
E. coli (CFU mL^{-1})	5700	2600	280	330	550	330	640
Giardia spp. (cysts g^{-1})	356	2551	2534	830	607	346	3302
Cryptosporidium spp. (oocysts g^{-1})	6	11	211	nd	nd	nd	24

Notes: TS: total solids; TSS: total suspended solids; FSS: fixed suspended solids; VSS: volatile suspended solids; nd: not detected. I and III: completed maintenance of the filters, after 64th and 140th days of operation; II: maintenance of the non-woven blanket from DGFs, after 121st day of operation.

456 Table 5– HSSF sludge characteristics

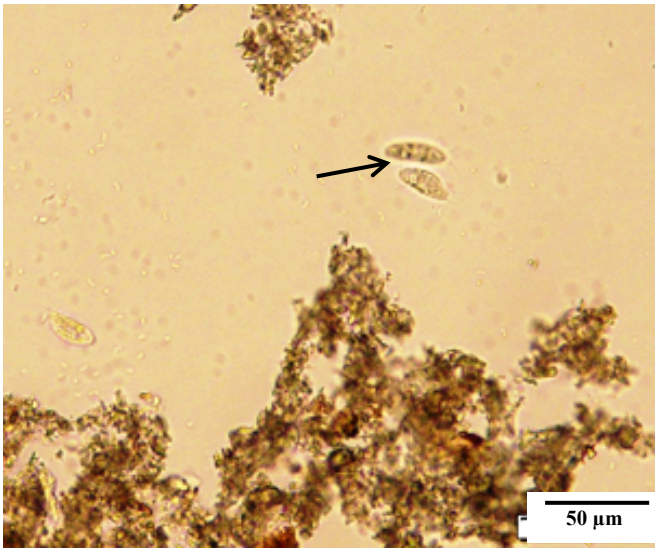
Parameter	Non-woven blanket				Top sand layer			
	HSSF1		HSSF2		HSSF1		HSSF2	
	I	III	I	III	I	III	I	III
Apparent colour (HU)	855	1060	965	1850	1090	3460	1340	4080
Turbidity (NTU)	720	894	485	1160	590	2060	1060	1960
TS (mg L^{-1})	858	1160	746	1424	914	5000	1244	5480
TDS (mg L^{-1})	268	274	266	244	277	2900	167	3380
TSS (mg L^{-1})	590	886	480	1180	637	2100	1077	2100
FSS (mg L^{-1})	425	705	347	880	510	1650	847	1630
VSS (mg L^{-1})	165	182	133	300	127	450	230	470
VSS/TSS (%)	28	21	28	25	20	21	21	22
E. coli (CFU mL^{-1})	170	7	3	10	910	1200	1400	320
Giardia spp. (cysts g^{-1})	163	483	509	2598	44	2920	241	2117
Cryptosporidium spp. (oocysts g^{-1})	70	nd	27	1025	22	120	nd	2263

Notes: TS: total solids; TSS: total suspended solids; FSS: fixed suspended solids; VSS: volatile suspended solids; nd: not detected. I and III: completed maintenance of the filters, after 64th and 140th days of operation; II: maintenance of the non-woven blanket from DGFs, after 121st day of operation.

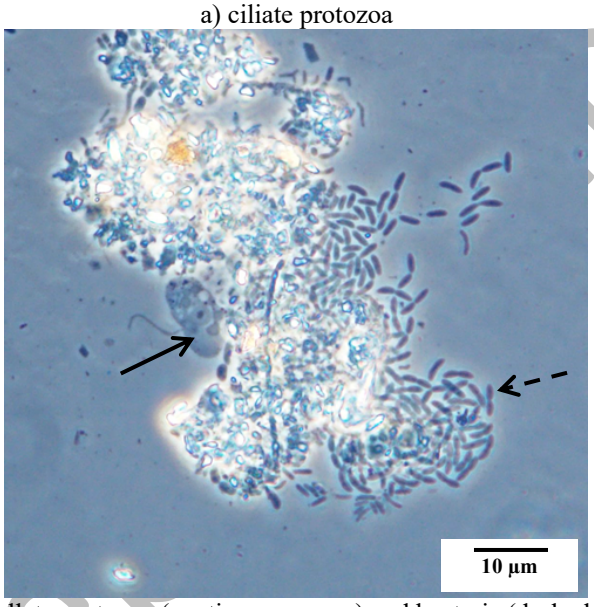
457

458 Solid retention was observed mainly in the DGF1 blanket and inside the DGFs´
459 beds. In HSSFs, blanket and top sand layer showed high concentrations of total suspended
460 solids, apparent colour and turbidity. VSS concentration increase was found between
461 periods I and III for all the filters, except for DGF1 (between periods II and III) and this
462 can be a result of microorganism accumulation (i.e. bacteria, free-living protozoa, fungi)
463 in the *schumutzdecke*, blankets and inside the DGFs' beds, according to Figure 6.

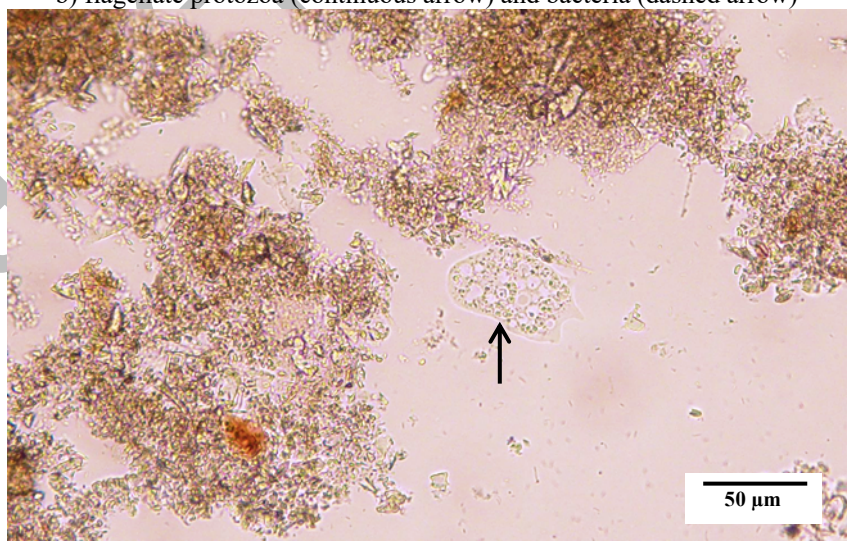
464



a) ciliate protozoa



b) flagellate protozoa (continuous arrow) and bacteria (dashed arrow)



c) amoebae

465

466 Figure 6 - Microorganisms present in the blankets

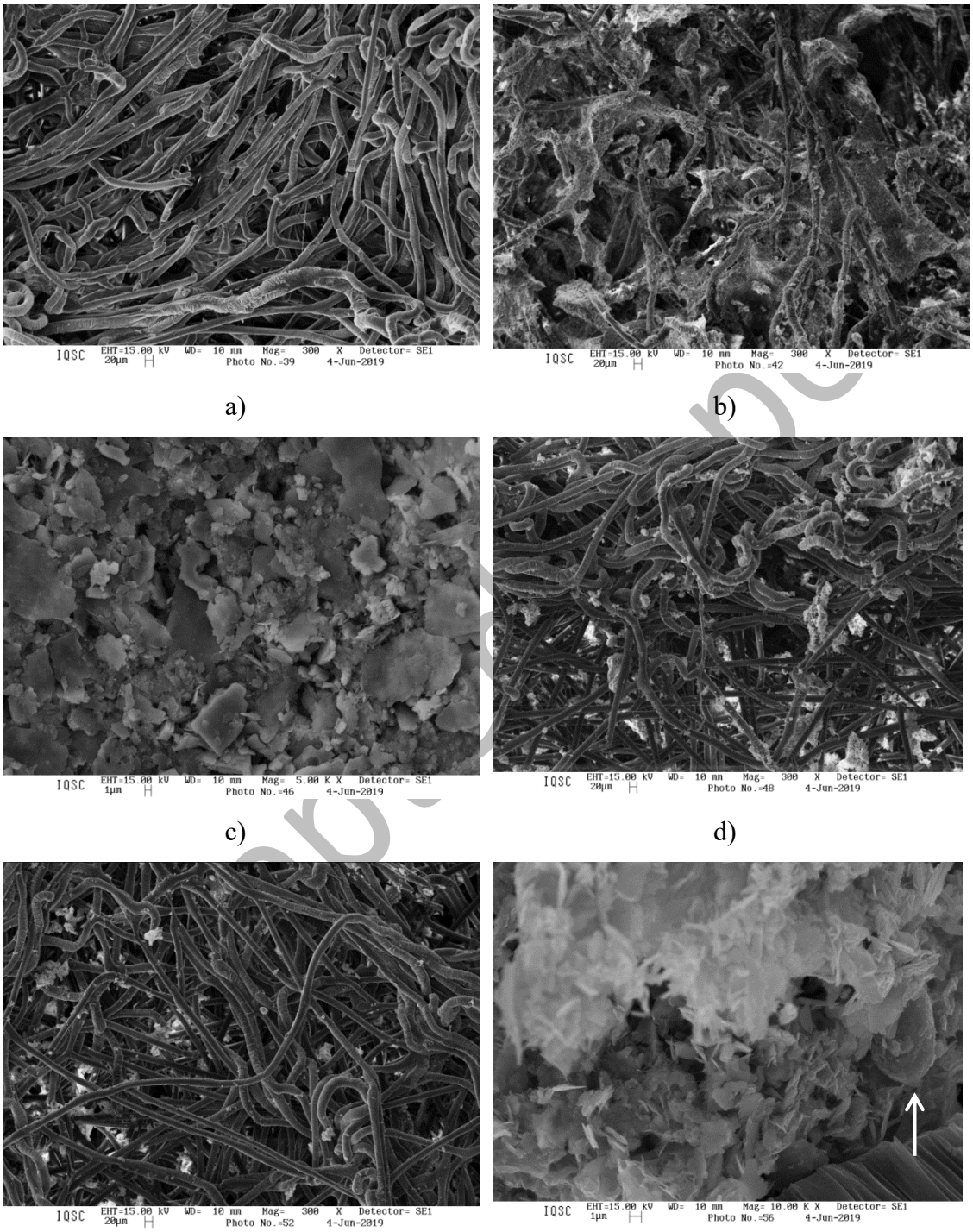
In the blankets, microorganisms morphologically similar to ciliate protozoa (Figure 6a) were found, as well as flagellates (Figure 6b – continuous arrow), amoebae (Figure 6c) and a great amount of bacteria (i.e. cocci, bacilli, isolates and colonials, Figure 6b – dashed arrow) and some fungal hyphae. The number of microorganisms visualised in the blankets followed the relation DGF1 > HSSF2 > HSSF1. The presence of zooplankton as ciliate protozoa, amoebae and rotifers is associated with the greater oocyst removal at the top sand bed (Hijnen et al., 2007). Some authors identified rotifers (Bichai et al., 2014) and ciliate protozoa (Siqueira-Castro et al., 2016) as predators of *Giardia* cysts and *Crypstosporidium* oocysts.

The blankets, mainly in DGF1, showed potential for protozoa removal. The HSSF2 blanket presented a higher concentration of cysts and oocysts per gram compared with the HSSF1 blanket. This fact can be explained by the DGF1 blanket role in protozoa retention. However, this might also be interpreted as a warning for careful and safe planned handling of the blankets when conducting filter maintenance to avoid any unnecessary biological risk exposure of the filters' operator. SEM images for the blankets are shown in Figure 7.

Images display solids accumulation in the blankets for DGF1 (Figure 7b), HSSF1 (figure 7d) and HSSF2 (Figure 7e) compared to its original state (Figure 7a). Figures 7c and 7f show a large amount of kaolinite in the DGF1 blanket and a possible oocyst retained in the HSSF2 blanket as well (arrow in Figure 7f).

A positive aspect of the blankets is to facilitate the filter maintenance, especially on a household scale (Souza Freitas and Sabogal-Paz, 2019; Terin and Sabogal-Paz, 2019). Blankets can also extend the filter run time since they protect the sand bed from particle deposition and the sand compaction (Faria Maciel and Sabogal-Paz, 2018; Modal

491 et al., 2007). However, the presence of blanket in DGF1 generated higher head loss,
492 requiring two blanket cleanings, besides the complete maintenance.



493 Figure 7 - SEM images for blankets (a, b, d and e: 300 x; c: 5,000 x; f: 10,000 x).

494

The DGF2 bed showed higher *E. coli* and protozoa retentions than the DGF1 bed, as a result of the blanket installed in DGF1 that retained part of these microorganisms, not allowing their penetration in the filter bed. The HSSF top sand layer was able to retain part of the protozoa and *E. coli* which passed through the DGFs.

4. Conclusions

HMSF removed turbidity (> 60%), *E. coli* (>1.5 log) and protozoa (>2 log) from influent water; but it was not efficient for colour removal. On the other hand, HMSF was not enough to generate drinking water according to World Health Organisation guidance. Consequently, further studies are needed to optimise the technology.

There were few correlations according to statistical tests between operating parameters. Nonetheless, operation time must be evaluated as a filter ripening parameter since it influenced *E. coli* and protozoa removals.

Non-woven blankets acted as a physical and microbiological barrier, improving *E. coli* and cyst and oocyst retention and turbidity removal.

HMSFs with a non-woven blanket is a clear example of the multi-barrier concept, in which there is more than one treatment stage to improve water quality, with gradual removal of particles and microorganisms.

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6. Statement

Authors hereby declare previous originality check, no conflict of interest and open access to the repository of data used in this paper for scientific purposes.

7. Supplementary Material

Statistical analysis used in the study is provided.

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