Influence of PPCPs on the performance of intermittently operated slow sand filters for household water purification

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ABSTRACT

Removal of pharmaceuticals and personal care products (PPCPs) from drinking water is usually enhanced by advanced oxidation which is not affordable in low income countries. Slow sand filtration has been found to be capable of removing anti-inflammatory compounds, and its low maintenance costs and easy operation make it an attractive technology for treating drinking water in many parts of the world. In addition, slow sand filters can be used at large scale and household levels. The biofilm (i.e. *schmutzdecke*) developed on the top of the sand and within the upper layers of the sand is acknowledged to be responsible for the water purification. However, it is possible that the PPCPs may affect the *schmutzdecke* development and microbial community within the filters, and consequently the performance of the filter. This study investigated two household slow sand filters (for water purification) operated intermittently with and without contamination by six PPCPs. Eleven parameters were monitored in the affluent and effluent water, including bacterial species present and *schmutzdecke* biomass development. Results demonstrated that the household slow sand filter performance was not affected by the 2 μg L^{-1} of PPCPs in the water. There was no significant difference between filters for total coliforms and *E. coli* removal, but there was considerable difference between sampling times. Biomass considerably increased with the number of filtrations in both filters and there was no significant difference between filter biomass. However, it was found that more bacterial species were present in the period with no contamination than during the contamination period. *Bacillus anthracis* and *Exiguobacterium* sp. showed to be resistant to the effects of the PPCPs. These suggest there are effects of PPCPs on bacterial species within the filter. However, the effect of the PPCPs on biomass was not conclusive in this study and needs to be further investigated.

Keywords: slow sand filters; pharmaceuticals and personal care compounds; biomass; biofilm; bacteria sequencing.

66 ## 1. INTRODUCTION

67 Despite efforts of achieving the Millennium Development Goal (MDG 7) on
68 drinking-water, globally around 1.9 billion people use a drinking-water source
69 contaminated with pathogens (WHO, 2016). In addition to pathogens, chemical
70 compounds have been found worldwide in surface water, drinking water and groundwater
71 at concentration levels of ng L^{-1} to µg L^{-1} (Sui et al., 2015). Among these compounds are
72 antibiotics, anti-inflammatory, analgesics, steroids, antidepressants, antipyretics,
73 stimulants, antimicrobials, fragrances, cosmetics, and many other pharmaceuticals and
74 personal care products (PPCPs). These have been widely detected in surface water and
75 wastewater in many countires such as UK (Lyons G., 2014), China (Chen et al., 2012),
76 Spain (Gomez et al., 2007), Romania (Moldovan, 2006), South Korea (Yoon et al., 2010),
77 U.S.A (Hageman et al., 2016), Canada (Chan et al., 2014), and many others. On the other
78 hand, there is very little information on the occurrence of PPCPs within the aquatic
79 environment of low-income countries (Sorensen et al., 2015). However, in Africa, the use
80 of use of synthetic organic compounds in the domestic context, within agriculture and
81 industry is increasing (Breivik et al., 2011). In addition, 70% of the total urban population
82 in many large African cities is estimated not to be connected to a sewerage system and
83 80% of wastewater is discharged untreated to surface waters or the soil (Nyenje et al.,
84 2010). These facts illustrate how PPCPs pose a risk to surface and ground water quality,
85 and public health in developing countries.

86

87 PPCPs are biologically active and designed to interact with specific pathways and
88 processes in humans and animals (Boxall et al., 2012); but they may exert their activity
89 at low concentrations (Vulliet and Cren-Olivé, 2011), and potentially have an impact on
90 drinking-water supplies (Jones et al., 2005). Adverse effects caused by pharmaceutical
91 compounds include aquatic toxicity, development of resistance in pathogenic bacteria,

92 genotoxicity and endocrine disorders in humans and aquatic life (Kummerer, 2008).
93 Therefore, PPCPs presents a threat to the ecosystem and human health.

94 Conventional water treatment processes usually use coagulation with alum or
95 ferrate followed by flocculation, sedimentation, filtration, and disinfection. This process
96 achieves high removal of microorganisms, but very modest removal of dissolved organic
97 carbon (DOC) (Rigobello et al., 2013). On the other hand, advanced water treatment using
98 oxidation and activated carbon usually enhance the removal of DOC. For example, Qiao
99 et al. (2011) investigated the occurrence and fate of PPCPs in drinking water and found
100 15 and 12 PPCPs at concentrations of 0–36 ng L^{-1} in source water and 0–20 ng L^{-1} in
101 treated water, respectively. Conventional water treatment processes were observed to
102 remove various PPCPs by an average of 30% and above 50%. However, advanced water
103 treatment processes were more efficient in the removal of most PPCPs, being types and
104 concentrations reduced by 50% and approximately 90%, respectively. One of the
105 drawbacks of advanced treatment processes is their high capital and operational costs.
106 This makes the removal of PPCPs unfeasible in developing countries where financial
107 resources are limited. Therefore a more cost-effective treatment alternative is required.

108 Biodegradation has been suggested as one of the mechanisms responsible for the
109 removal of organic trace contaminants (Halle, 2009; Qiao et al., 2011, Bertelkamp et al.,
110 2014). Slow sand filtration (SSF) which is a biological process showed to be an efficient
111 process in removing anti-inflammatory compounds such as Diclofenac, Naproxen and
112 Ibuprofen (Erba et al., 2014). Therefore, it seems a viable alternative, since it is easy to
113 operate and maintain – it does not require chemical coagulants and it can be used at both
114 large and household scales. An example of SSF at household scale is the biosand filter
115 (BSF) which is operated interminttently (CAWST, 2012). Kennedy et al. (2013)
116 illustrates the potential of household SSF by investigating the estrone, estriol, and 17α-

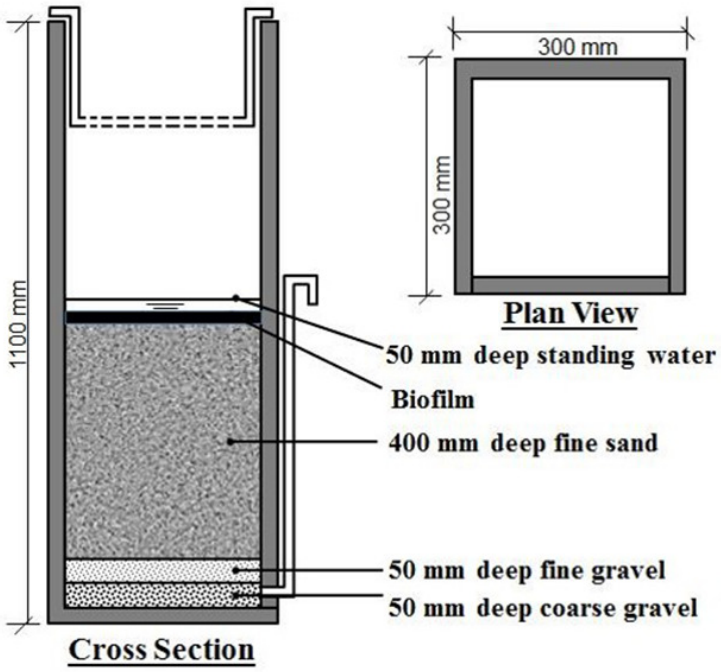
117 ethinyl estradiol (endocrine disrupting compounds - EDCs) by BSF. Very low removal
118 rate (< 15%) was observed for these EDCs, however, the removal was increased (> 98%)
119 by adding household bleach to the effluent water of the BSF.

120 The high efficiency of water treatment achieved by SSF is partly explained by the
121 slow filtration rate (0.1–0.4 mh^{-1}) and fine effective size of the sand (0.1–0.3 mm). But it
122 is also attributed to biological processes in the layer of biofilm that accumulates above
123 the sand (*schmutzdecke*) and within the upper layers of the sand bed (Huisman and Wood,
124 1974). Nakamoto, (2014) explains the importance of the biofilm in the treatment system
125 and refers to SSF as ecological filtration emphasizing the important role of the biological
126 layer in the top of the sand.

127 Many studies have focused on the investigation of the biomass and microbial
128 community in SSF (Campos et al., 2002; Rooklidge et al., 2005; Unger and Collins, 2008;
129 Wakelin et al., 2011; Hwang et al., 2014; Haig et al., 2014 and 2015) but none of them
130 investigated the effect of chemical compounds on the biofilm. However, there have been
131 some studies demonstrating the effects of pharmaceutical compounds on bacterial species
132 present in the aquatic biofilms. Cited effects are supressed biomass, respiration, and
133 photosynthesis (Rosi-Marshall et al., 2013), increased drug resistance (Drury et al., 2013),
134 and toxicicity (Harada et al., 2008). More recently, Rosi-Marshall et al. (2015) reviewed
135 the literature on the ecological effects of illicit drugs on aquatic organisms and conclude
136 that a wide array of aquatic organisms such as bacteria and algae have receptors that make
137 them potentially sensitive to these compounds. Therefore, PPCPs may affect the
138 development of the *schmutzdecke* and microbial community in slow sand filters, and
139 consequently influence the performance of the filter.

140 In this work the development of the biofilm, including the bacterial species and
141 biomass growth, in intermittently operated household SSF treating water contaminated

142 by PPCPs was investigated. In addition, the effect of hydraulic detention time on filter
143 performance was evaluated.
144
145 ## 2. MATERIALS AND METHODS
146 ### *2.1.Raw water and slow sand filter setup*
147 Two physically identical household SSFs (Figure 1) were used for this study. The
148 filters contained 50 mm of underdrain gravel, 50 mm of medium sized support gravel,
149 and 400 mm of sand. The effective size (d_{10}) of the sand was 0.210 mm with uniformity
150 coefficient of 1.40. The values are within the typical range of grain size between 0.15 mm
151 and 0.30 mm and uniformity coefficient of less than 4 for use in slow sand filtration
152 (Huisman and Wood, 1974).
153 Raw water was collected from the Regent's Park Lake in London, UK which has
154 on average turbidity of less than 10 NTU. Once a week, a total of 100 L of water were
155 collected from the lake and a volume of 24 L was filtered by each filter twice a week. The
156 experimental work was divided into two phases, resulting in a total of 21 filtrations in
157 phase 1, and 11 filtrations in phase 2 (see Table 1). Filter F2 received water contaminated
158 by PPCPs during phase 1, and filter F1 worked as control. During phase 2, there was no
159 contamination at all.
160 To investigate the effect of hydraulic detention time on water quality, the filters
161 were operated with tap so that the water filtration was paused during phase 1, while in
162 phase 2 filters were operated without pause.
163



164

165 **Figure 1:** Plan view and cross section of constructed household SSF.

166

167 *2.2. PPCPs solution*

168 During phase 1, the affluent water of filter F2 was contaminated with a mix of 6
169 PPCPs (i.e. paracetamol, diclofenac, naproxen, ibuprofen, methylparaben and
170 benzophenone-3). For this, the standard compounds were weighed in precision weighing
171 balances and dissolved in methanol. A stock solution was prepared and from this, an
172 aliquot was withdrawn and mixed into the raw water to be filtered by F2, in a final
173 concentration of 2 µg L^{-1} for all of them, which is usually the mean value found in surface
174 water (Sui et al., 2015). All the PPCPs used (Table A.3 Appendix material) were
175 analytical standard ≥ 98 % purity from Sigma-Aldrich, UK.

176

177 *2.3.Water Sample Collection*

178 For water quality analysis, 100 mL of affluent and filtered water were collected in
179 triplicate during phase 1 and phase 2 after each filtration (Figure 2). In phase 1, once

filtration started, filtered water samples were collected at 10 min (S1) - representing the water from the previous filtration, and at 4 hours and 30 min (S2) - representing the water from the same day (Campos and Outhwaite, 2014). In phase 2, S2 samples were collected after 90 min representing water from the same filtration day, and S1 represented the water from the previous filtration (See Figure 2).

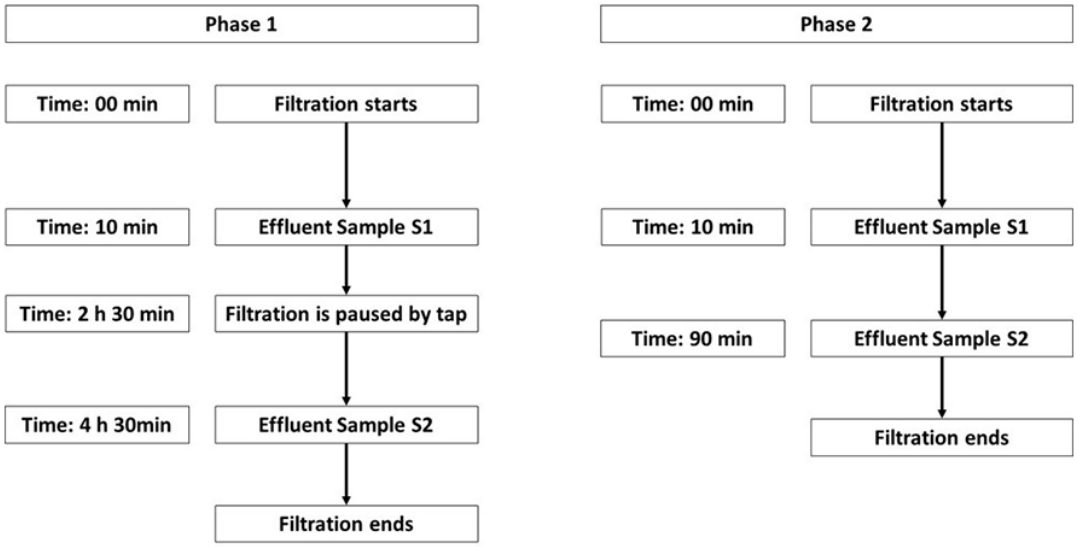


Figure 2: Sampling procedure household filters in phases 1 and 2.

To identify and quantify the PPCPs in water at phase 1, 300 mL of filtered water were collected in two different times:- (1) at the end of the filtration procedure in the day (S2) and (2) 24 hours after the contamination (S1), in duplicate. Six measurements for each time point were performed by the LC-MS analysis. A sample from raw water was collected to investigate if the Regent's Park water was contaminated with the PPCP compounds used in this study.

2.4. Water quality parameters

196 Turbidity, dissolved oxygen (DO), specific ultraviolet absorbency, pH,
197 temperature, conductivity, total organic carbon (TOC), nitrite, nitrate, and phosphate
198 were the physico-chemical parameters monitored. Their determination followed standard
199 methods (APHA, 2005). The treated water quality was compared with the parameters set
200 by the World Health Organization (WHO, 2011) for drinking-water quality (Table A.1).
201 Phosphate, nitrite, and nitrate which were determined by ion chromatography
202 (KS-1100 Dionex). The method used the AS23 4 x 250 mm carbonate eluent anion-
203 exchange column (Dionex). Anion mode analysis was carried out according to the
204 manufacturer's recommendations, using a mobile phase of 4.5 mM Na_2CO_3. The flow
205 rate was set at 1 mL min^{-1}, with a total run time of 30 min and temperature held at 30 °C.
206 Cation analysis was undertaken using an IonPac CS 16-5 µm (5 x 250 mm) column with
207 30 mM Methanesulfonic acid as eluent. The flow rate was also set at 1 mL min^{-1}, with a
208 total run time of 25 min and temperature held at 40 °C. Detection of ion peaks in both
209 conditions was undertaken by suppressed conductivity measurements at 25 mA. The
210 spectra were analysed using a set of standards and software provided by Dionex.
211 Bacteria was measured as total coliforms and *E. coli* using the m-ColiBlue24®
212 ampules according to manufacturer's instructions. Before analysis, the raw water had to
213 be diluted at the ratio of 1:10 for bacterial counting as initial concentration was larger
214 than the limit of the test (more than 200 colonies per plate).
215
216 *2.5. Identification and quantification of PPCPs in water*
217 To quantify the compounds, the water samples were passed through the Solid
218 Phase Extraction (SPE) cartridges, the final eluate from the SPE cartridge was pre-
219 concentrated and injected onto the capillary liquid chromatography mass spectrometry
220 (cap LC-MS) instrument.

221

222 *2.5.1. Solid Phase Extraction (SPE)*

223 Prior to the extraction, the water samples (300 mL) were filtered through
224 Whatman no.1 filter paper and the pH was adjusted to 3.0 (using hydrochloric acid,
225 1:1,v/v). The SPE C_{18}-E cartridges 200 mg per 6 ml were from Extrata-X (Phenomenex)
226 (Catalogue number 8B-S100–FCH). The SPE cartridge was first conditioned twice with
227 6 mL each of methanol following with 6 ml of milli-Q water and then with 6 ml of water
228 acidified to pH 3.0 with HCl by gravity using a vacuum manifold (Phenomenex,
229 California, USA). This followed by the application of the water sample through the SPE
230 cartridge. The SPE was washed with 6 ml of water and subsequently, the cartridges were
231 dried for 15 min, then the analytes were eluted twice with 4 ml of methanol. The solvent
232 from the final eluate was evaporated to dryness under a stream of N_2 and reconstituted to
233 a final volume of 300 µl with methanol-water (20:80, v/v).

234

235 *2.5.2. Direct infusion capillary ESI mass spectrometry*

236 For the analysis of reference compounds (20 µg L^{-1}) infused at 10 µL min^{-1} at the
237 LTQ. MS and MS^2 spectra were recorded. The MS^2 isolation width was set at 2 to allow
238 the selection of monoisotopic precursor ions. The LTQ was calibrated externally prior to
239 each analytical session, and mass accuracy was 50 ppm. The *m/z* scale of the instrument
240 was calibrated with a solution polypropylene glycol (PPG). The *m/z* values were set for
241 use in SIMs mode. For the identification of each pharmaceutical standard, MS^2
242 acquisitions were set up to determine its fragmentation patterns to confirm its identity.

243

244 *2.5.3. Capillary Liquid Chromatography Mass Spectrometry (capLC-MS, -MS^2)*

245 Liquid chromatographic separation was performed on an Accela HPLC system
246 utilising a Hypersil GOLD reversed-phase column (1.9 µm particles, 150 x 2.1 mm) from
247 Thermo Electron Corp (San Jose, CA).

248 The LC system consisted of an Accela solvent rack, Accela 600 pressure dual
249 gradient pumping system with an on-line solvent degasser and an Accela autosampler.
250 Mobile phase A consisted of 1 % methanol (MeOH), and mobile phase B was acetonitrile
251 (ACN) and MeOH (50:50, v/v), all containing 0.1 % formic acid. After 0.5 min at 1 % B,
252 the proportion of B was raised to 70 % B over the next 5 min, and then gradually increased
253 to 80 % B over the next 10 min. This was followed by an increase to 99 % B in 0.5 min,
254 and remaining at 99 % B for 2 min 30 s before returning to 1 % B in 6 s and re-
255 equilibration for a further 2 min 50 s, giving a total run time of 22 min. The flow rate was
256 maintained at 200 µL min and eluent directed to the capillary ESI source of a LTQ mass
257 spectrometer performed on a Finnigan LTQ (Thermo Electron Corp, San Jose, CA) with
258 a linear ion-trap (LIT) analyser. Ions from the ESI source operated in positive and
259 negative ESI modes and were analysed in either MS or SIMs modes. The mass
260 spectrometer was operated under the following settings: -spray voltage 4.5 kV, capillary
261 temperature 280 °C, sheath gas flow rate 40, auxiliary gas flow rate 10. MS and SIMs
262 scans consisted of three averaged "microscans" each with a maximum injection time of
263 200 ms. For the acquisition of MS^2 spectra the collision energy setting was 35 Volt using
264 argon as the collision gas. These MS^2 scans were used for a conformation of
265 pharmaceuticals. Full mass spectra were acquired at *m/z* 120 - 400. Retention times, SIMs
266 and MS^2 scans were utilised for identification and confirmation of pharmaceuticals.

267 For LC-MS and LC-MS^2 analysis of the reference compounds, each sample (30
268 µg L^{-1} in 60 % MeOH) was injected (15 µL) onto the reversed-phase column and eluted
269 into the LTQ at a flow-rate of 200 µL min^{-1}. Naproxen, methylparaben and diclofenac

were analysed in negative mode, whereas paracetamol, benzophenone-3 and ibuprofen were analysed in positive ESI mode. For the quantitation of pharmaceutical compounds a selected ion monitoring (SIM) scan was used. The set-up *m/z* values of each compound are at Table A.4.

For the analysis of pharmaceuticals extracted from the cartridge, 10 µL of the sample was acidified to 0.5 % v/v with formic acid. The sample was centrifuged at 13,000 xg for 3 min and 10 µL injected onto the LC column.

The analytical batch consisted of six pharmaceutical standards to establish the calibration curves in the range from 10 to 1000 µg L^{-1}, following sample analyses and blank injection between each sample. Quality control samples were analysed after 15 LC-MS runs of samples to check the instrumentation performance. The sample needle and injection loop were washed with methanol to eliminate sample carry-over, and each sample was analysed in duplicate. Each sample was analysed twice in positive and negative ESI modes.

Limit of detection (LOD) and limit of quantification (LOQ) were determined in ranges from 0.2 to 0.7 µg L^{-1} and 1.8 to 5.1 µg L^{-1}, respectively, using the signal-to-noise ratios (Table A.4). More information about are in the Appendix material.

2.6. Microbiological analysis of the schmutzdecke

Two *schmutzdecke* samples (around 10 g each) from both filters were collected in triplicate and randomly from the top of the sand at end of phases 1 and 2. The samples were stored in -80 °C in vials with 10 % glycerol.

The samples were mixed with PBS buffer (Phosphate-buffered saline) and vortexed. Serial ten-fold dilutions were performed and cultured on R2A agar which is recommended for bacteria in water, especially potable water (Oxoid, UK), at 25 °C for

48 h. Different types of colony morphologies were isolated. The DNA from each morphology was extracted using the QIAamp DNA mini kit ® (QIagen, UK). Extracted DNA was amplified using the 16S rRNA gene universal primers 27F (5'AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'GGTTACCTTGTTACGACTT-3') (Lane et al., 1985). The amplified DNA was purified using the QIAquick PCR purification kit (QIAGEN, UK). Sequencing of the 16S rRNA gene purified amplicons was carried out by Eurofins Genomics, UK. Forward and reverse sequences were edited and aligned using EDISEQ Software and Megalign. The nearest phylogenetic neighbour for each isolate was found using the Basic Local Alignment search (BLAST) tool from NCBI (National Centre for Biotechnology Information, Bethesda, MD, USA). A presence/absence matrix looking at the presence of all identified organisms in all samples was created. The matrix was then used to perform principal component analysis (PCA) which makes a comparison between samples with many variables. The PCA was carried out using Multiple Variable Statistical Package (Kovach Computing Services, UK).

2.7. Biomass measurements

Three samples of 100 g each were taken randomly from both filter sand surfaces (i.e. *schmutzdecke* and few top centimetres of sand) for biomass determination. Biomass during phase 1 was measured only once at the end of phase 1 due to resource constrains and in phase 2, samples were collected in the second filtration of each week. In total, 6 samples were collected. The sand sample collection was limited to avoid affecting the performance of the filters since the biomass is an essential component for the water purification by SSF (Nakamoto, 2014). After collected, the samples were immediately analysed for biomass determination by an adapted fumigation procedure (Campos et al., 2002).

321 *2.8. Statistics analysis*

322 Statistical test of normality for the water quality parameters was carried out using
323 the software Originlab origin 8. All parameters showed to be normally distributed by the
324 Kolmogorov-Smirnov test at the 0.05 level (95 %). Therefore t-test statistic was done to
325 examine the relationships between F1 (control) and F2 (contaminated) for sampling times
326 S1 and S2, and between sampling times (S1 and S2) of F1 and F2. Values were significant
327 at $p < 0.05$.

328

329 **3. RESULTS AND DISCUSSION**

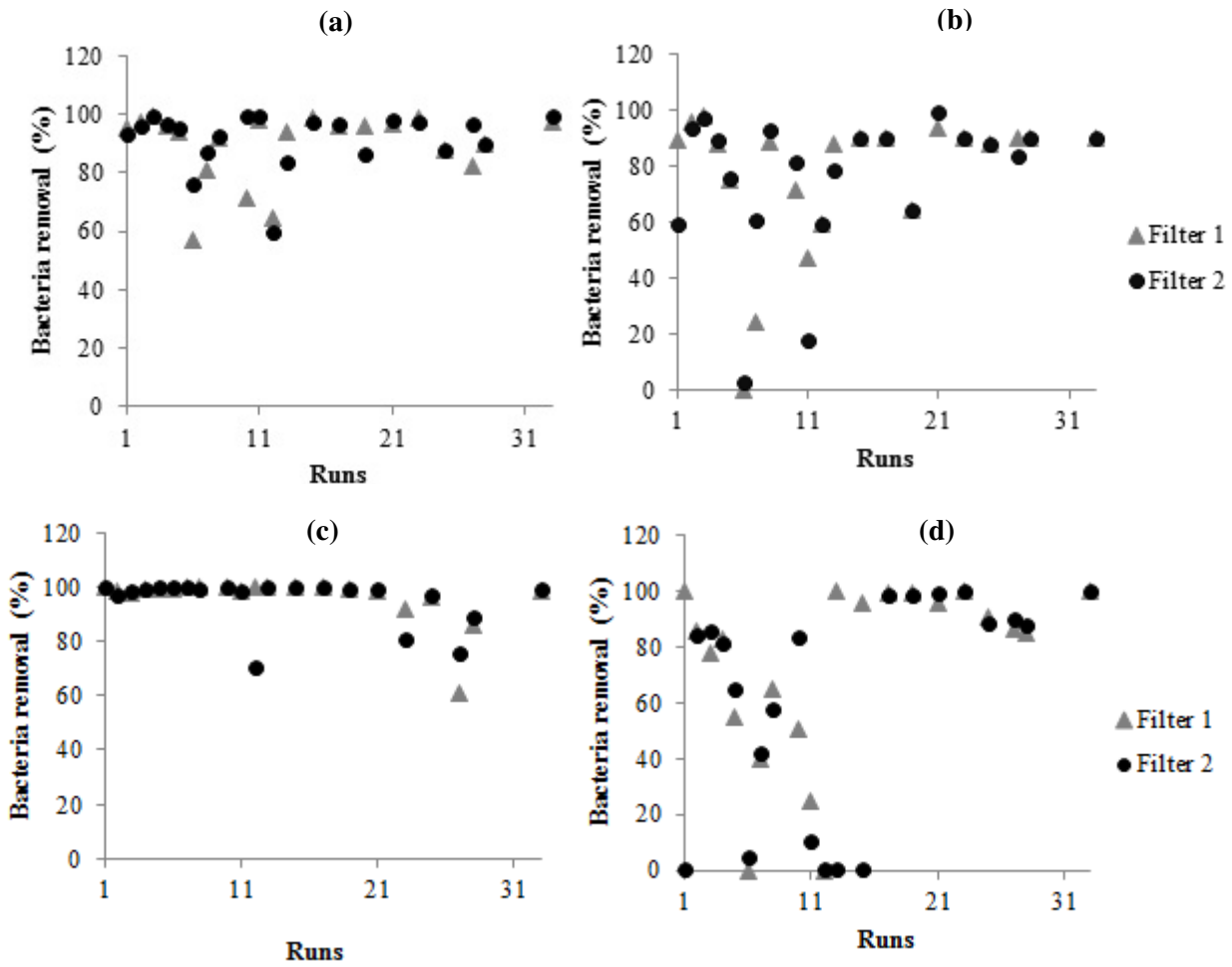
330

331 *3.1.Water quality measurements*

332 Mean values of the monitored parameters of water quality and the concentration
333 of total coliforms and *E. coli* from the affluent water are presented in Table A.2. Overall
334 turbidity of the raw water was on average 6.94 to 6.55 NTU in phase 1 and phase 2
335 respectively. Dissolved oxygen in phase 2 (5.77 mg L^{-1}) was a bit lower than in phase 1
336 (7.86 mg L^{-1}) and this was probably due to the presence of algae bloom. As result,
337 conductivity, phosphates and TDS were higher in phase 2 than in phase 1. Also total
338 coliforms and *E. coli* concentrations were much higher in phase 2 than in phase 1.

339 After filter maturation – achieved after 10 filtrations within a period of 20 days --
340 analysis of the water quality parameters showed that the household SSFs met the quality
341 parameters (Table A.1) set by the World Health Organization (WHO, 2011), except for
342 total coliform and *E. coli* removal which exceeded the limit of absence in 100 mL (Figure
343 3b and 3d). However, both filters presented excellent removal of total coliforms and *E.*
344 *coli* (≥ 99 %) at sampling time S1 (Figure 3a and 3c) as expected (Ngai et al., 2007,
345 Mwabi et al., 2012). In addition, there was significant difference ($p < 0.05$) between S1
346 and S2 (sampling time) for total coliforms and *E. coli*. However, there wasn't any

significant difference ($p = 0.80$) between F1 and F2. This indicates that the retention time influence the removal of bacteria and that PPCPs did not affect it.



Figures 4 and 5 show the average results of all the water quality parameters, at the different time of sample collection for phases 1 and 2. It can been seen that F1 (control) and F2 (contaminated) had significant differences between affluent and effluent for the same parameters. This indicates that the PPCPs seemed not to affect the removal performance of the physico-chemical parameters. However, the pause of the flow caused by the tap in phase 1 seemed to improve the removal of turbidity and absorbance, while dissolved oxygen was reduced significantly ($p < 0.05$) from 7.81 mg L^{-1} to 9.93 mg L^{-1} and 3.80 mg L^{-1} in F1 and F2, respectively, due to microbial respiration. But these levels are above the recommended value of 3 mg L^{-1} for SSF (Huisman and Wood, 1974). Nitrate levels were increased significantly ($p < 0.05$) from 0.29 mg L^{-1} to 2.40 mg L^{-1} and

385 2.28 mg L^{-1}, while phosphate increased moderately from 8.61 mg L^{-1} to 11.53 mg L^{-1} and
386 11.36 mg L^{-1} in F1 and F2 respectively, in phase 1 for sampling time S1. The decrease in
387 oxygen and increase in nitrite and phosphates may be due to algae respiration which
388 converts algal phosphorus to inorganic phosphorus and algal nitrogen to inorganic
389 nitrogen (Brown and Barnwell, 1987).

390 It was also observed that the water quality of the Regent's Park Lake had higher
391 levels of conductivity, TDS, and phosphate in phase 2 than in phase 1 (Figure 4 and 5).
392 This was due to the fact phase 2 was carried out during summer in the presence of an
393 algal bloom. Consequently, the removal efficiency of the filters in phase 2 was smaller
394 than in phase 1. However, another reason might have been the fact that 3 samples of
395 *schmutzdecke* plus few centimetres of the sand were collected every two weeks during
396 phase 2 for biomass determination.

397 There was no significant difference between the filters and sampling time, in both
398 phases, for temperature, conductivity, TDS, pH, turbidity, TOC, nitrate and phosphate (p
399 = 0.1 to 0.9). These results confirmed that the effect of increased residence time after
400 filter maturation did not influence filter performance as found previously (Campos and
401 Outhwaite, 2014). Results also showed that the presence of PPCPs in the affluent did not
402 affect the efficiency of F2 (contaminated) in removing turbidity, total coliforms and *E.*
403 *coli* during phase 1 (removal > 90 %).
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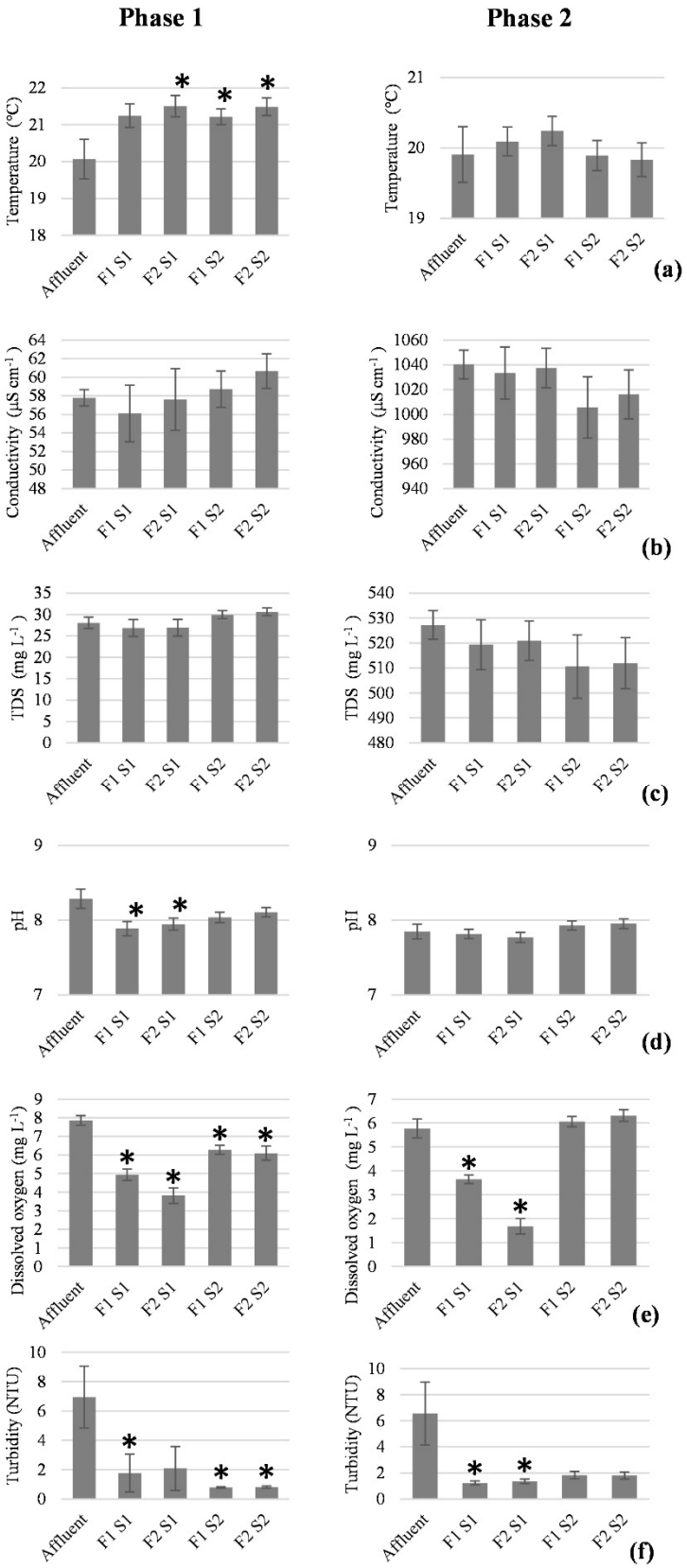
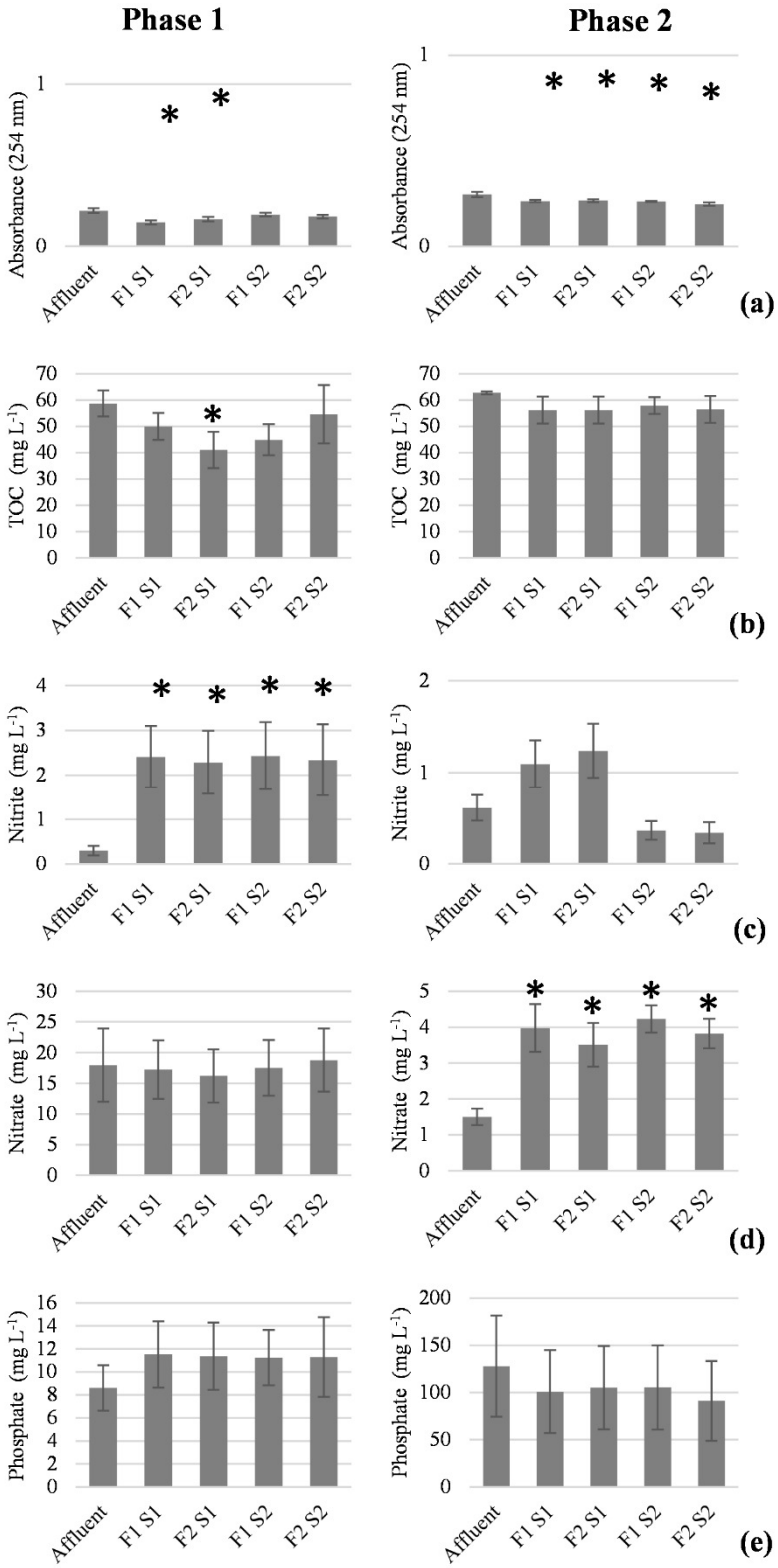


406

407 **Figure 4:** Mean values of: a) temperature; b) conductivity; c) TDS; d) pH; e) Dissolved oxygen;
408 f) turbidity, measured during phases 1 and 2, standard deviation and *p* values showing the
409 relationships between affluent and effluent water, represented as * for significant *p*-values ($p <$
410 0.05) .

411



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413 **Figure 5:** Mean values of: a) absorbance; b) TOC; c) nitrite; d) nitrate; e) phosphate, measured
414 during phases 1 and 2, standard deviation and *p* values showing the relationships between
415 affluent and effluent water, represented as * for significant *p*-values ($p < 0.05$).
416

417 However, there was considerable difference between and within phases for
418 absorbance, nitrite and DO. For absorbance, there was considerable difference in F1
419 between sampling time S1 and S2 ($p = 0.006$) in phase 1 for operation with tap. But there
420 was no difference between the filters in phase 1. The increased residence time (S1)
421 increased the filters performance. This is in agreement with the findings of Elliott et al.
422 (2008) for *E. coli* removal by BSF. Regarding nitrite, a significant difference between
423 both F1 and F2 occurred only in phase 2 for both filters operated without tap and sampling
424 time S1 ($p = 0.01$) and S2 ($p = 0.008$).
425 For DO measured in phase 1, there was no significant difference between the
426 filters ($p = 0.70$), however, a significant difference was observed between sampling times
427 being DO in S1 well below than in S2. And this was most significant in the contaminated
428 filter F2 ($p = 0.0009$) than in F1 ($p \leq 0.05$). The average concentrations for F1 in S1 and
429 S2 were 4.93 mg L^{-1} and 6.28 mg L^{-1} respectively, while for F2 in S1 and S2 were 3.80
430 mg L^{-1} and 6.09 mg L^{-1} respectively. This was probably caused by the pause of the flow
431 by the tap and consequently S1 – i.e. the water from the previous filtration - presented the
432 lowest DO caused by the increased detention time. This indicates that the biofilm may
433 have started to decay due to the lower oxygen diffusion into the supernatant water caused
434 by the idle time (Lea, 2014).
435 A similar DO behaviour was observed in phase 2 as there was no significant
436 difference between the filters F1 and F2 in S2 (sample from the same day filtration). But
437 there was a significant difference between the effluent DO of both filters for sampling
438 time S1, being the average effluent DO (3.65 mg L^{-1}; $p = 0.003$) in F1 higher than in F2
439 (1.67 mg L^{-1}, $p = 0.0009$). It can also be noticed that a significant difference between the
440 sampling times for both filters for F1 ($p < 0.00001$), and for F2 ($p < 0.00001$). Regardless
441 of the filter operation mode, standing supernatant water in the filters for more than 24

442 hours caused low amounts of DO in the effluent water. This leads to low activity of
443 microorganisms that require the DO for their basic activities, may change the bacterial
444 activity from aerobic to anaerobic in the filter, as Young-Rojanschi and Madramootoo
445 (2014) found when comparing intermittent and continuous operation of biosand filters.

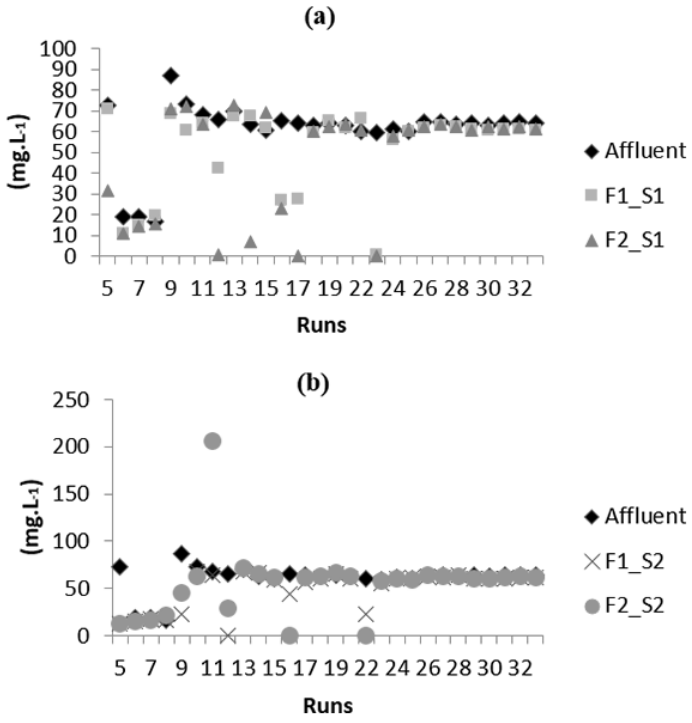
446 The *p* value for total coliform removal (Figures 3a and 3b) shows that for phase
447 1, there was no significant difference between filters F1 and F2, but there was a difference
448 between sampling times S1 and S2 ($p = 0.0041$ for F1; $p = 0.0042$ for F2). Total coliforms
449 were better removed at S1 (F1 = 89.2 %, F2 = 91.1 %) than S2 (F1 = 68.7 %, F2 = 72.1
450 %). These results are in agreement with Campos and Outhwaite (2014). Although
451 increased retention time seems to improve total coliform removal, removal improved with
452 the increased number of filtrations and time. For example, in phase 2, there was no
453 significant difference between F1 and F2 and sampling times S1 and S2. However, in all
454 cases in phase 2, the average total coliform removal was > 85 %. The difference between
455 the sampling times ceased to exist when more number of filtrations were performed. The
456 improvement of water quality parameters with time by household SSFs has been reported
457 elsewhere (Kaiser et al., 2002, Ngai et al., 2007, Mwabi et al., 2012). For example Mwabi
458 et al. (2012) found that the removal of turbidity by biosand filters increased with time,
459 when the filters were maturated, decreasing turbidity on average from 40.0 NTU to 2
460 NTU.

461 For removal of *E. coli* there was a significant difference only between sampling
462 times for F2 ($p = 0.0048$), during phase 1, where the average removal was 97.6 % at S1
463 and 47.8 % at S2. In phase 2, there was no significant difference between F1 and F2 and
464 sampling times S1 and S2 where removal of *E. coli* was > 87 % in all cases. Both total
465 coliform and *E. coli* removal were variable in phases 1 and 2 for F1 and F2 at sampling
466 time S2 i.e. water samples from the same filtration day, although it became constant after

467 the 16th filtration. Overall, the removals of coliforms and *E. coli* are in agreement with
468 published work on household biosand filter (Ngai et al., 2007; Mwabi et al., 2012).

469 It can be seen from Figure 6 that the effluent TOC concentrations were variable
470 in phase 1 (up to filtration 21), while it was kept constant in phase 2 (from filtration 22).
471 This was probably due to the PPCPs contamination that makes TOC variable at phase 1.
472 However, no differences were observed in the effluent TOC between F1 and F2. These
473 results are in agreement with Campos et al., (2002) who found no differences in the water
474 quality of effluents between large-scale covered and uncovered filters despite large
475 differences in the biological properties of the covered and uncovered filter beds. It can be
476 seen (Figure 5b) there was no significant difference between the filters in S1 ($p = 0.30$ for
477 phase 1; $p = 0.99$ for phase 2) in S2 ($p = 0.44$ for phase 1; $p = 0.80$ for phase 2); and
478 between the sampling times for F1 ($p = 0.49$ for phase 1; $p = 0.78$ for phase 2) in F2 ($p =$
479 0.31 for phase 1; $p = 0.97$ for phase 2).

480



481

482 **Figure 6:** TOC in phase 1 (Filtrations 1-21) and phase 2 (Filtrations 22-33) for sampling
483 time (a) S1 and (b) S2.

3.2 Quantitation of PPCPs by LC-MS analyses

Initially to establish the LC-MS methodology for analysis of PPCPs, six pharmaceutical standards by the direct infusion ESI using the LTQ mass spectrometer were analysed. This was perfomed in order to establsih their mass spectrometry signals under ESI conditions. MS spectra of some compounds are shown in Appendix material (Figures A.1 – A.5), with shows, for example, $[M-H]^-$ ions at *m/z* 294 corresponding to diclofenac (Figure A.1). The isotopic pattern confirms the presence of two chlorine molecules present in diclofenac. Table A.4 summarises mass spectrometry results.

After the direct infusion experiments the standards were injected on the reversed phase column coupled to the LTQ mass spectrometer. The reconstructed ion chromatogram (RIC) are in Appendix material (Figures A.6 - A.8).

In the Regent's Park raw water only benzofenone-3 and paracetamol were detected, while the other PPCPs were not detected by the LC-MS analysis. The samples were collected in May, June and July at phase 1. Table 2 shows the measured amounts of benzofenone-3 and paracetamol. As there is also possibility that the Regents Park water may contain some compounds which might have the identical molecular weight to benzophenone and therefore this compound will behave identical under LC conditions and ESI ionisation to benzophenone-3, the compounds would suppress or enhance signal of analytes. To identify if this was the matrix effect, the MS^2 analysis of this LC chromatographic peak eluted at 11.4 min from the Regents River sample was performed. The MS^2 spectrum shows an identical fragmentation pattern to the pure standard of benzophenone injected on the LC-MS system under the same operational conditions. Also, from literature reports up to 125 ng L^{-1} of benzophenone-3 have been measured in surface water in a lake in Switzerland (Poiger et al., 2004). In the UK benzophenone-3 was detected in river water at concentration of 17 ng L^{-1} (Kasprzyk-Hordern et al., 2008).

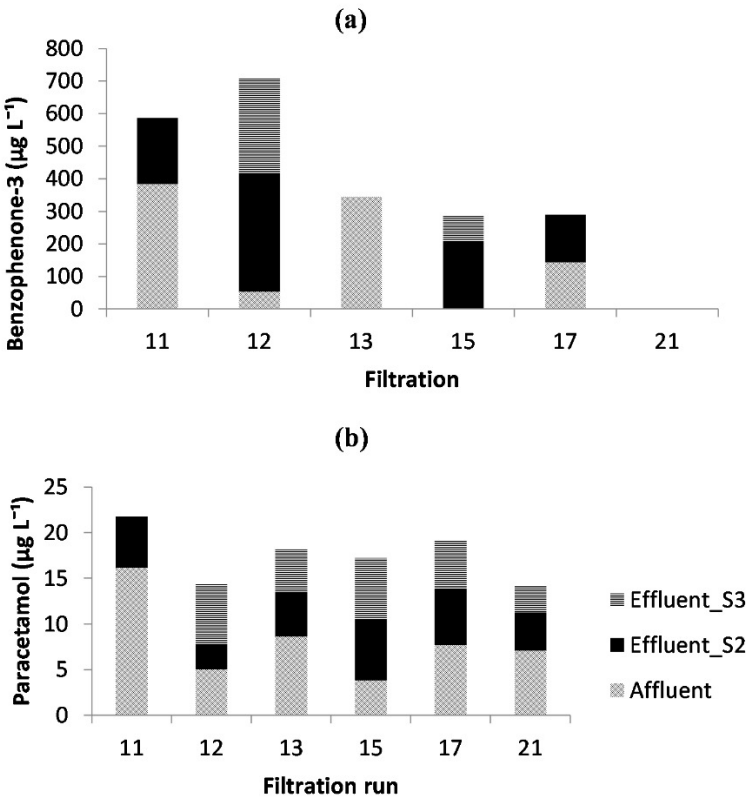
509 Paracetamol was measured at the concentration from 1.86 to 14.17 μg L^{-1}. The
510 identity of paracetramol in the sample water was conformed by MS/MS analysis, its
511 retention time on the C18 column and its corresponding *m/z* value. It was ranked as one
512 of the top three drugs prescribed in England, and the mass of paracetamol through
513 prescription alone totaled more than 400 tons in that year (Sebastine and Wakeman,
514 2003). Paracetamol has been reported in UK waters at concentrations of 0.555 μg L^{-1} by
515 Bound and Voulvoulis (2006). Kasprzyk-Hordern et al. (2008) detected paracetamol in
516 two rivers at Wales, UK, at maximum concentration of 2.38 μg L^{-1}.

517 Diclofenac, naproxen, ibuprofen and methylparaben were not detected in the
518 filtered water from the contaminated filter (F2), indicating that the 2 μg L^{-1} were removed
519 by household slow sand filter, while benzophenone-3 and paracetamol were found at
520 varied concentrations.

521 During filtration 11, benzophenone-3 had 47.5 % of removal by F2 at S2 (after 4h
522 30 min of pause), while paracetamol presented the highest removal (65.2 %). The removal
523 of paracetamol was similar in both, S2 and S1. These results contradict Erba et al. (2012)
524 who reported removal of 80 % for paracetamol by ecological filters. Although they
525 worked with similar initial concentrations of 2 μg L^{-1} of drug mix solution of four
526 pharmaceuticals, this discrepancy might be because the filters were operated continuously
527 and had developed greater biofilm as the filters were kept outdoors, while this study was
528 carried out using intermittent operation, and filters were kept in the laboratory.

529 During filtration 12, the concentrations of these compounds in the filtered water
530 were greater than the affluent concentration. This also occurred for TOC concentration
531 (Figure 6). It can be seen (Figure 7) that on average, at S1 (24 hours after contamination),
532 the concentrations of the compounds were lower than S2.

533 Benzophenone-3 (Figure 7a) was not detected in the filtered water of the
534 household slow sand filter (F2) in filtrations 13 and 21 at the sampling time S2, suggesting
535 that it was totally removed by the filter. In the sampling time S1, benzophenone was not
536 detected at filtrations 13, 17 and 21. However, in filtrations 12 and 15, benzophenone-3
537 was not removed by the filter at sampling times S2 and S1. Some PPCPs are
538 biodegradable, such as ibuprofen and clofibric acid in river biofilms (Winkler et al.,
539 2001). The biodegradation of benzophenone was reported in the activated sludge of an
540 sewage treatment plant (Fujii and Kikuchi, 2005), and by 16 fungi (Takita et al., 2005).
541 Chen et al. (2015) reported natural biodegradation of benzophenone-3 by
542 photodegradation and microbial degradation. The biodegradation of paracetamol (90–100
543 %) was also reported during activated sludge process (Joss et al., 2006 and 2005) and was
544 also reported biodegradation by aerobic granules (Hu et al., 2012). Nugrohoi et al. (2010)
545 reported removal of paracetamol by sand filtration and related with biodegradation
546 (affluent 0.34 and effluent 0.06 μg L^{-1}), using low concentration in inffluent water.



547

548 **Figure 7:** Detection of the concentration in affluent and filtered water from F2 in S2 (4h 30min
549 after contamination) and S1 (24 hours after contamination) for (a) Benzophenone-3 and (b)
550 Paracetamol.
551

552 *3.2. Comparing bacterial diversity from two different operating systems in household*
553 *slow sand filters*

554

555 Samples from the *schmutzdecke* in three random locations (1, 2 and 3) in both
556 filters were collected in order to compare the community of bacteria that developed in
557 phase 1 and phase 2 (see Table 3). It is worth noting again that F 1 worked as control, and
558 F2 was contaminated only in phase 1.

559 The BLAST of the sequences was distributed in presence and absence of the
560 microorganisms in each sampling point. The first option in the BLAST was taken with
561 similarity 99.9 % for all.

562 According to other studies on slow sand filtration, bacterial community was
563 particularly rich in species within the biofilm i.e. *schmuztdecke* (Petry-Hansen et al.,

564 2006; Wakelin et al., 2010 and 2011), but many of them were uncultivable (Hugenholtz
565 et al., 1998; Calvo-Bado et al., 2003), and the filter material was a key factor in
566 determining the occurrence of microbial species (Wakelin et al., 2010). It is worth noting
567 that the data shown here may be biased because only culture methods were used in this
568 work (Head et al., 1998). However, based on the filtration methodology adopted in this
569 study, in total (phases 1 and 2), it was possible to identify 22 species of bacteria in the
570 biofilm samples cultured during this research, three could not be isolated - Strain S8, S15,
571 S18 (Table 3).

572 Two species, namely *Bacillus anthracis* and *Exiguobacterium* sp., were found in
573 both filters F1 (control) and F2 (contaminated filter) during phase 1. This suggests that
574 these species seemed resistant to 2 $\mu g\ L^{-1}$ of PPCPs mix applied to F2. *Bacillus pumilus*
575 and *Enterobacterium bacterium* developed only in F1 during phase 1, while the others
576 six species developed only in F2 (i.e. *Bacillus mycoides*, *Serratia ureilytica*,
577 *Chryscobacterium* sp., No iso, *Stemotrophomonas rhizophila*, *Bacillus* sp. – Strais S5 to
578 S10) (Table 3). Interestingly, the bacteria species in F1 were different from F2 in phase
579 1, however, to confirm if this difference relates to the presence of PPCPs, more tests
580 should be conducted for this purpose.

581 In phase 2, without PPCP contamination, 12 species were separated, 10 isolated
582 species and identified and two no isolated, that is more than in phase 1. From the total,
583 only two species that were present in phase 1 persisted to phase 2 (i.e. *B. anthracis*, and
584 *Exiguobacterium* sp.). The other 10 species were different, so these results indicate that
585 the bacteria community in phase 1 was different from those in phase 2. However, it does
586 not necessarily mean that this difference was related to the operation mode of the filters
587 but it may be related associated to age of the filters. According to Haig et al. (2015), the
588 microbial community compositions of the SSFs were significantly different, depending

589 on several factors such as: sample location, month of sample collection, depths at which
590 samples were taken; being the age of the filter the most significant parameter in
591 explaining changes in the microbial community and a water quality variable. In addition,
592 the diversity of species of bacteria colonizing the biofilm and their composition depends
593 also on the affluent water quality. For example, Calaway et al. (1952) and Brink (1967)
594 using wastewater, with higher nutrient loading found quite low diversity of bacteria. On
595 the other hand, Bahgat et al. (1999) found a greater diversity of bacteria species in the
596 biofilm of SSF supplied with primary-treated wastewater from those found by Wakeling
597 et al. (2011) who used rainwater as affluent.

598 The *B. anthracis* and *Exiguobacterium* sp. that were present in phases 1 and 2
599 were the same that showed resistance to 2 $\mu g\ L^{-1}$ of PPCPs when comparing the isolated
600 bacteria in F1 and F2 during phase 1 (Table 3), suggesting the adaptation and resistance
601 abilities of these species to contamination factor and operation time.

602 The principal component analysis (PCA), considering phases 1 and 2 and location
603 of *schmutzdecke* sampling in each filter (Table 3), summed up 64.1 % of the joint
604 variability of the data in its first two axis (Figure 8). On the positive side of the X axis
605 are grouped F1 and F2 sampling points of the biofilm collected in phase 1. It indicates
606 that these samples are similar to each other, showing no difference between contaminated
607 and no contaminated filter. On the negative side of the Y axis are some biofilm samples
608 related to F1 and F2 sampling in phase 2 (F1.7, F1.9, F2.10 and F2.12), showing
609 simillarity between them.

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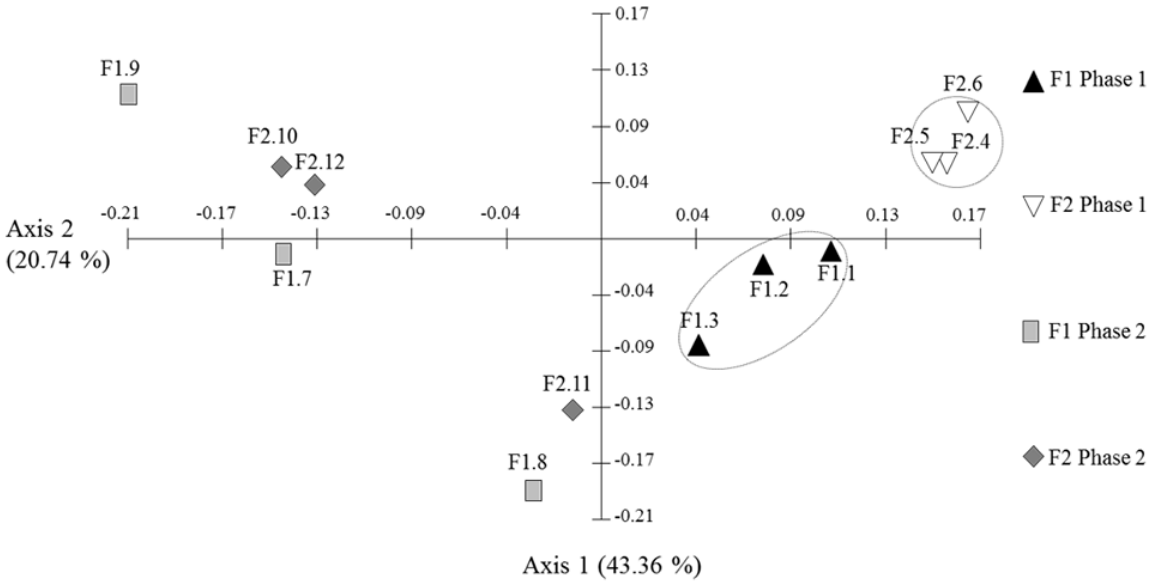

Figure 8: PCA graph Ordination biplot by principal component analysis (PCA). Note: F1.1 to F1.3 correspond to F1 in phase 1, F2.4 to F2.6 correspond to F2 in phase 1; F1.7 to F1.9 correspond to F1 in phase 2, and F2.10 to F2.12 correspond to F2 in phase 2.

On the negative side of the Y axis are grouped F1.8 and F2.11 sampling in phase 2. Then there is similarity between F1.7 and F1.9; F2.10 and F.12; F1.8 and F2.11 in phase 1. This shows there was similarity between random locations of biofilm sampling in both phases. However, the bacteria community in phase 1 was different from those in phase 2, as F1.1, F1.2, F1.3, F2.4, F2.5 and F2.6 (all sampling points during phase 1) grouped at the right side of the graph, in contrast with F1.7, F1.8, F1.9, F2.10, F2.11 and F2.12 (phase 2) grouped at left side of the PCA graph, which is in agreement to Haig et al. (2015), about differences in the microbial community compositions in biofilms of SSFs. In the present study, the grouping of species may be occurred because of age of filters, raw quarter quality or presence or absence of PPCPs, as Shaw et al. (2015) demonstrate effect of PPCPs in lentic biofilms.

3.3.Biomass determination

633 The biomass concentration increased significantly with number of filtrations and
634 was summarised by an exponential growth function ($p < 0.0001$ for F1, $p < 0.001$ for F2)
635 in both filters, but there was no substancial difference between them ($p = 0.76$). However,
636 during phase 1 the biomass concentration (F1 = 46.45 µg C g^{-1}; F2 = 50.08 µg C g^{-1}) was
637 smaller than the average in phase 2 (F1 = 94.96 µg C g^{-1}; F2 = 90.93 µg C g^{-1}) (Figure 9)
638 in both filters. These values are higher than those observed by Campos et al. (2002) in
639 large scale SSF and this may be due to the affluent water quality. While the present study
640 was carried out using raw water from Regent's Park Lake, Campos et al. (2002) collected
641 *schmutzdecke* samples from large scale SSF which was part of an advanced water
642 treatment involving pre-treatment by reservoir, pre-ozonation, flotation, rapid filtration,
643 intermediate ozonation, and granular activated carbon. Also during phase 2 (Filtrations
644 22 - 32), an algal bloom was observed in the Regent's Park lake and this explains the
645 considerable increased of biomass from phase 1 (Filtration 21) to phase 2 (Filtrations 22
646 - 32). It is important to note that the filters were not cleaned between phases as headloss
647 development was not significant during operation time.
648 Filter F2 (contaminated) showed slightly higher biomass concentration at the end
649 of phase 1 (see Filtration 21) than F1 (control) (F1 = 46.45 µg C/g; F2 = 50.08 µg C/g).
650 This is in agreement with the genetic sequencing of bacteria performed (Figure 8), in
651 which most number of species were isolated and identified in filter F2 during phase 1.
652 However, the fitted biomass in F2 was smaller than the one in F1 in phase 2, although
653 phase 2 did not receive contamination. Overall this is in agreement with various work
654 which demonstrated the effect of pharmaceuticals on the structure of aquatic communities
655 (Munoz et al., 2009; Drury et al., 2013, Roise-Marshall et al., 2013) as well as the
656 behaviour of aquatic organisms (Brodin et al., 2013; Jonsson et al., 2014). However, the

fact the small PPCP concentrations used in the present study (i.e. 2 µg L^{-1}) may explain the reason there was no significant difference between filter biomass (p = 0.001).

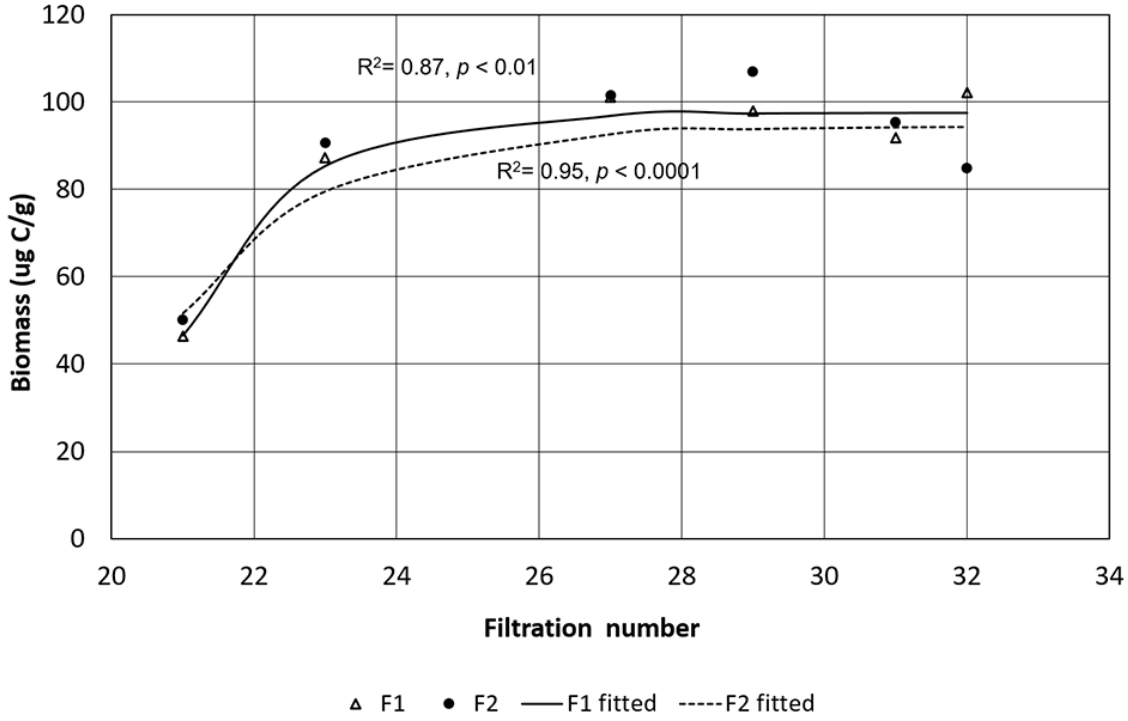

Figure 9: Biomass measurement in µg C/g in phase 1 (Filtrations 1-21), and phase 2 (Filtrations 22-32) for filters (a) F1 (control), and (b) F2 (contaminated with PPCPs from Filtrations 11-21). Note: Filtration 25 data was omitted due to error.

3.4. Overall filter performance

Overall the both filters had similar performance and the PPCPs contamination seemed not to affect the removal of the physico-chemical parameters. However, it was observed the total coliforms and E. *coli* removals were higher for those water samples related to the previous filtration (S1) than those from the same day filtration (S2). Also it was observed that DO content was reduced in the samples from previous filtration, showing that high detention time may lead to biofilm decay. It is highlighted here that although the recommendations for household BSF by CAWST (2009) that "*pause period should be a minimum of 1 hour after the water has stopped flowing up to a maximum of 48 hours"* were not followed for the purpose of this research, overall all water quality parameters

were within the recommended values by WHO (2011). This shows the robustness of the slow sand filter as a household device for water purification.

Finally, the bacteria community in phase 1 was different from those in phase 2 in both filters, and biomass in phase 1 was slightly smaller than in phase 2 ($p = 0.001$). This is in agreement with the review findings by Rosi-Marshall et al. (2013), although the amount of PPCPs used is the present study was much smaller (i.e. 2 µg L^{-1}) than the work cited by Rosi-Marshall et al. (2013) which varied from 1-4 g L^{-1}. However, the observed change in composition of the bacterial community between phases 1 and 2 in this study, could be related with the age of the filters (the filters were not cleaned between phases), the quality of raw water, the presence of PPCPs, and operation with and without pauses. Comparing the filters, we realize that the biological community even among identical filters can be different.

4. CONCLUSIONS

The performance of the household slow sand filters operated intermittently was not affected by the presence of 2 µg L^{-1} of PPCPs in the water. Only two of the PPCPs (i.e. benzophenone-3 and paracetamol) were found at Regent's Park Lake water and in the filtered water. The benzophenone-3 was better removed by the SSF (F2) at sampling time S1 (24 hours after contamination), similarly to the total coliforms and *E. coli* removals for the same sampling time, reaffirming that the increased residence time (S1) increases the filters performance.

More number of bacteria species were present in the phase 2 (normal operation) than in phase 1 which received PPCPs and had flow paused. *B. anthracis* and *Exiguobacterium* sp. seemed resistant to the 2 µg L^{-1} of PPCPs applied to F2, as they were both found in filters F1 (control) and F2 (contaminated filter) during phase1 (1-21 filtrations). All other bacteria species were different from F1 to F2. However, it is not

700 clear that the PPCPs mix affected the bacteria species, once another influential factors
701 could be temperature, light, water quality, and filter operation with pause or not.
702 Filter biomass concentration increased significantly with filter time and was
703 summarised by an exponential growth function in both filters, but there was no substantial
704 difference between them. But F2 (contaminated) during phase1 showed slightly higher
705 biomass concentration at the end of phase 1 (filtration 21) than F1 (control). This is in
706 agreement with the genetic sequencing of bacteria performed, in which most number of
707 species were isolated and identified in filter F2 during phase 1.
708

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717

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721

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