REMOVAL OF BACTERIA AND PHARMACEUTICALLY ACTIVE COMPOUNDS DURING NATURAL FILTRATION

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To my parents and grandparents

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Abstract

Natural filtration represents a sustainable technology to enhance the quality of the source water. Slow sand filtration (SSF) and riverbank filtration (RBF) are examples of natural filtration that have been used. SSF is a low-cost water treatment technology often used by small municipalities and households. It can be used to improve the quality of the source water in the aftermath of a natural disaster as well as in underdeveloped countries and rural areas. RBF, on the other hand, can be used as a primary treatment or a pre-treatment of water for municipalities and regional authorities. Similar removal processes, such as filtration, biodegradation, and adsorption, occur during both natural filtration technologies. However, during RBF the additional processes of chemical precipitation, redox reactions and mixing can occur. SSF and RBF showed similar removal efficiency in terms of turbidity (20−90% vs. 70−90%), total coliforms and *E. coli* (1−3 logs in both filtration technologies).

Two SSF units, disposed in parallel or in series, coupled with two post treatment point-of-use (POU) devices (an ultraviolet water treatment, UV unit, or an activated carbon impregnated with silver nanoparticles, AC unit) were used to examine the ability to treat source water with high levels of turbidity (> 20 NTU), high bacterial content (E+6 to E+7 MPN/100 mL of total coliforms and *E. coli*), and pharmaceutically active compounds (PhACs, at concentration of 50 μg/L). Six PhACs, caffeine, carbamazepine, 17-β estradiol (E2), estrone (E1), gemfibrozil, and phenazone were selected using a multi-step approach based on: i) occurrence in the environment, ii) toxicity and pharmaceutical class, iii) environmental fate, iv) behavior under different redox conditions, and v) availability of analytical standards and adequate instrumentation.

Turbidity removal ranging from 40% to 80% and removal of total coliforms and *E. coli* greater than 95% was consistently achieved by SSF regardless of the configuration (series vs. parallel) of the units, the hydraulic loading rate, the starting turbidity of the source water and the presence or absence of PhACs. However, the presence of a post treatment unit, such as an UV unit, is desirable to further enhance the quality of the source water, especially in the presence of bacterial loads greater than 10^6 MPN/100 mL. SSF can also

be used to treat water with a high concentration of PhACs such as a wastewater treatment plant spill or a pharmaceutical industry spill. However, the nature and concentrations of the PhACs, the duration of the spill, and the age of the SSF may significantly impact the overall performance of the filtration unit in terms of bacterial removal. Among the selected PhACs, complete removal of caffeine, and partial removal of E2 and E1 (11−92%) were achieved by both SSF units. Adsorption and biodegradation are the main removal mechanisms for the selected PhACs. None to limited (< 10%) removal of carbamazepine, gemfibrozil, and phenazone occurred.

Columns and two side-by-side sandbox were used to investigate the role of oxygen (aerobic vs. anoxic), temperature (summer vs. winter), and level of organic matter (TOC = 3, 10, 20 mg/L) on the removal of selected PhACs during simulated RBF. The same PhACs investigated during SSF were also used for the simulated RBF. RBF can be effectively used to remove most of the PhACs present in surface waters. However, the geochemistry of the RBF site is expected to play a key role in their removal. Depending on the compound, removal of PhACs may predominantly occur due to biodegradation, but environmental variables such as oxygen and temperature may enhance or limit biodegradation. Limited and slower removal of selected PhACs may occur during the winter temperature conditions. Limited removal of carbamazepine (< 10%) and gemfibrozil (< 30%) occurred regardless of the different environmental conditions. Among the different PhACs, removal of phenazone occurred only under aerobic conditions, while removal of caffeine was highly impacted by the level of organics as well as by the temperature. The occurrence of air beneath the riverbed can enhance the development of locally present aerobic conditions, that leads to an enhanced removal of redox sensitive PhACs.

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CHAPTER 1. INTRODUCTION

1.1. NATURAL FILTRATION

Natural filtration in the context of this thesis refers to:

- Slow sand filtration (SSF) which is a low-cost water treatment technology often used by small municipalities and households. It can be used to improve the quality of the feed water in the aftermath of a natural disaster as well as in underdeveloped countries and rural areas.
- Riverbank filtration (RBF) which can be used as primary treatment or pre-treatment of drinking water for municipalities and regional water authorities.

Table 1 summarizes key design parameters, removal processes, removal efficiency, advantages and limitations of both natural filtration systems.

Table 1: Key design parameters, removal processes, removal efficiency, advantages and limitations of slow sand filtration and river bank filtration.

	Slow Sand Filtration (SSF)[1]	**River Bank Filtration (RBF)[2]**
Key design parameters		
Media type	Sand	Native subsurface material, mostly sand
Media Depth	0.5 – 1.5 m	10 – 600 m (travel distance)
EBCT[a]	3 – 12 hr	1-3 d
HLR[b]	0.1 – 0.3 m/hr	0.03 – 1 m/hr
Removal processes		
Major removal processes	• Filtration • Biodegradation • Adsorption	• Filtration • Biodegradation • Adsorption • Chemical precipitation • Redox reactions • Mixing
Removal efficiency		
Turbidity	20 – 90%	70 – 90%

	Slow Sand Filtration (SSF)	River Bank Filtration (RBF)
Total coliforms	1 – 3 logs	1 – 3 logs
E. coli	1 – 2 logs	1 – 2 logs
DOC	8 – 30%	40 – 80%
Advantages and Limitations		

Advantages	• Low capital investiments
	• Low operating costs
	• Lack of chemical pretreatment
	• Ability to remove:
	  • suspended particles
	  • biodegradable compounds
	  • bacteria and viruses
		• Low capital investiments
		• Low operating costs
		• Lack of chemical pretreatment
		• Ability to remove:
		  • suspended particles
		  • biodegradable compounds
		  • bacteria and viruses
		• Ability to buffer possible fluctuation in the quality of the water produced

Limitations	• Clogging
	• Increasing in head losses
	• Temperature
		• Development of an unsaturated zone near the collector wells
		• Riverbed clogging
		• Increase in:
		  • hardness
		  • ammonium
		  • dissolved iron and manganese
		• Possible formation of malodorous sulfur compounds

[a] Empty bed contact time; [b] hydraulic loading rate; [1] Huisman and Wood, 1974; Cleasby, 1991;Weber-Shirk and Dick, 1997a-b; Longsdon et al., 2002; [2] Hiscock and Grischeck, 2002; Ray et al., 2002.

1.1.1. Background on slow sand filtration (SSF)

Slow sand filtration (SSF) represents one of the earliest forms of an engineered potable water treatment. The first known SSF plant was installed in Paisley, Scotland in 1804 and since then SSF has been used primarily in Europe (Huisman and Wood, 1974). SSF systems were first built in North America at the end of the twentieth century, but the advent of effective coagulation, sedimentation, and rapid filtration resulted in a declining interest in SSF (Logsdon et al., 2002). At the end of the twentieth century SSF became

more popular in the USA due to the growing concern about viruses, *Giardia* cysts, and *Cryptosporidium* oocysts, and to the ability of SSF to remove these pathogens (Logsdon et al., 2002).

The effectiveness of SSF can be seen by the role it played during the 1892 cholera epidemic in Hamburg, Germany. Two cities, Hamburg and Altona, were using the River Elbe as a source of drinking water. Although the water intake for the city of Altona was located downriver from the sewer outfalls of Hamburg, the water was filtered through SSF prior to use. In Hamburg, however, there was no preliminary water treatment (Gainey and Lord, 1952). Hamburg had a death rate of 1344 per 100000 and Altona had a death rate of 230 per 100000. Most of the cholera deaths that occurred in Altona were due to infections that occurred in Hamburg (Gainey and Lord, 1952).

Today, the simplicity of its construction and maintenance and its low cost make SSF an attractive technology to improve water quality after a natural disaster and to improve drinking water in the developing world (WHO, 2008).

SSF is accomplished by passing untreated water through a bed of sand. The effective size (ES), or D_{10}, of the sand used in most SSF units ranges between 0.15 and 0.4 mm in diameter (Huisman and Wood, 1974; Thanh and Hettiaratchi, 1982). In order to maximize the efficiency of the treatment, a hydraulic loading rate between 0.1 to 0.3 m/h is recommended (Huisman and Wood, 1974). The depth of sand required usually ranges between 0.5 and 1.5 m (Huisman and Wood, 1974). To treat water efficiently using SSF, the feed water should have a turbidity less than 5 NTU, be free of high concentrations of algae, have a chlorophyll *a* content of less than 0.05 μg/L and limited concentrations of iron and manganese (0.3 mg/L and 0.05 mg/L) (Cleasby, 1991). Temperature can also impact the performance of SSF. A cold climate limits the presence of nutrient in the feed water, and consequently reduces the development of the biolayer (Longsdon et al., 2002). In order to avoid this scenario, SSF are usually covered when they are used in cold climates where supernatant water could freeze (Longsdon et al., 2002). SSF beds need to be periodically cleaned by replacing the top layer of sand. The frequency of cleaning is primarily determined by the turbidity of the feed water. Fast clogging of the SSF may

occur using feed water with high turbidity (> 50 NTU). In a practical sense, these are the limitations of an SSF system.

SSF has been widely used to remove turbidity and bacteria from stream (Cleasby et al., 1984; Bellamy et al., 1985; Aydin, 1997) and lake waters (Weber-Shirk and Dick, 1997a, b) as well as secondary (Farooq and Al-Yousef, 1993; Logsdon et al., 1993) and tertiary effluent (Langenbach et al., 2009). During the past decade, however, SSF has also been used to treat a broad variety of water such as rain water (Neto et al., 2012), rural prairie water (Gottinger et al., 2011), wastewater from mariculture facilities (Palmer, 2010), and effluent from an up-flow anaerobic sludge blanket (Tyagi et al., 2009). Another use of SSF is as pre- or post- treatment for other types of filtration or treatment. For example, Zheng et al. (2009) used SSF as the preliminary treatment for secondary effluent prior to ultra-filtration, and Aslan (2008) reported SSF used as post-treatment for drinking water after a biodenitrification unit.

Besides being used to remove turbidity and bacteria, SSF can also remove chemical oxygen demand (COD), total phosphorous (TP), and total nitrogen (TN) from slightly contaminated water (Lwesya and Li, 2010), and iron and manganese from groundwater (Pacini et al., 2005; Gottinger et al., 2011).

During the past decade, SSF has been used occasionally to remove PhACs and inconsistent results were achieved. For example, in a study conducted by Ternes et al. (2002), a municipal SSF was unable to remove bezafibrate, clofibric acid, diclofenac, carbamazepine, and primidone. SSF was partially able to remove the organic compounds 2-methylisoborneol (MIB) and geosmin. More than 50% of MIB and geosmin were degraded by the microbial population on the sand surface of an SSF unit (Hsieh et al., 2010). Limited removal of atrazine and its metabolites diethylatrazine (DEA), deisoprophylatrazine (DIA), and diethylhydroxylamine (DEHA) was achieved using SSF alone (Coelho and Di Bernardo 2012). However, higher removal of atrazine was achieved using an SSF unit with an intermediate layer of granular activated carbon, suggesting that sorption to the activated carbon plays a key role in the removal of atrazine and its metabolites. Rooklidge et al. (2005) investigating the removal of antimicrobials in a

multistage filtration (roughing and SSF) system at a municipal water treatment facility, reported that at the end of a 14-day simulation period, 100% removal of tylosin, 99% removal of trimethoprim, < 25% removal of lincomycin, and < 4% removal of a sulfonamide class of antimicrobial was achieved with an SSF unit. Turbidity removal by SSF is based upon straining and adsorption that occurs between the sand in a filter and the suspended particles in the feed water (Huisman and Wood, 1974; Weber-Shirk and Dick, 1997a-b). Straining is due to the interception and retention of particles too large to pass through the interstices between the grains of sand. When a sand filter is newly constructed, straining is predominant at the surface of the filter, but with the maturation of the filter, straining occurs throughout the entire filtration unit.

The removal efficiency of bacteria is affected by the status (partially or completely developed) of the biolayer, and the age of the SSF unit. Several studies have suggested that bacterial removal is due to a combination of bacterivory by zooplankton, straining and/or adsorption (Huisman and Wood, 1974; Weber-Shirk and Dick, 1997a-b) primarily occurring at the biolayer. As water is passed through the sand, a biolayer or *schmutzedcke* forms at the sand water interface. The biolayer which appears as a reddish, slimy layer may contain sand, algae, bacteria, humus, and protozoa and represents one of the predominant mechanisms involved in the filtration process of the SSF.

However, during the maturation of the filter, organic material may be deposited not only on the filter surface but also on the individual grains. These deposits can become a breeding ground of bacteria and other microorganisms, which produce a slimy material known as zooglea that consists of living bacteria, their wastes and dead cells, and partly assimilated organic materials (Huisman and Wood, 1974). The zooglea forms a sticky gelatinous film on the surface of the biolayer and sand grains, increasing the opportunity for particles contained in the feed water to be trapped and adsorbed (Huisman and Wood, 1974).

Adsorption of particles onto a sand grain surface once they have made contact is due to a combination of electrostatic attraction, van der Waals forces and adherence (Huisman and Wood, 1974).

1.1.2. Background on riverbank filtration (RBF)

Riverbank filtration (RBF) systems consist of a series of abstraction wells in the vicinity of a stream or lake resulting in groundwater depletion that forces river or lake water to infiltrate into the subsurface towards the abstraction wells (Hoppe-Jones et al., 2010). RBF wells can be either vertical or radial (like collector wells). The application of one type versus the other is related to the characteristics of the aquifer (Ray et al., 2002).

In the United States, most RBF systems are comprised of collector wells constructed in alluvial aquifers located along riverbanks. Ideal conditions for RBF typically include coarse-grained, permeable water-bearing deposits that are hydraulically connected with riverbed materials (Ray et al., 2002). RBF effectively recharges the groundwater that is extracted for drinking water purposes with river water, and naturally improves the quality of the river water through a variety of physicochemical and biological processes (Schubert, 2002). Factors influencing the performance of RBF systems are primarily related to the quality of the surface water, infiltration, soil passage, and management of the site and facilities (Hiscock and Grischek, 2002).

RBF has been used to provide drinking water to communities for more than a century in Europe and for half a century in the United States (Ray et al., 2002). RBF provides about 50% of the potable water in the Slovak Republic, 45% in Hungary, 16% in Germany and 5% in The Netherlands (Dash et al., 2008). RBF facilities are also located in the USA mainland (i.e., Kentucky, Nebraska, and California) (Table 2). Many other countries around the world including India, China, Korea, Jordan, and Egypt recently have started to evaluate the feasibility of using RBF for water treatment (Ray, 2008; Ray and Shamrukh, 2011).

Table 2: Riverbank filtration sites: location, type of well and design capacity of well field. RBF sites are listed by their design capacity (highest to lowest).

Site location	River	Well type	Design capacity (m^3/s)	Reference
Csepel Island (Hungary)	Danube	Horizontal vertical	3.470	Ray et al., 2002
Dusseldorf (Germany)	Rhine	Horizontal vertical	2	Eckert and Irmscher, 2006; Hunt et al., 2002a
Cincinnati (OH)	Great Miami	Vertical	1.750	Ray et al., 2002
Columbus (OH)	Scioto/Big Walnut	Horizontal	1.750	Ray et al., 2002
Kansas City (KS)	Missouri	Horizontal	1.750	Ray et al., 2002
Torgau (Germany)	Elbe	Vertical	1.737	Ray et al., 2002
Lincoln (NE)	Platte	Horizontal, vertical	1.530	Ray et al., 2002
Mockritz (Germany)	Elbe	Vertical	1.260	Ray et al., 2002
Cedar Rapids (IA)	Cedar	Horizontal vertical	1.25	Hunt et al. 2002b
Louisville (KY)	Ohio	Horizontal vertical	0.875	Ray et al., 2002
Maribor (Slovenia)	Drava	Vertical	0.750	Ray et al., 2002
British Columbia (Canada)	Nechao	Horizontal	0.66	Hunt et al., 2002a
Independence (MO)	Missouri	Horizontal	0.656	Ray et al., 2002
Terre Haunte (IL)	Wabash	Horizontal	0.525	Ray et al., 2002
Sacramento (CA)	Sacramento	Horizontal	0.438	Ray et al., 2002
Galesburg (IL)	Mississippi	Horizontal	0.438	Ray et al., 2002
Jacksonville (IL)	Illinois	Horizontal	0.350	Ray et al., 2002
Kalama (WA)	Kalama	Horizontal	0.114	Ray et al., 2002
Kennewick (WA)	Columbia	Horizontal	0.131	Ray et al., 2002
Mt Carmel, (IL)	Wabash	Vertical	0.095	Ray et al., 2002

The design and operation of bank filtration sites are largely related to the different treatment objectives. For example, in Europe RBF is designed to remove pathogens, trace organic pollutants, and biodegradable dissolved organic carbon from surface water. In the United States, RBF is designed to remove pathogens, reduce turbidity, and dissolved organic carbon (Grünheid et al., 2005). The retention time represents a key distinction. Retention time of several weeks or even months is common in Europe, while in the USA the retention time usually ranges between a few hours to a few days (Grünheid et al., 2005). The advantages of RBF compared to other water technologies are related to the low capital investments and low operating costs, as well as the ability to remove suspended particles, biodegradable compounds, bacteria, and viruses (Ray et al., 2002). Compared to other water technologies, RBF can also buffer possible fluctuations in the quality of the water produced (Ray et al., 2002).

The two major limitations to the effectiveness of the RBF are the development of an unsaturated zone (Su et al., 2007) near the collector wells and riverbed clogging (Schubert, 2002). Other possible limitations of RBF are related to increases in hardness, ammonium, dissolved iron and manganese, and the possible formation of malodorous sulfur compounds due to changing redox conditions (Ray et al., 2002; Schubert, 2002).

The development of an unsaturated zone beneath the riverbed may occur if the pumping rate is higher than the recharge rate, or if the recharge from a losing river is less than the saturated hydraulic conductivity of the aquifer. This unsaturated zone can reduce the hydraulic conductivity, limit the water production, and also affect the redox conditions at the riverbank filtration site. Field studies along the Russian River (Sonoma County, California, USA), showed that, among the different factors that may impact the development of the unsaturated zone beneath the riverbed, the contrast between the riverbed permeability and aquifer permeability as well as the aquifer anisotropy ratio are key factors (Zhang et al., 2011).

Riverbed clogging can be due to physical, biological, and/or chemical processes (Bouwer, 2002). Physical clogging is due to the accumulation of inorganic and organic suspended solids in the recharge water. Biological clogging includes the accumulation of

algae and bacterial floc in the water onto the infiltrating surface and the growth of micro-organisms on and in the soil to form biofilms and biomass that block pores and/or reduce pore size (Bouwer, 2002). Chemical clogging is related to the precipitation of calcium carbonate, gypsum, phosphate, and other chemicals on and in the soil. Sometimes, these precipitations are induced by pH increases due to algae as they remove dissolved CO_2 from the water for photosynthesis. Bacteria can also produce gases (nitrogen and methane) that block pores and accumulate below the clogging layers to create vapor barriers to infiltration. Gas is also formed in the soils below the recharge basins when the recharge water contains entrapped air or dissolved air and/or is cooler than the soil or the aquifer itself. The water then warms up in the soil or aquifer; air goes out of the solution and forms entrapped air, which reduces the hydraulic conductivity. Due to the reduced permeability of the clogging layers compared to the natural soil material, the infiltration rate is reduced (Bouwer, 2002).

A key aspect in RBF is related to the development of redox conditions since they may cause the mobilization of undesired metals such as iron (Fe^{2+}) and manganese (Mn^{2+}) as well as copper, zinc, cadmium, and lead. In addition, redox conditions affect the pH and calcite solution capacity of the infiltrated water (Tufenkji et al., 2002). Redox processes are primarily driven by organic matter degradation in the presence of terminal electron acceptors (TEAs) such as oxygen (O_2), nitrate (NO_3^-), manganese (Mn^{4+}), and iron (Fe^{3+}) oxides and hydroxides, and sulphates (SO_4^{2-}) which are typically consumed in a sequential order from the highest to the lowest energy yield (Greskowiak et al., 2005). The consequent formation of distinguishable redox zones (Table 3) have often been observed in aquifers recharged by RBF (Bourg and Bertin, 1993; Dousson et al., 1997; Massmann et al., 2004 and 2008).

Table 3: Different redox zones occurring at RBF sites.

$CH_2O + O_2 \rightarrow CO_2 + H_2O$	Aerobic Respiration Zone
$CH_2O + 8H^+ + 4Fe(OH)_3 \rightarrow 4Fe^{2+} + 11H_2O + CO_2$ $CH_2O + 2MnO_2 + 4H^+ \rightarrow 2Mn^{2+} + 3H_2O + CO_2$ $CH_2O + \frac{4}{5}NO_3^- + \frac{4}{5}H^+ \rightarrow \frac{2}{5}N_2 + \frac{7}{5}H_2O + CO_2$	Anoxic Fe^{3+}, Mn^{4+}, NO_3 Reduction Zone
$CH_2O + \frac{1}{2}SO_4^{2-} + \frac{1}{2}H^+ \rightarrow \frac{1}{2}HS^- + H_2O + CO_2$	SO_4^{2+} Reduction Zone
$CH_2O + \frac{1}{2}CO_2 \rightarrow \frac{1}{2}CH_4 + CO_2$	CH_4 Fermentation zone

Depth ↓

Gradient of electron acceptors e⁻ ↓

1.1.3. Presence of pharmaceutically active compounds (PhACs) and impact of environmental conditions on their removal

During the last few decades, the presence of trace organic chemicals (TrOCs), such as personal care products (PCPs), household chemicals, and pharmaceutically active compounds (PhACs) dramatically increased in the environment (Ternes, 1998; Kolpin et al., 2002; Snyder et al., 2007; Benotti et al., 2009). The presence of these chemicals has been reported in surface water and groundwater in the United States (Kolpin et al., 2002; Benotti et al., 2009) and in Europe (Ternes, 1998). Conventional wastewater treatments are not adequate to fully remove certain groups of TrOCs that can then be discharged into rivers (Heberer, 2002; Yu et al., 2006). Traces of organic micropollutants have also been found at different RBF sites around the world and some of these micropollutants were partially removed (Table 4). Some of these chemicals, even at concentrations of a few ng/L may have carcinogenic effects on the aquatic and terrestrial organisms (Thiele-Bruhn and Beck, 2005; Vajda et al., 2008; Madureira et al., 2011) and potential adverse effects on human health (Boxall, 2004; Fent et al., 2006).

Table 4: Occurrence and environmental fate of selected pharmaceutically active compounds (PhACs) at RBF sites and in column experiments.

Co (μg/L)	Redox	Residence time (d)	Study	Removal efficiency (%)	Reference
CAFFEINE					
>50	oxic		Lab Column	98	Maeng, 2010
>50	oxic		Lab Column	97	Maeng, 2010
>50	anoxic		Lab Column	93	Maeng, 2010
>50	anoxic		Lab Column	97	Maeng, 2010
CARBAMAZEPINE					
<50-885	aerobic		Brighton, RBF	0-10	Hoppe-Jones, 2010
<0.002-0.56	aerobic	7–20	BF Rhine	no reduction	Schmidt et al., 2007
0.393	aerobic	4–50	AGR Lake Tegel	13–20	Zühlke, 2004
0.11	aerobic	7–20	BF Rhine	no reduction	Brauch et al., 2000
0.11	aerobic	40–60	BF Rhine	20	Brauch et al., 2000
0.11	aerobic	60–100	BF Rhine	40	Brauch et al., 2000
0.036–0.2	suboxic	12–60	BF Rhine	no reduction	Schmidt et al., 2007
>50	oxic		Lab Column	0	Maeng, 2010
>50	anoxic		Lab Column	0	Maeng, 2010
10	anoxic	< 1h	Lab Column	0–17	Mersmann et al., 2002
0.315	anoxic	20–65	BF Wannsee	0–20	Heberer et al., 2002, 2004
0.325	anoxic	20–120	BF Lake Tegel	0–40	Heberer et al., 2004
0.061–0.34	anoxic	45–300	BF Elbe	33–51	Schmidt et al., 2007
0.036–0.13	anaerobic	5–15	BF Ruhr	> 97	Schmidt et al., 2007
ESTRONE (E1)					
0.0011	aerobic	3–50	BF Lake Tegel	>91	Verstraeten et al., 2005
0.0009	anoxic	20–90	BF Lake Tegel	>89	Verstraeten et al., 2005
GEMFIBROZIL					
<25–395	aerobic		Brighton, RBF	0–60	Hoppe–Jones, 2010
>50	oxic		Lab Column	97	Maeng, 2010
>50	oxic		Lab Column	28	Maeng, 2010
>50	anoxic		Lab Column	98	Maeng, 2010
>50	anoxic		Lab Column	12	Maeng, 2010

Co (μg/L)	Redox	Residence time (d)	Study	Removal efficiency (%)	Reference
PHENAZONE					
0.344	aerobic	4–25	AGR Lake Tegel	> 85	Zühlke, 2004
0.15	oxic→ anoxic	15	Lake Tegel	66%	Massmann et al., 2008
0.15	anoxic		Lab Column	33	Massmann et al., 2008
0.15	anoxic	30	Lake Tegel	10	Massmann et al., 2008

There are still uncertainties regarding the environmental fate of these micropollutants. The behavior of selected PhACs at bank filtration sites is impacted by the physico-chemical properties of the compounds, and by the local conditions, such as redox conditions, temperature and travel time (Table 4). For example, Hoppe-Jones et al. (2010) investigating the removal efficacy to remove PhACs at three full-scale RBF sites in the U.S. (Brighton, Cedar Rapids, and Louisville) observed that gemfibrozil and ibuprofen were more efficiently removed during the summer. During the winter, additional travel time was required to reduce the concentrations of these compounds to the level found in summer (Hoppe-Jones et al., 2010). On the other hand, Massmann et al. (2006) while investigating phenazone type analgesic at an artificial recharge near Lake Tegel (Berlin, Germany) observed that the PhACs were eliminated more in winter than in summer. They found that phenazone was redox sensitive and required aerobic conditions for microbial degradation. The aerobic conditions were encountered near the shore in winter when the temperatures were very low.

Field and laboratory experiments with sulfamethoxazole yielded contrasting results. Field data suggested a better degradation of this compound under anaerobic (Schmidt et al., 2004), and anoxic (Grünheid et al., 2005; Heberer et al., 2008) conditions, while column simulations suggested that sulfamethoxazole should be more efficiently removed under aerobic conditions (Jekel and Grünheid, 2008; Baumgarten et al., 2011). There are also some PhACs, such as estrogens, (i.e., 17-β estradiol, E2, and estrone, E1) that are

highly removed in the presence of both aerobic and anaerobic conditions, as well as under different temperatures (Verstraeten et al., 2005) (Table 1).

1.2. RESEARCH OBJECTIVES AND STRUCTURE OF THE THESIS

The overall objective of this thesis is to evaluate the ability of two natural filtration systems, SSF and RBF, to improve the quality of the source water especially in terms of PhACs. The ability to remove PhACs and factors that may affect this removal, such as local redox conditions, temperature and level of organic matter in the source of water, are investigated. Results obtained can be used to better understand the mechanisms involved during the removal of PhACs occurring at RBF sites as well as during SSF.

This thesis is divided into five chapters. A brief review of SSF and RBF, occurrence of PhACs in surface waters and possible environmental fate of these compounds is provided in chapter 1. Chapters 2, 3, 4, and 5 include: abstract, introduction, materials and methods, results and discussion.

Specific goals of this thesis are to investigate (1) the ability of SSF to remove high levels of turbidity or high counts of bacteria in feed water, (2) the impact of selected PhACs on the bacterial removal efficiency by SSF units, and the ability of SSF to remove PhACs, (3) the impact of redox conditions, temperature and organic matter on the removal of PhACs during simulated RBF, and (4) the impact of clogging and infiltrated air on the dynamic of redox conditions as well as on the removal of PhACs in a simulated RBF system.

1. Ability of SSF to remove high levels of turbidity or high counts of bacteria in feed water (Chapter 2)

Several studies (Aydin, 1997; Weber-Shirk and Dick, 1997a-b) have investigated the impact of different parameters (i.e., effective size of sand, hydraulic loading rates, and thickness of sand required) on turbidity and bacterial removal by SSF. Field scale investigations (Hijnen et al., 2004; Gottinger et al., 2011) as well as laboratory experiments (Bellamy et al., 1985, 1995; Hijnen et al., 2004) have demonstrated the

ability of SSF to reduce turbidity and bacteria in feed water. However, the different types of feed water (stream water, stream water containing very low concentrations of sewage, and lake water) used in the studies were generally characterized by low to medium levels of turbidity (0.6 to 20 NTU) and bacteria (i.e., total coliforms ranging between 1.0 E+1 to 9.9 E+4 MPN/100 mL) (Cleasby et al., 1984; Ellis and Aydin, 1995; Elliott et al., 2008; Gottinger et al., 2011).

Although previous studies (Hijen et al., 2004; Gottinger et al., 2011) the studies highlight the efficiency of SSF to remove total coliforms, and *E. coli* (1 to 3 logs removal were achieved), it is not clear whether SSF can also be used with feed water having high turbidity or high bacterial loads. High levels of turbidity and bacteria can occur as a result of sewage spills during and after a natural disaster or may be the usual state of the available water sources and in underdeveloped countries. There is an existing need to evaluate the ability of SSF to treat feed water with high bacterial loads.

The objective of this study is to examine the performance of an SSF unit at different hydraulic loading rates, and with high levels of turbidity (> 20 NTU) or bacteria (E+6 to E+7 MPN/100 mL of total coliforms and *E. coli*) in the feed water.

The results of this study have been submitted to the *Environmental Technology.*

2. Impact of selected PhACs on the bacterial removal by SSF units, and ability of SSF to remove PhACs (Chapter 3)

Besides being used to remove turbidity and bacteria, SSF can be used to remove chemical oxygen demand (COD), total phosphorous (TP), and total nitrogen (TN) from slightly contaminated water (Lwesya and Li, 2010), and iron and manganese from groundwater (Pacini et al., 2005; Gottinger et al., 2011). The occurrence of TOrCs such as PhACs, PCPs, surfactants, and pesticides have been significantly increasing in the environment during the past few decades (Kolpin et al., 2002; Focazio et al., 2008). There are limited and contrasting studies on the effect of TOrCs on the performance of SSF units (Woudneh et al., 1997; Ternes et al., 2002; Rooklidge et al., 2005; Oszako et al., 2013).

The hypothesis that the presence of PhACs from wastewater treatment plants or pharmaceutical industry spills can affect the bacterial populations in the biolayer as well as in the sand, and consequently the bacterial removal ability of a SSF unit is tested.

A microbial community analysis is used to better understand the impact of PhACs on the bacterial removal by the SSF units, as well as on the ability of the SSF to remove PhACs.

The first objective of this study is to examine the impact of selected PhACs on the bacterial removal by an SSF unit. The second objective is to examine the ability of SSF to remove a variety of PhACs (i.e., stimulant, hormones, antipyretic) present in the feed water. Such scenarios may occur in surface water impacted by wastewater treatment plants or spills from pharmaceutical industries, particularly in situations following natural disasters. The third objective is to investigate the impact that feed water (i.e., stream water alone or stream water with 1% primary effluent) may have on the development and dynamics of the biolayer in two SSF units.

The results of this study have been submitted to *PlusOne.*

3. Impact of redox conditions, temperature and organic matter on the removal of PhACs during simulated RBF (Chapter 4)

RBF can be used as possible treatment to remove PhACs (Ray et al., 2002; Grünheid et al., 2005). However, given the number of PhACs that may be present in environmental waters and the number of parameters that can affect their removal, there are still uncertainties regarding the environmental fate of these micropollutants. Laboratory (Rauch-Williams et al., 2010; Hoppe-Jones et al., 2012; Onesios and Bouwer, 2012) and field studies (Hoppe-Jones et al., 2010; Patterson et al., 2011; Benotti et al., 2012) have been conducted to evaluate the behavior of selected PhACs under different environmental conditions. Some PhACs (i.e., dichlofenac, gemfibrozil, phenazone) are more efficiently removed under aerobic conditions than anaerobic conditions (Rauch-Williams et al., 2010; Maeng et al., 2011; Teerlink et al., 2012), while some PhACs (i.e., iodinated X-ray contrast agents, sulfamethoxazole**)** are preferentially removed under anaerobic conditions

(Grünheid et al., 2005; Heberer et al., 2008). Limited removal of PhACs was observed during the winter (Hoppe-Jones et al., 2010).

Knowing the redox conditions present at the RBF sites is crucial to better understand the environmental fate of PhACs.

The objectives of this study are to investigate the role of 1) level of oxygen – aerobic vs. anoxic, 2) temperature – summer vs. winter, and 3) level of organic matter (humic acid) on the removal of selected PhACs during simulated RBF.

The results of this study have been submitted to *Chemosphere*

4. Impact of clogging and infiltrated air on the dynamic of redox conditions as well as on the removal of PhACs in simulated RBF (Chapter 5)

The development of an unsaturated zone beneath the riverbed may occur in the presence of a pumping rate higher than the recharge rate, or if the recharge from a losing river is less than the saturated hydraulic conductivity of the aquifer. This unsaturated zone can reduce the hydraulic conductivity, limit the water production, and also affect the redox conditions at the RBF site (Su et al., 2007). The change in redox conditions may also impact the removal of selected PhACs.

The objective of this study is to investigate the impact of air present below the clogging layer on the dynamics of redox conditions and on the removal of six selected PhACs - caffeine, carbamazepine, E2, E1, gemfibrozil, and phenazone. The impact of organic matter (humic acid) on the removal of selected PhACs was also investigated.

The results of this study are being finalized for publication.

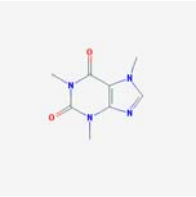
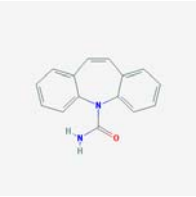
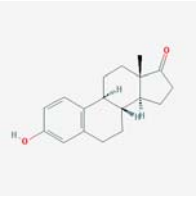
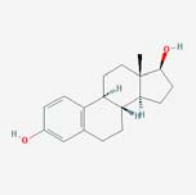
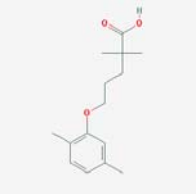
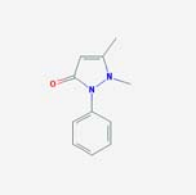
1.2.1. Selection of limited number of PhACs

According to the Physicians' Desk Reference (2008) there are more than 3000 pharmaceuticals that are currently approved for prescription in the U.S., and hundreds of others are approved for over-the-counter use, are used in personal care products, or are

used as adjuncts in the formulation of these materials. In order to reduce the number of possible PhACs investigated during this study, the flowing multistep selection was developed. The PhACs were selected based on: (i) occurrence in the environment and public interest, (ii) toxicity, (iii) environmental fate, (iv) behavior under different redox conditions, (v) pharmaceutical class, and (vi) availability of analytical standards and adequate instrumentation.

Caffeine, carbamazepine, estrone, 17-β estradiol, gemfibrozil, and phenazone were selected. Their physico-chemical and toxicological properties are shown in Table 5.

Table 5: Physico-chemical and toxicological properties of selected PhACs.

Selected chemicals	**Chemical structure**	**Log K_{ow}**	**Log D**	**Class**
Caffeine ($C_8H_{10}N_4O_2$) Stimulant (*carcinogenicity, tumor promotion, mutagenicity, tumor inhibition*)		-0.07	-0.45	Hydrophilic neutral
Carbamazepine ($C_{15}H_{12}N_2O$) Anticonvulsant (*carcinogenicity, mutagenicity*)		2.45	2.58	Hydrophobic neutral
Estrone ($C_{18}H_{22}O_2$) Estrogen (*carcinogenicity, tumor promotion, mutagenicity*)		3.13	3.46	Hydrophobic neutral
17-β estradiol ($C_{18}H_{24}O_2$) Estrogen (*carcinogenicity, tumor promotion, mutagenicity*)		4.01	3.94	Hydrophobic neutral
Gemfibrozil ($C_{15}H_{22}O_3$) Antilipemic, blood lipid regulator (*carcinogenicity*)		4.77	2.2	Hydrophobic neutral
Phenazone ($C_{11}H_{12}N_2O$) Analgesic and antipyretic (*carcinogenicity, tumor promotion, mutagenicity*)		0.38	0.45	Hydrophilic neutral

The target compounds selected for this study include several of the most frequently detected compounds in the USGS national survey in streams (Table 6, Kolpin et al., 2002) as well as in U.S. drinking water facilities (Table 7, Benotti et al., 2009).

Table 6: Compounds with high frequency of detection in the U.S. streams (Source: Kolpin et al., 2002).

Chemical	**freq**	**max**	**use**
	%	µg/L	
Veterinary and Human Antibiotics			
erythromycin-H2O	21.5	1.7	erythromycin metabolite
lincomycin	19.2	0.73	antibiotic
sulfamethoxazole	12.5	1.9	antibiotic
trimethoprim	12.5	0.71	antibiotic
tylosin	13.5	0.28	antibiotic
Prescription Drugs			
codeine	10.6	1.0	analgesic
dehydronifedipine	14.3	0.03	antianginal
diltiazem	13.1	0.049	antihypertensive
Nonprescription Drugs			
acetaminophen	23.8	10	antipyretic
caffeine	70.6	5.7	stimulant
cotinine	38.1	0.9	nicotine metabolite
1-7-dimethylxanthine	28.6	3.1d	caffeine metabolite
Steroids and Hormones			
cis-androsterone	14.3	0.214	urinary steroid
cholesterol	84.3	60	plant/animal steroid
coprostanol	85.7	150	fecal steroid
17α-ethynyl estradiol	15.7	0.831	ovulation inhibitor
17β-estradiol	10.6	0.2	reproductive hormone
estriol	21.4	0.051	reproductive hormone
estrone	7.1	0.112	reproductive hormone
mestranol	10	0.407	ovulation inhibitor
19-norethisterone	12.8	0.872	ovulation inhibitor

Table 7: Occurrence of selected pharmaceuticals and endocrine disruptive compounds (EDCs) in source water, finished water, and distribution systems (Source: Benotti et al., 2009).

		Source , n = 19			Finished, n = 19			Distribution, n = 19		
ng/L	**MRL**	**max.**	**med.**	**no.**	**max.**	**med.**	**no.**	**max.**	**med.**	**no.**
pharmaceuticals										
atenolol	0.25	36	2.3	12	18	1.2	8	0.84	0.47	8
atorvastatin	0.25	1.4	0.8	3	<MRL	<MRL		<MRL	<MRL	
carbamazepine	0.5	51	4.1	15	18	6	8	10	6.8	6
diazepam	0.25	0.47	0.43	2	0.33	0.33	1	<MRL	<MRL	
diclofenac	0.25	1.2	1.1	4	<MRL	<MRL		<MRL	<MRL	
fluoxetine	0.5	3	0.8	3	0.82	0.71	2	0.64	0.64	1
gemfibrozil	0.25	24	2.2	11	2.1	0.48	7	1.2	0.43	4
o-hydroxy atorvastatin	0.5	1.2	0.7	3	<MRL	<MRL		<MRL	<MRL	
p-hydroxy atorvastatin	0.5	2	1	3	<MRL	<MRL		<MRL	<MRL	
meprobamate	0.25	73	8.2	16	42	5.7	14	40	5.2	11
naproxen	0.5	32	0.9	11	<MRL	<MRL	<MRL	<MRL	<MRL	
norfluoxetine	0.5	<MRL	<MRL		<MRL	<MRL	0.77	0.77	1	
phenytoin	1	29	5.1	14	19	6.2	10	16	3.6	10
risperidone	2.5	<MRL	<MRL		<MRL	<MRL		2.9	2.9	1
sulfamethoxazole	0.25	110	12	17	3	0.39	4	0.32	0.32	1
triclosan	1	6.4	3	6	1.2	1.2	1	<MRL	<MRL	
trimethoprim	0.25	11	0.8	11	<MRL	<MRL		<MRL	<MRL	
known or potential EDCs										
atrazine	0.25	870	32	15	870	49	15	930	50	12
17β-estradiol	0.5	17	17	1	<MRL	<MRL		<MRL	<MRL	
estrone	0.2	0.9	0.3	15	<MRL	<MRL		<MRL	<MRL	
17R-ethynylestradiol	1	1.4	1.4	1	<MRL	<MRL		<MRL	<MRL	
bisphenol A	5	14	6.1	3	25	25	1	<MRL	<MRL	
butylbenzyl phthalate	50	54	53	2	<MRL	<MRL	<MRL	<MRL		
diethylhexyl phthalate	120	170	150	2	<MRL	<MRL	<MRL	<MRL		
galaxolide	25	48	3	4	33	31	2	<MRL	<MRL	
linuron	0.5	9.3	4.1	5	6.2	6.1	2	<MRL	<MRL	
nonylphenol	80	130	100	8	100	93	2	110	97	2
progesterone	0.5	3.1	2.2	4	0.57	0.57	1	<MRL	<MRL	
testosterone	0.5	1.2	1.1	2	<MRL	<MRL		<MRL	<MRL	

MRL = method reported limit, max = maximum concentration, med = median concentration, no = number of detections. Source of water sample = sample taken proximate to but before any drinking water treatment process, finished water sample: sample taken after final disinfection exiting the drinking water treatment plant, distribution system sample: sample taken at customer's tap at least several hours after leaving the drinking water treatment plant.

CHAPTER 2. SLOW SAND FILTRATION: IMPACT OF CONFIGURATION TYPE, STARTING TURBIDITY, AND BACTERIAL LOAD ON TURBIDITY AND BACTERIAL REMOVAL EFFICIENCY[1]

Abstract

Providing a reliable supply of potable water of consistent quality in the aftermath of a natural disaster or in a developing country is a challenge. Two slow sand filtration (SSF) units (55 cm internal diameter and 55 cm height) coupled with one of two point-of-use (POU) devices (ultraviolet water disinfection, UV, unit and activated carbon, AC, filter impregnated with silver nanoparticles) were tested in different configurations (series and parallel) using feed water with high turbidity (> 20 NTU) and high bacterial load (E+6 to E+7 MPN/100 mL of total coliforms and *E. coli*).

Regardless of the configuration of the SSF units and the hydraulic loading rates, turbidity removal was highly impacted by the turbidity of the feed water. High turbidity removal (> 75%) was observed when the turbidity of the feed water was moderate to high (> 9 NTU). Removal of total coliforms and *E. coli* ranged between 96% and 100% in the presence of feed water with E+5 MPN/100 mL, regardless the configuration and the hydraulic loading rate. However, switching from low to high hydraulic loading rate showed an initial drop in the removal efficiency of the SSF unit. SSF can also be effectively used (approximately 90% removal) to treat feed water with high bacterial loads (E+6 to E+7 MPN/100 mL of total coliforms and *E. coli*). Approximately 60% of the bacterial removal occurred in the top 5 cm of the SSF unit. Bacterial removal efficiency was related not only to the quality of the biolayer (i.e., whether it was completely or partially developed) but also to the age of the SSF unit (i.e., whether the unit was relatively new or old and more biologically mature).

[1] This chapter has been submitted to *Environmental Technology*: Matteo D'Alessio, Gabriel El-Swaify, Bunnie Yoneyama, Chittaranjan Ray. Slow sand filtration: Impact of configuration type and bacterial load on turbidity and bacteria removal efficiency.

The ability of the biolayer to remove bacteria does not seem to depend on the water used to develop the biolayer. Similar overall removal of total coliforms and *E. coli* was achieved in the presence of two different sources of water (Manoa Stream alone and Manoa Stream with 1% primary effluent).

When the two SSF units were placed in series, limited (< 10%) to no additional removal was achieved by the second unit, particularly in terms of total coliforms and *E. coli*. The lack of nutrients in the feed water of the second unit (effluent from the first unit) may limit the formation of the biolayer and prevent the development of a sticky gelatinous film around the sand grains.

The UV unit, used after an SSF unit, represents a more efficient and flexible POU device compared with the AC filter impregnated with silver nanoparticles. The UV unit was able to produce larger quantities of higher quality water (total coliforms and *E. coli* constantly below detection limits) compared with the AC impregnated with silver nanoparticles.

2.1. INTRODUCTION

Providing a reliable supply of potable water of consistent quality in the aftermath of a natural disaster and in undeveloped countries is a challenge. According to the World Health Organization (WHO, 2005, 2008) 7 liters per capita per day (Lpcd) of water represent the minimum "survival" allocation sufficient for a few days during emergencies. Approximately 1.1 billion people in the developing world lack access to safe sources of drinking water. Most of them live in rural, dispersed, and often remote communities (WHO, 2008).

Due to its simplicity of construction and maintenance and its low cost, slow sand filtration (SSF) is a suitable technology to improve water quality for affected populations during emergencies, and to improve drinking water in the developing world (WHO, 2008).

SSF is accomplished by passing untreated water through a bed of sand of 0.15 to 0.4 mm in diameter (Huisman and Wood, 1974). As water is passed through the sand, a biolayer or *schmutzedcke* forms at the water-sand interface. This layer is slimy and typically consists of sand, algae, humus and protozoa. The *schmutzedcke* represents one of the predominant mechanisms involved in bacterial removal during SSF.

Physical-chemical mechanisms are significant in the removal of particles between 0.75 and 10 μm (Weber-Shirk and Dick, 1997a). Biological mechanisms such as bacterivory are important for the removal of particles smaller than 2 μm (Weber-Shirk and Dick, 1997b). Turbidity removal by SSF is based upon straining and adsorption of particles in the input or feed water by the sand (Huisman and Wood, 1974). Several studies have suggested that bacterial removal is due to a combination of bacterivory by zooplankton, straining and/or adsorption (Huisman and Wood, 1974; Weber-Shirk and Dick, 1997a-b).

There are a number of commercially available point-of-use (POU) devices, such as activated carbon (AC) impregnated with silver nanoparticles or UV light, which can be effectively used to improve the quality of drinking water. Their popularity is due to their

ability to remove bacteria without causing significant taste and odor problems, and also easy dosage quantification and absence of disinfection by-products. The effectiveness of an AC unit is based on the adsorptive properties of the AC and the bactericidal effect of the silver nanoparticles when they are in direct contact with the cell wall of an organism (Larimer et al., 2010). The bactericidal effect of UV systems is based on the ability of UV light to penetrate the cells of microorganisms and disrupt the structure of their DNA (Wegelin et al., 1994; USEPA, 1996). The effectiveness of a UV device depends on the light transmissivity and on the contact time (USEPA, 1996).

Several studies (Aydin, 1997; Weber-Shirk and Dick, 1997a-b) have investigated the impact of different parameters (i.e., effective size of sand, hydraulic loading rates, and thickness of sand required) on turbidity and bacteria removal by SSF. Field scale investigations (Hijnen et al., 2004; Gottinger et al., 2011) as well as laboratory experiments (Bellamy et al., 1985, 1995; Hijnen et al., 2004) have demonstrated the ability of SSF to reduce turbidity and bacteria in the feed water. Most of these studies used stream water (Aydin, 1997; Elliott et al., 2008), lake water (Weber-Shirk and Dick, 1997b) or stream water containing very low concentrations of sewage (Ellis and Aydin, 1995) as feed water. The different types of feed water used were generally characterized by low to medium levels of turbidity (0.6 to 20 NTU) and bacteria (i.e., total coliforms ranging between 1.0 E+1 to 9.9 E+4 MPN/100 mL) (Cleasby et al., 1984; Elliott et al., 2008; Gottinger et al., 2011). Although the studies highlight the efficiency of SSF, it is not clear whether SSF can also be used with feed water having high turbidity (> 20 NTU) or high bacterial loads (E+7 MPN/100 mL). High levels of turbidity and bacteria can occur as a result of sewage spills during and after a natural disaster, or may be the usual state of available water sources in underdeveloped countries. There is an existing need to evaluate the ability of SSF to treat feed water with high bacterial loads.

The objective of this study is to examine the performance of an SSF unit at different hydraulic loading rates, and in the presence of feed water with high levels of turbidity or high bacterial load.

2.2. MATERIALS AND METHODS

2.2.1. Packing materials

Silica sand (0.15–0.30 mm, Orange County #60) with a uniformity coefficient of 1.6 was used as the main packing material, while pea gravel (effective size of 9.5 mm) was used as the underlying supporting material. The AC impregnated with silver nanoparticles contained 1.05% silver (Bestech Inc., Pompano Beach, FL).

2.2.2. Feed water

Feed water was obtained from Manoa Stream (21°18’35.04” N, 157°48’37.22W, Honolulu, HI) (Fig. A1-Appendix A), which runs by the University of Hawaii’s main campus. This stream is subject to limited urban pollution. The different influent turbidities and bacterial concentrations seen in the study are reflections of the changing conditions in the stream. Primary effluent used in the experiments was collected from the Honouliuli Wastewater Treatment Facility in Ewa Beach, Oahu, Hawaii, stored at 4 °C, and settled prior to use.

2.2.3. Experimental setup

Two polyethylene barrels (57 cm inner diameter, 88 cm height) were used to construct the SSF units. Two sampling ports were installed at 5 and 35 cm below the water-sand interface to collect samples immediately after the biolayer and from the middle of the unit (Fig. A2-Appendix A).

A 10 cm layer of pea gravel was placed at the bottom of each barrel to support 57 cm of silica sand. Prior to packing the two filters, the sieved sand was repeatedly washed with tap water to remove fine particles. Two stainless steel wire cloths with 0.014-mm openings (TWP, Berkeley, CA) were placed on top of the gravel in order to support the sand and to prevent possible migration of fine particles. To achieve a homogenous distribution of sand, small amounts of sand were added in increments of 5 cm to each barrel. Prior to beginning the experiment, tap water was pumped upwards from the bottom of the unit to further remove fine particles present in the sand. Following this,

Manoa Stream water was introduced to the top of the filters. A constant head of feed water (approximately 10 cm) over the top of the surface of the sand filter was maintained throughout the study using an overflow valve. To achieve a uniform flow distribution of the feed water across the surface of the two barrels, two PVC pipes with multiple holes were placed on top of each barrel. A dual head peristaltic pump (Cole-Parmer, Vernon Hills, IL) was used to simultaneously deliver feed water from the same reservoir to both sand filters; or deliver feed water from two separate reservoirs, but at the same hydraulic loading rate. A constant depth of the filter medium was maintained by replacing any sand removed during filter cleaning with sand of identical specification. The top 1 to 2 cm of biolayer was removed when a 10 to 15% reduction in the output of the filters was observed. The filters were operated continuously from June 2011 until July 2012. The temperature ranged between 20 °C and 30 °C during the study.

A filter packed with AC impregnated with silver nanoparticles and an in-line UV disinfection device were used in this study as examples of POU devices that might improve the quality of the SSF effluent. A flow-through PVC column (7 cm internal diameter and 20 cm height) was packed with AC impregnated with silver nanoparticles. A stainless steel wire cloth with 0.014-mm openings (TWP, Berkeley, CA) was placed at the bottom of the column to prevent possible migration of fine particles. In order to compact and to achieve an even distribution of the packing material, small amounts of AC were incrementally added to the PVC column and mixed with a vortex mixer (VWR, Thorofare, NJ). Prior to being used, the column was flushed with Manoa Stream water for 24 h.

A Sun-Pure Ust-200 Ultraviolet System (Freshwater Systems, Greenville, SC) was used as an in-line UV device. This device contains a low-pressure mercury lamp (2 cm internal diameter and 24 cm length) in a polypropylene chamber (5 cm internal diameter and 30 cm length) with a stainless steel lining, and an 0.625 cm inlet and outlet. The UV unit can operate between 4.4 °C and 40.5 °C, and has a maximum hydraulic loading rate of 3.6 L/min, in the presence of a 30 mJ/cm^2 dose (*http://www.freshwatersystems.com/p-4969-sun-pure-ust-200-ultraviolet-system-1-gpm-120v-w-electronic-ballast.aspx*). A voltage of

120 V, frequency of 50–60 Hz and power consumption of 6 W are the main electrical specifications of this device.

2.2.4. Experimental conditions

Table 8 summarizes the different experimental conditions as well as when the two POU devices were tested.

Table 8: Experimental configurations, level of turbidity and bacteria in the feed water, and presence or absence of the two point-of-use devices.

Case (configuration)	Objective	Turbidity (NTU)	Hydraulic loading rate (m/h)	Total coliforms (MPN/100 mL)	*E. coli* (MPN/100 mL)	POU devices
BASELINE (parallel)	Development of the biolayer	< 4	0.04	$10^4 – 10^5$	$10^2 – 10^3$	UV, AC
CASE 1 (parallel)	Impact of hydraulic loads	2 – 20 2 – 20	0.04 to 0.12 0.12 to 0.04	$10^4 – 10^5$ $10^4 – 10^5$	$10^3 – 10^4$ $10^3 – 10^4$	UV AC
CASE 2 (series)	Impact of configuration and starting turbidity	< 5 15 – 35	0.1 0.1	$10^3 – 10^4$ $10^3 – 10^4$	$10^2 – 10^3$ $10^2 – 10^3$	AC AC
CASE 3 – HBI (parallel)	Impact of high bacteria loads and type of feed water	< 2	0.05	$10^6 – 10^7$ (S) $10^4 – 10^5$ (FO)	10^6 (S) $10^2 – 10^3$ (FO)	–
CASE 3 – HBII (parallel)		2 – 12	0.05	$10^6 – 10^7$ (S) $10^4 – 10^5$ (FO)	10^6 (S) $10^2 – 10^3$ (FO)	UV

Note: S = spike, FO = flush-out, HB = high bacteria; UV = UV light; AC = activated carbon impregnated with silver nanoparticles.

At the beginning of the study, the two barrels (units B1 and B2) were conditioned in parallel using stream water having a low turbidity (< 4 NTU) and delivered at a low hydraulic loading rate of 0.04 m/h (Table 8, Baseline). Stream water stored in a common reservoir was simultaneously pumped to both barrels. Once the biolayers were established, and 1 to 2 logs of bacteria removal was achieved in both sand filters, different experimental conditions were tested. At the end of each experiment, the biolayer was removed and re-established under the new conditions (Fig. A3-Appendix A).

The effect of hydraulic loading rate (Table 8, Case 1) on the performance of the sand filters was tested by acclimating unit B1 at 0.04 m/h, a low hydraulic loading rate, measuring the turbidity and bacterial removal; and then switching to 0.12 m/h, a higher

hydraulic loading rate, and measuring removal efficiency again. Unit B2 was treated in reverse – it was acclimated at 0.12 m/h and switched to 0.04 m/h.

The impact of high bacteria numbers in the feed water was investigated only when the two barrels were placed in parallel (Table 8 - Case 3, HBI and HBII). Two tests, HBI and HBII, were performed during which unit B1 received a high bacterial load (stream water and 20% primary sewage effluent) while unit B2 acted as a control, receiving only stream water. The first test HBI was designed to evaluate the ability of the SSF unit to treat feed water with high bacterial counts. The second test HBII was designed to determine if the type of water used to establish the biolayer impacted the ability of the biolayer to remove bacteria.

Prior to the start of HBI, both units were conditioned with stream water. Once both units showed 1 to 2 logs of bacterial removal, unit B1 received a mixture of stream water and 20% primary effluent for 24 h, while unit B2 was fed with stream water alone. After the 24 h spike of stream water with primary effluent, both units received only stream water. This mimics what an SSF unit might encounter if there was a sewage spill into the stream. Prior to the start of HBII, the biolayer of unit B1 was developed using stream water with 1% primary effluent. Once the biolayer was established and unit B1 achieved 1 to 2 logs of bacterial removal, unit B1 received a mixture of stream water and 20% primary effluent for 24 h. Subsequently, unit B1 received stream water with 1% primary effluent. Unit B2 as the control received only stream water for the entire test. The elevated level of chloride found in the primary effluent relative to the stream water was used as a non-reactive tracer to estimate the travel time of the feed water through the filtration unit.

2.2.5. Analysis of the influent and effluent of the filtration units

Influent and effluent from the sand filters and effluent from the POU devices were collected and analyzed for anions and cations, total organic carbon (TOC), turbidity, total coliforms and *E. coli*.

Anions and cations were measured with an ion chromatograph (IC) (Dionex, Bannockburn, IL) having a detection limit of 0.1 mg/L. TOC was measured (Method 5310B) using a TOC analyzer (Shimadzu, Columbia, MD) coupled with a total nitrogen (TN) detector. Turbidity was measured using a portable turbidimeter (HACH, Loveland, CO).

Total coliforms and *E. coli* were quantified using a commercial Most Probable Number (MPN) test, Colilert 18 with a Quanti-Tray 2000 from IDEXX Laboratories (Westbrook, ME) (ISO, 2012). This system can detect concentrations of total coliforms and *E. coli* from less than 1 MPN/100 mL to more than 2419.6 MPN/100 mL without sample dilution (*http://www.idexx.com/view/xhtml/en_us/water/products/quanti-tray.jsf*).

Colilert 18 utilizes a Defined Substrate Technology (DST) to detect total coliforms and *E. coli.* Samples were collected aseptically and 100 mL, or an appropriate dilution of the sample, was mixed with the reagent, poured into sterile trays, heat sealed, and incubated at 35 °C for 18 h. Total coliforms appeared yellow while *E. coli* appeared blue under 365-nm UV light. Bacterial removal was always determined by comparing the number of total coliforms and *E. coli* in the influent (raw water) against the effluent from the SSF or the port samples, or from the two POU devices.

Turbidity removal is reported through the entire paper without decimal digits, while bacterial removal is expressed using two decimal digits.

2.3. RESULTS

2.3.1. Baseline performance

Baseline performance was established with the two SSF units B1 and B2 in parallel with feed water of low turbidity (< 4 NTU) delivered at low hydraulic loading rate (0.04 m/h). The hydraulic loading rate of 0.04 m/h is within the range of the lowest hydraulic loading rate suggested for SSF units (Huisman and Wood, 1974).

In both B1 and B2 units, the average turbidity in the SSF effluent was below 2.3 NTU (Table 9; Fig. 1a). Unit B1 achieved an average turbidity removal of 41%, while unit B2 achieved 65% removal (Table 10).

TOC in the feed water ranged between 0.92 and 1.01 mg/L in Manoa Stream water alone and between 0.97 and 1.51 mg/L in Manoa Stream water with 1% primary effluent. Approximately 30% removal of TOC was observed in both SSF units (data not shown). EC in the feed water ranged between 286 μS (Manoa Stream water alone) to 384 μS (Manoa Stream water with 1% primary effluent). There were limited or no changes in EC and pH with passage through the sand filter during the baseline period as well as during the different experimental conditions (data not shown).

The feed water to both units contained an average concentration of 2.0 E+4 MPN/100 mL of total coliforms (ranging from 1.2 E+3 to 9.6 E+4 MPN/100 mL) (Table 9; Fig. 1b). High and similar removal greater than 99.00% of total coliforms was observed in both B1 and B2 units (Table 10). The average concentration of *E. coli* in the feed water to unit B1 was 1.0 E+3 MPN/100 mL (2.0 E+1 to 3.9 E+3 MPN/100 mL) (Fig. 1c) and passage through the unit resulted in an average removal of 95.92%.

Unit B2 was fed with the same water. Average concentration of *E. coli* in the feed water to B2 was 1.1 E+3 MPN/100 mL (ranging from 1.5 E+2 to 3.9 E+3, Table 9) and passage through B2 removed an average of 99.04% of the *E. coli* (Table 10).

Table 9: Turbidity, total coliforms, and *E. coli* during baseline in the presence of low hydraulic loading rate (0.04 m/h) and low turbidity (< 4 NTU) (n = 13). The two slow sand filtration (SSF) units (B1 and B2) were run in parallel.

SSF unit	Stage	Minimum	Maximum	Average	Standard deviation
TURBIDITY (NTU)					
B1	Inflow	1.26	3.89	2.30	0.79
	After Sand	0.44	1.94	1.26	0.57
	After UV	0.27	1.85	1.17	0.58
B2	Inflow	1.28	3.37	2.20	0.68
	After Sand	0.21	1.33	0.65	0.32
	After AC	0.25	0.41	0.34	0.08
TOTAL COLIFORMS (MPN/100 mL)					
B1	Inflow	1.2E+3	9.6E+4	2.0E+4	2.8E+4
	After Sand	1.0E-1	4.7E+2	8.0E+1	1.5E+2
	After UV	1.0E-1	7.4E+0	1.3E+0	2.3E+0
B2	Inflow	1.1E+3	9.6E+4	2.0E+4	2.8E+4
	After Sand	2.0E+0	4.4E+2	8.4E+1	1.4E+2
	After AC	1.0E-1	9.6E+1	3.1E+1	4.4E+1
E. COLI (MPN/100 mL)					
B1	Inflow	2.0E+1	3.9E+3	1.0E+3	1.2E+3
	After Sand	1.0E-1	1.7E+1	5.8E+0	7.6E+0
	After UV	1.0E-1	1.0E-1	1.0E-1	0.0E+0
B2	Inflow	1.5E+2	3.9E+3	1.1E+3	1.2E+3
	After Sand	1.0E-1	3.7E+1	6.7E+0	1.2E+1
	After AC	1.0E-1	1.4E+1	7.1E+0	5.9E+0

Note: AC = activated carbon impregnated with silver nanoparticles, UV = UV light.

Table 10: Baseline: Turbidity, total coliforms and *E. coli* reductions in the presence of low hydraulic loading rate (0.04 m/h) and low turbidity (< 4 NTU) (n = 13). The two slow sand filtration (SSF) units (B1 and B2) were run in parallel.

SSF unit	Stage	Overall removal (%)			Standard deviation
		Minimum	Maximum	Average	
TURBIDITY					
B1	After Sand	0	87	41	30
	After UV	0	91	45	31
B2	After Sand	14	94	65	24
	After AC	76	92	86	7
TOTAL COLIFORMS					
B1	After Sand	98.06	100	99.44	0.70
	After UV	99.94	100	99.98	0.02
B2	After Sand	96.65	99.99	99.31	1.01
	After AC	99.45	100	99.76	0.28
E. COLI					
B1	After Sand	70.80	100	95.92	9.25
	After UV	99.50	100	99.91	0.16
B2	After Sand	95.71	100	99.04	1.43
	After AC	95.77	100	98.67	1.95

Note: AC = activated carbon impregnated with silver nanoparticles, UV = UV light; removal after sand, AC and UV are expressed in terms of values measured in feed water.

Between the two POU devices, turbidity removal increased by 21% after AC (from 65% to 86%), while UV had no significant impact on turbidity (Table 10). After AC, the average total coliforms removal increased very slight from 99.31% to 99.76%; after UV the average removal of total coliforms increased from 99.44% to 99.98%. In the case of *E. coli*, passage through AC did not increase removal. After the sand filter, there was 99.04% removal, and after AC there was 98.67%. UV, however, increases *E. coli* removal from 95.92% to 99.91%.

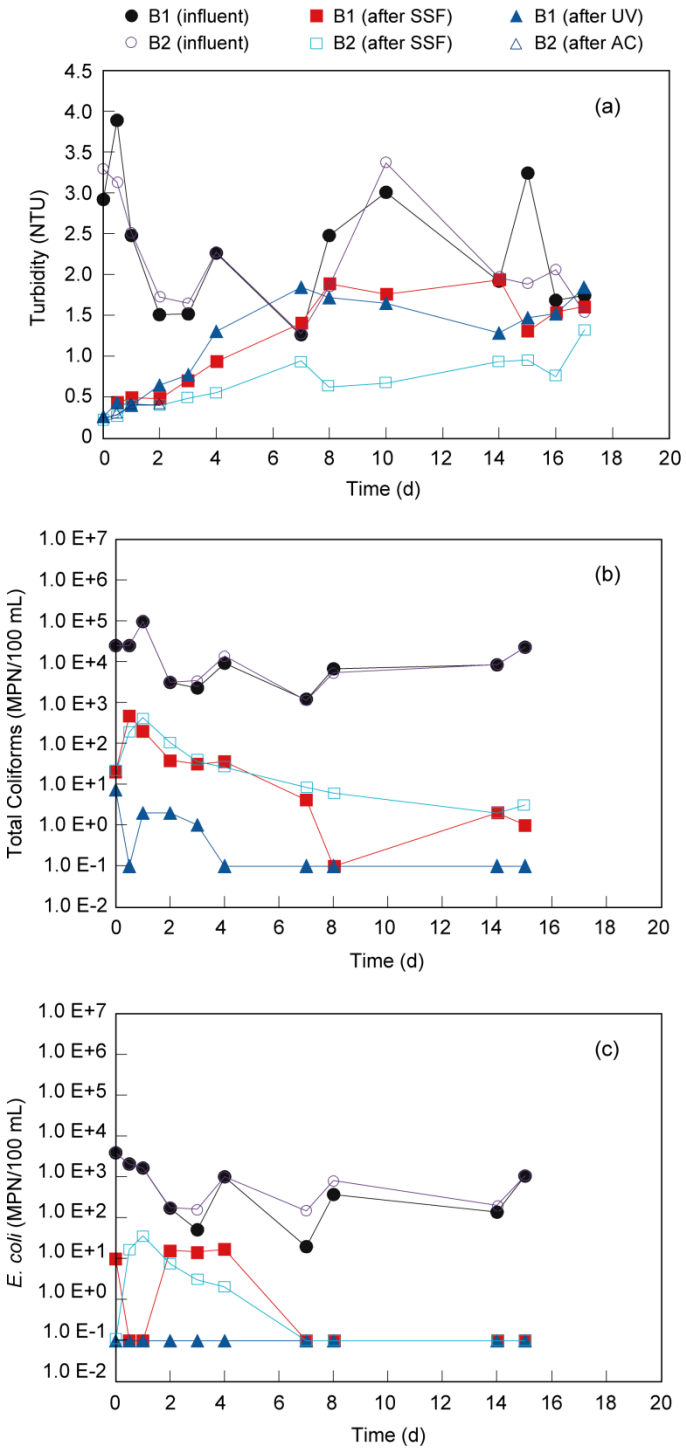


Figure 1: Baseline: (a) turbidity, (b) total coliforms, and (c) *E. coli* reductions in the presence of low hydraulic loading rate (0.04 m/h) and low turbidity (< 4NTU). Unit B1 and unit B2 were run in parallel.

2.3.2. Case 1: Effect of hydraulic loading rates on turbidity and bacteria removal in SSF units in parallel configuration

Unit B1was fed with stream water at a hydraulic loading rate of 0.04 m/h for 14 days. The feed water had an average turbidity of 9.8 NTU (ranging from 3.3 to 17.4 NTU) (Table 11; Fig. 2a). After 14 days, the hydraulic loading rate was increased to 0.12 m/h, but the turbidity of the feed water remained approximately the same with an average of 11.7 NTU (ranging between 2.4 and 19.2 NTU) (Table 11; Fig. 2a). Under the lower hydraulic loading rate, the average turbidity removal was 83%, while under high hydraulic loading rate 74% turbidity removal was achieved (Table 12). Under lower hydraulic loading rate, the average number of total coliforms in the input water was 4.4 E+4 MPN/100 mL (Table 11) and the average removal was 99.87% (Table 12). When the hydraulic loading rate was increased to 0.12 m/h, the average concentration of total coliforms in the feed water was 3.3 E+3 MPN/100 mL (Table 11) and removal decreased to 99.20% (Table 12). *E. coli* showed a similar trend where removal dropped from 99.86% under low hydraulic loading rate to 97.87% under high hydraulic loading rate (Table 12).

B2 was fed with stream water at 0.12 m/h (high hydraulic loading rate) and received feed water having an average turbidity of 11.0 NTU (ranging from 2.8 to 19.2 NTU) (Table 11; Fig. 2a). Once the hydraulic loading rate was lowered to 0.04 m/h, the average turbidity of the feed water was 16.1 NTU (ranging from 2.7 to 57.2 NTU) (Table 11; Fig. 2a). During both the high and the low hydraulic loading rates, 82% turbidity removal was achieved (Table 12). The feed water contained an average of 4.4 E+4 MPN/100 mL and 4.1 E+4 MPN/100 mL of total coliforms during the high and low hydraulic loading rate phases, respectively (Table 11; Fig. 2b). In the presence of a high hydraulic loading rate, B2 achieved 99.75% removal of total coliforms; after the switch to a low hydraulic loading rate, 99.96% removal was achieved (Table 12). Removal of *E. coli* was similar. Under a high hydraulic loading rate, the feed water contained an average of 2.7 E+3 MPN/100 mL of *E. coli* and at a low hydraulic loading rate 1.4 E+3 MPN/100 mL (Table

11; Fig. 2c). During the high and low hydraulic loading rate phases, 99.28% and 99.57% of *E. coli* removal was achieved (Table 12).

Table 11: Turbidity, total coliforms and *E. coli* during Case 1 when the two slow sand filtration (SSF) units were placed in parallel. The biolayer of two SSF units (B1 and B2) was developed at a low hydraulic loading rate (0.04 m/h) and at a high hydraulic loading rate (0.12 m/h), respectively. After 14 days the hydraulic loading rate was switched between the two units (n = 14 [Phase I], n = 6 [Phase II]).

SSF Unit	**Stage**	**Minimum**	**Maximum**	**Average**	**Standard deviation**
TURBIDITY (NTU)					
	Inflow	3.31	17.40	9.78	5.74
B1 – Phase I	After Sand	0.72	2.86	1.33	0.69
	After AC	0.67	1.43	0.91	0.28
	Inflow	2.40	19.20	11.73	12.99
B1 – Phase II	After Sand	0.74	3.25	1.67	0.97
	After UV	0.91	2.92	1.59	0.87
	Inflow	2.76	19.20	10.99	6.22
B2 – Phase I	After Sand	0.76	3.89	1.45	0.93
	UV	0.75	3.93	1.24	0.88
	Inflow	2.66	57.20	16.10	22.15
B2 – Phase II	After Sand	0.74	1.28	0.90	0.20
	After AC	0.71	1.38	0.99	0.23
TOTAL COLIFORMS (MPN/100 mL)					
	Inflow	1.7E+4	9.1E+4	4.4E+4	2.1E+4
B1 – Phase I	After Sand	4.1E+0	3.8E+2	6.3E+1	1.1E+2
	After AC	4.1E+0	9.8E+0	6.7E+0	2.9E+0
	Inflow	9.6E+3	7.6E+4	3.3E+4	1.9E+4
B1 – Phase II	After Sand	2.0E+1	3.0E+2	1.9E+2	9.5E+1
	After UV	1.0E-2	1.0E-2	1.0E-2	0.0E+0
	Inflow	1.3E+4	9.6E+4	4.4E+4	2.6E+4
B2 – Phase I	After Sand	8.6E+0	9.1E+2	1.1E+2	2.4E+2
	After UV	1.0E-2	8.6E+0	2.3E+0	3.3E+0
	Inflow	9.1E+3	9.6E+4	4.1E+4	2.3E+4
B2 – Phase II	After Sand	1.5E+0	1.9E+1	7.8E+0	1.1E+1
	After AC	1.0E-2	8.6E+0	3.1E+0	3.3E+0
E. COLI (MPN/100 mL)					
	Inflow	5.2E+2	1.0E+4	3.2E+3	2.6E+3
B1 – Phase I	After Sand	1.0E-2	1.4E+1	2.5E+0	3.8E+0
	After AC	1.0E-2	4.1E+0	1.2E+0	1.4E+0
	Inflow	4.4E+2	1.0E+4	3.1E+3	2.6E+3
B1 – Phase II	After Sand	1.0E-2	2.9E+1	7.8E+0	1.2E+1
	After UV	1.0E-2	1.0E-2	1.0E-2	0.0E+0
	Inflow	4.1E+2	6.7E+3	2.7E+3	1.9E+3
B2 – Phase I	After Sand	1.0E-2	9.1E+1	9.8E+0	2.4E+1
	After UV	1.0E-2	3.1E+0	2.5E-1	8.6E-1
	Inflow	5.0E+1	4.6E+3	1.4E+3	1.8E+3
B2 – Phase II	After Sand	1.0E-2	3.1E+0	1.2E+0	1.1E+0
	After AC	1.0E-2	1.0E+0	3.4E-1	5.1E-1

Note: AC = activated carbon impregnated with silver nanoparticles, UV = UV light.

During both experiments (low to high and high to low hydraulic loading rate) the two POU devices achieved very limited increases in turbidity removal. Regardless of the hydraulic loading rate, very little additional removal of turbidity, total coliforms or *E. coli* occurred with passage through the AC unit or the UV unit.

Table 12: Case 1: Turbidity, total coliforms and *E. coli* reductions when the two slow sand filtration (SSF) units were placed in parallel. The biolayer of two SSF units (B1 and B2) was developed at a low hydraulic loading rate (0.04 m/h) and at a high hydraulic loading rate (0.12 m/h), respectively. After 14 days the hydraulic loading rate was switched between the two units (n = 14 [Phase I], n = 6 [Phase II]).

SSF unit	**Stage**	**Overall removal (%)**			**Standard deviation**
		Minimum	**Maximum**	**Average**	
TURBIDITY					
B1 – Phase I	After Sand	63	94	83	9
	After AC	57	92	78	11
B1 – Phase II	After Sand	52	92	74	16
	After UV	58	92	75	15
B2 – Phase I	After Sand	48	96	82	13
	UV	66	96	85	9
B2 – Phase II	After Sand	71	98	82	12
	After AC	68	98	80	13
TOTAL COLIFORMS					
B1 – Phase I	After Sand	99.48	99.99	99.87	0.17
	After AC	99.97	99.99	99.99	0.01
B1 – Phase II	After Sand	97.88	99.79	99.20	0.61
	After UV	100	100	100	–
B2 – Phase I	After Sand	99.00	99.98	99.75	0.42
	After UV	99.25	99.91	99.69	0.20
B2 – Phase II	After Sand	99.87	99.99	99.96	0.05
	After AC	99.81	100	99.98	0.04
E. COLI					
B1 – Phase I	After Sand	99.48	100	99.86	0.17
	After AC	99.93	100	99.97	0.02
B1 – Phase II	After Sand	95.38	100	97.87	1.79
	After UV	100	100	100	–
B2 – Phase I	After Sand	94.81	100	99.28	1.58
	After UV	100	100	100	–
B2 – Phase II	After Sand	98.00	100	99.57	0.78
	After AC	98.00	100	99.64	0.81

Note: AC = activated carbon impregnated with silver nanoparticles, UV = UV light; removal after sand, AC and UV are expressed in terms of the values measured in feed water.

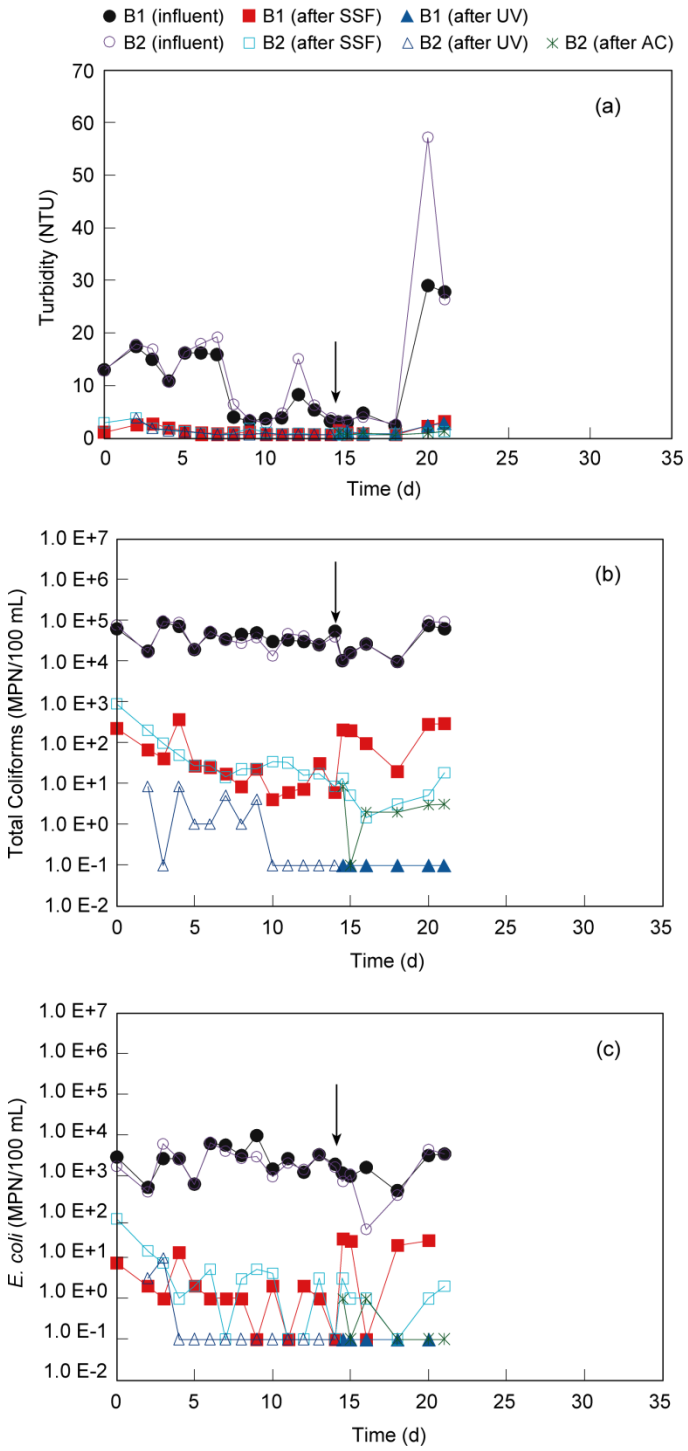


Figure 2: Case 1: (a) turbidity, (b) total coliforms, and (c) *E. coli* reductions when the biolayer of the unit B1 and unit B2 was developed at low (0.04 m/h) and high (0.12 m/h) hydraulic loading rate, respectively. After 14 days (vertical arrow) the hydraulic loading rate was switched between the two units. Unit B1 and unit B2 were run in parallel.

2.3.3. Case 2: Effect of series configuration on turbidity and microorganism removal

In an attempt to increase the efficiency of the SSF units, B1 and B2 were placed in series. The effluent from unit B1 became the feed water for unit B2. During the first 25 days, the SSF units received stream water with a low turbidity of less than 5 NTU (except on days 12 and 21) (Table 13; Fig. 3a). For the next 15 days, they received stream water with an average turbidity of 23.4 NTU (ranging from 11.1 to 33.0 NTU) (Table 13; Fig. 3a). During the low turbidity feed water condition, turbidity decreased from an average of 4.5 NTU to 1.4 NTU after the first unit B1, and to 1.0 NTU after the second unit B2 (Table 13). Turbidity removal of 57% occurred in unit B1, and an additional 14% removal was observed after unit B2 (Table 14). When the average turbidity of the water was 23.4 NTU (Table 13), an average 80% removal was observed after unit B1, and an additional 9% removal was observed after unit B2 (Table 14).

The low turbidity feed water contained an average of 7.5 E+3 MPN/100 mL of total coliforms and 8.0 E+2 MPN/100 mL of *E. coli* (Table 13; Figs. 3b-c). After unit B1, an average 96.80% removal of total coliforms was achieved, and an additional 2% removal was observed after unit B2 (Table 14). A similar removal was also observed in terms of *E. coli* (Table 14). A similar pattern–high removal within the first unit and limited removal after the second unit–was observed in the presence of feed water with high turbidity. High turbidity feed water contained 1.1 E+4 MPN/100 mL of total coliforms and 2.2 E+3 MPN/100 mL of *E. coli* (Table 13). After unit B1, an average 97.82% and 99.34% removal of total coliforms and *E. coli* occurred. Limited additional removal (< 1%) of total coliforms and *E. coli* was achieved after B2 (Table 14).

AC was the only POU device used. There was no additional removal of turbidity (Table 14). Passage through the AC caused very little change in removal of total coliforms and *E. coli* (Table 14).

Table 13: Turbidity, total coliforms and *E. coli* during Case 2 when two SSF units, B1 and B2, were run in series, and in the presence of a low hydraulic loading rate (0.04 m/h), low turbidity (< 5 NTU, n = 6) and high turbidity (11–33 NTU, n = 15).

SSF Unit	**Stage**	**Minimum**	**Maximum**	**Average**	**Standard deviation**
TURBIDITY (NTU)					
Low NTU	Inflow	1.05	19.80	4.52	4.59
	After B1	0.78	2.67	1.38	0.52
	After B2	0.44	2.00	1.05	0.47
	After AC	0.57	2.11	1.10	0.46
High NTU	Inflow	11.13	33.00	23.37	9.17
	After B1	1.98	4.39	3.47	0.88
	After B2	0.97	3.03	2.20	0.77
	After AC	0.93	3.05	2.16	0.72
TOTAL COLIFORMS (MPN/100 mL)					
Low NTU	Inflow	5.4E+2	2.4E+4	7.5E+3	5.3E+3
	After B1	1.7E+1	6.6E+2	2.0E+2	1.6E+2
	After B2	8.5E+0	1.7E+2	7.0E+1	4.8E+1
	After AC	2.0E+0	3.9E+1	1.4E+1	1.2E+1
High NTU	Inflow	2.4E+3	2.4E+4	1.1E+4	1.0E+4
	After B1	1.1E+1	2.2E+2	1.0E+2	8.4E+1
	After B2	4.1E+0	1.3E+2	6.8E+1	4.9E+1
	After AC	1.0E-1	3.9E+1	2.0E+1	1.9E+1
E. COLI (MPN/100 mL)					
Low NTU	Inflow	1.0E+1	2.7E+3	8.0E+2	7.1E+2
	After B1	1.0E-1	9.1E+1	2.2E+1	2.5E+1
	After B2	1.0E+0	3.3E+1	8.4E+0	8.9E+0
	After AC	1.0E-1	2.7E+1	5.4E+0	7.4E+0
High NTU	Inflow	4.8E+2	4.0E+3	2.2E+3	1.5E+3
	After B1	3.1E+0	3.5E+1	1.2E+1	1.2E+1
	After B2	1.0E+0	1.5E+1	7.2E+0	4.9E+0
	After AC	0.0E+0	8.4E+0	4.0E+0	3.4E+0

Note: AC = activated carbon impregnated with silver nanoparticles, UV = UV light.

Table 14: Case 2: Turbidity, total coliforms and *E. coli* reductions when two SSF units, B1 and B2, were run in series, and in the presence of a low hydraulic loading rate (0.04 m/h), low turbidity (< 5 NTU, n = 6) and high turbidity (11–33 NTU, n = 15).

SSF unit	**Stage**	**Overall removal (%)**			**Standard deviation**
		Minimum	**Maximum**	**Average**	
TURBIDITY					
	After B1	6	94	57	22
Low NTU	After B2	52	94	71	12
	After AC	52	89	70	11
	After B1	63	91	80	10
High NTU	After B2	84	97	89	5
	After AC	82	97	89	6
TOTAL COLIFORMS					
	After B1	90.44	99.29	96.80	2.34
Low NTU	After B2	95.37	100	98.70	1.19
	After AC	98.93	100	99.71	0.31
	After B1	94.58	99.95	97.82	1.97
High NTU	After B2	97.21	99.98	98.82	1.12
	After AC	98.57	100	99.55	0.60
E. COLI					
	After B1	80.78	99.52	96.49	4.65
Low NTU	After B2	90.00	99.83	98.01	2.58
	After AC	90.00	99.97	98.66	2.49
	After B1	98.94	99.91	99.34	0.44
High NTU	After B2	98.83	99.97	99.50	0.53
	After AC	99.35	100	99.74	0.26

Note: B1 = first SSF unit, B2 = second SSF unit, AC = activated carbon impregnated with silver nanoparticles, UV = UV light; removal after B1, B2, AC and UV are expressed in terms of the values measured in feed water.

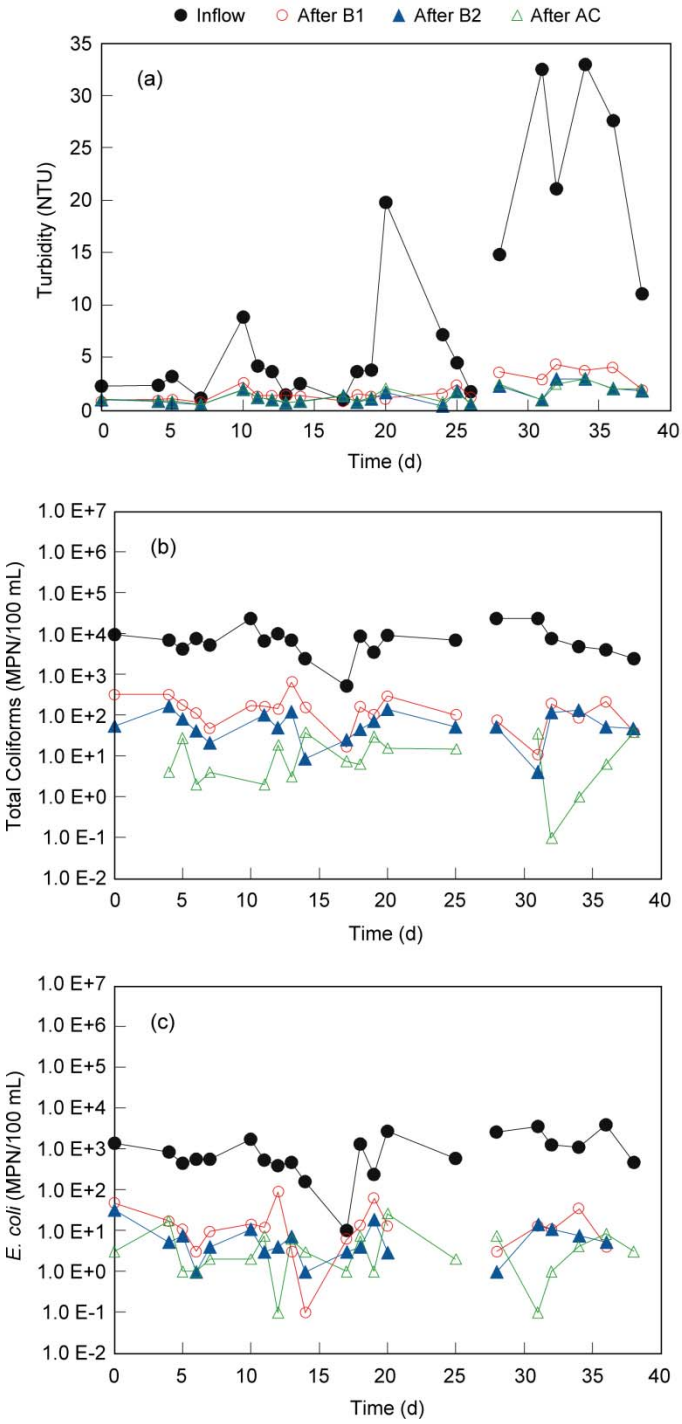


Figure 3: Case 2: (a) turbidity, (b) total coliforms, and (c) *E. coli* reductions when unit B1 and unit B2 were run in series. After 25 d (vertical arrow) the turbidity of the feed water was increased from low (< 4 NTU, left side) to high (11–33NTU, right side). A low hydraulic loading rate (0.04 m/h) was used throughout the study.

2.3.4. Case 3 (HBI and HBII): Effect of high bacterial loads on removal of turbidity and microorganisms by SSF

During the first test, HBI, the biolayers of units B1 and B2 were established with stream water alone. During the second test, HBII, the biolayer of unit B1 was established with stream water containing 1% primary effluent and the biolayer of unit B2 with stream water alone. During HBI and HBII, unit B1 received stream water with 20% primary effluent and unit B2 stream water only. Units B1 and B2 were placed in parallel.

Travel time through the filter was determined using the chloride in the primary effluent as a non-reactive tracer. Travel time from the feed water reservoir to the top port, which was 5 cm below the water-sand interface, was 1 h; to the middle port, 35 cm below the interface, was 6 h; and to the bottom of the filter was 11 h.

During the HBI test, the turbidity of the stream with 20% primary effluent mix averaged 1.5 NTU (ranging from 0.6 to 2.4 NTU) (Table 15; Fig. 4a). The amount of total coliforms and *E. coil* in the feed water was 2.9 E+6 MPN/100 mL and 6.7 E+5 MPN/100 mL, respectively (Table 15; Fig. 4b). Unit B1, which received this high bacterial feed water, achieved 54% removal of turbidity, 84.53% removal of total coliforms and 91.83% removal of *E. coli* (Table 16). Data from the top sampling port of unit B1 indicated that approximately 70% of the overall removal of total coliforms and *E. coli* occurred within the first five centimeters of the unit. Middle port data indicated that the upper half of the filter removed an additional 20% of total coliforms and *E. coli*.

After 24 h, the high bacterial feed water was restored to stream water alone. Average turbidity removal decreased from 54% to 22% (Table 15). Port data indicated that 32% of turbidity removal was due to the biolayer and decreased in the upper portion of the filter to 8% removal due to the release of particles occurring within the unit (Table 15). The average removal of total coliforms and *E. coli* after the filtration unit decreased to 75.81% and 21.35%, respectively (Table 16).

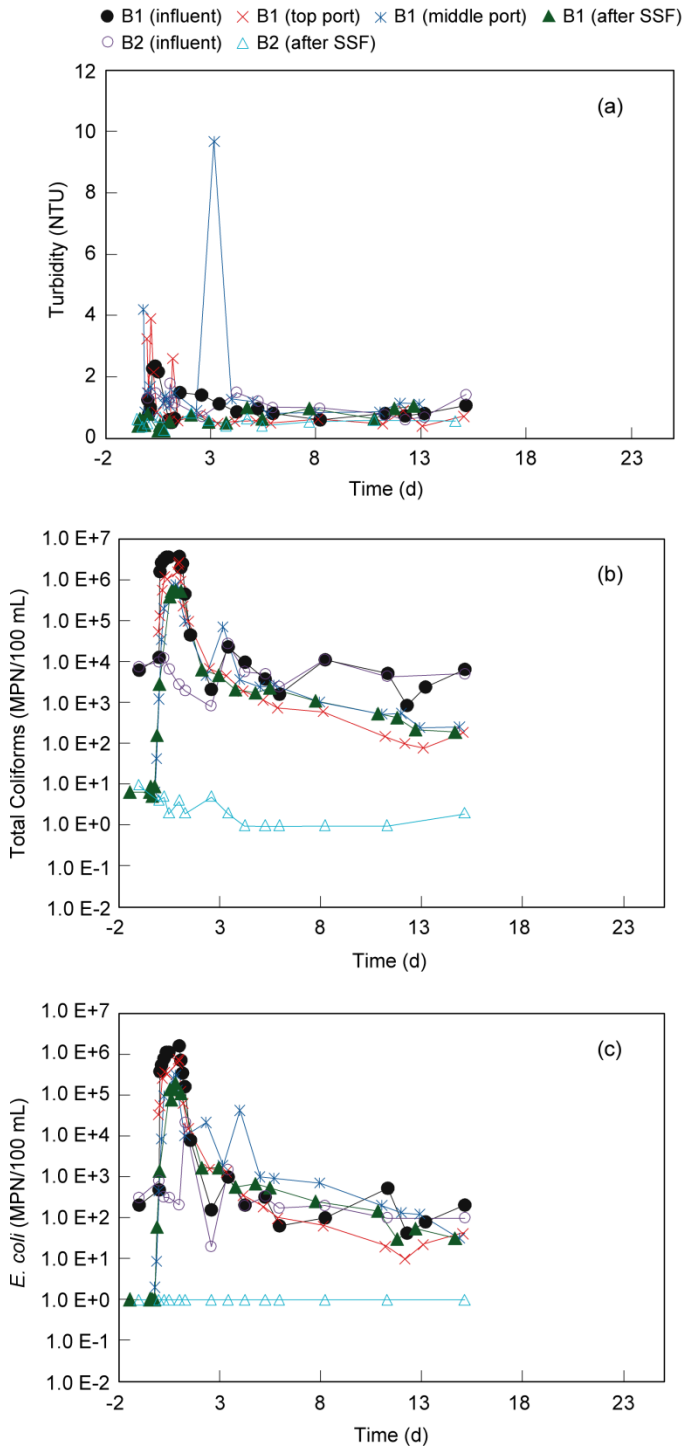


Figure 4: Case 3 (HBI): (a) turbidity, (b) total coliforms, and (c) *E. coli* reductions in the presence of high bacterial loads. Unit B1 and unit B2 were run in parallel. Unit B1 was stressed using Manoa stream water with 20% primary effluent. Unit B2 was used as a control – no primary effluent was added. The biolayer in both units was developed using only Manoa Stream water.

Table 15: Turbidity, total coliforms and *E. coli* during Case 3 (HBI) in the presence of high bacterial loads. The two SSF units, B1 and B2, were run in parallel. Unit B1 was stressed using Manoa Stream water with 20% primary effluent. Unit B2 was used as a control–no primary effluent was added. The biolayer in both units was developed using only Manoa Stream water (n = 8 [during the spike], n = 14 [during the flush-out]).

SSF Unit	**Stage**	**Minimum**	**Maximum**	**Average**	**Standard deviation**
TURBIDITY (NTU)					
B1 - spike	Inflow	0.59	2.36	1.48	0.76
	Top Port	0.52	3.91	1.71	1.27
	Middle Port	0.69	1.68	1.24	0.32
	After Sand	0.25	0.78	0.43	0.21
B1 – flush-out	Inflow	0.53	1.48	0.89	0.30
	Top Port	0.40	2.60	0.74	0.74
	Middle Port	0.54	9.68	1.80	2.63
	After Sand	0.49	1.06	0.79	0.21
B2 – spike	Inflow	0.71	1.78	1.21	0.44
	After Sand	0.28	0.69	0.48	0.21
B2 – flush-out	Inflow	0.61	1.49	1.02	0.33
	After Sand	0.40	0.83	0.57	0.14
TOTAL COLIFORMS (MPN/100 mL)					
B1 – spike	Inflow	1.6E+6	3.8E+6	2.9E+6	8.7E+5
	Top Port	5.4E+4	2.7E+6	1.2E+6	9.8E+5
	Middle Port	1.2E+3	7.7E+5	3.8E+5	3.4E+5
	After Sand	2.8E+3	5.6E+5	4.2E+5	2.1E+5
B1 – flush-out	Inflow	8.6E+2	4.4E+5	4.6E+4	1.2E+5
	Top Port	7.8E+1	8.8E+5	9.4E+4	2.5E+5
	Middle Port	2.4E+2	9.8E+4	1.7E+4	3.4E+4
	After Sand	1.9E+2	6.3E+3	1.9E+3	2.0E+3
B2 – spike	Inflow	1.9E+3	6.6E+3	3.7E+3	2.5E+3
	After Sand	2.0E+0	4.1E+0	2.7E+0	1.2E+0
B2 – flush-out	Inflow	8.0E+2	2.7E+4	7.7E+3	8.5E+3
	After Sand	1.0E+0	5.2E+0	1.8E+0	1.5E+0
E. COLI (MPN/100 mL)					
B1 - spike	Inflow	4.9E+2	1.6E+6	6.7E+5	4.9E+5
	Top Port	3.4E+4	7.5E+5	3.9E+5	2.8E+5
	Middle Port	4.4E+2	3.1E+5	1.1E+5	1.2E+5
	After Sand	1.4E+3	2.0E+5	1.1E+5	6.7E+4
B1 – flush-out	Inflow	4.1E+1	7.9E+3	9.6E+2	2.3E+3
	Top Port	1.0E+1	1.2E+5	1.6E+4	1.6E+4
	Middle Port	2.0E+1	4.2E+4	6.1E+3	1.3E+4
	After Sand	3.0E+1	1.7E+3	5.6E+2	6.2E+2
B2 – spike	Inflow	2.1E+2	2.1E+4	4.6E+3	9.3E+3
	After Sand	1.0E+0	1.0E+0	1.0E+0	0.0E+0
B2 – flush-out	Inflow	2.0E+1	1.5E+3	3.3E+2	4.8E+2
	After Sand	1.0E+0	1.0E+0	1.0E+0	0.0E+0

Note: HB = high bacterial loads, top port = 5 cm below the sand/water interface, middle port = 35 cm below the sand/water interface.

Table 16: Case 3 (HBI): Turbidity, total coliforms and *E. coli* in the presence of high bacterial loads. The two SSF units, B1 and B2, were run in parallel. Unit B1 was stressed using Manoa Stream water with 20% primary effluent. Unit B2 was used as a control–no primary effluent was added. The biolayer in both units was developed using only Manoa Stream water (n = 8 [during the spike], n = 14 [during the flush-out]).

SSF unit	**Stage**	**Overall removal (%)**			**Standard deviation**
		Minimum	**Maximum**	**Average**	
TURBIDITY					
B1 - spike	Top Port	0	64	12	24
	Middle Port	0	69	17	29
	After Sand	5	83	54	28
B1 – flush-out	Top Port	0	62	32	23
	Middle Port	0	50	8	18
	After Sand	0	62	22	23
B2 – spike	After Sand	41	61	54	11
B2 – flush-out	After Sand	11	61	41	19
TOTAL COLIFORMS					
B1 – spike	Top Port	28.04	96.59	69.57	23.05
	Middle Port	79.62	100	91.11	9.04
	After Sand	72.66	99.83	84.53	10.13
B1 – flush-out	Top Port	0.00	97.20	69.60	34.24
	Middle Port	0.00	96.17	52.84	39.63
	After Sand	40.73	97.12	75.81	19.96
B2 – spike	After Sand	99.85	99.97	99.91	0.06
B2 – flush-out	After Sand	99.35	99.99	99.90	0.22
E. COLI					
B1 - spike	Top Port	51.86	90.99	67.22	15.64
	Middle Port	76.98	99.88	90.74	9.30
	After Sand	84.77	99.64	91.38	5.07
B1 – flush-out	Top Port	0.00	96.15	43.54	39.09
	Middle Port	0.00	90.00	28.19	39.87
	After Sand	0.00	84.50	21.35	32.30
B2 – spike	After Sand	99.53	100	99.75	0.18
B2 – flush-out	After Sand	95.00	99.63	98.88	1.60

Note: HB = high bacterial loads, top port = 5 cm below the sand/water interface, middle port = 35 cm below the sand/water interface; removal after top port, middle port and after sand are expressed in terms of values measured in feed water.

During HBI, unit B2 received only stream water. The turbidity of the stream water averaged 1.2 NTU (ranging from 0.7 NTU to 1.8 NTU) (Table 15; Fig. 4a). Turbidity removal was 54% exactly as seen in unit B1. Total coliforms and *E. coli* in the stream water were 3.7 E+3 MPN/100 mL and 4.6 E +3 MPN/100 mL, respectively (Table 15; Fig. 4c). Unit B2 achieved 99.91% removal of total coliforms and 99.75% removal of *E. coli* (Table 16). When feed water was switched in unit B1, unit B2 continued to receive

stream water. After unit B2 overall turbidity removal decreased to 41%, but the total coliforms and *E. coli* removals continued to be 99% or greater (Table 16).

For the second test, HBII, the biolayer of unit B1 was established using stream water with 1% primary effluent. Otherwise, the procedure was the same as the HBI test. During HBII, the feed water to unit B1, consisting of stream water with 20% primary effluent, had an average turbidity of 5.6 NTU (ranging from 4.3 to 8.3 NTU), 2.2 E+6 MPN/100 mL (ranging from 8.0 E+5 to 3.3 E+6 MPN/100 mL) total coliforms and 9.3 E+5 MPN/100mL *E. coli* (ranging from 4.1 E+3 to 2.2 E+6 MPN/100 mL) (Table 17; Fig. 5a,b,c). Table 18 shows that overall turbidity removal in unit B1 was 84%, total coliforms removal was 94.24% and *E. coli* was 95.83%. Approximately 60% of the overall bacterial removal occurred within the first five centimeters of the filter (Table 18).

Table 17: Turbidity, total coliforms, and *E. coli* during Case 3 (HBII) in the presence of high bacterial loads. Unit B1 and unit B2 were run in parallel. Unit B1 was stressed using Manoa Stream water with 20% primary effluent. Unit B2 was used as a control − no primary effluent was added. The biolayer in unit B1 was developed using Manoa Stream water with 1% of primary effluent, while the biolayer in unit B2 was developed using only Manoa Stream water (n = 10 [during the spike], n = 21 [during the flush-out]).

SSF Unit	**Stage**	**Minimum**	**Maximum**	**Average**	**Standard deviation**
TURBIDITY (NTU)					
B1 – spike	Inflow	4.31	8.34	5.64	1.30
	Top Port	0.98	4.67	2.39	1.26
	After Sand	0.77	0.99	0.84	0.07
	After UV	0.45	0.77	0.64	0.14
B1 – flush-out	Inflow	2.35	11.70	6.87	3.46
	Top Port	0.61	3.37	1.96	0.87
	After Sand	0.40	2.46	1.39	0.46
	After UV	0.39	1.78	1.09	0.98
B2 – spike	Inflow	2.10	6.47	2.81	0.65
	After Sand	0.72	2.38	1.33	0.41
B2 – flush-out	Inflow	1.05	6.47	3.84	1.78
	After Sand	0.72	2.38	1.61	0.52
TOTAL COLIFORMS (MPN/100 mL)					
B1 – spike	Inflow	8.0E+5	3.3E+6	2.2E+6	8.8E+5
	Top Port	8.3E+3	2.4E+6	1.3E+6	8.1E+5
	After Sand	1.0E+0	2.4E+5	7.1E+4	1.1E+5
	After UV	1.0E-1	4.3E+1	8.0E+0	1.7E+1
B1 – flush-out	Inflow	1.6E+3	9.1E+5	7.9E+4	2.1E+5
	Top Port	9.8E+1	5.1E+4	9.5E+3	1.4E+4
	After Sand	3.1E+0	2.4E+5	1.9E+4	5.6E+4
	After UV	1.0E-1	1.0E-1	1.0E-1	0.0E+0
B2 – spike	Inflow	6.4E+3	2.4E+3	1.5E+2	2.1E+3
	After Sand	1.8E+0	8.4E+0	1.0E-1	2.5E+0
B2 – flush-out	Inflow	1.7E+3	4.0E+3	2.8E+3	1.5E+2
	After Sand	8.4E+0	1.7E+0	1.0E-1	1.0E-1
E. COLI (MPN/100 mL)					
B1 – spike	Inflow	4.1E+3	2.2E+6	9.3E+5	7.4E+5
	Top Port	3.0E+2	4.8E+6	7.2E+5	1.4E+6
	After Sand	1.0E+0	8.3E+4	3.2E+4	3.6E+4
	After UV	1.0E-1	1.1E+1	2.7E+0	4.2E+0
B1 – flush-out	Inflow	1.0E+2	2.4E+4	5.0E+3	6.0E+3
	Top Port	1.0E-1	1.5E+4	2.7E+3	4.4E+3
	After Sand	1.0E-1	9.1E+4	6.9E+3	2.1E+4
	After UV	1.0E-1	1.0E-1	1.0E-1	0.0E+0
B2 – spike	Inflow	2.0E+1	1.1E+3	4.5E+2	4.8E+2
	After Sand	1.0E+0	1.0E+0	1.0E+0	0.0E+0
B2 – flush-out	Inflow	4.1E+0	5.4E+2	2.1E+2	1.9E+2
	After Sand	1.0E-1	1.0E+0	2.1E-1	3.2E-1

HB = high bacterial loads, top port = 5 cm below the sand/water interface, middle port = 35 cm below the sand /water interface.

Table 18: Case 3 (HBII): Turbidity, total coliforms, and *E. coli* in the presence of high bacterial loads. Unit B1 and unit B2 were run in parallel. Unit B1 was stressed using Manoa Stream water with 20% primary effluent. Unit B2 was used as a control − no primary effluent was added. The biolayer in unit B1 was developed using Manoa Stream water with 1% of primary effluent, while the biolayer in unit B2 was developed using only Manoa Stream water (n = 10 [during the spike], n = 21 [during the flush-out]).

SSF unit	**Stage**	**Overall removal (%)**			**Standard deviation**
		Minimum	**Maximum**	**Average**	
TURBIDITY					
	Top Port	26	83	59	22
B1 – spike	After Sand	73	90	84	5
	After UV	84	94	89	4
	Top Port	0	100	66	28
B1 – flush-out	After Sand	14	89	75	18
	After UV	53	83	68	22
B2 – spike	After Sand	31	74	51	16
B2 – flush-out	After Sand	31	73	53	13
TOTAL COLIFORMS					
	Top Port	27.44	100	61.07	32.32
B1 – spike	After Sand	78.77	100	94.24	6.97
	After UV	99.99	100	100	–
	Top Port	0.00	99.73	65.15	28.91
B1 – flush-out	After Sand	0.00	99.73	78.89	28.91
	After UV	100	100	100	–
B2 – spike	After Sand	94.27	100	99.29	2.00
B2 – flush-out	After Sand	94.00	100	99.23	2.00
E. COLI					
	Top Port	0.00	99.91	53.29	28.51
B1 – spike	After Sand	73.12	100	95.83	7.83
	After UV	100	100	100	–
	Top Port	0.00	99.58	65.07	42.90
B1 – flush-out	After Sand	0.00	99.61	69.16	40.03
	After UV	100	100	100	–
B2 – spike	After Sand	95.00	99.91	98.53	2.36
B2 – flush-out	After Sand	75.61	99.98	95.83	9.91

HB = high bacterial loads, top port = 5 cm below the sand/water interface, middle port = 35 cm below the sand /water interface; removal after top port, after sand, and UV are expressed in terms of the values measured in the feed water.

After 24 h, the high bacterial feed water to unit B1 was replaced with stream water with 1% primary effluent that contained an average of 6.9 NTU (ranging from 2.4 to 11.7 NTU), 7.9 E+4 MPN/100 mL of total coliforms (ranging from 1.6 E+3 to 9.15 E+5 MPN/100 mL) and 5.0 E+3 MPN/100 mL of *E. coli* (ranging from 1.0 E+2 to 2.4 E+4 MPN/100 mL) (Table 17; Fig. 5). During this period, unit B1 had an overall turbidity

removal of 75%, total coliforms removal of 78.89% and *E. coli* removal of 69.16% (Table 18).

As in HBI, unit B2 received only stream water throughout test HBII. The feed water turbidity averaged 3.8 NTU (ranging from 1.1 to 6.5 NTU) (Table 17; Fig. 5). The total coliforms and *E. coli* concentrations were 2.8 E+3 MPN/100 mL (ranging from 1.7 E+3 to 4.0 E+3 MPN/100 mL) and 2.1 E+2 MPN/100 mL (ranging from 4.1 E+0 to 5.4 E+2 MPN/100 mL), respectively. Turbidity removal was 53% and total coliforms and *E. coli* removal was at least 96% throughout the experiment (Table 18).

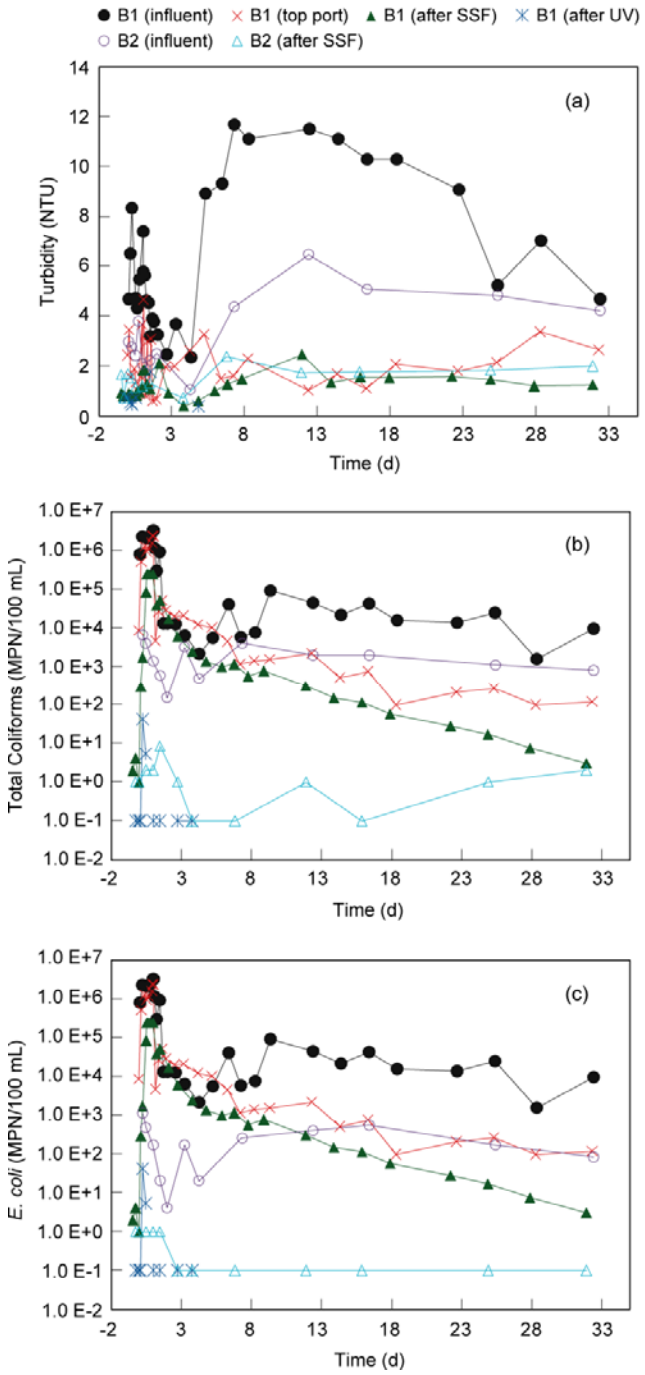


Figure 5: Case 3 (HBII): (a) turbidity, (b) total coliforms, and (c) *E. coli* reductions in the presence of high bacterial loads. Unit B1 and unit B2 were run in parallel. Unit B1 was stressed using Manoa Stream water with 20% primary effluent. Unit B2 was used as control – no primary effluent was added. The biolayer in unit B1 was developed using Manoa Stream water with 1% of primary effluent, while the biolayer in unit B2 was developed using only Manoa Stream water.

2.4. DISCUSSION

2.4.1. Turbidity removal

The straining and adsorption that occurs between the sand in a filter and the suspended particles in the feed water are two of the dominant processes involved in turbidity removal (Huisman and Wood, 1974; Weber-Shirk and Dick, 1997a). Straining is due to the interception and retention of particles too large to pass through the interstices between the grains of sand. When a sand filter is newly constructed, straining is predominant at the surface of the filter, but with the maturation of the filter, straining occurs throughout the entire filtration unit.

The pores within a tightly packed bed of spherical sand grains of uniform size are sufficiently small to prevent the passage of particles that are one-seventh the diameter of the sand grains used (Huisman and Wood, 1974). In this study, using silica sand with a grain size ranging between 150 and 300 μm (coefficient of uniformity of 1.6), the smallest pores are slightly over 20 μm in diameter and are unable to intercept colloidal particles.

Adsorption of particles onto a sand grain surface once they have made contact is due to a combination of electrostatic attraction, van der Waals forces, and adherence (Huisman and Wood, 1974). During the maturation of the filter, organic material may be deposited on the filter surface and on the individual grains. These deposits can become a breeding ground for bacteria and other microorganisms, which produce a slimy material known as zooglea that consists of living bacteria, their wastes and dead cells, and partly assimilated organic materials (Huisman and Wood, 1974). The zooglea forms a sticky gelatinous film on the surface of the biolayer and sand grains, increasing the opportunity for particles contained in the feed water to be trapped and adsorbed (Huisman and Wood, 1974).

Outflow turbidity after the SSF units was always lower than 4 NTU but removal varied depending on the turbidity of the feed water. For feed water with turbidity less than 5 NTU, approximately 41–65% turbidity removal was achieved (Table 10 and Table 12).

Higher removals of 75–90% were consistently achieved when feed waters had turbidity greater than 9 NTU (Table 12). A similar pattern was also observed by Bellamy et al. (1985) and Tyagi et al. (2009) in pilot-scale SSF units. For example, using effluent from an up-flow anaerobic sludge blanket (USAB) reactor with an average starting turbidity of 56.5 NTU (35–60 NTU), Tyagi et al. (2009) observed an average turbidity removal of 91.6% (outflow turbidity ranged between 1.6 and 6.2 NTU). On the other hand, using input water collected from Horsetooth Reservoir (Colorado) with starting turbidities between 6 and 8 NTU, Bellamy et al. (1985) observed only 25–35% turbidity removal (outflow turbidity ranged between 3 and 5 NTU).

According to Bellamy et al. (1985) the lower turbidity removal was related to the large fraction of small clay particles in the feed water (30% of the turbidity was due to particles smaller than 0.45 μm). On the other hand, in feed water having high turbidity, possible interactions between the particles may enhance turbidity removal by the unit (Weber-Shirk and Dick, 1997a). Data from the two sampling ports on unit B1 suggested that turbidity removal occurred throughout the sand filter and not just in the biolayer. During HBI when the feed water was < 2 NTU, 11% of the turbidity removal occurred within the biolayer, an additional 5% occurred in the upper half of the filter and 38% occurred in the lower half of the filter. The overall turbidity removal (54%) was consistent with the theory that low turbidity input water means low/moderate percent turbidity removal (10–60%). During HBII the biolayer had been developed using stream water with 1% primary effluent, and the turbidity of the feed water ranged between 2 and 12 NTU. Measurements made at the top port showed that the biolayer was responsible for 59% of turbidity removal and the rest of the filter accounted for an additional 25% removal. The overall removal (84%) was consistent with the high average turbidity of the input water.

During HBI and HBII, reductions in turbidity removal occurred in unit B1 when the high bacterial feed water was replaced with stream water alone or stream water with 1% primary effluent. During HBI, there was limited removal of turbidity in unit B1 for approximately 10 days following the switch of feed water. Turbidity removal of 32% occurred at the top port, but only 8% at the middle port, and 22% in the effluent after the

filter unit. This reduction in turbidity removal in the samples from the middle and the end of the filter may be due to the detachment of particles once the mixture of stream water with 20% primary effluent was replaced with stream water alone. Particles may be detaching from the biolayer as well as from the packing material and may be moving slowly through the SSF unit (Hijnen et al., 2004). There may have been a charge reversal in the sand particles with the release of particulates of similar charge.

The effect of the feed water switch was more pronounced in HBI than HBII. The reduction in turbidity removal lasted 10 days in HBI and one day in HBII. The difference observed may be related to the fact that the biolayer was developed using stream water with 1% primary effluent in HBII rather than stream water alone.

2.4.2. Bacterial removal

Biological mechanisms are important in the removal of particulates < 2 μm in size (Weber-Shirk and Dick, 1997a). During the operation of a sand filter, biological growth occurs on the surface and throughout the sand bed. The biological growth, consisting of among other things bacteria, their waste products, and partly metabolized organic matter, can enhance the trapping and attachment of other bacterial particles. The trapped or adsorbed bacteria can be consumed, they can remain permanently adsorbed and eventually metabolized or they can be released. For example, input water may affect the charge on the sand particles and cause the negatively charged bacteria to be repelled rather than attracted to the sand particles (Huisman and Wood, 1974).

Studies of SSF units show that bacterial removal ranged from 90% to 99.99% or from 1 log to 4 logs (Hijnen et al., 2004; Tyagi et al., 2009; Gottinger et al., 2011). In the present study, bacterial removal can range from 90% to 99.9% even under different configurations (parallel and series), different hydraulic loading rates (0.04 m/h and 0.12 m/h), and different starting concentration of bacteria (Tables 10–14). The lowest bacterial removal occurred at the beginning of the study and is an example of the importance of the maturity of the filter and the biolayer in bacterial removal. Bellamy et al. (1985, 1995) observed that the microbiological maturity of the sand bed is the most important variable

in the removal of total coliforms and *Giardia*. When the sand filter was initially constructed, the silica sand was clean and had a negative charge. This negative charge attracts positively charged particles and repels negatively charged particles.

Results from the two tests conducted with high bacterial loads provided an idea of the ability of SSF to treat feed water having high loads of total coliforms and *E. coli* and emphasize the impact of the biolayer on bacteria removal. With high bacterial loads of approximately 3.0 E+6 MPN/100 mL of total coliforms, the SSF was able to remove approximately 1.0 E+6 MPN/100 mL. The biolayer played a crucial role in terms of bacteria removal since about 60% of the removal occurred within the top 5 cm of the SSF units, with some additional removal observed in the upper 25 cm of the unit (Tables 16 and 18). Calvo-Bado et al. (2003) studied an SSF unit used for filtering horticultural irrigation water and found that the top layer (1 cm) of the SSF unit was dominated by a variable and active microbial population, whereas the middle (50 cm) and the bottom (80 cm) layers were dominated by less active and diverse bacterial populations. Ellis and Aydin (1995), using an SSF column with 1.2 m layer of sand covered by 1.5 m depth of water, observed a noticeable reduction in the number of bacteria below the depth of 400 mm, suggesting a limited bacterial removal compared with the upper section of the filtration unit.

In HBII the results obtained from the top sampling port, located 5 cm below the water and sand interface, showed that the type of water used to develop the biolayer appeared to play a marginal role in the overall bacterial removal. There was no significant difference in bacterial removal with a biolayer developed with stream water alone versus a biolayer developed with stream water containing 1% primary effluent. The biolayer developed with only stream water removed 70% of total coliforms and 67% of *E. coli*, while the biolayer developed with stream water containing 1% primary effluent removed 61% of total coliforms and 53% of *E. coli* (Tables 16 and 18).

During the flush-out (i.e., replacing the stream water with 20% primary effluent with stream water alone [HBI] and stream water with 1% primary effluent [HBII]), which occurred after the 24 h injection of stream water with 20% primary effluent, a decline in

bacterial removal was observed after both SSF units (Tables 16 and 18). This may have been caused by the detachment of microorganisms due to electrostatic charge reversals on particle occurring during this transition period. The decrease in bacteria removal was more evident in terms of *E. coli* compared to total coliforms, especially after HBI. In fact, in HBI during the flush-out, *E. coli* removal decreased from 92% to 21%, while the removal of total coliforms decreased from 85% to 76%. In HBII, during the second restoring period when feed water containing stream water with 20% primary effluent was replaced with stream water having 1% primary effluent, *E. coli* removal dropped from 96% to 69% while the removal of total coliforms dropped from 94% to 78%.

Regarding the POU devices, UV can effectively treat the effluent from a SSF unit having high bacterial loads (up to 8.0 E+4 MPN/100 mL) (Figs. 4b-c, 5b-c), while AC can only treat the effluent from a SSF unit with lower bacterial loads (1.0 E+3 MPN/100 mL) (Fig. 4b). UV also represents a more dynamic device compared to the AC impregnated with silver nanoparticles. UV is able to treat a larger volume of water with greater bacterial removal (constantly > 99.9%). AC needs to be replaced more often than UV and possible leaching of silver nanoparticles from the packing material may occur.

2.5. CONCLUSIONS

- Across the different experimental scenarios using series and parallel configurations, SSF proved to be a valuable technology to enhance the quality of the raw water. Turbidity removal ranging from 40% to 80% was observed and higher removal of total coliforms and *E. coli* (> 95%) was consistently achieved.
- The duration of filter times and declining hydraulic loading rate is mostly related to the hydraulic loading time. In the presence of higher hydraulic loading rates, quicker declining hydraulic loading rates are expected and scraping of the filtration units is required more frequently.
- The time required to produce the biolayer is a function of the amount and quality of water passed through the SSF. A rate of 0.04 m/h represents the minimum hydraulic loading rate that can be used for the SSF.

- The removal efficiency of the SSF unit is mostly related to the quality of the biolayer and to the maturity of the filter unit. Higher removal is achieved by more biologically mature units.

- Regardless of the configuration of the SSF units and the proposed hydraulic loading rates, turbidity removal was highly impacted by the initial turbidity of the feed water. High removal (75–90%) was observed for feed water having turbidity above 9 NTU.
- Most of the bacterial removal occurred within the first few centimeters of the SSF.
- When the two units were placed in series, limited to no additional removal by the second unit, particularly of total coliforms and *E. coli* was observed. The lack of nutrients in the effluent of the first SSF unit (influent to the second unit) may limit the development of the biolayer as well as the formation of the sticky gelatinous film around the sand grains in the second SSF unit.
- SSF can be used for water with high bacterial loads.
- The type of feed water used to develop the biolayer plays a limited role in terms of bacterial removal.
- The UV unit, used after an SSF filter with feed water having turbidity lower than 4 NTU, represents a more efficient and flexible POU device compared with AC impregnated with silver nanoparticles. The UV unit can produce larger quantities of higher quality water (total coliforms and *E. coli* below detection limits) compared with the AC impregnated with silver nanoparticles.

CHAPTER 3. IMPACT OF SELECTED PHARMACEUTICALLY ACTIVE COMPOUNDS (PhACs) ON SLOW SAND FILTER (SSF) BACTERIAL REMOVAL AND REMOVAL OF PhACs BY SSF[1]

Abstract

Slow sand filtration (SSF) has been widely used for potable water supply due to its low cost and minimum maintenance. The aim of this work was to examine the impact of selected pharmaceutically active compounds (PhACs) on the removal of bacteria by an SSF unit and to examine the ability of SSF to remove these PhACs. Such scenarios may occur in surface waters impacted by wastewater treatment plants or spills from pharmaceutical industries.

Two polyethylene barrels (57 cm inner diameter, 88 cm height) were used to construct the two SSF units. Unit B1 was fed with stream water with 1% primary effluent, while unit B2 with stream water alone. Once the biolayers were established, and at least 2 logs of bacterial removal were achieved in both units, a mixture of a maximum of 6 of the selected PhACs was added to the feed water of each unit (referred to as a spike). Three such spiking events were performed. At the end of each spike, feed water without PhACs was introduced until the SSF units achieved approximately 2 logs of bacterial removal.

The presence of selected PhACs in the feed water, especially 17-β estradiol (E2), impacted the biolayer of the SSF units and the bacterial removal efficiency of the SSF units. However, in a "biologically mature" SSF unit (unit that has been used for an extensive period of time in which a "sticky" and gelatinous film has developed throughout the packing material), regardless of the nature of the feed water, high removal (> 99%) of total coliforms and *E. coli* was achieved even in the presence of a "compromised biolayer" were limited, < 15%, removal was achieved after the biolayer.

[1] This chapter has been submitted to *PlusOne*: Matteo D'Alessio, Bunnie Yoneyama, Marek Kirs, Veljo Kisand, Chittaranjan Ray. Impact of Selected Pharmaceutically Active Compounds (PhACs) on Slow Sand Filter (SSF) Bacterial Removal Efficiency and removal of PhACs by SSF.

At the end of the study, the microbial community within the sand of the SSF units was significantly more diverse than the community in the biolayer. Proteobacteria, predominantly class Gammaproteobacteria, and Bacteroidetes were the dominant phyla in the biolayer samples while Actinobacteria, Bacteroidetes, and several classes of Proteobacteria (Alpha-, Beta-, Delta-, and Gamma-proteobacteria) were prevalent in the sand samples.

In both SSF units there was no to limited removal (< 10%) of carbamazepine, gemfibrozil, and phenazone, partial removal (11–92%) of E2 and estrone (E1) and complete removal of caffeine was achieved by both SSF units. Sorption and biodegradation are the main removal mechanisms of the selected PhACs. The different removal of caffeine during the three spikes reflects the change in the presence of *Pseudomonas* during the entire study. *Pseudomonas* was the predominant group of bacteria present in the biolayer during the third spike in which at least 75% removal of caffeine was achieved within the biolayer of both SSF units.

3.1. INTRODUCTION

Slow sand filtration (SSF) has been widely used due to its low costs and the relative simplicity of construction and maintenance of the filtration system. The effective size of the sand particles, the depth of the sand bed, the hydraulic loading rates, and the quality of the raw water are the main factors impacting turbidity and bacterial removal by SSF (Huisman and Wood, 1974; Thanh and Hettiaratchi, 1982). SSF has been used to treat high quality surface water, such as stream (Cleasby et al., 1984; Bellamy et al., 1985; Aydin, 1997) and lake waters (Weber-Shirk and Dick, 1997a-b) as well as secondary (Farooq and Al-Yousef, 1993; Logsdon et al., 1993) and tertiary effluent (Langenbach et al., 2009). During the past decade, however, SSF has also been used to treat a broad variety of water such as rain water (Neto et al., 2012), rural prairie water (Gottinger et al., 2011), wastewater from mariculture facilities (Palmer, 2010), and effluent from an up-flow anaerobic sludge blanket (Tyagi et al., 2009). SSF can also be used as pre- or post-treatment for other types of filtration or treatment. For example, Zheng et al. (2009) used SSF as the preliminary treatment for secondary effluent prior to ultra-filtration, and Aslan (2008) reported SSF used as post-treatment for drinking water after a biodenitrification unit.

Besides being used to remove turbidity and bacteria, SSF is used to remove chemical oxygen demand (COD), total phosphorous (TP), and total nitrogen (TN) from slightly contaminated water (Lwesya and Li, 2010), and iron and manganese from groundwater (Pacini et al., 2005; Gottinger et al., 2011). The occurrence of trace organic contaminants (TOrCs) such as pharmaceutically active compounds (PhACs), personal care products (PCPs), surfactants, and pesticides have been significantly increasing in the environment during the past decades (Kolpin et al., 2002; Focazio et al., 2008). Multiple studies (Ternes, 1998; Clara et al., 2005; Nakada et al., 2006) have revealed the limited ability of conventional wastewater treatment to completely remove these compounds. PhACs at low concentrations are capable of disrupting the endocrine systems of many organisms (Manning, 2005; Vajda et al., 2008). Therefore, the presence of these compounds in surface water is a matter of concern because of their potential to be present in potable

water. PhACs, and more in general TOrCs, may also impact the bacterial removal by natural filtration processes such as SSF. There are limited and contrasting studies on the effect of TOrCs on the performance of SSF units (Woudneh et al., 1997; Oszako et al., 2013). For example, no significant deterioration of microbial removal by an SSF unit occurred during a dosage of 2,4-D herbicide (Woudneh et al., 1997), but reduced removal of bacteria was observed in the presence of feed water polluted with a mineral fertilizer and the fungicide pentachloronitrobenzene (PCNB) (Oszako et al., 2013). However, this different trend may also be related to the different level of toxicity between 2,4-D and PCNB.

There is still uncertainty regarding the ability of an SSF unit to remove TOrCs. Coelho and Di Bernardo (2012) reported that limited removal of atrazine and its metabolites diethylatrazine (DEA), deisoprophylatrazine (DIA), and diethylhydroxylamine (DEHA) was achieved using SSF alone. However, higher removal of atrazine was achieved using SSF with an intermediate layer of granular activated carbon, suggesting that sorption to the activated carbon plays a key role in the removal of atrazine and its metabolites. In a pilot SSF, 2,4-D was removed and the presence of microorganisms in the bed was a precondition for the removal of the herbicide (Woudneh et al., 1997). SSF was also able to partially remove the organic compounds 2-methylisoborneol (MIB) and geosmin. More than 50% of MIB and geosmin were degraded by the microbial population on the sand surface of an SSF unit (Hsieh et al., 2010).

The ability of SSF to remove PhACs is still being investigated. For example, in a study conducted by Ternes et al. (2002), a municipal SSF was unable to remove bezafibrate, clofibric acid, diclofenac, carbamazepine, and primidone. On the other hand, Rooklidge et al. (2005) investigating the removal of antimicrobials in a multistage filtration (roughing and SSF) system at a municipal water treatment facility, reported that at the end of a 14-day simulation period, 100% removal of tylosin, 99% removal of trimethoprim, < 25% removal of lincomycin, and < 4% removal of a sulfonamide class of antimicrobial was achieved with an SSF unit. These results suggested that multistage filtration was an ineffective treatment for antimicrobials with low filter-media-sorption

coefficients, while antimicrobials with high sorption coefficients can accumulate in roughing filter waste and biolayer.

The presence of PhACs from wastewater treatment plants or pharmaceutical industry spills may affect the bacterial population in the biolayer as well as in the sand. The first objective of this work was to examine the impact of selected PhACs on bacteria removal by an SSF unit. A second objective was to examine the ability of SSF to remove a variety of PhACs (i.e., stimulant, hormones, antipyretic) present in the feed water. Such scenarios may occur in surface water impacted by wastewater treatment plants or spills from pharmaceutical industries, particularly in situations following natural disasters. The third objective was to investigate the impact that feed water (i.e., stream water alone or stream water with 1% primary effluent) may have on the development and dynamics of the biolayer in two SSF units. Results from this third objective were used to better understand the impact of PhACs on the bacterial removal by the SSF units, as well as on their ability to remove PhACs.

3.2. MATERIALS AND METHODS

3.2.1. Chemicals

Caffeine (100% pure), carbamazepine (100% pure), estrone (E1, 99% pure), 17-β estradiol (E2, 100% pure), gemfibrozil (99.9% pure), and phenazone (99.9% pure) were purchased from Sigma-Aldrich (St. Louis, MO); HPLC-grade methanol and acetonitrile were purchased from EMD Chemicals Inc. (Gibbstown, NJ). Physico-chemical properties of the selected PhACs used in this study are provided in Table 5.

3.2.2. Packing materials

Silica sand (0.015–0.030 cm, Orange County # 60) with a uniformity coefficient of 1.6 was used as the main packing material for the sand filters. Pea gravel with an effective size of 0.95 cm was used as the underlying supporting material for the sand in the SSF units.

3.2.3. Feed water

Feed water was obtained from Manoa Stream water (21°17'35.6" N, 157°48'45.8" W; Honolulu, HI) (Fig. A1-Appendix A), which runs by the University of Hawaii's main campus. The different influent bacterial concentrations observed during this study are reflections of the changing conditions in the stream. Primary effluent was collected from the Honouliuli Wastewater Treatment Facility in Ewa Beach, Oahu, Hawaii, and stored at 4 °C prior to use.

3.2.4. Experimental setup

Two polyethylene barrels (57 cm inner diameter, 88 cm height) were used to construct the two SSF units, referred to as unit B1 and unit B2. Each barrel had two stainless steel sampling ports located at 5 and 35 cm from the water–sand interface (Fig. A2-Appendix A). Ten centimeters of pea gravel covered by a stainless steel wire cloth (TWP, Berkeley, CA) with 0.014 mm openings was located at the bottom of each barrel to support 57 cm of silica sand. Prior to packing the two barrels, the sieved sand was repeatedly washed with tap water to remove fine particles. A dual head peristaltic pump (Cole-Parmer, Vernon Hills, IL) was used to simultaneously deliver feed water to both sand filters from two separate reservoirs, each representing a different source water (stream water alone and stream water with 1% primary effluent). Once a 10–15% reduction in the output flow rate was observed, maintenance of the SSF units was done by scraping and removing the top 1–2 cm of the sand bed, which consisted primarily of the biolayer. A constant depth of the filter medium was maintained by replacing any sand removed during the filter cleaning with sand of identical specification. However, no reduction in the output flow rate occurred throughout the entire study.

3.2.5. Experimental conditions

For 25 days unit B1 was conditioned with stream water with 1% primary effluent and unit B2 with stream water alone. Once the biolayers were established, and at least 2 logs of bacterial removal were achieved in both units, a mixture of maximum 6 selected

PhACs was added to the feed water of each unit (referred to as a spike) to evaluate (1) the effect of the PhACs on the ability of the two SSF units to remove total coliforms and *E. coli*, (2) the ability of the two SSF units to remove the selected PhACs present in the feed water, and (3) the changes in the microbial population present in the biolayer of both SSF units. Three such spiking events occurred. During each of the three spikes, PhACs at a concentration of approximately 50 μg/L each were added to the feed water of the SSF units for three consecutive days. After this period of continuous injection of these PhACs, the feed water reverted to stream water with 1% primary effluent or stream water alone. The period after the spike when feed water contained no PhACs is referred to as flush-out. The next set of PhACs was not introduced until the SSF units achieved approximately 2 logs of bacterial removal.

A summary of the experimental conditions is given in Table 19. The first spike consisted of caffeine, a stimulant, and E2, an estrogen, neither of which was expected to affect the biolayer of the SSF units. During the second spike four additional PhACs (carbamazepine, E1, gemfibrozil and phenazone) were added to caffeine and E2 used during the first spike. Since carbamazepine, gemfibrozil and phenazone are an anticonvulsant, an antilipemic and an antipyretic, respectively, they may impact the biolayer. E1 is an estrogen and is the breakdown product of E2 (Lee and Liu, 2002). The third spike of caffeine alone was conducted to verify the limited impact of caffeine on the two SSF units and to clarify the fate of caffeine. To prevent the degradation of the selected PhACs within the reservoir, the stock solution was prepared daily.

A hydraulic loading rate of 0.04 m/h was used throughout the entire study. Potassium chloride (KCl) was selected as a non-reactive tracer during the three spikes because it appeared to have no observable impact on the biolayer and bacterial removal by the SSF units. Approximately 50 mg/L KCl were used throughout the study.

Table 19: Summary of experimental conditions – duration of each spike and flush-out and the pharmaceutically active compounds (PhACs) present in each spike.

Spike	Duration (d)	PhACs used
SPIKE 1	3 (spike) 6 (flush-out)	Caffeine, E2 -
SPIKE 2	3 (spike) 15 (flush-out)	Caffeine, Carbamazepine, E1, E2, Gemfibrozil, Phenazone -
SPIKE 3	3 (spike) 6 (flush-out)	Caffeine -

Spike = selected PhACs were added to the feed water.
Flush-out = PhACs were removed from the feed water, and the two SSF units were flushed with stream water with 1% primary effluent or stream water alone. KCl was used as conservative tracer during the three spikes.

3.2.6. Analysis of the influent, effluent, and sampling ports of the two SSF units

Influent, effluent, and sampling port samples were collected and analyzed for turbidity, total coliforms, *E. coli*, KCl, and selected PhACs.

Turbidity was measured using a portable turbidimeter (HACH, Loveland, CO). Total coliforms, and *E. coli* were quantified using a commercial Most Probable Number (MPN) test, Colilet 18, with a Quanti-Tray 2000 from IDEXX Laboratories (Westbrook, ME) (ISO, 2012). This method can measure from less than 1 MPN/100 mL to more than 2419.6 MPN/100 mL without sample dilution (*http://www.idexx.com/view/xhtml/en_us/water/products/quanti-tray.jsf*). KCl was measured with an ion chromatograph (IC) (Dionex, Bannockburn, IL) having a detection limit of 0.1 mg/L. Analyses of selected PhACs were performed using an HPLC system (Thermo Finnigan,Waltham, MA) coupled with a photo diode array (PDA) detector (Shimadzu, Columbia, MD) having a detection limit of 0.1 μg/L. Acetonitrile and deionized (DI) water were used as the mobile phase.
Bacterial removal was always determined by comparing the number of total coliforms and *E. coli* in the influent (feed water) against the effluent from the SSF or the two

sampling ports. Bacterial removal is expressed using only two decimal digits if it is greater than 99%. Recovery of select PhACs is expressed as mass recovered in the effluent compared to the mass injected.

The level of oxygen in the effluent was periodically monitored with an oxygen probe (Ocean Optics, Dunedin, FA) placed in a flow-through cell. The probe used the fluorescence of a chemical complex in a sol-gel to measure the partial pressure of oxygen. A pulsed blue LED sends light, at ~475 nm, to an optical fiber which carries the light to the probe (http://www.oceanopticssensors.com/theory.com).

3.2.7. DNA extraction and characterization of the biolayer

The biolayer of each SSF unit was divided into 114 quadrants of 0.5 × 0.5 cm, and 14 quadrants were randomly sampled with a sterile spatula and mixed to provide a representative sample of the biolayer. Samples from the biolayer were collected during the equilibration time (3 days and 13 days from the start of the study), after the first and second spike, before and during the third spike, and at the end of the study. Also at the end of the study, each SSF unit was sectioned and sand samples from the barrel were collected at 3, 10, 20, and 45 cm below the water-sand interface.

Microbial community DNA was extracted from the biolayer and sand samples using a PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA) according to the manufacturer's protocol, except bead beating was conducted for two minutes on a Mini-Beadbeater-8 (Biospec Products Inc., Bartlesville, OK). The variable V4 region of the 16S RNA gene was amplified using primers 515F and 806R containing barcodes (12-base Golay code) and sequencing platform (Illumina) specific adapter sequences as specified in Caporaso et al. (2010). Triplicate 25 µl PCR reactions of each sample contained 2 µl of DNA template, 0.5 µl of each forward and reverse primer (200 nM final concentration), 10 µl of 2.5X Prime HotMasterMix (5 Prime, Hamburg, Germany) (1X final concentration), and 12 µl of PCR grade water. After an initial denaturation for 2 min at 94 °C, the reactions were cycled for 35 cycles (94 °C for 45 sec, 50 °C for 30 sec, and 65 °C for 90 sec) on an CFX 96 (Bio-Rad Laboratories, Inc., Hercules, CA), followed by the final extension at 65 °C for 10 min. Triplicate PCR reactions were pooled, cleaned

using an UltraClean® PCR cleanup kit (Mo Bio Laboratories, Inc., Carlsbad, CA), visually examined on 2% agarose gel, and quantified using Qubit® dsDNA HS Assay Kit (Life Technologies, Grand Island, NY) according to the manufacturers' protocols. All samples were pooled at an equimolar ratio into a single tube, purified on 2% agarose gel using a Wizard® VS Gel and PCR Clean-Up Kit (Promega, Madison, WI), and paired-end sequenced (2 × 250 bp) on a MiSeq Benchtop Sequencer (Illumina Inc., San Diego, CA) at the Keck DNA Sequencing Facility, Yale University. After barcode based demultiplexing, low quality reads were filtered out and paired-end reads aligned and clustered using modified script allowing zero mismatches between the paired reads using cd-hit-otu (Li et al., 2012). Phylogenetic affiliation of the operational taxonomic units (OTUs) was determined at > 97% identity, the closest match with the SILVA reference database (*http://www.arb-silva.de/*) using the SINA aligner (Pruesse, et al., 2012). The Vegan package in R (*http://vegan.r-forge.r-project.org/*) was applied to evaluate alpha rarefaction, and beta diversity, as well as to conduct detrended correspondence analysis (DCA) and identify associations (linear combinations by envfit) between the microbial community composition, time, and depth. OTU abundance data was log-transformed prior to the DCA and parameters were considered significant when the P-value < 0.001.

3.3. RESULTS AND DISCUSSIONS

Prior to conducting this study, units B1 and B2 were used for approximately one year. This may have led to the formation of a more "biologically mature" system, compared with the barrels packed with clean sand.

Turbidity of the feed water ranged between 0.47 and 4.33 NTU (average = 1.60 NTU), while over the course of this study the turbidity for both SSF units ranged between 0.15 and 2.01 NTU (average = 0.56 and 0.50 NTU) (data not shown). The presence of PhACs did not impact the removal efficiency in terms of turbidity of both SSF units throughout the entire study.

The level of oxygen present in the effluent ranged between 2 and 3 mg/L during the study.

3.3.1. Microbial community composition in the biolayer and sand

Overall microbial richness was high despite most of the sequencing data not passing the quality threshold (no mismatches allowed between paired end reads in overlapping region [~250 bp, > 99% sequence length after primers were removed]). In total, 236,033 sequences (1.3% of total) passed the threshold and were assigned to 2067 OTUs. More specifically, the sequencing effort yielded 3,799 to 53,185 sequences per sample, which translated into 9 to 914 distinct OTUs per sample. Rarefraction curves were saturated, indicating sufficient sequence coverage in all the samples analyzed (Fig. 6).

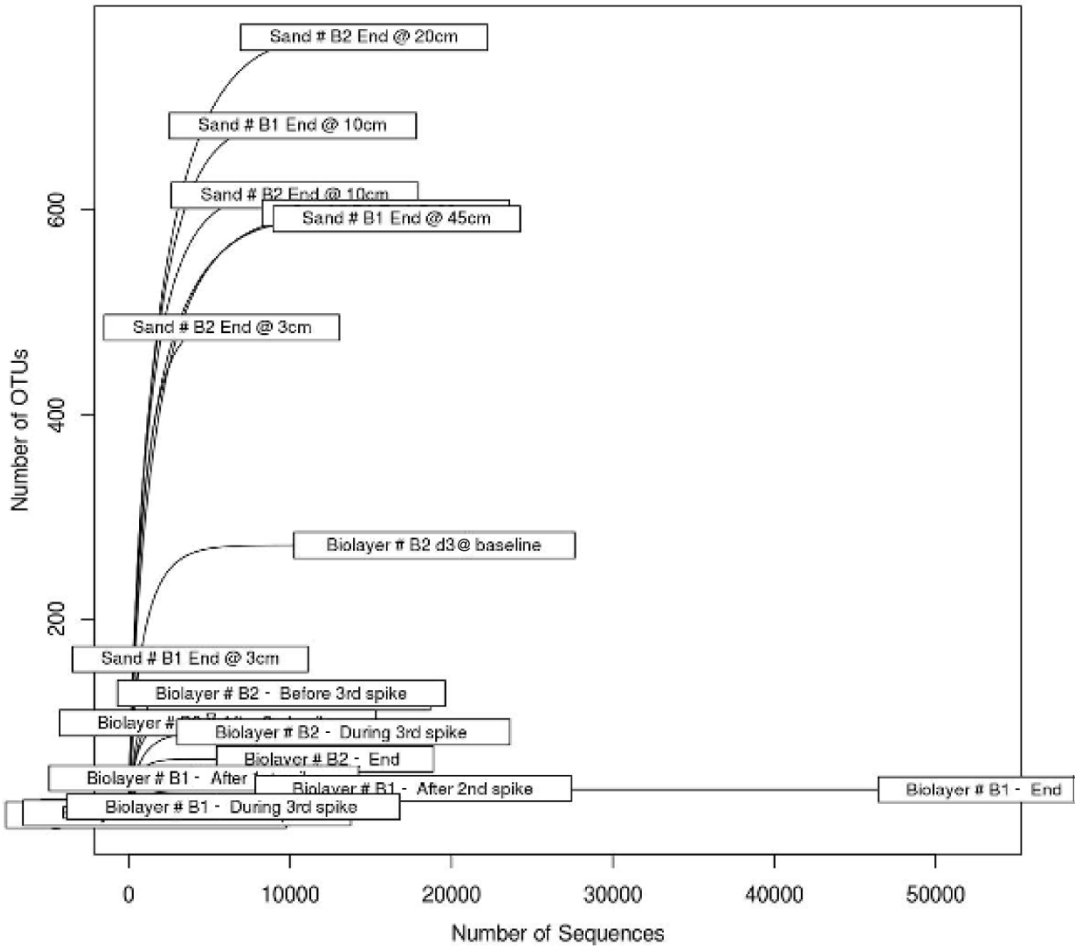


Figure 6: Rarefraction curves. Unit B1 received stream water with 1% primary effluent, while unit B2 received only stream water throughout the entire study.

Microbial communities in the sand were significantly more diverse when compared to the communities in the biolayer (r^2=0.8945, P < 0.001). Proteobacteria (27–99%), predominantly class Gammaproteobacteria (9–99%), and Bacteroidetes were the dominant phyla in the biolayer samples while Actinobacteria (10–18%), Bacteroidetes (10–26%) and several classes of Proteobacteria (37–50%) (Alpha-, Beta-, Delta- and Gammaproteobacteria) were prevalent in the sand samples (Fig. 7).

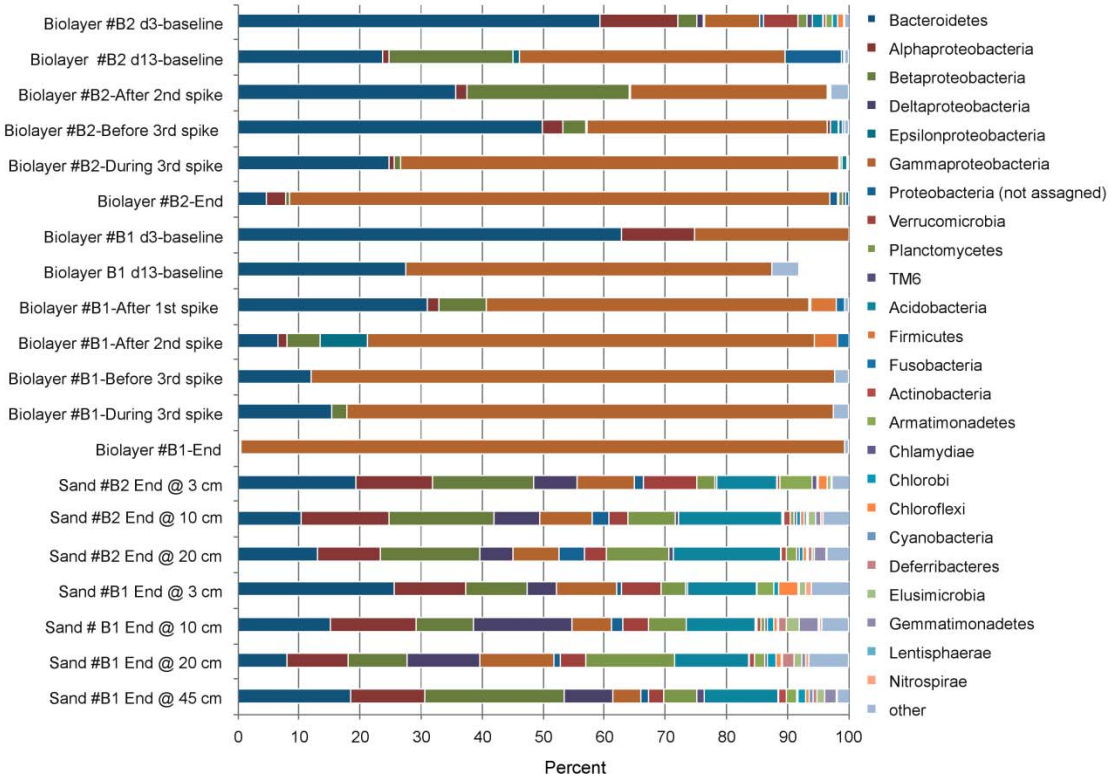


Figure 7: Bacterial community composition in the biolayer and sand samples based on the paired-end sequencing (2 x 250 bp) of the variable V4 region of the 16S RNA gene on MiSeq Benchtop Sequencer. Biolayer samples were collected before and after PhACs spikes, while sand samples were collected at the end of the experiment at different depths as indicated.

A total of 1710 and 512 OTUs were found in the sand and biolayer samples, respectively, of which 155 OTUs (7.5%) were shared between both matrixes (Fig. 8).

The percentage of OTUs shared between the sand samples of units B1 and B2 was 38% (n=651) and 15% (n=76) in the biolayer samples (Fig. 8).

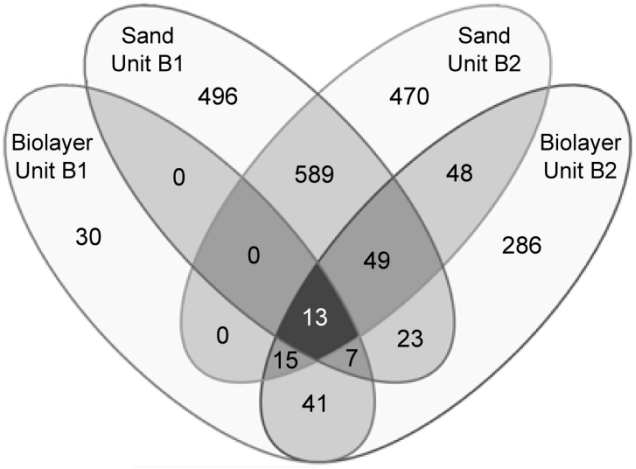


Figure 8: Number of OTUs shared between sand and biolayer samples collected from both units. Unit B1 received stream water alone with 1% primary effluent and unit B2 received stream water alone.

Changes in the microbial community composition were significant over time (r^2=0.8437, $P < 0.001$). In general, Bacteroidetes was replaced by Proteobacteria in the biolayer of both units over the course of the study. The relative abundance of Bacteriodetes decreased over time from 63% to less than 1%, and from 59% to 5% within the biolayer of units B1 and B2, respectively (Fig. 7). At the same time, the relative proportion of Proteobacteria increased from 37% to 99% and 27% to 93% in units B1 and B2, respectively (Fig. 7). This increase was primarily due to Gamma-proteobacteria, which was the predominant group in the biolayer samples at the end of the experiment (99% for unit B1, 88% for unit B2).

The microbial community in the biolayer appeared to be sensitive to the three spikes of PhACs. Throughout the study no OTU persisted at detectable levels in all of the biolayer samples in unit B1, while only 6 OTUs (1.2%) of 482 OTUs identified persisted in unit

B2. These 6 OTUs belonged to following taxa: *Flexibacter* (n=1), *Pseudomonas* (n=2), *Rhodobacter* (n=1), uncultured *Saprospiraceae* (n=1), and *Methylophilaceae* (n=1).

Overall, *Pseudomonas* and *Flavobacterium* were the two most dominant bacterial genera identified in the biolayer samples from both units when taxonomic identification at genus rank was possible. The majority of biolayer sequences in unit B1 (19.4%, n=16, 581) and unit B2 (95.7%, n=67, 580) were identified as *Pseudomonas*. The next most abundant genus, *Flavobacterium*, contributed with 7,836 biolayer sequences (9.1%) in unit B1 and 535 biolayer sequences (0.7%) in unit B2. *Flavobaterium* was not detected in any of the samples in unit B2 after spiking. A rapid decrease in diversity and a relative increase in abundance of Bacteroidetes was observed (Fig. 7).

Shannon's diversity index for the biolayer samples in unit B1 averaged 1.991 (standard deviation = 0.69) and for biolayer samples in unit B2 averaged 3.279 (standard deviation = 0.51), indicating lower diversity in the biolayer of unit B1. Several taxa, such as Acidobacteria, Burkholderiales, Cytophagales, and Verrumicrobia, were common in the biolayer of unit B2, but were not detected in the biolayer of unit B1. The change in microbial composition over time was manifested by a decrease in diversity. Shannon's diversity index decreased from 2.583 and 3.475 prior to the first chemical spike to 1.782 and 2.399 by the end of the experiment in units B1 and B2, respectively. A similar trend was indicated by Simpson's diversity index, although both indexes fluctuated over time. Decreasing diversity, coupled with overall higher diversity in unit B2, indicates healthier and more resilient biofilm formation in the presence of stream water alone (unit B2).

Microbial communities in the sand samples collected at the end of the experiment at different depths (3, 10, 20, and 45 cm) in the SSF units, had the lowest diversity at 3 cm depth (Shannon's diversity index = 3.396 [unit B1] and 4.967 [unit B2]) and the highest diversity at 10 cm depth (Shannon's diversity index = 5.677 [unit B1] and 5.579 [unit B2]). However, the changes were not significantly correlated with the depth (r^2=0.5705, P=0.188) or the nature of the feed water (r^2=0.1051, P=0.460).

At the start of the current study the biolayers of unit B1 and unit B2 were removed and re-established. This was probably one of the major factors contributing to the low diversity at 3 cm depth. Depletion of nutrients under the biofilm and transport of bacteria into the deeper layers might also have been contributing factors. While taxonomic representation and relative abundance of major microbial phyla was comparable to the previous studies, the microbial diversity estimated in the sand layer were elevated when compared to the finger printing based estimates in an earlier study (Hunter et al., 2013). Although this discrepancy may result from multiple factors, it can indicate that better resolution is achieved using massively parallel sequencing technology. Li et al. (2013) using similar DNA extraction, pyrosequencing and sequence analysis procedure investigated the microbial community evolution during simulated managed aquifer recharge and achieved 807,804 sequences for 118 sediments samples. Proteobacteria was also found to be the major phyla present (Li et al., 2013).

3.2.2. Impact of selected PhACs on bacterial removal

During the first spike of caffeine and E2, unit B1 (receiving Manoa Stream water with 1% primary effluent) achieved an average 99% removal of total coliforms (Table 20). The concentration of total coliforms in the feed water ranged between 1.6 E+5 and 2.5 E+5 MPN/100 mL (Table 21). Despite the high removal achieved after the SSF unit, the concentration of total coliforms progressively increased in the effluent from 2.0 E+1 on day one to 4.8 E+3 MPN/100 mL on day three (Fig. 9a). Results from the two sampling ports showed that an average of 95% removal (ranging from 85% to 99.47%) was achieved after the biolayer (first sampling port) and 99% (ranging between 96% and 99.94%) within the upper half of the barrel (second sampling port) (Table 20). The amount of total coliforms after the biolayer and after the second sampling port increased during the spike (Fig. 9a).

Once the injection of the two PhACs was suspended and the flush-out started, 84% removal of total coliforms was achieved within one day, while 93% and 99.78% were achieved after the second and third day, respectively (Fig. 9a). During the 6 days of flush-out, an average 96% removal of total coliforms was achieved (Table 20). Total coliforms

in the feed water were still constant, ranging between 2.0 and 6.0 E+4 MPN/100 mL, while in the output they progressively decreased from 4.8 E+3 on day one of flush-out to 9.1 E+0 MPN/100 mL on day 6 (Fig. 9a). An average of 84% removal (ranging between 66% and 98%) was achieved within the biolayer while 97% removal (ranging between 92% and 99.64%) of total coliforms was achieved in the upper half of the barrel (Table 20). The decline in bacterial removal occurring at the beginning of the flush-out may have been caused by the detachment of microorganisms due to electrostatic charge reversals on particles occurring during this transition period.

Table 20: Spike 1: Removal (%) of total coliforms and *E. coli* when caffeine, and E2 were added to the feed water.

SSF Unit	**Stage**	**Overall removal (%)**			**Standard Deviation**
		Minimum	**Maximum**	**Average**	
TOTAL COLIFORMS					
	After Biolayer (4)	84.88	99.47	94.36	6.58
B1 – spike	After 2nd port (4)	95.69	99.94	98.52	2.00
	After Sand (4)	97.01	99.99	98.85	1.41
	After Biolayer (6)	65.77	97.88	83.91	11.05
B1 – flush-out	After 2nd port (6)	92.33	99.64	97.36	2.73
	After Sand (6)	84.00	99.96	96.08	6.48
	After Biolayer (4)	90.00	97.74	94.65	3.64
B2 – spike	After 2nd port (4)	97.00	99.96	98.77	1.37
	After Sand (4)	97.14	99.97	99.19	1.37
	After Biolayer (6)	71.11	98.23	89.75	9.89
B2 – flush -out	After 2nd port (6)	97.11	99.95	99.06	1.04
	After Sand (6)	93.78	99.76	98.57	2.36
E. COLI					
	After Biolayer (4)	7.87	99.36	74.72	44.61
B1 – spike	After 2nd port (4)	69.51	99.97	89.31	14.36
	After Sand (4)	55.53	100.00	86.49	21.12
	After Biolayer (6)	65.83	91.60	83.01	10.39
B1 – flush-out	After 2nd port (6)	93.87	99.87	98.51	2.30
	After Sand (6	84.00	99.97	95.81	6.67
	After Biolayer (4)	37.25	92.98	75.12	25.85
B2 – spike	After 2nd port (4)	79.66	99.99	93.14	9.15
	After Sand (4)	88.77	99.99	96.39	5.21
	After Biolayer (6)	90.00	99.00	93.64	4.11
B2 – flush -out	After 2nd port (6)	90.00	99.95	96.16	4.78
	After Sand (6)	90.00	99.95	97.64	3.81

Removals achieved after the biolayer, after the second sampling port and after sand are expressed in terms of the feed water. First sampling port was located at 5cm from the water sand-interface and it is referred to as biolayer, while the second sampling port was located at 35cm from the water-sand interface, and it is approximately half way between the biolayer and the output. KCl was used as conservative tracer. () = number of samples used to estimate minimum, maximum, average and standard deviation.

Table 21: Occurrence of total coliforms and *E. coli* during Spike 1 when caffeine, and E2 were added to the feed water.

SSF Unit	Stage	Minimum	Maximum	Average	Standard deviation
TOTAL COLIFORMS (MPN/100 mL)					
B1 – spike	Inflow (4)	1.6E+5	2.5E+5	2.0E+5	4.8E+4
	After Biolayer (4)	1.3E+3	2.4E+4	9.6E+3	1.0E+4
	After 2nd port (4)	1.5E+2	6.9E+3	2.4E+3	3.2E+3
	After Sand (4)	2.0E+1	4.8E+3	1.9E+3	2.2E+3
B1 – flush-out	Inflow (6)	2.0E+4	6.0E+4	3.5E+4	1.7E+4
	After Biolayer (6)	1.3E+3	7.6E+3	4.6E+3	2.5E+3
	After 2nd port (6)	1.9E+2	2.3E+3	7.3E+2	7.9E+2
	After Sand (6)	9.1E+0	4.8E+3	1.1E+3	1.9E+3
B2 – spike	Inflow (4)	1.9E+4	1.5E+5	7.1E+4	6.1E+4
	After Biolayer (4)	4.3E+2	9.6E+3	3.7E+3	4.0E+3
	After 2nd port (4)	1.1E+1	2.4E+3	8.1E+2	1.1E+3
	After Sand (4)	6.3E+0	5.5E+2	2.6E+2	2.7E+2
B2 – flush -out	Inflow (6)	2.0E+3	1.9E+4	8.1E+3	7.3E+3
	After Biolayer (6)	1.3E+2	1.8E+3	6.6E+2	7.1E+2
	After 2nd port (6)	9.7E+0	1.3E+2	4.1E+1	4.5E+1
	After Sand (6)	1.1E+1	2.8E+2	7.2E+1	1.0E+2
E. COLI (MPN/100 mL)					
B1 – spike	Inflow (4)	2.2E+3	6.6E+4	2.9E+4	2.8E+4
	After Biolayer (4)	4.2E+2	2.0E+3	1.1E+3	8.1E+2
	After 2nd port (4)	2.0E+1	1.8E+3	6.3E+2	8.3E+2
	After Sand (4)	3.1E+0	1.4E+3	6.0E+2	7.0E+2
B1 – flush-out	Inflow (6)	5.0E+3	1.5E+4	1.0E+4	5.1E+3
	After Biolayer (6)	7.0E+2	2.2E+3	1.5E+3	6.4E+2
	After 2nd port (6)	2.0E+1	5.8E+1	3.5E+1	1.8E+1
	After Sand (6	2.3E+0	4.1E+1	1.9E+1	1.6E+1
B2 – spike	Inflow (4)	9.9E+1	7.6E+2	4.3E+2	3.0E+2
	After Biolayer (4)	4.2E+1	7.4E+1	5.8E+1	1.3E+1
	After 2nd port (4)	1.0E-1	2.0E+1	1.2E+1	9.2E+0
	After Sand (4)	1.0E-1	1.1E+1	5.4E+0	4.8E+0
B2 – flush -out	Inflow (6)	1.0E+1	2.0E+2	8.0E+1	9.1E+1
	After Biolayer (6)	1.0E-1	8.2E+0	3.1E+0	3.6E+0
	After 2nd port (6)	1.0E-1	1.0E+0	3.3E-1	4.5E-1
	After Sand (6)	1.0E-1	1.0E-1	1.0E-1	0.0E+0

First sampling port was located at 5cm from the water sand-interface and it is referred to as biolayer, while the second sampling port was located at 35cm from the water-sand interface, and it is approximately half way between the biolayer and the output. KCl was used as conservative tracer. () = number of samples used to estimate minimum, maximum, average and standard deviation.

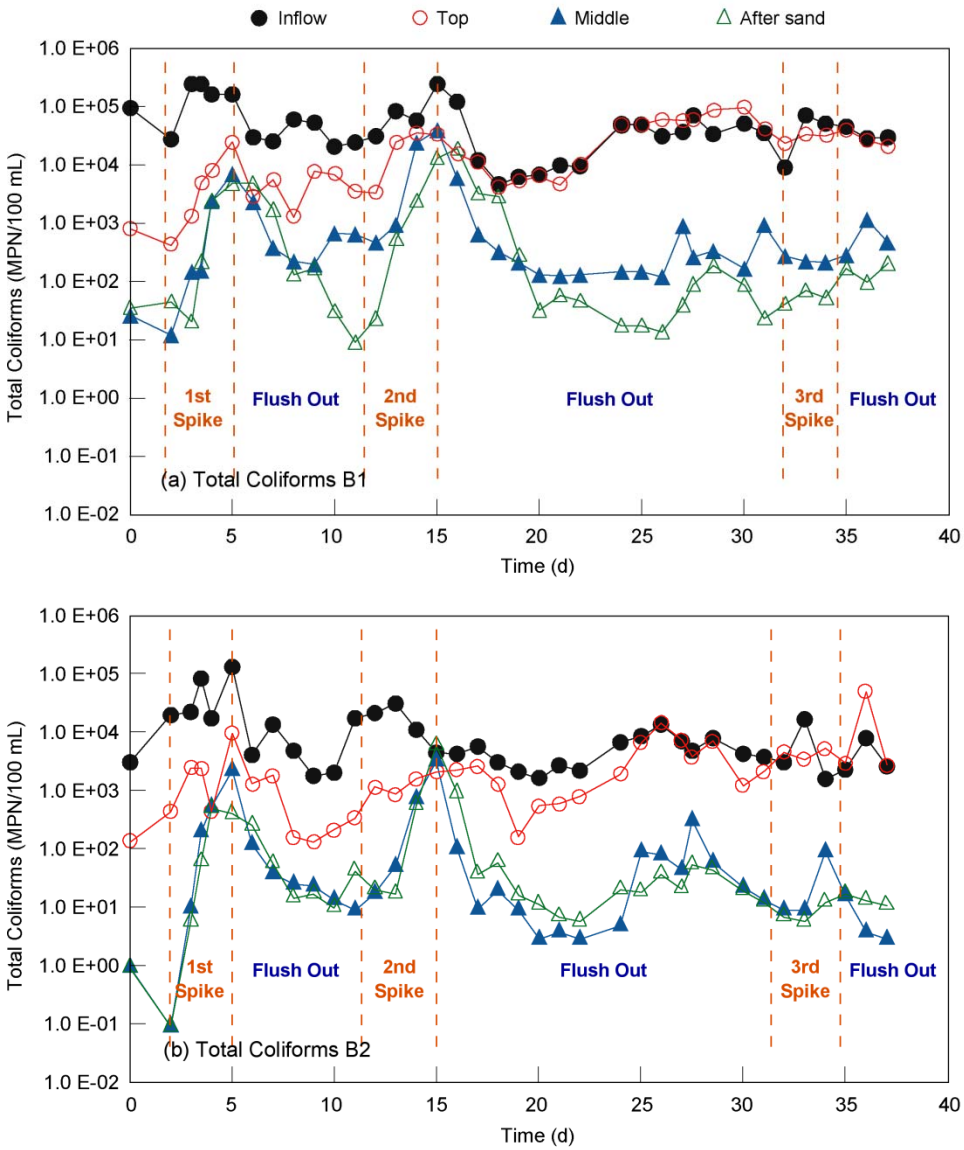


Figure 9: Impact of selected PhACs on the removal of total coliforms after SSF (a) unit B1 and (b) unit B2. Unit B1 received stream water with 1% primary effluent, while unit B2 received only stream water throughout the entire study. During the first spike, ~ 50 μg/L of caffeine and 17-β estradiol (E2) were added; during the second spike, ~ 50 μg/L of caffeine, carbamazepine, E2, estrone (E1), gemfibrozil and phenazone were added. During the third spike only caffeine was added. KCl was used as a non-reactive tracer during the three spikes with PhACs.

During the second spike of 6 PhACs, unit B1 achieved an average of 98% removal of total coliforms (Table 22). The concentration of total coliforms in the feed water ranged between 3.1 E+4 and 9.3 E+4 MPN/100mL, while in the effluent of the SSF unit total coliforms progressively increased from 2.3 E+1 on day one to 2.4 E+4 MPN/100 mL on day three (Table 23; Fig. 9a). Results from the two sampling ports showed that an average of 67% removal was achieved after the biolayer (first sampling port) and 85% within the upper half of the barrel (second sampling port) (Table 22). Total coliforms increased from 3.3 E+3 to 3.4 E+4 MPN/100 mL after the biolayer and from 4.6 E+2 to 2.4 E+4 MPN/100 mL after the second sampling port (Fig. 9a).

Once the injection of the 6 PhACs was suspended and the flush-out started, the concentrations of total coliforms remained stable after the first sampling port, while it progressively decreased after the second sampling port and after the filtration unit. Although the removal of total coliforms after unit B1 decreased continuously for 72 hr reaching a minimum of 73% (Fig. 9a), 95% removal was reached during the next 48 hr (Fig. 9a). During the 15 days of flush-out, an average 96% removal of total coliforms was achieved (Table 22). Total coliforms in the feed water ranged between 4.7 E+3 and 2.4 E+5 MPN/100 mL, while in the outflow, they decreased from 1.9 E+4 on day one of the flush-out to 1.3 E+1 MPN/100 mL on day fifteen of the flush-out (Table 23; Fig. 9a). Data from the two sampling ports showed that an average of 18% removal was achieved within the biolayer while 97% removal of total coliforms was achieved in the upper half of the barrel (Table 22). Total coliforms ranged between 4.0 E+3 and 9.6 E+4 MPN/100 mL after the first sampling port (biolayer) and decreased from 5.9 E+3 (beginning) to 1.7 E+2 MPN/100 mL (end) after the second sampling port.

Table 22: Spike 2: Removal (%) of total coliforms and *E. coli* when caffeine, carbamazepine, E1, E2, gemfibrozil and phenazone were added to the feed water.

SSF Unit	Stage	Overall removal (%)			Standard deviation
		Minimum	Maximum	Average	
TOTAL COLIFORMS					
B1 – spike	After Biolayer (3)	40.14	89.51	66.83	24.93
	After 2nd port (3)	57.91	98.90	85.11	23.56
	After Sand (3)	95.79	99.93	98.36	2.24
B1 – flush-out	After Biolayer (10)	0.00	87.12	18.09	31.06
	After 2nd port (10)	83.60	99.70	96.91	4.21
	After Sand (10)	72.66	99.96	95.99	9.26
B2 – spike	After Biolayer (3)	86.80	97.47	93.18	5.63
	After 2nd port (3)	93.46	99.92	97.74	3.71
	After Sand (3)	94.80	99.95	98.22	2.96
B2 – flush -out	After Biolayer (10)	2.23	93.28	52.03	27.98
	After 2nd port (10)	27.99	99.93	94.29	18.40
	After Sand (10)	70.01	99.79	97.70	5.24
E. coli					
B1 – spike	After Biolayer (3)	22.70	98.88	65.53	38.97
	After 2nd port (3)	75.15	98.84	90.63	13.41
	After Sand (3)	94.28	99.93	97.76	3.05
B1 – flush-out	After Biolayer (10)	0.00	95.73	64.50	30.08
	After 2nd port (10)	66.52	99.94	94.90	8.74
	After Sand (10)	0.00	99.99	86.45	29.01
B2 – spike	After Biolayer (3)	90.00	99.05	93.44	4.90
	After 2nd port (3)	96.90	99.95	98.52	1.53
	After Sand (3)	99.00	100.00	99.65	0.56
B2 – flush -out	After Biolayer (10)	0.00	91.15	49.45	41.35
	After 2nd port (10)	75.61	99.90	96.74	6.76
	After Sand (10)	75.61	99.96	96.56	6.78

Removals achieved after the biolayer, after the second sampling port, and after sand are expressed in terms of the feed water. First sampling port was located at 5cm from the water sand-interface and it is referred toas biolayer, while the second sampling port was located at 35cm from the water-sand interface, and it is approximately half way between the biolayer and the output. KCl was used as conservative tracer. () = number of samples used to estimate minimum, maximum, average and standard deviation.

Table 23: Occurrence of total coliforms and *E. coli* during Spike 2 when caffeine, carbamazepine, E1, E2, gemfibrozil and phenazone were added to the feed water.

SSF Unit	Stage	Minimum	Maximum	Average	Standard Deviation
TOTAL COLIFORMS (MPN/100 mL)					
B1 – spike	Inflow (4)	3.1E+4	8.3E+4	5.7E+4	2.6E+4
	After Biolayer (4)	3.3E+3	3.4E+4	2.1E+4	1.6E+4
	After 2nd port (4)	4.6E+2	2.4E+4	8.5E+3	1.4E+4
	After Sand (4)	2.3E+1	2.4E+3	9.9E+2	1.3E+3
B1 – flush-out	Inflow (6)	4.7E+3	2.4E+5	4.8E+4	6.2E+4
	After Biolayer (6)	4.0E+3	9.6E+4	3.6E+4	3.1E+4
	After 2nd port (6)	1.2E+2	4.0E+4	3.3E+3	1.0E+4
	After Sand (6)	1.3E+1	1.9E+4	2.6E+3	5.7E+3
B2 – spike	Inflow (4)	1.2E+4	3.4E+4	2.4E+4	1.1E+4
	After Biolayer (4)	8.7E+2	1.6E+3	1.2E+3	3.7E+2
	After 2nd port (4)	1.9E+1	7.9E+2	2.9E+2	4.4E+2
	After Sand (4)	1.9E+1	6.3E+2	2.2E+2	3.5E+2
B2 – flush -out	Inflow (6)	1.9E+3	1.5E+4	5.9E+3	3.5E+3
	After Biolayer (6)	1.6E+2	1.5E+4	3.5E+3	3.9E+3
	After 2nd port (6)	3.1E+0	3.6E+3	3.0E+2	9.2E+2
	After Sand (6)	6.3E+0	6.6E+3	5.3E+2	1.7E+3
E. COLI (MPN/100 mL)					
B1 – spike	Inflow (4)	1.2E+4	3.1E+4	2.0E+4	1.0E+4
	After Biolayer (4)	1.3E+2	2.4E+4	9.4E+3	1.3E+4
	After 2nd port (4)	1.3E+2	4.0E+3	1.6E+3	2.1E+3
	After Sand (4)	8.5E+0	9.1E+2	4.0E+2	4.6E+2
B1 – flush-out	Inflow (6)	1.5E+1	6.0E+4	1.1E+4	1.7E+4
	After Biolayer (6)	1.5E+1	1.2E+4	2.3E+3	3.4E+3
	After 2nd port (6)	1.5E+1	2.0E+4	1.5E+3	5.2E+3
	After Sand (6	1.5E+1	8.8E+3	1.0E+3	2.4E+3
B2 – spike	Inflow (4)	1.0E+2	2.1E+3	8.1E+2	1.1E+3
	After Biolayer (4)	1.0E+1	2.0E+1	1.7E+1	5.8E+0
	After 2nd port (4)	1.0E+0	3.1E+0	2.4E+0	1.2E+0
	After Sand (4)	1.0E-1	1.0E+0	4.0E-1	5.2E-1
B2 – flush -out	Inflow (6)	1.5E+1	2.3E+2	8.3E+1	5.1E+1
	After Biolayer (6)	1.5E+1	1.0E+2	4.1E+1	4.3E+1
	After 2nd port (6)	5.8E+0	1.0E+1	2.2E+0	4.0E+0
	After Sand (6)	1.2E+0	1.0E+1	2.1E+0	4.1E+0

First sampling port was located at 5cm from the water sand-interface and it is referred to as biolayer, while the second sampling port was located at 35cm from the water-sand interface, and it is approximately half way between the biolayer and the output. KCl was used as conservative tracer. () = number of samples used to estimate minimum, maximum, average and standard deviation.

During the third spike of caffeine alone, removal of total colifoms for unit B1 was steady throughout the spike with an average removal of 99.79% as well as during the flush-out with an average removal of 99.63% (Table 24). The concentration of total coliforms in the feed water, during the spike, ranged between 8.8 E+3 and 6.9 E+4 MPN/100mL, and after the SSF unit, ranged between 2.3 E+1 MPN/100 mL on day one and 7.0 E+1

MPN/100 mL on day fifteen (Table 25). During the flush-out, the concentration of total coliforms in the feed water ranged between 2.9 E+4 and 5.0 E+4 MPN/100 mL, while a constantly low concentration of total coliforms, between 5.2 E+1 and 2.0 E+2 MPN/100 mL was achieved after the filtration unit (Table 25; Fig. 9a). In contrast with the first two spikes, no reduction of total coliforms removal was observed at the beginning of the flush-out (Table 24; Fig. 9a). Results from the two sampling ports showed that an average 22% removal (ranging between 9% and 38%) was achieved within the biolayer, while 98% removal was achieved within the upper half of the barrel (ranging between 96% and 99.58%) (Table 24).

The concentration of total coliforms, during this spike and flush-out, ranged between 2.1 E+4 and 4.2 E+4 MPN/100 mL after the first sampling port, and between 2.1 E+2 and 1.1 E+3 MPN/100 mL after the second sampling port (Table 25; Fig. 9a). These results showed that during the second spike, the high bacteria removal occurred within the sand rather than the biolayer.

Similar behavior was also observed in unit B2 fed with Manoa Stream water alone. The concentrations of total coliforms in the feed water as well as after unit B2, were comparable to those observed in unit B1 (Fig. 9b). Similar behavior in terms of *E. coli* removal was observed throughout the three spikes (Tables 20–24; Fig. 10a-b).

Table 24: Spike 3: Removal (%) of total coliforms and *E. coli* when only caffeine was added to the feed water.

SSF ID	Stage	Overall removal (%)			Standard deviation
		Minimum	Maximum	Average	
TOTAL COLIFORMS					
	After Biolayer (3)	0.00	52.39	17.46	30.25
B1 – spike	After 2nd port (3)	96.91	99.69	97.98	1.50
	After Sand (3)	99.54	99.93	99.79	0.22
	After Biolayer (4)	8.70	37.55	21.53	14.91
B1 – flush-out	After 2nd port (4)	96.10	99.58	98.37	1.59
	After Sand (4)	99.33	99.90	99.63	0.23
	After Biolayer (3)	0.00	81.76	43.44	41.12
B2 – spike	After 2nd port (3)	99.65	99.95	99.77	0.16
	After Sand (3)	99.68	99.97	99.81	0.15
	After Biolayer (4)	0.00	0.00	0.00	0.00
B2 – flush -out	After 2nd port (4)	94.51	99.95	98.42	2.64
	After Sand (4)	99.25	99.83	99.49	0.28
E. COLI					
	After Biolayer (3)	0.00	93.15	61.81	53.53
B1 – spike	After 2nd port (3)	90.00	99.97	96.32	5.50
	After Sand (3)	99.90	99.98	99.94	0.04
	After Biolayer (4)	0.00	92.29	63.19	42.63
B1 – flush-out	After 2nd port (4)	98.00	99.92	99.17	0.93
	After Sand (4)	98.32	99.97	99.53	0.81
	After Biolayer (3)	0.00	95.00	31.67	54.85
B2 – spike	After 2nd port (3)	99.00	99.95	99.62	0.53
	After Sand (3)	99.00	99.95	99.62	0.53
	After Biolayer (4)	0.00	67.74	16.94	33.87
B2 – flush -out	After 2nd port (4)	99.00	99.97	99.48	0.56
	After Sand (4)	99.00	99.97	99.48	0.56

Removals achieved after the biolayer, after the second sampling port and after sand are expressed in terms of the feed water. First sampling port was located at 5cm from the water sand-interface and it is referred to as biolayer, while the second sampling port was located at 35cm from the water-sand interface, and it is approximately half way between the biolayer and the output. KCl was used as conservative tracer. () = number of samples used to estimate minimum, maximum, average and standard deviation.

Table 25: Occurrence of total coliforms and *E. coli* during Spike 3 when only caffeine was added to the feed water.

SSF Unit	Stage	Minimum	Maximum	Average	Standard deviation
TOTAL COLIFORMS (MPN/100 mL)					
B1 – spike	Inflow (3)	8.8E+3	6.9E+4	3.7E+4	2.5E+4
	After Biolayer (3)	2.3E+4	4.2E+4	3.2E+4	7.7E+3
	After 2nd port (3)	2.1E+2	9.1E+2	4.7E+2	3.4E+2
	After Sand (3)	2.3E+1	7.0E+1	4.5E+1	2.0E+1
B1 – flush-out	Inflow (4)	2.9E+4	5.0E+4	3.8E+4	1.1E+4
	After Biolayer (4)	2.1E+4	4.0E+4	2.9E+4	8.1E+3
	After 2nd port (4)	2.1E+2	1.1E+3	5.2E+2	4.1E+2
	After Sand (4)	5.2E+1	2.0E+2	1.3E+2	6.7E+1
B2 – spike	Inflow (3)	3.4E+3	1.9E+4	8.8E+3	7.9E+3
	After Biolayer (3)	2.2E+3	4.6E+3	3.4E+3	1.4E+3
	After 2nd port (3)	9.8E+0	1.5E+1	1.1E+1	4.3E+1
	After Sand (3)	6.3E+0	1.4E+1	9.1E+0	3.8E+0
B2 – flush -out	Inflow (4)	1.8E+3	8.8E+3	4.0E+3	3.2E+3
	After Biolayer (4)	2.7E+3	5.0E+4	1.5E+4	2.3E+4
	After 2nd port (4)	3.1E+0	9.8E+1	3.1E+1	4.5E+1
	After Sand (4)	1.2E+1	1.9E+1	1.5E+1	3.0E+0
E. COLI (MPN/100 mL)					
B1 – spike	Inflow (3)	1.0E+2	1.6E+4	5.7E+3	8.5E+3
	After Biolayer (3)	1.0E+2	1.2E+3	4.7E+2	6.4E+2
	After 2nd port (3)	4.0E+0	1.5E+1	9.5E+0	5.3E+0
	After Sand (3)	1.0E-1	3.1E+0	1.4E+0	1.5E+0
B1 – flush-out	Inflow (4)	3.1E+2	1.1E+4	6.4E+3	5.0E+3
	After Biolayer (4)	3.1E+2	2.7E+3	1.1E+3	1.1E+3
	After 2nd port (4)	6.2E+0	5.2E+1	1.9E+1	2.2E+1
	After Sand (4)	3.1E+0	6.3E+0	5.0E+0	1.3E+0
B2 – spike	Inflow (3)	1.0E+1	2.0E+2	1.0E+2	9.5E+1
	After Biolayer (3)	1.0E+1	1.0E+2	4.0E+1	5.2E+1
	After 2nd port (3)	1.0E-1	1.0E-1	1.0E-1	1.7E-17
	After Sand (3)	1.0E-1	1.0E-1	1.0E-1	1.7E-17
B2 – flush -out	Inflow (4)	1.0E+1	3.1E+2	1.6E+2	1.7E+2
	After Biolayer (4)	1.0E+1	4.1E+2	1.3E+2	1.9E+2
	After 2nd port (4)	1.0E-1	1.0E-1	1.0E-1	0.0E+0
	After Sand (4)	1.0E-1	1.0E-1	1.0E-1	0.0E+0

First sampling port was located at 5cm from the water sand-interface and it is referred to as biolayer, while the second sampling port was located at 35cm from the water-sand interface, and it is approximately half way between the biolayer and the output. KCl was used as conservative tracer. () = number of samples used to estimate minimum, maximum, average and standard deviation.

Results from the three spikes suggested that some of the selected PhACs can significantly impact the bacterial removal by the SSF units. Prior to the first spike, bacterial removal > 99% was constantly achieved after both SSF units and approximately 91% removal occurred within the biolayer. However, during the first two spikes, as well as at the beginning of the flush-out, bacterial removal progressively decreased after both filtration

units, especially after the biolayer. Consequently, the decrease in bacteria resulted in an increase in total coliforms in the effluent from approximately E+1 to E+4 MPN/100 mL (Fig. 9a-b). Contrasting results were observed during the third spike in which only caffeine was added to the feed water. During the third spike, the concentration of total coliforms in the effluent was consistently low (ranging from 2.3 E+1 to 7.0 E+1 MPN/100 mL).

These results suggested that the presence of E2 in the feed water may lead to a decrease in the bacterial removal efficiency of the filtration unit. The extended presence of E2 led to a decreasing ability of the biolayer to positively treat the feed water. The biolayer results suggest that it is sensitive to the presence of certain PhACs in the feed water (Fig. 9a-b). However, a “biologically mature” SSF unit can attenuate the impact of selected PhACs, especially if a spike is of limited temporal duration. Even in the presence of a severely damaged biolayer (limited [~15%] to no removal observed after the first sampling port located 5 cm below the water-sand interface) (after spike 2), at least 99.33% removal was achieved after the filtration unit (at the end of the study).

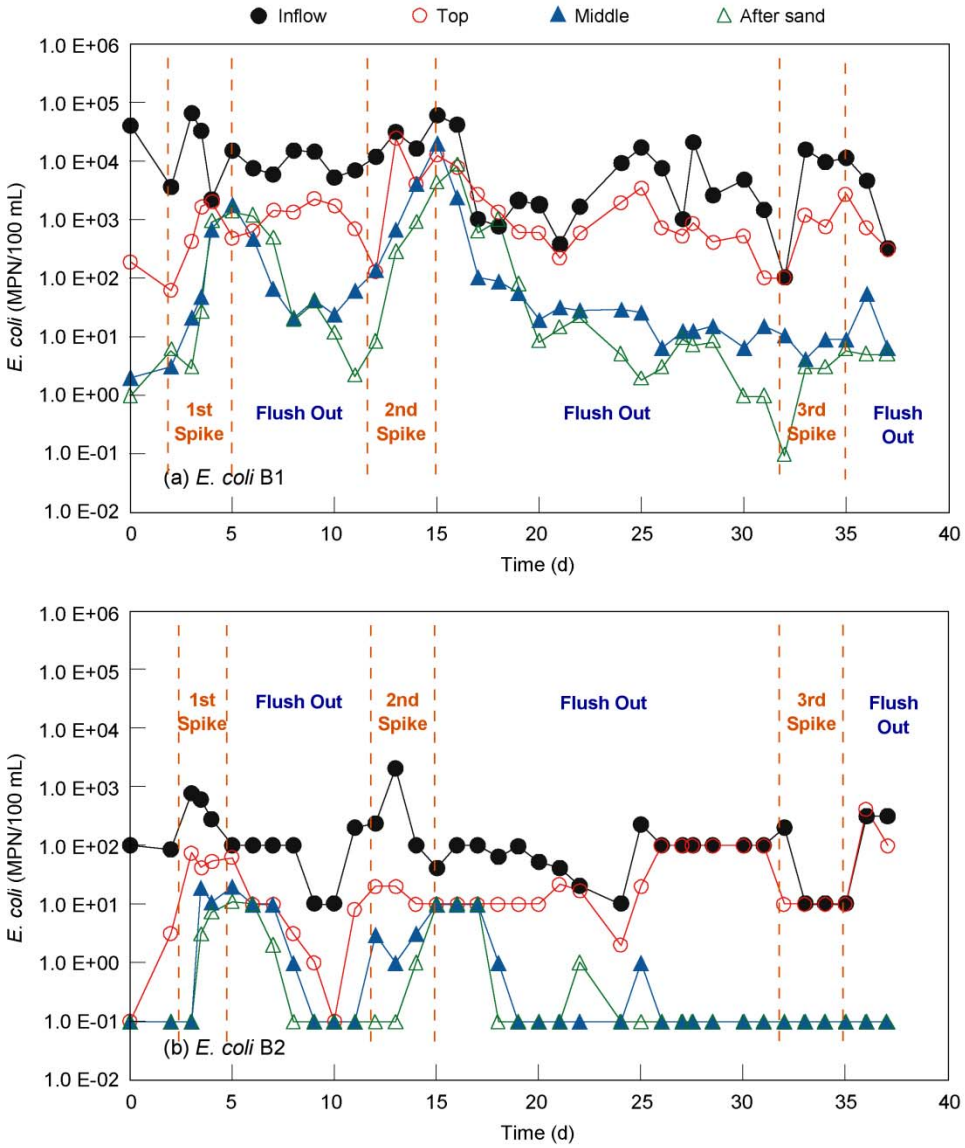


Figure 10: Impact of selected PhACs on removal of *E. coli* after SSF (a) unit B1 and (b) unit B2. Unit B1 received stream water with 1% primary effluent, while unit B2 received only stream water throughout the entire study. During the first spike, ~ 50 µg/L of caffeine, and 17-β-estradiol (E2) were added; during the second spike, ~ 50 µg/L of caffeine, carbamazepine, E2, estrone (E1), gemfibrozil and phenazone were added. During the third spike only caffeine was added. KCl was used as a non reactive tracer during the three spikes with PhACs.

3.3.3. Removal of PhACs using SSF

The PhACs used in the feed water exhibited different behaviors during passage through the SSF units. Carbamazepine, phenazone and gemfibrozil showed no to limited removal while caffeine, E1 and E2 were partially or completely removed.

Carbamazepine

Carbamazepine (Fig. 11a-b) behaved as the non-reactive tracer, KCl, in both SSF units (Fig. 12a-b). The concentration of carbamazepine at the two sampling ports, and in the effluent from both SSF units increased rapidly (Fig. 11a-b), and the C_i/C_0 ratio was constantly one during the entire spike. After the addition of the PhACs was stopped and units B1 and B2 were flushed with stream water with 1% primary effluent and stream water alone, respectively, the concentration of carbamazepine decreased rapidly to zero.

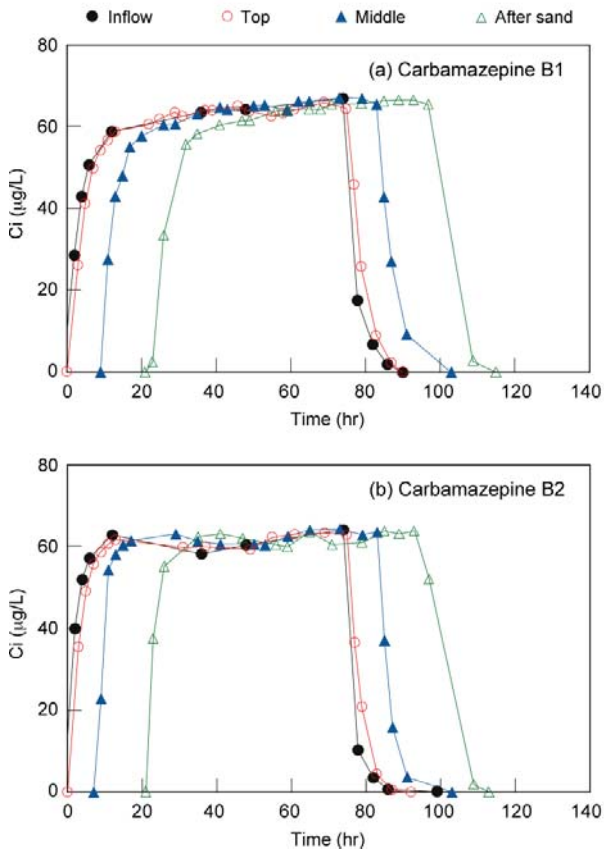


Figure 11: BTCs of carbamazepine in (a) unit B1 and (b) unit B2. Unit B1 received stream water with 1% primary effluent, while unit B2 received only stream water throughout the entire study.

The symmetric shape of the breakthrough curve (BTC), a C_i/C_0 ratio reaching one, and the fact that 100% of the amount added was recovered from the leachate fraction (Table 26), shows that no removal of carbamazepine occurred. The persistence of carbamazepine in the environment was also observed at riverbank filtration sites (Massmann et al., 2006; Hoppe-Jones et al., 2010) as well as during conventional wastewater treatment (Leclercq et al., 2009). No to limited removal of carbamazepine (0–20%) was achieved under aerobic (Hoppe-Jones et al., 2010; Schmidt et al., 2004), suboxic (Schmidt et al., 2004) and anoxic (Heberer et al., 2004) conditions.

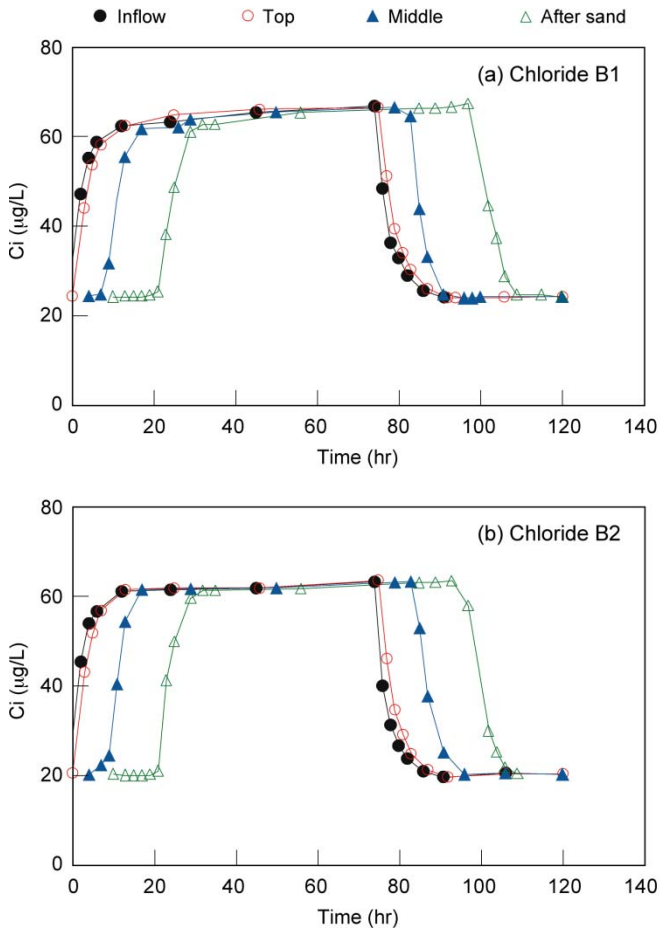


Figure 12: BTCs of KCl in (a) unit B1 and (b) unit B2. Unit B1 received stream water with 1% primary effluent, while unit B2 received only stream water throughout the entire study.

Table 26: Recovery (%) of carbamazepine, gemfibrozil, and phenazone after spike 2.

Experimental conditions	**Carbamazepine (%)**			**Gemfibrozil (%)**			**Phenazone (%)**		
	A bio	**A 2nd**	**AS**	**A bio**	**A 2nd**	**AS**	**A bio**	**A 2nd**	**AS**
UNIT B1 Spike 2	100	102	103	98	97	97	102	102	103
UNIT B2 Spike 2	100	104	105	95	88	92	100	102	104

Unit B1 received stream water with 1% primary effluent (100:1), unit B2 received only stream water. A bio = after Biolayer (1st sampling port); A 2nd = after second sampling port; AS = after sand filter. First sampling port was located at 5cm from the water-sand interface and it is referred to as biolayer, while the second sampling port was located at 35cm from the water-sand interface, and it is approximately half way between the biolayer and the output.

Phenazone

Phenazone showed a behavior similar to carbamazepine in both SSF units. The rapid appearance of phenazone from all the sampling ports as well as in the effluent (Fig. 13a-b), symmetric shape of the BTC, and 100% recovery (Table 26) suggested that phenazone was not degraded by SSF. Phenazone is a very polar substance, difficult to degrade, and shows limited removal (less than 30%) in a conventional wastewater treatment plant (Zuehlke et al., 2006). Phenazone can be degraded during bank filtration under aerobic conditions (Massmann et al., 2006; Massmann et al., 2008), but limited removal was observed under anaerobic conditions (Massmann et al., 2006; Massmann et al., 2008). In the SSF units, aerobic conditions were probably preserved only within the upper few centimeters of the sand bed. Immediately below this region, oxygen depletion may occur leading to no removal of phenazone. The presence of anaerobic bacteria few centimeters below the first sampling port, and the low level of oxygen (< 3mg/L) observed in the effluent of the SSF unit supports the occurrence of reduced conditions in the filtration unit.

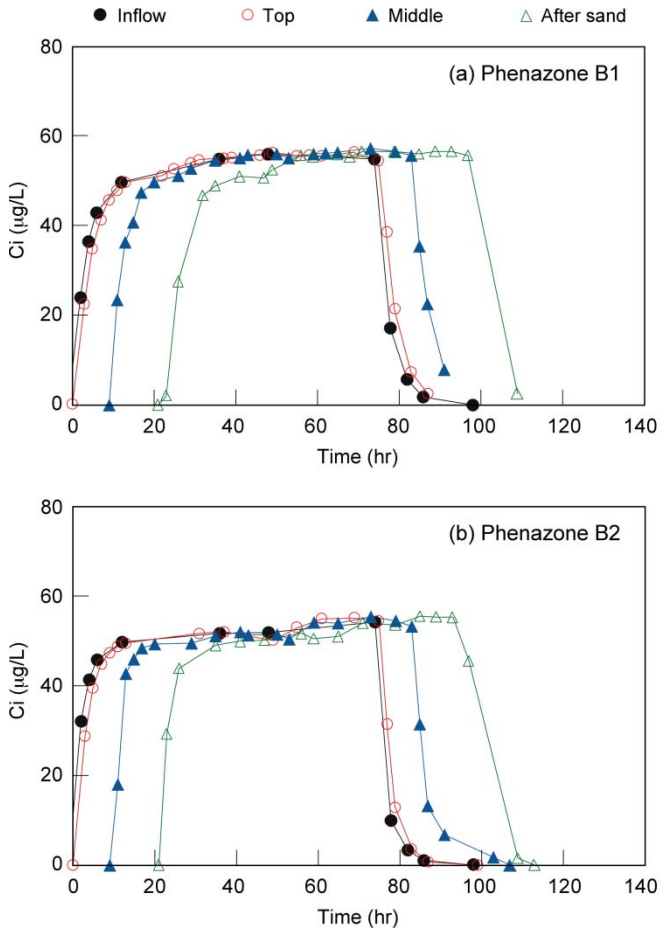


Figure 13: BTCs of phenazone in (a) unit B1 and (b) unit B2. Unit B1 received stream water with 1% primary effluent, while unit B2 received only stream water throughout the entire study.

Gemfibrozil

Like carbamazepine and phenazone, gemfibrozil was introduced into the feed water. The concentration of gemfibrozil at each sampling port, as well as in the outflow of the SSF increased rapidly (Fig. 14a-b). C_i/C_0 ratio reached, on average 0.95 and 0.90 after unit B1 and unit B2, respectively. Mass balance of input and output values revealed that approximately 97% of gemfibrozil was recovered in the outflow of unit B1, while a slightly lower recovery of 92% was achieved after unit B2 (Table 26). Data obtained from the two sampling ports in B1 showed that a limited removal of 3% was achieved

within the biolayer and no further removal was observed throughout the entire unit. In unit B2, 5% removal was achieved within the biolayer and an overall removal of 8% was achieved after the filtration unit (Table 26). Enhanced removal of gemfibrozil was observed during field (Hoppe-Jones et al., 2010) as well as laboratory (Hoppe-Jones et al., 2012; Rauch-Williams et al., 2010) investigations, probably due to longer adaptation time and the extended presence of viable soil biomass.

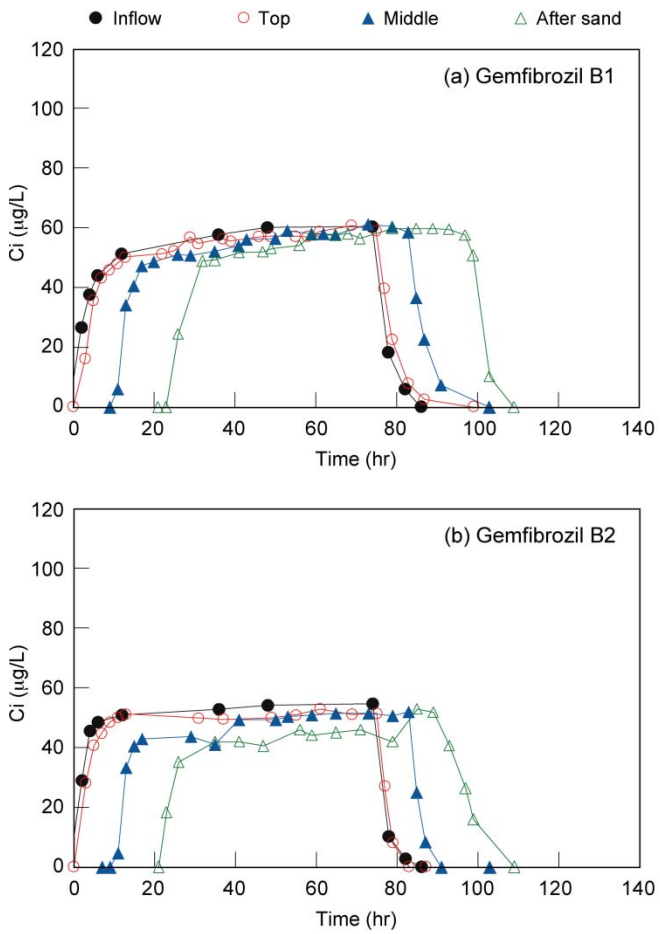


Figure 14: BTCs of gemfibrozil in (a) unit B1 and (b) unit B2. Unit B1 received stream water with 1% primary effluent, while unit B2 received only stream water throughout the entire study.

Caffeine

During the three spikes caffeine showed a different behavior compared to carbamazepine, gemfibrozil and phenazone. Caffeine was partially removed during the first two spikes, and complete removal occurred during the third spike in both units (Fig. 15 a-f).

The slow appearance of caffeine in the two sampling ports and in the effluent compared to the non-reactive tracer, asymmetric shape of the BTC curves, and a recovery constantly lower than 100% in both SSF units during the three spikes, suggested removal of caffeine mostly due to biodegradation (Fig. 15a-b; Table 27). During the first spike of caffeine and E2, 57% and 77% recovery were achieved after units B1 and B2, respectively (Table 27). Data from the two sampling ports, showed lower recovery (77% and 68%) in unit B1 than in unit B2 (97% and 94%). During the second spike of 6 PhACs, 73% and 66% recovery were achieved after unit B1 and unit B2, respectively. Data from the two sampling ports showed similar recovery in both units, 84% and 81% in unit B1 and 86% and 76% in unit B2. During the third spike of caffeine alone, no caffeine was recovered after either unit. Data from the two sampling ports showed that only 4% recovery was achieved after the biolayer of unit B1, and no recovery was achieved in the upper half of this unit. Higher, but still limited recoveries of caffeine 26% and 11% were seen after the two sampling ports in unit B2 (Table 27).

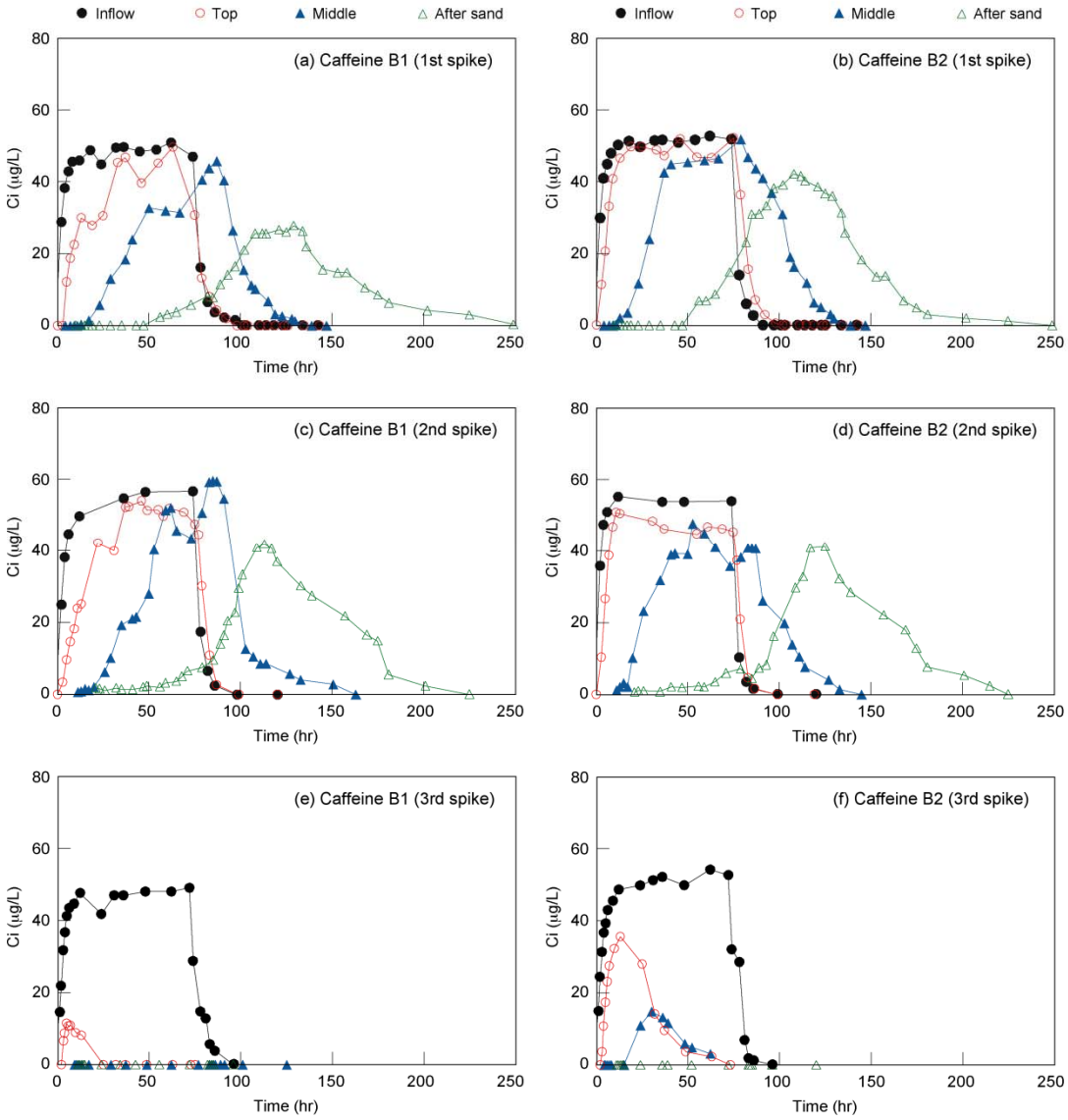


Figure 15: BTCs of caffeine in unit B1 and unit B2 during the: (a,b) first spike, (c,d) second spike, and (e,f) third spike. Unit B1 received stream water with 1% primary effluent, while unit B2 received only stream water throughout the entire study. During the first spike, caffeine and E2 were added into the feed water. During the second spike, 6PhACs were added into the feed water, while during the third spike only caffeine was added into the feed water.

Table 27: Recovery (%) of caffeine after spike 1, spike 2, and spike 3.

Experimental conditions	**Caffeine (%)**		
	A bio	**A 2nd**	**AS**
UNIT B1			
Spike 1	77	68	57
Spike 2	84	81	73
Spike 3	4	0	0
UNIT B2			
Spike 1	97	94	77
Spike 2	86	76	66
Spike 3	26	11	0

Unit B1 received stream water with 1% primary effluent (100:1), unit B2 received only stream water. A bio = after Biolayer (1st sampling port); A 2nd = after second sampling port; AS = after sand filter. The first sampling port was located at 5cm from the water- sand interface and it is referred to as biolayer, while the second sampling port was located at 35cm from the water-sand interface, and it is approximately half way between the biolayer and the output.

During the first spike of caffeine and E2, in unit B1, 23% removal of caffeine occurred within the biolayer, with an additional 10% in the upper half as well as in the lower half of the filtration unit (Table 27). During the second spike of 6 PhACs, a similar trend was observed, however a limited overall removal (27%) was achieved after unit B1. During the third spike, 96% of caffeine was removed within the biolayer, and complete removal occurred in the upper half of unit B1 (Table 27).

During the first and second spike, caffeine in unit B2 exhibited a slightly different behavior than caffeine in unit B1 (Fig. 15a-b). During the first spike, 3% and 6% removal occurred within the biolayer and in the upper half of the unit, while 23% occurred in the lower half of the unit. During the second spike, 14% and 24% removal occurred after the two sampling ports, with an additional 10% removal occurring after the filtration unit. During the third spike, caffeine was completely removed after unit B2. 74% removal occurred within the biolayer, while an additional 10% occurred in the upper and lower half of the filtration unit (Table 27).

Results from the three spikes suggested that caffeine can be completely removed using SSF, especially after sufficient exposure of the filtration unit to caffeine. Most of the removal occurred within the biolayer. The difference in caffeine removal between the

three spikes may reflect the changes in the population of the microbial community observed during the entire study. *Pseudomonas* is known to degrade caffeine (Blecher and Ligens, 1977; Yamoka-Yano and Mazzafera 1999; Gokulakrishnan et al., 2005). Prior to and during the third spike, *Pseudomonas* was the predominant group of bacteria present in the biolayer. This may be the reason for the extremely high removal of caffeine observed during the third spike. Among the two biolayers, unit B1 had the highest number of *Pseudomonas*, and this may lead to a more extensive removal of caffeine within the biolayer of unit B1 (96%) compared to the biolayer of unit B2 (74%).

E2 and E1

The slow appearance of E2 in the two sampling ports and in the effluent compared to the non-reactive tracer, asymmetric shape of the BTC curves, and a recovery constantly lower than 100% during the two spikes, suggested the removal of E2 by both SSF (Fig. 16a-b; Table 28). During the first spike of caffeine and E2, 35% and 8% recovery of E2 was achieved after unit B1 and B2, respectively (Table 28). Results from the two sampling ports showed 58% and 15% recovery within the biolayer of both units. The removal of E2 was primarily related to the production of E1. E1, the breakdown product of E2 (Lee and Liu, 2002), showed a behavior complementary to E2. During the first spike with caffeine and E2, E1 was not added into the feed water; therefore, the appearance of E1 was due to the degradation of E2. The degradation of E2 and production of E1 occurred in both reservoirs. E1 ranged between 11 and 20 μg/L in the feed water of unit B1, while it was constantly lower than 12 μg/L in the feed water of unit B2. The 133% and 165% recoveries of E1 achieved after unit B1 and B2, respectively, suggested that E1 was produced within both SSF units. The results from the top sampling port showed 161% and 393% recoveries of E1 compared to the E1 present in the feed water for units B1 and B2, respectively, suggested the production of E1 mainly occurred within the biolayer of both units (Table 28). Recoveries of 146% and 280% were achieved in the upper half of unit B1 and B2, respectively, suggesting limited removal of E1 within the barrel.

During the second spike of 6 PhACs in which E1 and E2 were simultaneously added to the feed water, E2 recoveries of 89% were achieved after unit B1 and 12% after unit B2 (Table 28). Results from the two sampling ports revealed that no removal of E2 occurred within the biolayer as well as in the upper half of unit B1. Unlike unit B1, E2 recoveries of 39% and 13% were achieved in the biolayer and in the upper half of unit B2, suggested removal of E2 and further production of E1.

Table 28: Recovery (%) of E1, E2, and E1+E2 after spike 1, and spike 2.

Experimental conditions	**E2 (%)**			**E1 (%)**			**E1 + E2 (%)**		
	A bio	**A 2nd**	**AS**	**A bio**	**A 2nd**	**AS**	**A bio**	**A 2nd**	**AS**
UNIT B1									
Spike 1	58	44	35	161	146	133	94	78	68
Spike 2	98	107	89	88	78	80	93	89	79
UNIT B2									
Spike 1	15	14	8	393	280	165	88	65	39
Spike 2	39	13	12	143	98	69	86	72	44

Unit B1 received stream water with 1% primary effluent (100:1), unit B2 received only stream water. A bio = after Biolayer (1st sampling port); A 2nd = after second sampling port; AS = after sand filter. The first sampling port was located at 5cm from the water-sand interface and it is referred to as biolayer, while the second sampling port was located at 35cm from the water-sand interface, and it is approximately half way between the biolayer and the output.

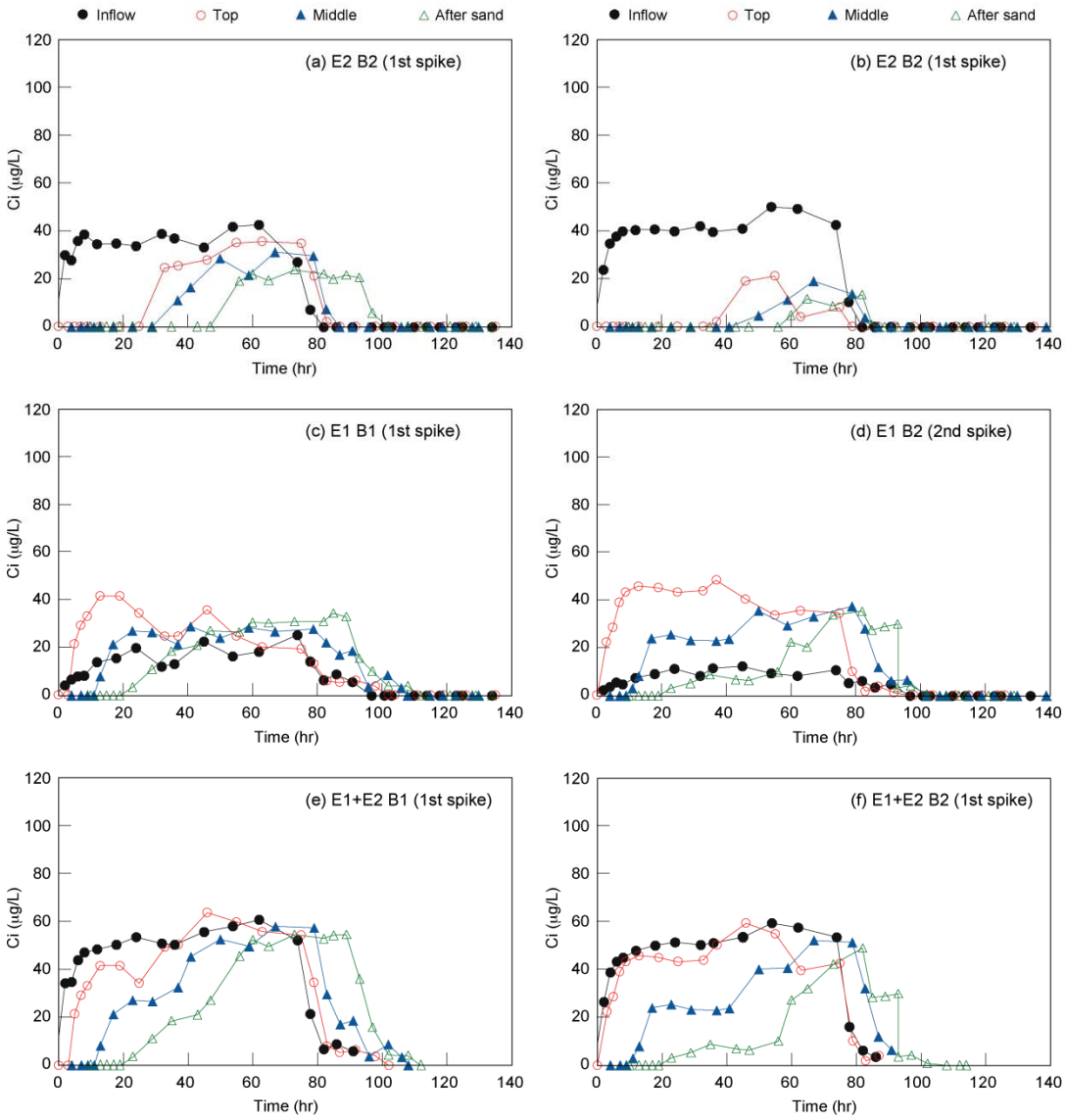


Figure 16: BTCs of: (a, c, e) E2, E1, and E1+E2 in unit B1 and (b, d, f) in unit B2 during the first spike of caffeine and E2 (no E1 was added in the feed water). Unit B1 received stream water with 1% primary effluent, while unit B2 received only stream water throughout the entire study.

During the second spike with 6 PhACs (E1 and E2 were simultaneously added in the feed water), recoveries of E1 achieved were 88% (after the biolayer), 78% (after the upper half), and 80% (after unit B1) (Table 28). These results suggested no to limited production as well as limited removal (< 20%) of E1 within unit B1. Contrasting results

were observed in unit B2 where recoveries of E1 were 143% (after the biolayer), 98% (after the upper half), and 69% (after unit B2). Production of E1 due to the degradation of E2 occurred only within the biolayer, while removal of E1 occurred in the remaining portion of unit B2 (Table 28).

During the first spike of caffeine and E2, E1 was constantly found in both reservoirs suggesting the degradation of E2, and consequently the production of E1. Higher production, almost double, of E1 was observed in the reservoir containing Manoa Stream water with 1% primary effluent, compared to the reservoir containing Manoa Stream water alone (Fig. 17a-b). This faster production was probably due to the higher overall number and different population of bacteria contained in the stream water with 1% primary effluent compared to stream water alone. Further production of E1 occurred in the biolayer of both SSF units. The overall recovery of E1 produced and E2 consumed, showed that 68% and 39% (E1 + E2) was achieved after units B1 and B2, respectively (Table 28).

During the second spike, due to the simultaneous presence of E1 and E2 in the feed water, limited production of E1 occurred within the biolayer of both SSF units. Overall recovery of E1 (79%) and E2 (44%) was achieved after both SSF units (Table 28).

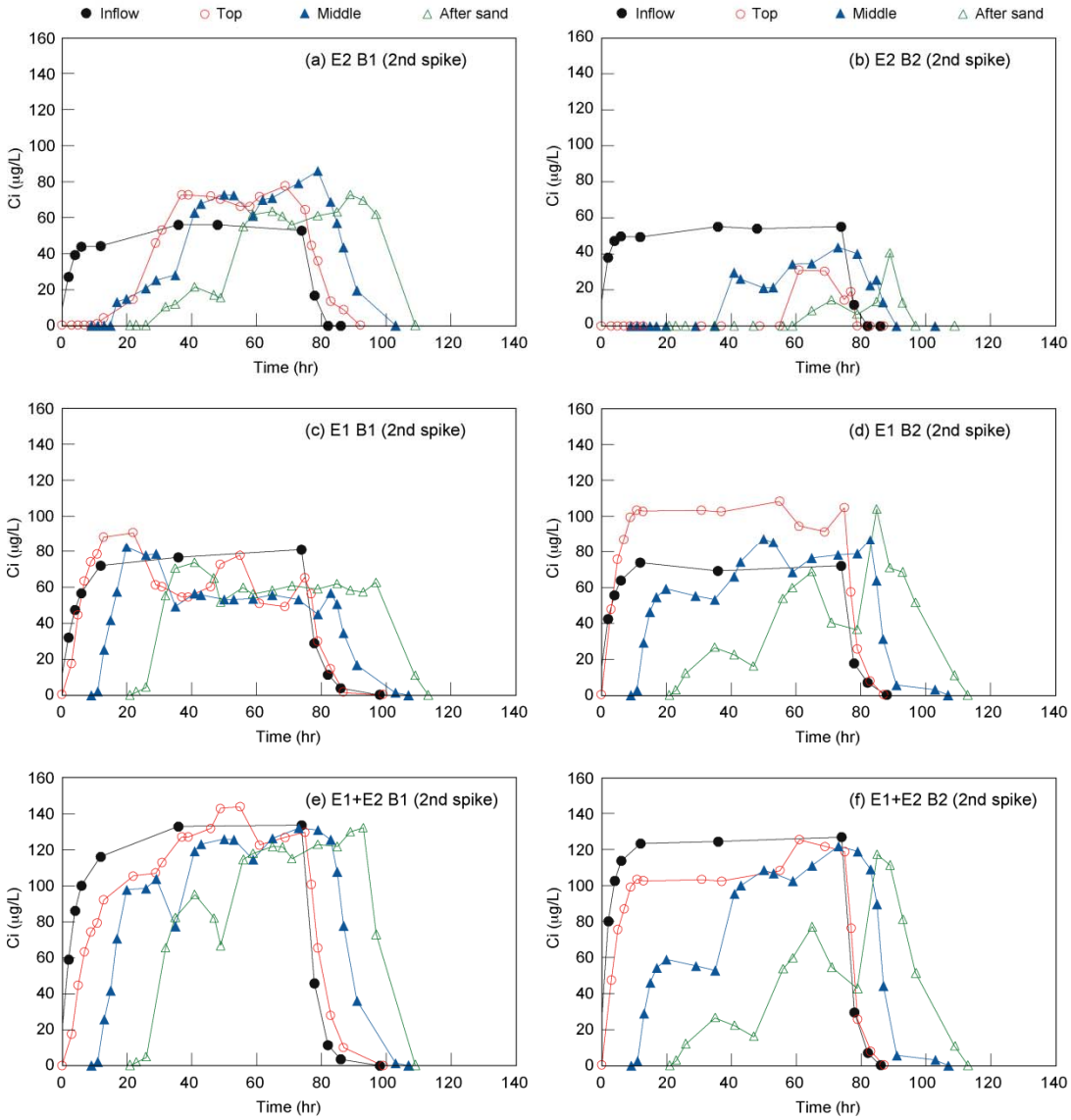


Figure 17: BTCs of: (a, c, e) E2, E1 and E1+E2 in unit B1 and (b, d, f) in unit B2 during the second spike with 6 PhACs (E1 and E2 were simultaneously added in the feed water). Unit B1 received stream water with 1% primary effluent, while unit B2 received only stream water throughout the entire study.

3.4. CONCLUSIONS

- The presence of selected PhACs, especially E2, may significantly impact the removal efficiency of an SSF unit in terms of total coliforms and *E. coli*. The removal of

both bacteria dropped approximately 3 logs during the second spike in the presence of E2.

- In the presence of a “biologically mature” SSF unit (unit that has been used for an extensive period of time, ~1 year depending on the feed water and on the hydraulic loading rate, in which a “sticky” and gelatinous film has been developed throughout the packing material), high removal (> 99%) of total coliforms and E. coli can be achieved in the presence of a “damaged biolayer” (limited [~15%] to no removal observed after the first sampling port located 5 cm below the water – sand interface) .
- Complete removal of caffeine, partial removal (11–92%) of E2 and E1, while no to limited removal (< 10%) of carbamazepine, gemfibrozil, and phenazone were achieved in the SSF units.
- The feed water, stream water with 1% primary effluent or stream water alone, used to develop the biolayer did not significantly impact the removal of the selected PhACs or their effect on the bacterial removal by the SSF units.
- The microbial community in the sand was significantly more diverse compared to the communities in the biolayer.
- Proteobacteria, predominantly class Gammaproteobacteria, and Bacteroidetes were the dominant phyla in the biolayer samples, while Actinobacteria, Bacteroidetes and several classes of Proteobacteria (Alpha-, Beta-, Delta-, and Gammaproteobacteria) were prevalent in the sand samples.
- Changes in the microbial community composition were significant over time. In general, Bacteroidetes was replaced by Proteobacteria in the biolayer independently from the SSF units.
- In the sand samples collected at the end of the experiment through the SSF profile (3, 10, 20, and 45 cm depth), microbial communities had lowest diversity at 3 cm depth and the highest diversity at 10 cm depth, but changes were not significantly correlated with the depth or the treatment.

- Results obtained from this study suggested that SSF can be positively used in the presence of high concentrations of PhACs from wastewater treatment plant spills or pharmaceutical industry spills. However, the nature (i.e., steroids vs. anticonvulsant vs. stimulant) and concentrations of the PhACs (5 μg/L vs. 50 μg/L), the duration of the spill (3d vs. 10d), and the age of the SSF (new vs. "biologically mature" unit) may significantly impact the overall performance of the filtration unit. In the presence of a longer spill or in the presence of a relatively new SSF unit, the overall removal achieved after a SSF unit may significantly deplete, while the required restoration time should significantly increase.

CHAPTER 4. FATE AND TRANSPORT OF SELECTED PHARMACEUTICALLY ACTIVE COMPOUNDS DURING SIMULATED RIVERBANK FILTRATION: THE IMPACT OF REDOX CONDITIONS, TEMPERATURE AND LEVEL OF ORGANICS[1]

Abstract

The occurrence of pharmaceutically active compounds (PhACs) in environmental waters has drastically increased during the past few decades. Riverbank filtration (RBF) can be a possible means of removal of PhACs. The objective of this study was to investigate the effect of oxygen, temperature, and organic matter on the removal of selected PhACs during simulated RBF. The behavior of six PhACs (caffeine, carbamazepine, 17-β estradiol [E2], estrone [E1], gemfibrozil, and phenazone) was evaluated by column experiments.

Results from the study suggested that RBF can be positively used to treat PhACs, but the geochemistry of the RBF site needs to be carefully investigated. Biodegradation and sorption represent the predominant mechanisms involved during the removal of the selected PhACs. However, there are few PhACs, such as carbamazepine, that are very persistent in the environment regardless of the environmental conditions occurring at the RBF site. All selected PhACs showed limited and slower removal during the winter. Partial removal (< 30%) of gemfibrozil occurred only during the summer under aerobic conditions. Phenazone was highly impacted by the redox conditions; complete removal occurred only under aerobic (DO ~8 mg/L) conditions. Caffeine was primarily impacted by the presence of humic acid in the feed water. E1 was completely removed only under aerobic conditions, and a partial slower removal occurred under anaerobic conditions. E2 was completely removed under all the different environmental conditions.

[1] This chapter has been submitted to *Chemosphere*: Matteo D'Alessio, Bunnie Yoneyama, Chittaranjan Ray. Fate and Transport of Selected Pharmaceutically Active Compounds During Simulated Riverbank Filtration: Impact of Redox Conditions, Temperature and Level of Organics.

4.1. INTRODUCTION

Trace organic compounds (TrOCs) such as personal care products, household chemicals, and pharmaceutically active compounds (PhACs) have dramatically increased in the environment during the last few decades (Ternes, 1998; Kolpin et al., 2002; Focazio et al., 2008). Conventional wastewater treatments are not adequate to fully remove TrOCs (Heberer, 2002; Yu et al., 2006). These chemicals, therefore, have been found in the surface water and groundwater of the United States and Europe (Ternes, 1998; Kolpin et al., 2002; Benotti et al., 2009). Some of the TrOCs, even at concentrations of a few ng/L, may have adverse effects on aquatic and terrestrial organisms (Thiele-Bruhn and Beck, 2005; Vajda et al., 2008; Madureira et al., 2011) and potential adverse effects on human health (Boxall, 2004; Fent et al., 2006). Riverbank filtration (RBF) systems consist of a series of abstraction wells in the vicinity of a stream or lake resulting in groundwater depletion that forces river or lake water to infiltrate into the subsurface towards the abstraction wells (Hoppe-Jones, et al., 2010). RBF can be used as a possible treatment to remove PhACs (Ray et al., 2002; Grünheid et al., 2005). However, there are many factors that can affect the removal which increase the uncertainty of the fate of these micropollutants.

Laboratory (Rauch-Williams et al., 2010; Hoppe-Jones et al., 2012; Onesios and Bouwer, 2012) and field studies (Hoppe-Jones et al., 2010; Patterson et al., 2011; Benotti et al., 2012) have been conducted to evaluate the behavior of selected PhACs under different environmental conditions. Some PhACs, such as dichlofenac, gemfibrozil, phenazone are more efficiently removed under aerobic conditions compared to anaerobic conditions (Rauch-Williams et al., 2010; Maeng et al., 2011; Teerlink et al., 2012), while some PhACs, such as iodinated X-ray contrast agents, sulfamethoxazole are preferentially removed under anaerobic conditions (Grünheid et al., 2005; Heberer et al., 2008). Generally, a limited removal of PhACs was observed during the winter (Hoppe-Jones et al., 2010). In a field study conducted at Lake Tegel (Germany) phenazone was completely removed only during the winter (Massmann et al., 2006). However, the complete removal of phenazone during the winter was related more to the level of oxygen

than the temperature, since aerobic conditions were achieved during the winter (Massmann et al., 2006). Physico-chemical properties of the PhACs, their travel time and local conditions such as redox conditions, temperature, and the presence of organic matter should be carefully investigated to better understand the behavior of these compounds at riverbank filtration sites.

The objectives of this study were to investigate the role of 1) the oxygen level – aerobic vs. anoxic, 2) temperature – summer vs. winter, and 3) the amount of organic matter on the removal of selected PhACs during simulated RBF.

4.2. MATERIALS AND METHODS

4.2.1. Selection of PhACs

There are more than 3000 pharmaceutical compounds that are currently approved for prescription in the U.S. (PDR, 2008), and hundreds of others that are approved for over-the-counter use. The PhACs investigated during this study were selected according to the following multistep approach: i) occurrence in the environment and public interest, ii) toxicity, iii) environmental fate, iv) behavior under different redox conditions, v) pharmaceutical class, and vi) availability of analytical standards and adequate instrumentation. Additional details related to the selection process of the PhACs are provided in Chapter 1.

4.2.2. Chemicals

Caffeine, carbamazepine, estrone (E1), 17-β estradiol (E2), gemfibrozil, phenazone, the sodium salt of humic acid (HA), sodium azide (NaN_3) (Sigma-Aldrich, St. Louis, MO), HPLC-grade methanol, acetone and acetonitrile (EMD Chemicals Inc., NJ) were used. Milli-Q (Billerica, MA) reagent grade DI water with a resistivity of 18.2 MΩ·cm (at 25 ºC) was used. The physico-chemical properties of the selected PhACs, including pharmaceutical class, toxicity, chemical structure, log K_{ow}, and factors impacting their environmental fate are shown in Table 5.

4.2.3. Packing materials

Silica sand (0.6 – 1.2 mm, Orange County # 16) with a uniformity coefficient of 1.57, and a porosity of 0.41, was used as the main packing material during this study. Sand was repeatedly rinsed with tap water and air-dried prior to being used.

4.2.4. Feed water

Feed water was collected from Lake Wilson (21º29'41" N, 158º01'29" W, Wahiawa State Freshwater Park, HI, USA) (Fig. A1-Appendix A). Lake Wilson is partially impacted by the Wahiawa Wastewater Treatment Plant (approximately 2mgd, http://www.state.hi.us/dlnr/pdf/lakewilson.pdf). Surface water samples were collected 1m away from the bank of the lake and stored at 4 °C prior to being used. Lake Wilson was selected as a feed water source due to its higher total organic carbon (TOC) content, 3 mg/L, compared to the TOC of most streams in Hawaii which are approximately 1 mg/L. Prior to being used as feed water for the columns, the lake water was pre-filtered through 11 µm paper filters (Whatman, GE Healthcare Life Sciences, Piscataway, USA), to remove large particles that may clog the columns.

4.2.5. Experimental set-up

Flow-through stainless steel columns, 4.75 cm internal diameter, and 14.50 cm long, were used (Fig. A4-Appendix A). Light-excluding stainless steel columns were selected to minimize the effect of photo-degradation of the selected PhACs as well as the potential adsorption of these compounds onto the internal wall of the column. A fine stainless steel wire cloth (TWP, Berkeley, CA, USA) with wire diameter of 40 µm was placed at the bottom of each column to support the packing material and to prevent any possible dispersion of fine materials from each column. All the different parts of the columns as well as the wire cloth were sterilized by autoclaving (Market Forge, Industries Inc., USA) for 30 min at 121 °C and 15 psi. The columns were operated under a re-circulating flow regime. The same water percolated repeatedly through the column and the inflow reservoirs also served as outflow containers. A four channel head peristaltic pump (Cole-

Parmer, Vernon Hills, IL, USA) was used to simultaneously introduce the different feed solutions to the different column setups at the same hydraulic loading rate of 0.2 mL/min. Samples were collected daily from each reservoir in order to evaluate the levels of the different PhACs.

Sand was pre-acclimated according to the different environmental conditions – aerobic and anaerobic, summer and winter, with and without HA - for approximately three months (Table 29). During this period the TOC/DOC removal by the sand in the columns reached a plateau. Once this plateau condition was achieved the selected PhACs were added. Aerobic conditions (oxygen: ~ 8 mg/L) were achieved by continuously bubbling air into the reservoir, while anaerobic-anoxic (oxygen < 2 mg/L) conditions were obtained by keeping the reservoir closed and by purging the reservoir with nitrogen. To verify the persistency of the aerobic and/or anoxic conditions throughout the study, oxygen probes (Ocean Optics, Dunedin, FA) were placed inside each reservoir. The impact of temperature – summer vs. winter – was investigated by performing the different simulations at 20 °C and 6 °C. One set of columns was placed on a laboratory bench top where the average ambient temperature was 20 – 21 °C, and another set of columns was operated inside an incubator (Isotherm Incubator, Thermo Fisher, Waltham, MA) set at 6 °C.

Table 29: Summary of experimental conditions.

Redox conditions	Temperature	Level of Organics	Control
Aerobic (DO ~ 8mg/L)	Summer (20 ºC – 21 ºC)	Low (TOC ~ 3 mg/L)	Sterile sand, and sterile water
Anoxic (DO < 2 mg/L)	Winter (6 ºC)	Moderate (TOC ~ 10 mg/L)	
		High (TOC ~ 20 mg/L)	

The impact of different levels of organic matter was evaluated by adding different amounts of HA to the water collected from Lake Wilson in order to produce feed water

with a TOC of 3, 10, and 20 mg/L. In order to increase the TOC of the feed water, HA was added. HA accounts for approximately 50-80% of dissolved organic matter and 30-40% of the aromatic carbon in natural waters (Aiken et al., 1985). HA tends to contain a variety of functional components including carboxylates and phenolic hydroxyls and has a refractory nature. Due to its complex structure, HA shows a strong tendency to retain organic compounds, hence reducing the potential of EDCs to be absorbed by the packing material (Zhang and Zhou, 2005).

An abiotic (sterile) control was used to evaluate the possible sorption of the selected PhACs by the column packing material (Table 30). Sand was washed with tap water, air dried, and autoclaved once a day, for three consecutive days at 121 °C and 15 psi for 30 minutes before being packed into the sterile column. The stainless steel columns and all tubing and fittings were sterilized by autoclaving at 121 °C and 15 psi for 30 minutes. Water collected from Lake Wilson was filtered through a 0.2 µm membrane (Pall, Port Washington, NY), autoclaved once at 121 °C and 15 psi for 30 minutes and supplemented with 1.5 mM sodium azide. The abiotic control was run under summer conditions. No abiotic control was used during the simulated winter conditions because low temperature usually limits microbial activity.

Table 30: Methodology used to achieve sterile conditions and summary of possible mechanisms involved in the presence of sterile and non-sterile conditions.

Lake water	Sand	Possible mechanisms involved
Non sterile Filtered through 11 μm filter paper	Non sterile Acclimated according to the different environmental conditions	• Biodegradation related to the microbial community in the lake water and in the sand; • Sorption
Sterile Filtered through 0.2 μm GHP membrane & autoclaved for 30 min at 121 ºC	Sterile Autoclaved 3x at 121 ºC for 30 min	• Sorption

4.2.6. Analysis of the effluent

During the acclimation period of the sand under the different environmental conditions, the effluent was analyzed in terms of TOC, DOC (dissolved organic carbon), and SUVA (specific ultraviolet absorbance). TOC was measured using a TOC analyzer (Shimadzu, Columbia, MD) coupled with a total nitrogen (TN) detector. Prior to measuring DOC using a TOC analyzer, samples were filtered through 0.45μm GHP syringe filter (Pall, Port Washington, NY).

SUVA is defined as the ratio between DOC and UV absorbance at 254 nm, represents the relative aromaticity of organic matter (Amy and Drewes, 2007) and it is used as a measurement of HA. The absorbance at 254 nm was measured with a spectrophotometer (HACH, Loveland, CO, USA).

Once the PhACs were added to the feed water, effluents from the columns were also analyzed for the selected PhACs according to the USEPA Method 1694 (USEPA, 2007) with an HPLC system (Thermo Finnigan, Waltham, MA) coupled with a photo diode

array (PDA) detector and a fluorescence detector (Shimadzu, Columbia, MD). The PhACs were separated using a reverse phase column (100.0 x 2.1 mm, 3.0 µm, Restek, Bellefonte, PA) protected by a guard column with a matching stationary phase. Both columns were kept at 30 °C during analysis. Acetonitrile and DI water were used as the mobile phase. Caffeine and carbamazepine were detected at 204 nm, while the remaining PhACs at 200 nm.

When possible, reaction rates and half-life of the selected PhACs were estimated. The half-life ($t_{1/2}$) was estimated using the following equation:

$$t_{1/2} = \frac{\ln(2)}{K}$$

where k = reaction rate.

To verify the persistency of the sterile conditions and to quantify the overall presence of bacteria in each reservoir during the different simulations, a spread plate method on mHPC media was used. 0.1 mL of the sample or the appropriate dilution was inoculated onto an mHPC agar plate and spread with a glass spreader. The plates were incubated at 35 °C for 48 hours (APHA, 2012). All operations were performed aseptically.

To verify the persistency of the aerobic and/or anoxic conditions, dissolved oxygen was measured using fiber optic T1000 oxygen probes (Ocean Optics, Dunedin, FA) coupled with temperature sensors. These oxygen probes use the fluorescence of a chemical complex in a sol-gel to measure the partial pressure of oxygen (*http://www.oceanopticssensors.com/theory.htm*).

4.3. RESULTS AND DISCUSSIONS

Approximately 30% removal of TOC and DOC was observed under the different experimental conditions. Most of the removal occurred within the first 7 days, after

which limited removal of TOC and DOC occurred (Fig. 18). No significant change in SUVA was observed during the entire acclimation period.

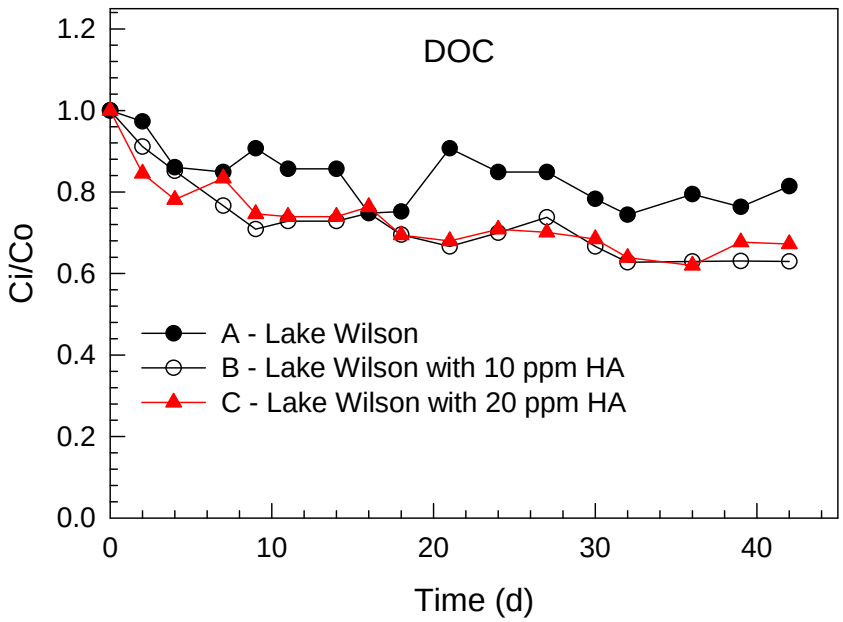


Figure 18: Removal of DOC in the presence of (a) lake water alone (TOC = 3mg/L), (b) lake water with HA (TOC = 10 mg/L), and (c) lake water with HA (TOC = 20 mg/L).

The selected PhACs used in this study were added to the feed water at higher concentrations (50 μg/L) than that normally found in surface water (Kolpin et al., 2002; Hoppe-Jones et al., 2010). Due to the sensitivity of the instrument and the high number of columns used, a higher concentration of the selected PhACs was used compared to that commonly detected in the environment. However, concentrations of PhACs in the range of a few μg/L have been detected in environmental water samples (Zühlke et al., 2004; Carrara et al., 2008).

The selected PhACs exhibited different behaviors after passage through the sand columns. Carbamazepine and gemfibrozil were persistent in the lake water under all the different environmental conditions simulated, while E2 was easily removed. Caffeine, E1, and phenazone were highly impacted by the different environmental conditions.

4.3.1. Sterile conditions

Sterile conditions were fully preserved during the first 40 days of each simulation. Table 31 and Figs. 19-24 (stable black line) showed the presence of sterility conditions. During this period (0 to 40 days), among the selected PhACs only caffeine (Fig. 22a-b) showed a small decrease (~ 10 %) in concentration, suggesting possible but limited sorption of caffeine to the packing material. These results suggested that sorption had a negligible role in the removal of the PhACs selected for the study.

The limited to no sorption of PhACs occurred under sterile conditions was also observed in other column studies (Rauch-Williams et al., 2010; Onesios and Bouwer, 2012). Patterson et al. (2011) observed partial removal of E2 under abiotic conditions, but they attributed this drop of E2 to a possible loss of sterility.

Table 31: Occurrence of bacteria in sterile control.

Time (d)	Summer Aerobic	Summer Anaerobic
0	0	0
3	0	0
7	0	0
12	0	0
30	0	0
45	75	50

4.3.2. PhACs persistent in the environment

Among the selected PhACs, carbamazepine and gemfibrozil were persistent under the different experimental conditions.

<u>Carbamazepine</u>

Less than 10% removal of carbamazepine occurred regardless of the level of oxygen, temperature and level of organic matter (Fig. 19a-d). No reduction of carbamazepine was seen in other column studies (Maeng et al., 2011; Rauch-Williams et al., 2010) or at RBF sites (Hoppe-Jone et al., 2010) or at wastewater treatment plants (Ternes, 1998; Clara et al., 2005). The present study confirms the persistency of carbamazepine in the

environment regardless of the redox state (Massmann et al., 2006; Teerlink et al., 2012), and the occurrence of abiotic or biotic conditions (Maeng et al., 2011).

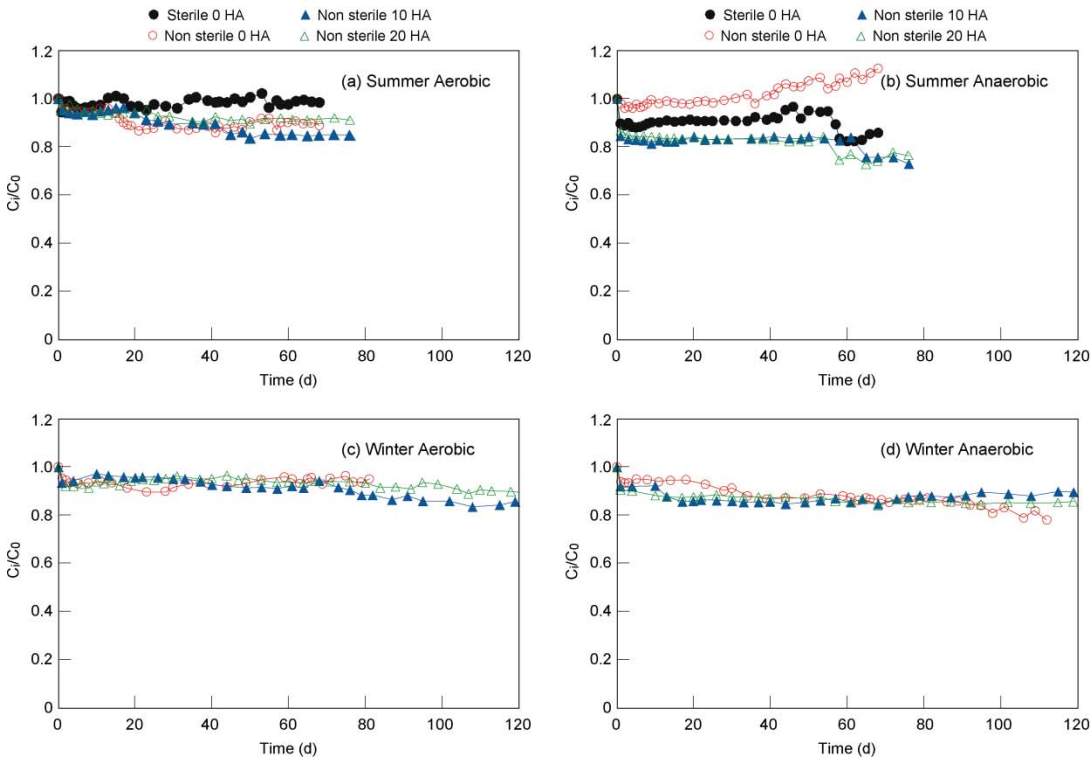


Figure 19: BTCs of carbamazepine during (a) summer and aerobic, (b) summer and anaerobic, (c) winter and aerobic, and (d) winter and anaerobic conditions.

Gemfibrozil

Gemfibrozil showed approximately 25% removal only in the presence of aerobic conditions, during the summer and with a low level of HA in the lake water. Under any of the other simulated environmental conditions, removal of gemfibrozil was constantly lower than 10% (Fig. 20a-d).

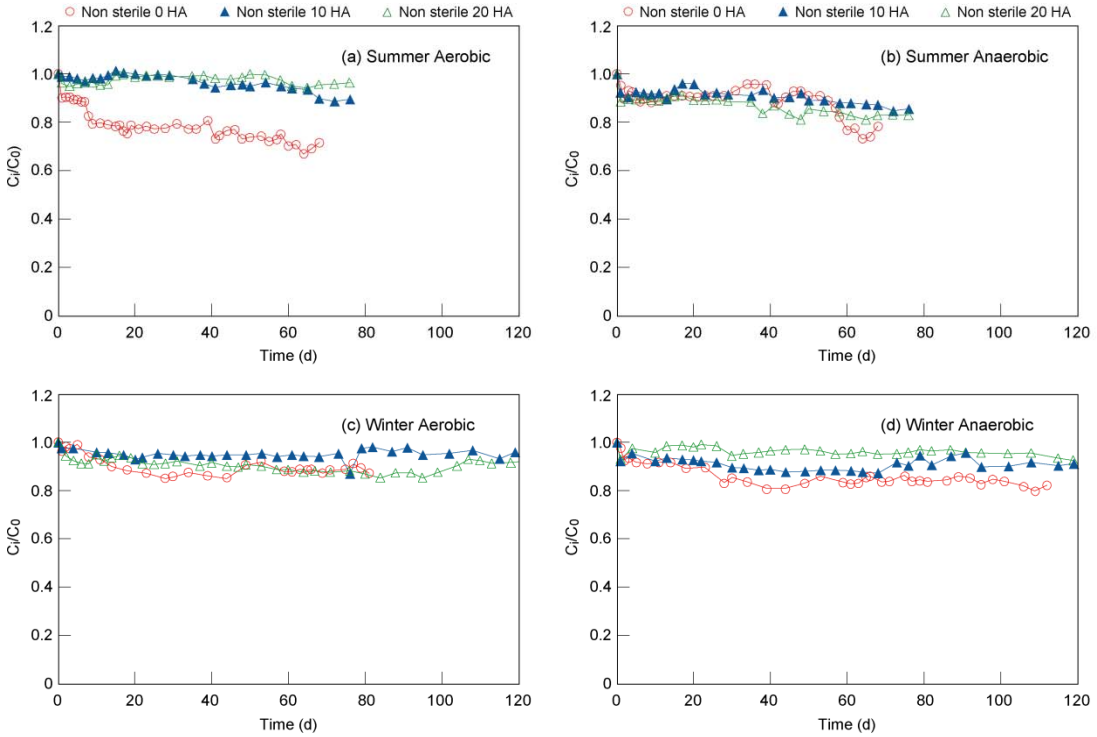


Figure 20: BTCs of gemfibrozil during (a) summer and aerobic, (b) summer and anaerobic, (c) winter and aerobic, and (d) winter and anaerobic conditions.

A similar trend – the enhanced removal of gemfibrozil during summer and under aerobic conditions – but with a higher removal (> 80%) was observed during field (Hoppe-Jones et al., 2010) as well as laboratory (Rauch-Williams et al., 2010; Hoppe-Jones et al., 2012; Onesios and Bouwer, 2012) investigations. The adaptation time of the packing material used during this study was shorter compared to previous studies (Rauch-Williams et al., 2010; Onesios and Bouwer, 2012). In both studies, the packing material was adapted to the different environmental conditions for one to two years. The shorter adaptation time and the possibly lower amount of viable soil biomass may have limited the removal of gemfibrozil. According to Rauch-Williams et al. (2010) the presence of more viable soil biomass on the column media led to enhanced removal of gemfibrozil.

4.3.3. PhACs impacted by environmental conditions

Phenazone

Complete removal of phenazone occurred only in the presence of aerobic conditions (Fig. 21a, c), while limited removal (< 20%) was observed under anaerobic conditions regardless of the season and the level of organics (Fig. 21b, d). Under aerobic conditions and in the presence of lake water alone (no HA added) faster removal (Table 32) occurred during the summer compared to the winter (Fig 21a, c). Complete removal of phenazone occurred within 22 days during the summer and approximately 80 days during the winter. These results suggested that a shorter acclimation time, 20 d, during the summer as opposed to 70 d, during the winter were needed to develop the microbial population able to degrade phenazone.

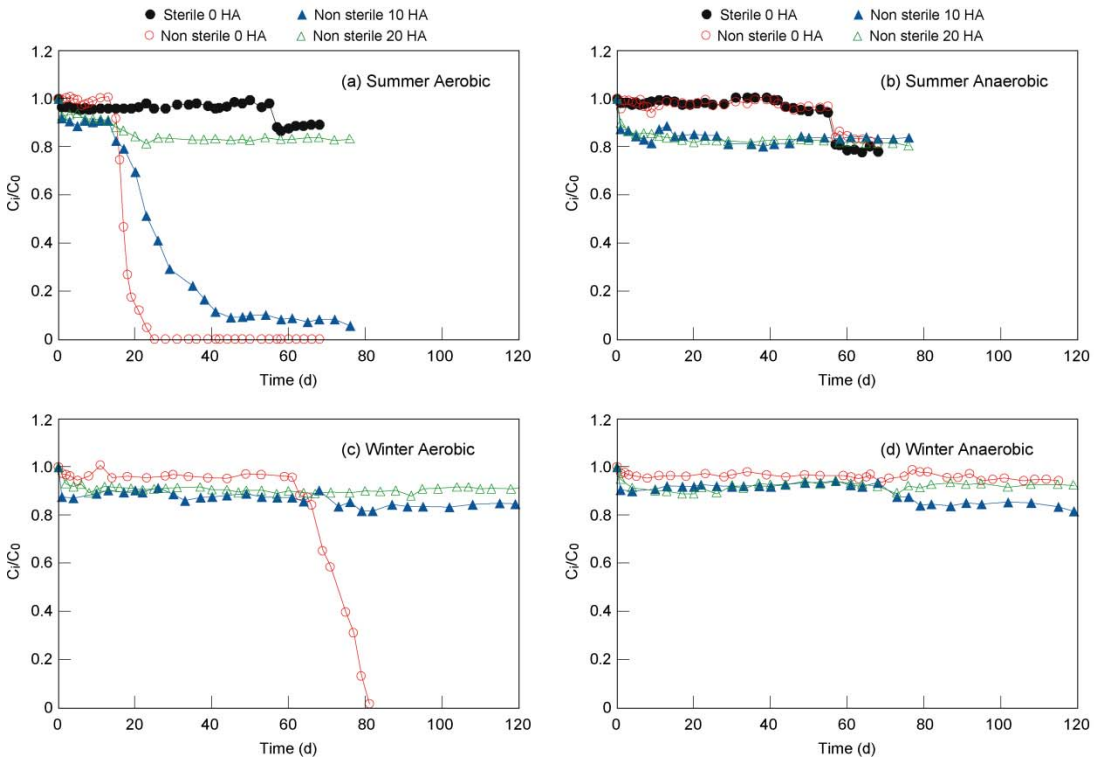


Figure 21: BTCs of phenazone during (a) summer and aerobic, (b) summer and anaerobic, (c) winter and aerobic, and (d) winter and anaerobic conditions.

Table 32: Equilibrium time, reaction rate, and half-life of phenazone in the presence of different environmental conditions.

	Summer Aerobic			Winter Aerobic		
	0 HA[a]	**10 HA[b]**	**20 HA[c]**	**0 HA[a]**	**10 HA[b]**	**20 HA[c]**
Equilibrium time (d)	18	18	-	70	-	-
Reaction rate (K)	0.32	0.06	-	0.09	-	-
Half-life ($t_{1/2}$ [d])	2.17	11.55	-	7.7	-	-
R^2	0.96	0.85	-	0.86	-	-

[a] 0 HA: TOC = 3mg/L; [b] 10 HA: TOC = 10 mg/L; [c] 20 HA: TOC = 20 mg/L

In the presence of feed water containing HA complete removal occurred only with a lower level of HA (TOC = 10 mg/L) under aerobic conditions during the summer. No removal was achieved under any other environmental conditions as well as in the presence of higher level of HA (TOC = 20 mg/L). However, faster removal occurred in the absence of HA in the feed water (0 HA vs. 10 HA).

Removal of phenazone was mainly due to biodegradation. No removal occurred under sterile conditions (Fig. 21a-b), suggesting that sorption plays a no to limited role in the removal of phenazone. The limited impact of sorption on phenazone removal was also shown by Zuehlke et al. (2006). The presence of HA may have precluded the development of favorable conditions for the biodegradation of phenazone.

Field studies (Massmann et al., 2006) as well as laboratory studies (Massmann et al., 2008; Zuehlke et al., 2006) confirmed the impact of redox conditions on the removal of phenazone-type PhACs. Zuehlke et al. (2006), using batch experiments, obtained complete removal of phenazone in the presence of aerobic conditions and with biologically active material collected from a location impacted by phenazone. No removal was observed in a column study conducted with clogged anaerobic sands from a bank filtration site (Massmann et al., 2006). A field investigation conducted at Lake Tegel, Germany, by Massmann et al., (2006) suggested that the presence of oxygen (influenced by the temperature) had greater impact on the removal of phenazone than the temperature itself.

According to the results of this study, redox conditions represented the predominant environmental factor affecting the removal of phenazone followed by the amount of HA and temperature. Phenazone was completely removed only in the presence of aerobic conditions. During the summer, a shorter acclimation period was needed to develop microorganisms able to fully remove phenazone.

Caffeine

The removal of caffeine was primarily impacted by the presence or absence of HA in the feed water (Fig. 22a-d). Both redox conditions and temperature, however, also affected the removal of caffeine. In the presence of HA, caffeine was removed regardless of the different environmental conditions, while in the presence of lake water alone (no HA added), complete removal of caffeine was achieved only during the summer under aerobic conditions (Fig. 22a). Regardless of the presence or absence of HA, the removal of caffeine was faster during the summer and in the presence of aerobic conditions (Table 33; Fig. 22a) than during the winter and under anaerobic conditions (Fig. 22d). High removal of caffeine (> 90%) was also observed by Benotti et al. (2012) in a pilot- and full scale RBF system in the presence of aerobic conditions.

Caffeine dropped at the beginning of each simulation regardless of the environmental conditions simulated as well as the sterility of the environment, suggesting sorption of caffeine to the packing material (Fig. 22a-b). However, progressive removal under non sterile conditions and no further removal under sterile conditions suggested that biodegradation represented the predominant removal mechanisms involved in lake water without HA (Fig. 22a-d). Biodegradation required a longer acclimation time during the winter (~ 120 d) than the summer (~ 10 d) and under anaerobic conditions compared to aerobic conditions (Fig. 22a-d). Complete removal of caffeine in the absence of HA occurred only during the summer and under aerobic conditions which are the most suitable conditions for biodegradation. Biodegradation was also the main mechanism involved during the removal of caffeine in a pilot- and full-scale RBF system (Benotti et al., 2012).

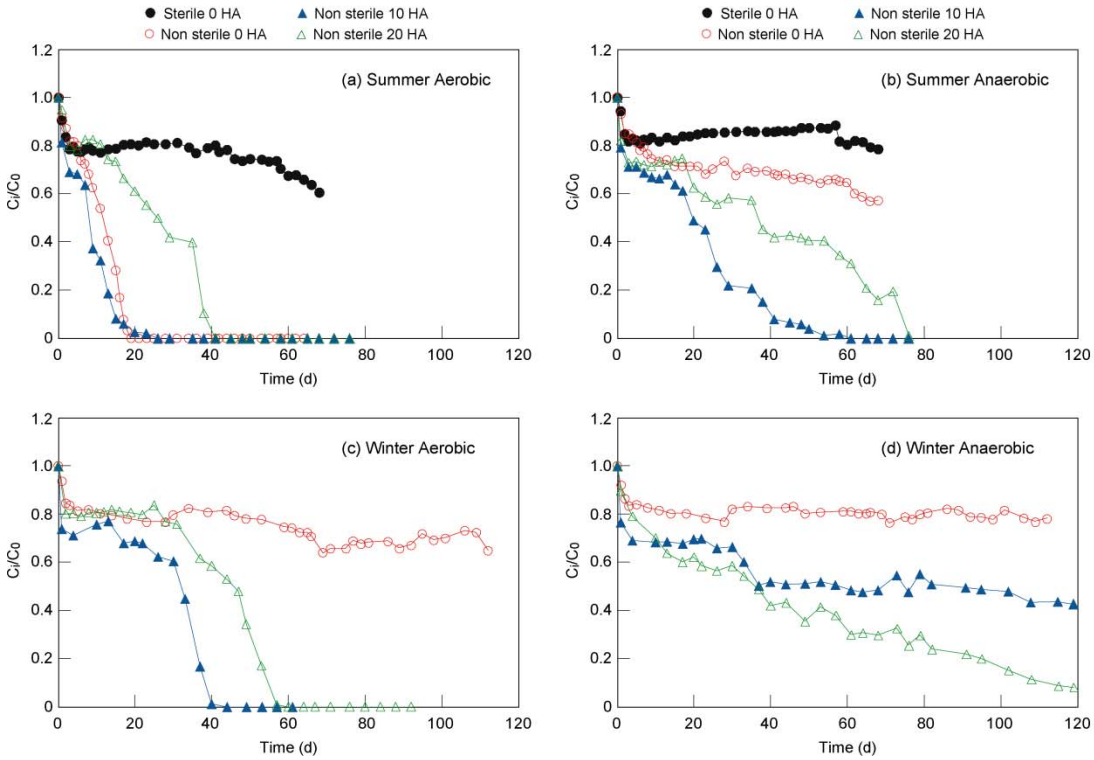


Figure 22: BTCs of caffeine during (a) summer and aerobic, (b) summer and anaerobic, (c) winter and aerobic, and (d) winter and anaerobic conditions.

Table 33: Equilibrium time, reaction rate, and half-life of caffeine in the presence of different environmental conditions.

	Summer Aerobic			**Winter Aerobic**			**Summer Anaerobic**			**Winter Anaerobic**		
	0 HA[a]	**10 HA[b]**	**20 HA[c]**	**0 HA[a]**	**10 HA[b]**	**20 HA[c]**	**0 HA[a]**	**10 HA[b]**	**20 HA[c]**	**0 HA[a]**	**10 HA[b]**	**20 HA[c]**
Equilibrium time (d)	-	-	-	-	30	35	-	-	-	-	-	-
Reaction rate (K)	0.091	0.182	0.053	-	0.0697	0.031	0.053	0.06	0.019	-	0.005	0.018
Half-life ($t_{1/2}$ [d])	7.62	3.81	13.08	-	9.94	22.36	130.19	11.55	36.67	-	141.46	38.94
R^2	0.86	0.94	0.9	-	0.70	0.90	0.71	0.94	0.88	-	0.75	0.95

[a] 0 HA: TOC = 3mg/L; [b]10 HA: TOC = 10 mg/L; [c]20 HA: TOC = 20 mg/L

In the presence of HA, complete removal of caffeine was observed during the winter and under anaerobic conditions both of which are limiting for biodegradation (Fig. 22a-d). During these limiting conditions for biodegradation no removal of caffeine was observed in the columns without HA. These results suggested that sorption to HA was the main removal mechanisms. However, this sorption also appeared to be affected by redox conditions and temperature. In the presence of winter conditions, greater removal occurred under aerobic than anaerobic conditions. Similarly, under anaerobic conditions, greater removal occurred during the summer rather than during the winter.

E2

Similar to caffeine, the removal of E2 was primarily impacted by the presence or absence of HA in the feed water (Fig. 23a-d). In the presence of lake water alone (no HA added) complete removal of E2 was achieved only under aerobic conditions (Fig. 23a, c), while in the presence of HA complete removal occurred regardless of the different environmental conditions (Fig. 23a-d).

In lake water alone, complete but slower removal occurred during the winter (Table 34; Fig. 23c-d), partial removal (approximately 80%) was achieved during the summer under anaerobic conditions (Fig. 23b). No removal was observed during the winter under anaerobic conditions (Fig. 23d).

The decrease of E2 in the sterile set-up that was observed after 40d was due to a loss of sterility in the water as revealed by the control (Table 31). These results suggested that in the absence of HA, removal of E2 was mostly related to biodegradation. Under conditions less favorable for biodegradation, winter and anaerobic, no removal of E2 was observed.

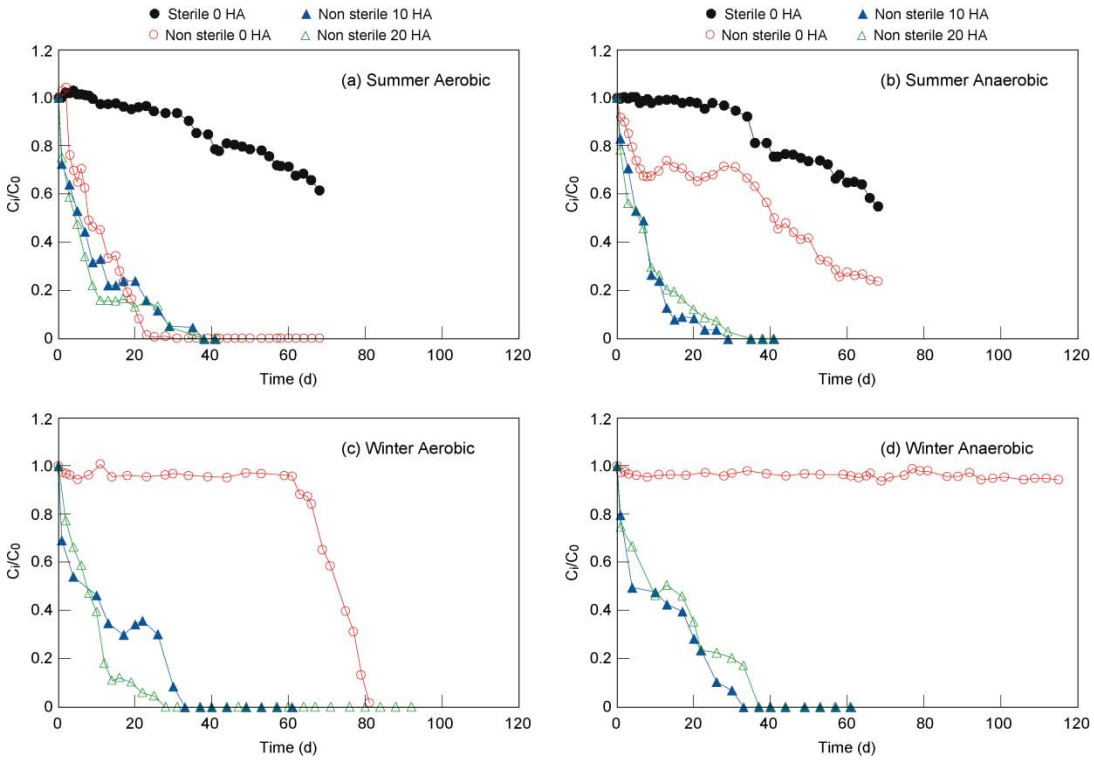


Figure 23: BTCs of E2 during (a) summer and aerobic, (b) summer and anaerobic, (c) winter and aerobic, and (d) winter and anaerobic conditions.

Table 34: Equilibrium time, reaction rate, half-life of E2 in the presence of selected environmental conditions.

	Summer Aerobic			**Winter Aerobic**			**Summer Anaerobic**			**Winter Anaerobic**		
	0 HA[a]	**10 HA[b]**	**20 HA[c]**	**0 HA[a]**	**10 HA[b]**	**20 HA[c]**	**0 HA[a]**	**10 HA[b]**	**20 HA[c]**	**0 HA[a]**	**10 HA[b]**	**20 HA[c]**
Equilibrium time (d)	-	-	-	-	30	35	-	-	-	-	-	-
Reaction rate (K)	0.091	0.182	0.053	-	0.0697	0.031	0.053	0.06	0.019	-	0.005	0.018
Half-life ($t_{1/2}$ [d])	7.62	3.81	13.08	-	9.94	22.36	130.19	11.55	36.67	-	141.46	38.94
R^2	0.86	0.94	0.9	-	0.70	0.90	0.71	0.94	0.88	-	0.75	0.95

[a] 0 HA: TOC = 3mg/L; [b]10 HA: TOC = 10 mg/L; [c]20 HA: TOC = 20 mg/L

In the presence of HA, E2 was removed regardless of the different environmental conditions, suggesting that sorption by HA played a crucial role in the removal of E2. A

relatively slower removal of E2 that occurred during the winter as opposed to the summer (Fig. 23c-d) suggested that temperature may also impact the adsorption of E2 by HA. Lee et al. (2011) showed that even if biodegradation was the predominant mechanism involved during E2 removal, sorption of E2 increased with an increasing concentration of HA. Sorption of E2 onto HA may also be expected due to the high log K_{ow} (Schwarzenbach et al., 1983).

E1

E1, the breakdown product of E2, was completely removed under most of the different environmental conditions but a slower removal than E2 was observed (Fig. 24a-d). This trend was probably related to the simultaneous degradation of E2 into E1. The level of E2 biodegradation and the production of E1 were impacted by the level of HA in the feed water. For example, under aerobic conditions during the summer maximum degradation of E2 was observed and consequently the concentration of E1 present in the feed water increased.

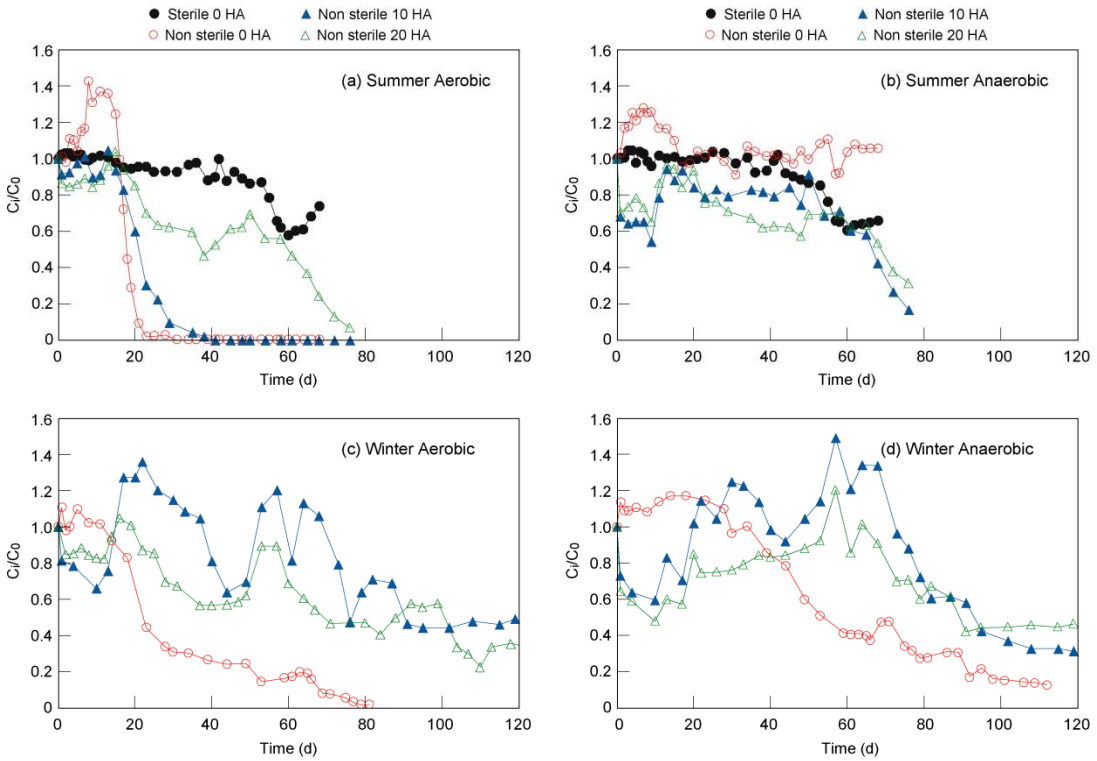


Figure 24: BTCs of E1 during (a) summer and aerobic, (b) summer and anaerobic, (c) winter and aerobic, and (d) winter and anaerobic conditions.

However, this trend occurred primarily in the presence of lake water alone (no HA). In the presence of HA, reduced biodegradation of E2 was observed (due to the predominant removal driven by sorption) and consequently limited production of E1 occurred. The limited transformation of E2 into E1 in the presence of HA was also observed by Lee et al. (2011).

4.4. CONCLUSIONS

- RBF can be effectively used to remove PhACs present in surface waters. However the geochemistry of the RBF site is expected to play a key role in their removal.
- Depending on the compound, removal of PhACs may predominantly occur due to biodegradation; however environmental variables, such as temperature, HA and oxygen may enhance or limit biodegradation.

- Depending on the compound, removal of PhACs may occur due to sorption to HA especially under conditions unfavorable to biodegradation.
- Limited and slower removal of selected PhACs may occur during winter temperature conditions.
- Limited removal of carbamazepine (< 10%) occured regardless of the different environmental conditions simulated. Limited removal of gemfibrozil (< 30%) was also observed. Temperature and level of organic matter may impact the behavior of gemfibrozil.
- Phenazone was highly impacted by the different environmental conditions according to the following order: level of oxygen > level of HA > temperature. Phenazone was completely removed only under aerobic conditions, and in the presence of a low to moderate levels of HA (TOC < 10 mg/L). Faster removal (4 x) occurred during the summer than during the winter.
- Caffeine was also impacted by the different environmental conditions according to the following order: level of HA > temperature > level of oxygen. In the presence of lake water alone (no HA) caffeine was completely removed only during the summer under aerobic condition. On the other hand, caffeine was also completely removed regardless of the different environmental conditions in the presence of HA. However, removal was twice as fast during the summer under aerobic conditions than winter. Biodegradation and sorption are the main mechanisms impacting the removal of caffeine.
- E2 was completely removed under the different environmental conditions expect under anaerobic conditions in the absence of HA.
- E1 was impacted by the different environmental conditions according to the following order: level of oxygen > level of HA > temperature. E1 was completely removed only under aerobic conditions. In particular, during the summer E1 was removed regardless of the presence or absence of HA. During the winter, E1 was completely removed only in the presence of a low level of HA.

CHAPTER 5. IMPACT OF INFILTERED AIR ON THE DYNAMIC OF REDOX CONDITIONS AS WELL AS ON THE REMOVAL OF PHARMACEUTICALLY ACTIVE COMPOUNDS IN SIMULATED RIVERBANK FILTRATION DURING CLOGGING CONDITIONS

Abstract

Riverbank filtration (RBF) has been used to provide drinking water to communities for more than a century in Europe and for half a century in the United States. The two major limitations to the effectiveness of the RBF are riverbed clogging and the development of an unsaturated zone near the collector wells. The development of an unsaturated zone beneath the riverbed can reduce the hydraulic conductivity due to compaction of the porous media, limit the water production and also affect the redox conditions at the riverbank filtration site. The change in redox conditions may also impact the removal of pharmaceutically active compounds (PhACs).

The objective of this study was to investigate the impact of air present below the clogging layer on the dynamics of redox conditions and on the removal of six selected PhACs, caffeine, carbamazepine, 17-β estradiol (E2), estrone (E1), gemfibrozil, and phenazone.

Results from the study suggested that RBF can be used to treat most of the PhACs, but the geochemistry of the RBF site needs to be carefully investigated. The occurrence of air beneath the clogging layer may create aerobic areas that enhance removal of PhACs.

All selected PhACs showed limited removal under reduced oxygen conditions (< 2mg/L). Carbamazepine and gemfibrozil showed no removal under all the different environmental conditions. The level of oxygen and the presence of humic acid (HA) impacted the removal of caffeine, E1, E2 and phenazone.

5.1. INTRODUCTION

Riverbank filtration (RBF) has been used to provide drinking water to communities for more than a century in Europe and for half a century in the United States (Ray et al., 2002). RBF provides about 50% of the potable water in the Slovak Republic, 45% in Hungary, 16% in Germany and 5% in The Netherlands (Dash et al., 2008). The RBF facilities in the continental USA are located in Kentucky, Missouri, Kansas, Nebraska, and California. Many other countries around the world including India, China, Korea, Jordan, and Egypt have recently started to evaluate the feasibility of using RBF for water treatment (Ray, 2008; Ray and Shamrukh, 2011).

The advantages of RBF compared to other water technologies are related to the low capital investments and low operating costs, as well as the ability to remove suspended particles, biodegradable compounds, bacteria, and viruses (Ray et al., 2002). RBF can also buffer possible fluctuations in the quality of the water produced (Ray et al., 2002).

The two major limitations to the effectiveness of the RBF are riverbed clogging (Schubert, 2002) and the development of an unsaturated zone near the collector wells (Su et al., 2007). Other possible limitations of RBF are related to the increases in hardness, ammonium, dissolved iron and manganese, and the possible formation of malodorous sulfur compounds all of which can be due to changing redox conditions.

Riverbed clogging can be due to physical, biological, and/or chemical processes (Bouwer, 2002). Physical clogging is the accumulation of inorganic and organic suspended solids in the recharge water. Biological clogging includes the accumulation of algae and bacterial floc in the water onto the infiltrating surface and the growth of micro-organisms on and in the soil to form biofilms and biomass that block pores and/or reduce pore size (Bouwer, 2002). Chemical clogging is related to the precipitation of calcium carbonate, gypsum, phosphate, and other chemicals on and in the soil.

The development of an unsaturated zone beneath the riverbed may occur if the pumping rate is higher than the recharge rate, or if the recharge from a losing river is less than the saturated hydraulic conductivity of the aquifer. This unsaturated zone can reduce the

hydraulic conductivity due to compaction of the porous media, limit the water production and also affect the redox conditions at the riverbank filtration site. The change in redox conditions may also impact the removal of pharmaceutically active compounds (PhACs).

PhACs (Holm et al., 1995; Ternes et al., 2004; Massmann et al., 2006), halogenated organic compounds (Kao et al., 2003; Grünheid et al., 2005), pesticides (Agertved et al., 1992; Patterson et al., 2002) and disinfection by-products (Pavelic et al., 2005) have all been shown to be impacted by different redox conditions and temperature variations.

The behavior of PhACs at bank filtration sites have been showed to be impacted primarily by their physico-chemical properties and by the local conditions, such as redox conditions, temperature and travel time (Table 1, Chapter 1). For example, phenazone-type analgesics were redox sensitive and were eliminated more efficiently under oxic conditions than anoxic conditions (Massmann et al., 2008). However, the redox conditions and thus the elimination of these chemicals are highly impacted by temperature. In fact, Massmann et al. (2008) showed that microbially mediated degradation of phenazone which was only effective under oxic conditions, and that these conditions were encountered only near the shore during the winter when the temperatures were very low. On the other hand, compounds such as gemfibrozil and ibuprofen, have been shown to be more efficiently removed during the summer than winter (Hoppe-Jones et al., 2010). During the winter, additional travel time was required to reduce the concentrations of these compounds to an amount equivalent to the reduction observed during the summer.

The objective of this study was to investigate the impact of air present below the clogging layer on the dynamics of redox conditions and on the removal of six selected PhACs - caffeine, carbamazepine, 17-β estradiol (E2), estrone (E1), gemfibrozil, and phenazone. The impact of organic matter (humic acid) on the removal of selected PhACs was also investigated.

During the passage of lake water through both sides of the sandbox, clogging can occur, leading to a reduction in flow. During the infiltration, as oxygen is depleted, a low redox

condition is expected and limited removal of the selected PhACs may occur. In the presence of a more developed clogging layer, more extended reduced conditions are expected. Local aerobic conditions would be developed by introducing air under the clogging layer, which may occur in field conditions under sustained pumpage when a clogging layer exists. Under these conditions, enhanced removal of selected PhACs such as E1, E2 and phenazone may occur.

5.2. MATERIALS AND METHODS

5.2.1. Selection of PhACs

The PhACs investigated during this study were selected according to the same multi-step approach used during chapter 4: i) occurrence in the environment and public interest, ii) toxicity, iii) environmental fate, iv) behavior under different redox conditions, v) pharmaceutical class, and vi) availability of analytical standards and adequate instrumentation. Additional information are provided in Chapter 1.

5.2.2. Chemicals

Caffeine (100% pure), carbamazepine (100% pure), estrone (E1 [99% pure]), 17-β estradiol (E2 [100% pure]), gemfibrozil (99.9% pure), phenazone (99.9% pure) and humic acid – sodium salt were purchased from Sigma-Aldrich (St. Louis, MO); HPLC-grade methanol and acetonitrile were purchased from EMD Chemicals Inc. (Gibbstown, NJ). The physico-chemical properties of the selected PhACs used in this study are provided in Table 5.

5.2.3. Packing materials

Silica sand (0.6–1.2 cm, Orange County # 16) with a uniformity coefficient of 1.57 and a porosity of 0.42 was used as the main packing material for the sandbox. Pea gravel with an effective size of 0.95 cm was used as the underlying supporting material for the sand in the sandbox.

5.2.4. Feed water

Feed water was collected from Lake Wilson (21º29'41" N, 158º01'29" W, Wahiawa State Freshwater Park, HI, USA) (Fig. A1-Appendix A). Lake Wilson is partially impacted by the Wahiawa Wastewater Treatment Plant (approximately 2mgd, http://www.state.hi.us/dlnr/pdf/lakewilson.pdf). Surface water samples were collected 1m away from the bank of the lake and stored at 4 °C prior to being used. Lake Wilson was selected due to its higher total organic carbon (TOC), approximately 3 mg/L, compared with most of the streams in Hawaii, which are approximately 1 mg/L TOC. Prior to being used as feed water during this study, the lake water was pre-filtered through 11 μm paper filters (Whatman, GE Healthcare Life Sciences, Piscataway, USA), to remove large particles that may clog the sandbox.

5.2.5. Experimental setup

A sandbox consisting of two side-by-side rectangular boxes (with width, height, and depth dimensions of 140x30x10 cm - each) made of transparent plexiglass sandbox were used throughout the study (Fig. A5–Appendix A). The sandbox was covered with a thick black cardboard in order to prevent any possible microbial growth due to light penetration through the plexiglass. The top of the sandbox was also covered in order to reduce possible evaporation and minimize the effect of the light. The sandbox was dry-packed with small increments of sand and manually compacted during this process. Five cm of pea gravel were placed at the bottom of the sandbox to support the sand. To minimize air entrapment, both sides of the sandbox were slowly wetted from the bottom by capillarity. A stainless steel mesh (TWP, Berkeley, CA) having a wire diameter of 0.5 mm was placed at the bottom of each side of the sandbox to support the packing material, while a fine stainless steel wire cloth (TWP, Berkeley, CA) having a wire diameter of 40 μm was placed above the 0.5 mm mesh to prevent possible release of fine particles. An overflow located 4 cm above the top layer of sand was used to provide a constant water level above the sand. The outflow from each side of the sanbox was constantly monitored throughout the study by using two automated balances connected to a datalogger (Campbell Scientific, Logan, UT). Each side of the experimental sandbox was equipped with oxygen probes (Ocean Optics, Dunedin, FA) and redox probes (HYPNOS III, The

Netherlands) for inline measurements, and sampling ports. Three oxygen probes were located 10, 50, and 105 cm below the water-sand interface on each side of the sandbox. Four redox probes were located 5, 15, 50, and 105 cm below the water-sand interface. Seven stainless steel sampling ports were located at 10, 15, 20, 40, 85, 135, and 140 cm, below the water-sand interface. The number of probes and sampling ports was higher in the top section of the sandbox to monitor the removal occurring just beneath the biological layer (*Schmutzdecke*) formed on the top surface of the sand. A dual-head peristaltic pump (Cole-Parmer, East Bunker Court Vernon Hills, IL) was used to simultaneously introduce the feed water to the two sides of the sandbox under down-flow conditions (feed water was not recirculated).

5.2.6. Experimental conditions

The experimental conditions selected for use with the sandbox were chosen based on the results from the column experiments presented in chapter 4. From the different combinations of oxygen and temperature, aerobic and summer conditions were selected. Lake water alone and lake water with HA (TOC = 20 mg/L) were selected.

For a month prior to the start of this study, the sandbox was fed from top to bottom with tap water at the hydraulic loading rate of 3.5 mL/min. The empty bed contact time approximately 24 hours.

For 16 days, both sides of the sandbox were conditioned with water collected from Lake Wilson and filtered through 11 μm filter paper (GE Healthcare Bio-Sciences, Pittsburgh, PA). On day 16, one side of the sandbox, referred to as LW, received unfiltered lake water alone (TOC = 3 mg/L), while the other side, referred to as LW-HA, received unfiltered lake water with HA (TOC = 20 mg/L). On day 21, a mixture of 6 PhACs (caffeine, carbamazepine, E1, E2, gemfibrozil, and phenazone) was added to the feed water of each side (referred to as a spike) to evaluate the ability of the system to remove the selected PhACs under different environmental conditions, and the impact of organic matter (HA) on the removal of PhACs. The spike was performed for approximately 30 days, after which the injection of PhACs was suspended for

approximately 60 days. During this period of no injection of PhACs LW received lake water alone, while LW-HA received lake water with HA. The particles present in the feed water caused a clogging layer to develop at the water-sand interface in LW and LW-HA which led to a reduction in the outflow. Prior to the start of the second spike, the outflow was increased to match the outflow occurred during the first spike, and air was introduced below the sand-water interface by placing multiple pipettes (open to the atmosphere) in the top 2 cm of the packing material. This led to the presence of locally aerobic areas. This new configuration was established to simulate the high pumping rate that occurs in the presence of developing clogging at a RBF site, and the possible entry of air from the bank. These conditions were observed at two riverbank filtration facilities: Sonoma County Water Agency (Su et al., 2007) and Louisville Water Company (personal communication). After new conditions (presence of air below the water-sand interface) were established in both sides of the sandbox, a second spike of 6 PhACs was conducted for approximately 30 days. During both spikes, PhACs at a concentration of approximately 50 μg/L were added to the feed water.

5.2.7. Analysis of the outflow

During the 21 days of acclimation of the sand in the sandbox, the effluent was analyzed in terms of total organic carbon (TOC), dissolved organic carbon (DOC), and specific ultraviolet absorbance (SUVS). TOC was determined using a TOC analyzer (Shimadzu, Columbia, MD) coupled with a total nitrogen (TN) detector. To measure DOC, samples were filtered through a 0.45 μm GHP syringe filter (Pall, Port Washington, NY) and analyzed using the TOC analyzer. The amount of organic matter was also evaluated in terms of SUVA. SUVA is defined as the ratio between DOC and UV absorbance at 254 nm, which represents the relative aromaticity of organic matter (Amy and Drewes, 2007) and can be used as a measure of HA. UV absorbance at a wavelength of 254 nm was determined using a DR/4000 Spectrophotometer (HACH, Loveland, Colorado, USA) equipped with a quartz cell. The sample was pre-filtered through a 0.45 μm duapore membrane filter (VWR, Arlington Heights, IL) using a 10 mL glass syringe and filter

head. Stains on the surface and air bubbles on the inner cell wall of the cuvette were carefully avoided. Between each reading the cuvette was flushed with Milli-Q water.

UV-254 was below 0.2 in LW and below 1.4 in LW-HA throughout the study.

Redox potential (Eh) in different portions of the sandbox was measured using HYPNOS III (The Netherlands). The device incorporates a control unit, a multiplexer, storage memory and Eh probes. The redox probes consisted of a fibreglass shell, having a diameter of 8mm, equipped with platinum wire tips that were partly buried in the fibreglass (PaleoTerra, Amsterdam, The Netherlands).

Dissolved oxygen was measured using fiber optic T1000 oxygen probes (Ocean Optics, Dunedin, FA) coupled with temperature sensors. These oxygen probes use the fluorescence of a chemical complex in a sol-gel to measure the partial pressure of oxygen. A pulsed blue LED sends light, at ~475 nm, to an optical fiber which carries the light to the probe. The distal end of the probe tip consists of a thin layer of a hydrophobic sol-gel material (*http://www.oceanopticssensors.com/theory.htm*). A sensor formulation is trapped in the sol-gel matrix, effectively immobilized and protected from water. The light from the LED excites the formulation complex at the probe tip. The excited complex fluoresces, emitting energy at ~600 nm. If the excited complex encounters an oxygen molecule, the excess energy is transferred to the oxygen molecule in a non-radiative transfer, decreasing or quenching the fluorescence signal (*http://www.oceanopticssensors.com/theory.htm*). The degree of quenching correlates to the level of oxygen concentration or to oxygen partial pressure in the film, which is in dynamic equilibrium with oxygen in the sample. The energy is collected by the probe, carried through the optical fiber to the spectrometer and this data is then converted to oxygen concentration.

Once the selected PhACs were added, samples from the sampling ports and the effluent were analyzed for PhACs according to the USEPA Method 1694 (USEPA, 2007) with an HPLC system (Thermo Finnigan, Waltham, MA) coupled with a photo diode array (PDA) detector and a fluorescence detector (Shimadzu, Columbia, MD). The PhACs

were separated using a reverse phase column (100.0 x 2.1 mm, 3.0 µm, Restek, Bellefonte, PA) protected by a guard column with a matching stationary phase. Both columns were kept at 30 °C during analysis. Acetonitrile and DI water were used as the mobile phase. Caffeine and carbamazepine were detected at 204 nm, and the remaining PhACs at 200 nm.

5.3. RESULTS AND DISCUSSIONS

5.3.1 Dynamics of oxygen and redox potential

The dynamics of oxygen in both sides of the sandbox are shown in Figure 25a-b. During the acclimation period, in the presence of filtered lake water alone, three separate zones occurred within each side of the sandbox. In LW, oxygen ranged between 7 and 9 mg/L in the 10 cm below the water-sand interface (first oxygen probe), between 2 and 5.2 mg/L 50 cm below the water-sand interface (second oxygen probe) and between 2 and 4.2 mg/L 105 cm below the water-sand interface (third oxygen probe) (Fig. 25a). Oxygen was relatively stable during the first 7-8 days, after which it began to drop throughout LW (Fig. 25a). Oxygen decreased from 7 to 3.7 mg/L at the first oxygen probe and from 3.7 to 2 mg/L at the second oxygen probe (Fig. 25a). A constant level of oxygen, 1.8 mg/L, was observed in the lower portion of LW (Fig. 25a).

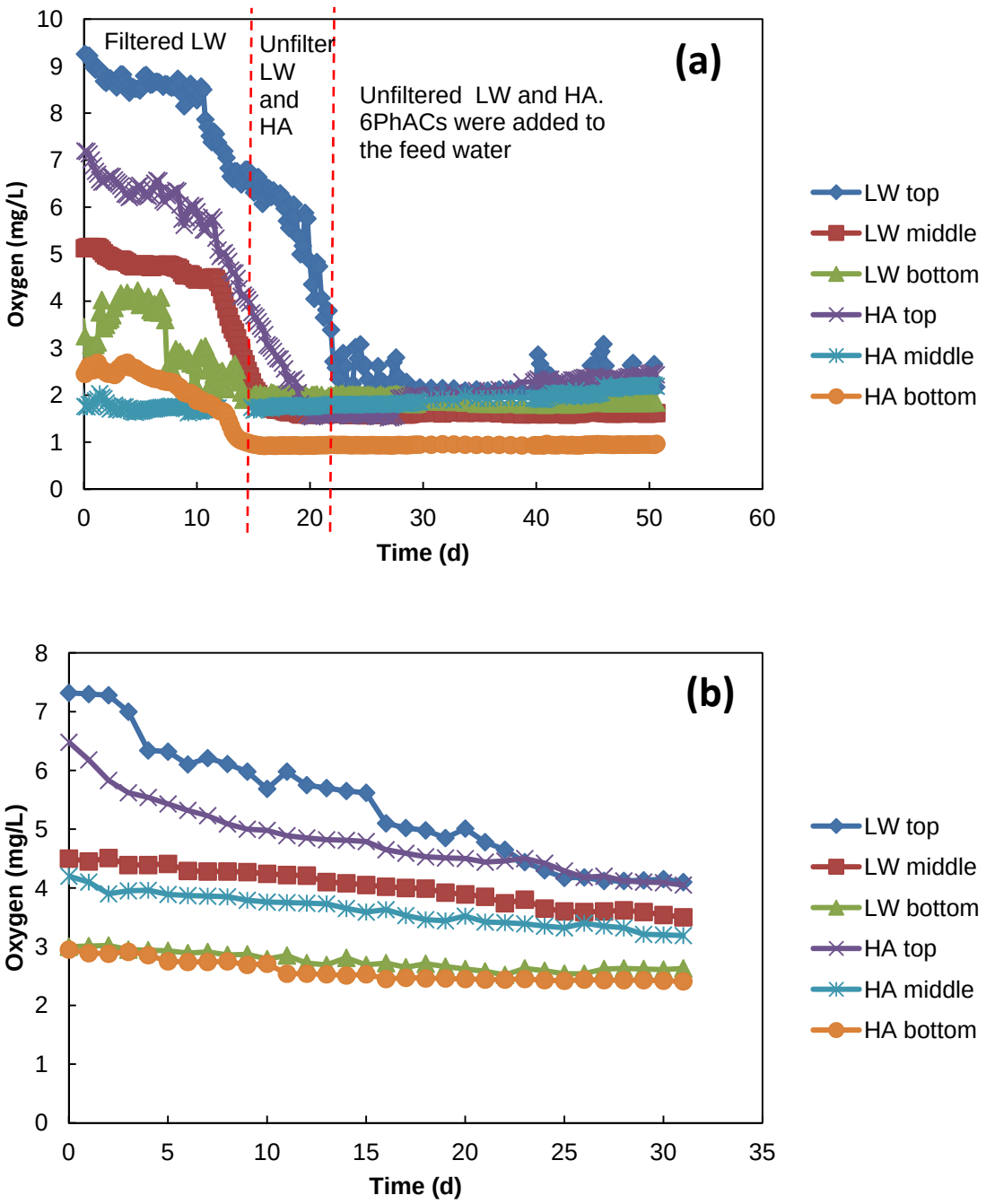


Figure 25: Dynamics of oxygen during the (a) first spike, and (b) second spike of selected PhACs.

During most of the first spike, a relatively low and stable level of oxygen, ranging between 2 mg/L near the water-sand interface and 1.8 mg/L at the bottom, occurred throughout LW.

A similar behavior was also observed in the second side of the sandbox, LW-HA, fed with lake water and HA (TOC = 20 mg/L). The starting levels of oxygen, however, were slightly lower in LW-HA than LW. Starting levels of oxygen of 7.2, 3, and 2 mg/L were observed at the first, second and third oxygen probe, respectively (Fig. 25a). During the acclimation period, a rapid depletion of oxygen occurred in the top 10 cm below the water-sand interface. Oxygen dropped from 7.2 to 3.7 mg/L (Fig. 25a). Further depletion occurred during the five days (day 17 to day 21) when LW-HA was fed with lake water ans HA. Prior to the start of the first spike, on day 21, the level of oxygen was below 2 mg/L at the first and second oxygen probe (Fig. 25a). An even lower level of oxygen (1 mg/L) was observed in the lower portion of LW-HA (third oxygen probe). Oxygen was stable during the spike, ranging from 1.8 mg/L (first two probes) to 0.9 mg/L (third probe) (Fig. 25a).

The dynamics of oxygen in both sides of the sandbox are shown in Figure 26.. During the acclimation period, high values of Eh (~500 mV) occurred throughout each side of the sandbox suggesting the presence of oxidizing conditions. After the injection of selected PhACs into the feed water, a progressive reduction of Eh was observed between day 25 and day 30 in the lower section of the sandbox. During this period, in LW, Eh dropped to 200, 60, 0, and -100 mV at the first (5cm below the water-sand interface), second (15 cm below the water-sand interface), third (50 cm below the water-sand interface) and forth (105 cm below the water-sand interface) redox probe, respectively. After this drop, Eh was relatively stable. A similar trend was also observed in LW-HA. These results suggested that reduced conditions were achieved in the sandbox. Reduction of manganese and iron may occur in the sandbox. In fact, reductions of manganese and iron are expected to occur at 200 mV, and between 100 and -100 mV, respectively (Mitsch and Gosselink, 2007).

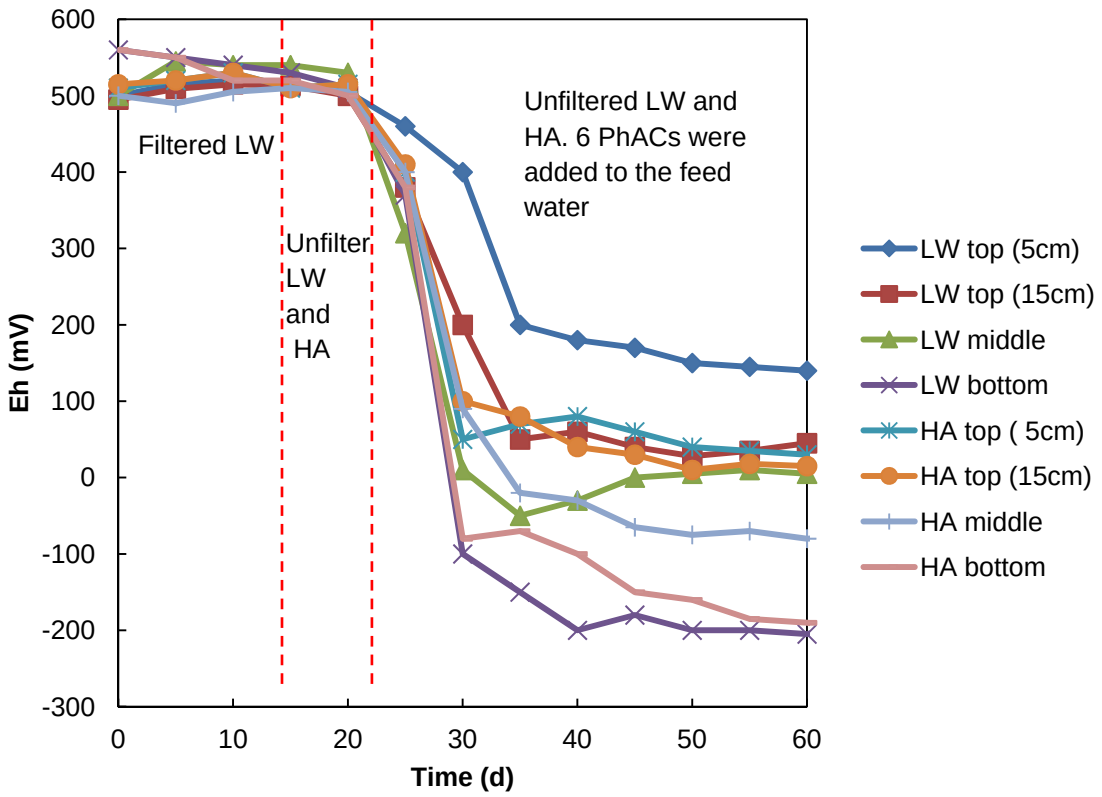


Figure 26: Dynamics of redox potential prior and during the first spike of selected PhACs.

Prior to starting the second spike, pipettes were used to introduce air into the top section (2-3 cm) of both sides of the sandbox. In LW, starting values of oxygen of 8.2, 4.5, and 3 mg/L were observed after the first, second, and third oxygen probe, respectively (Fig. 25b). During the spike, oxygen started to deplete primarily in the upper portion of LW, while a limited reduction occurred at the second and third probe. In LW, at the first oxygen probe which was 10 cm below the water-sand interface, the level of oxygen decreased to 5.8, 4.8, and 4 mg/L after 10, 20, and 30 days of the spike, respectively (Fig. 25b). During the same time, oxygen ranged between 4.5 and 3.7 mg/L at the second oxygen probe and between 3 and 2.5 mg/L at the third oxygen probe of LW (Fig. 25b). A similar trend was observed in LW-HA. The starting concentrations of oxygen were 6.5, 4.2 and 3 mg/L at the first, second, and third oxygen probe, respectively (Fig. 25b). Ten cm below the sand-water interface, oxygen decreased to 5, 4.5 and 4.1 mg/L after 10, 20,

and 30 days of the spike (Fig. 25b). No significant depletion of oxygen was observed at the second and third probe. Oxygen ranged between 4.2 and 3.2 mg/L at the second probe and from 3 to 2.5 mg/L in the lower portion of LW-HA (Fig. 25b).

Results obtained using the oxygen probes suggested that reduced oxygen conditions existed during the first spike. The introd ofuction external air before the second spike increased the level of oxygen at least in the upper portion of the sandbox immediately below the water-sand interface.

5.3.2. PhACs persistent in the environment

Travel time through the sandbox was determined using KCl as a non-reactive tracer. Travel time from the feed water reservoir to the 7 sampling ports was 9, 12, 15, 24, 49, 70, 72 hours. Two additional hours (total of 74 hours) are needed for the outflow.

Among the selected PhACs, carbamazepine and gemfibrozil were persistent under the different experimental conditions.

<u>Carbamazepine</u>

Carbamazepine behaved much like the non-reactive tracer, KCl, during the two spikes in both sides of the sandbox (Fig. 27a-d). The concentration of carbamazepine at the different sampling ports, and in the effluent from both sides of the sandbox increased rapidly (Fig. 26a-d), and the C_i/C_0 ratio was constantly one during the two spikes. These results showed that no to limited removal (< 10%) of carbamazepine was observed regardless of the presence or absence of HA and the level of oxygen (Fig. 27a-d). The result of no to limited removal was in agreement with the results obtained with the column experiments (Chapter 4) as well as with field studies (Hoppe-Jone et al., 2010) and other laboratory experiments (Rauch-Williams et al., 2010; Maeng et al., 2011).

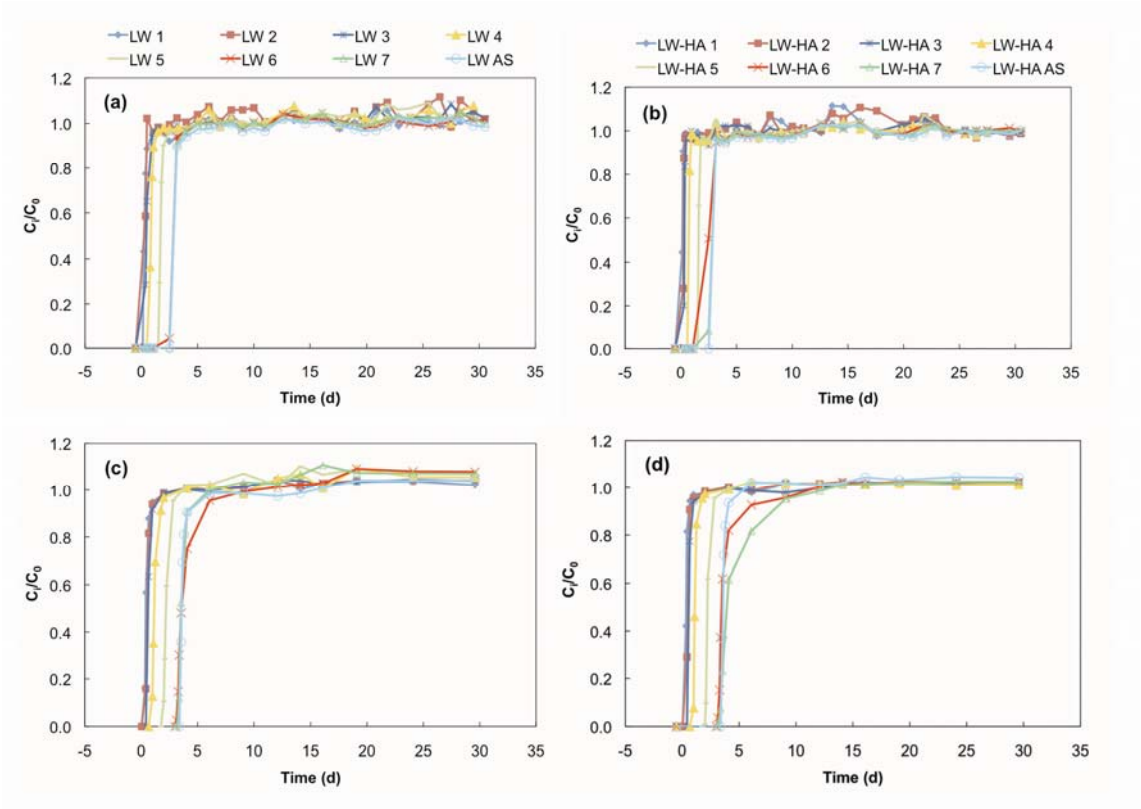


Figure 27 . BTCs of carbamazepine during the first spike in the presence of lake water alone (a), and lake water with humic acid (b); BTCs during the second spike in the presence of lake water alone (c), and lake water with humic acid (d).

Gemfibrozil

The concentration of gemfibrozil at each sampling port, as well as in the outflow of the sandbox increased rapidly (Fig. 28a-d) and the C_i/C_0 ratio was approximately one (plateau condition) during the two spikes. PhACs were added daily to the feed water, and this may have generated the irregularities of the BTCs during the plateau conditions. Gemfibrozil showed no to limited removal (10%) throughout the entire study (Fig. 28a-d). Results obtained with the column experiments in chapter 4 showed a limited removal, approximately 20%, of gemfibrozil only during summer and aerobic conditions. No removal occurred under any other environmental condition.

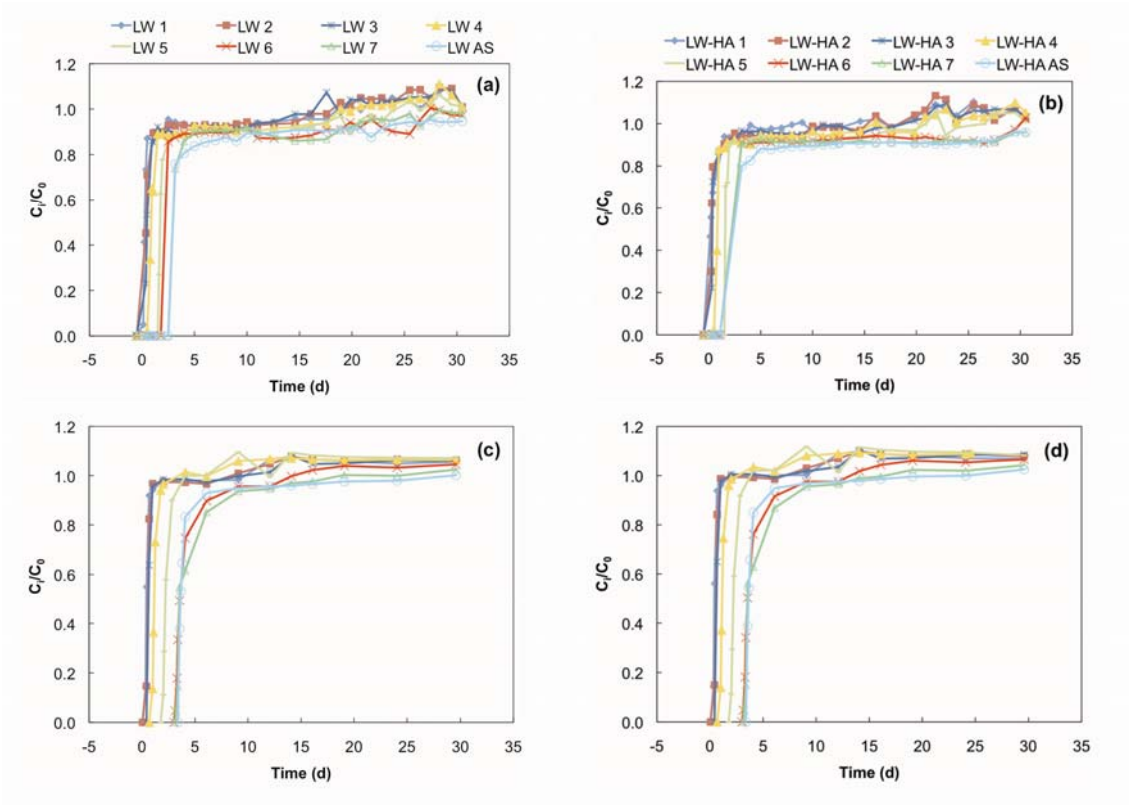


Figure 28. BTCs of gemfibrozil during the first spike in the presence of lake water alone (a), and lake water with humic acid (b); BTCs during the second spike in the presence of lake water alone (c), and lake water with humic acid (d).

Field (Hoppe-Jones et al., 2010) and laboratory study (Rauch-Williams et al., 2010; Hoppe-Jones et al., 2012; Onesios and Bouwer, 2012) have shown complete removal of gemfibrozil under summer and aerobic conditions. The oxygen levels in either side of the sandbox during both spikes indicated that even the portion near the water-sand interface was not saturated with oxygen. The reduced conditions present in the sandbox coupled with probably a low amount of viable biomass may be the reasons for the limited removal of gemfibrozil. Rauch-Williams et al. (2010) observed that enhanced removal of gemfibrozil occurred in columns with a large amount of viable biomass present in the column media.

5.3.3. PhACs impacted by the different redox conditions

<u>Phenazone</u>

During the first spike, regardless of the level of HA in the feed water, phenazone exhibited a behavior similar to carbamazepine and gemfibrozil (Fig. 29a-b). The rapid appearance of phenazone was observed in all the sampling ports as well as in the effluent (Fig. 29a-b). The C_i/C_0 ratio rapidly approached one and remained stable throughout the spike suggesting no to limited removal (< 10 %) of phenazone occurred in LW (Fig. 28a) and LW-HA (Fig. 28b). The small changes in the C_i/C_0 ratio were probably related to the fact that the PhACs mixture was prepared daily to reduce any possible degradation of the selected PhACs.

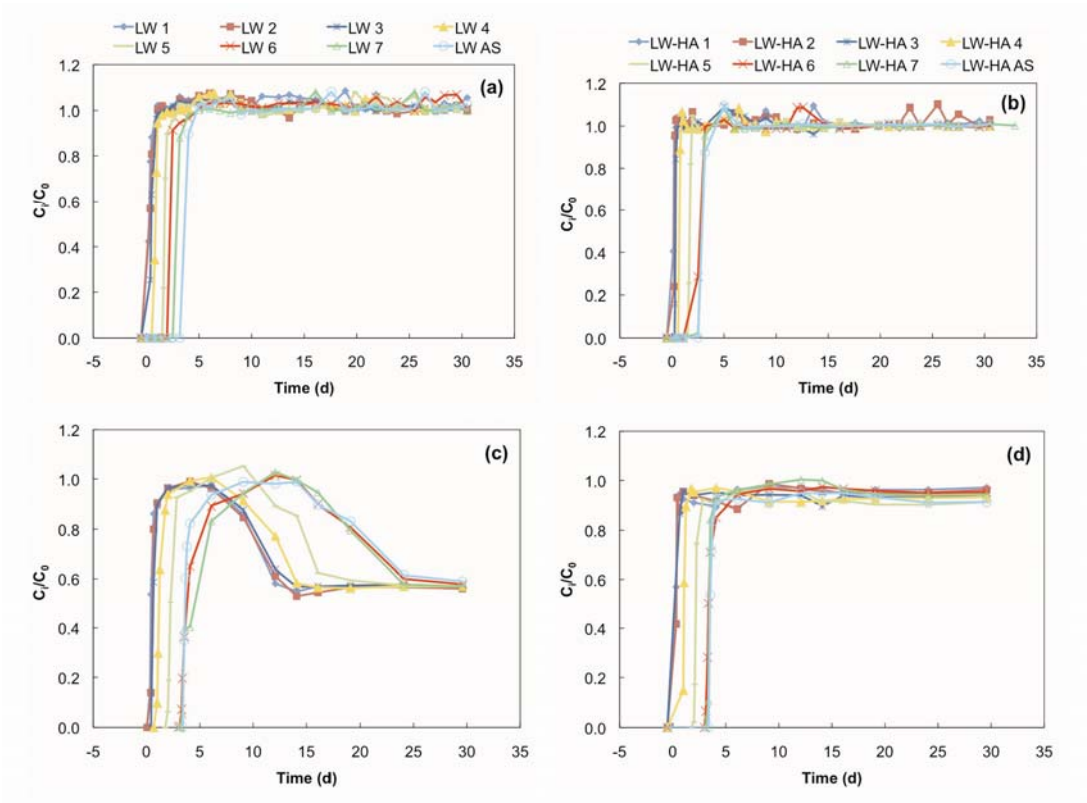


Figure 29. BTCs of phenazone during the first spike in the presence of lake water alone (a), and lake water with humic acid (b); BTCs during the second spike in the presence of lake water alone (c), and lake water with humic acid (d).

During the second spike in LW, which received lake water alone, there was a decrease in the C_i/C_0 at sampling ports 1, 2, and 3 that start at day six and plateau at 0.6 after 13 days (Fig. 29c). Gradually the C_i/C_0 ratio of phenazone at the remaining sampling ports also

reached 0.6 and remained stable throughout the study (Fig. 29c). These results suggested that the removal of phenazone started to occur in LW. In LW-HA, the side of the sandbox receiving lake water with HA, the C_i/C_0 ratio was constant at approximately one, suggesting that no removal of phenazone occurred (Fig. 29d).

Results obtained in chapter 4, in agreement with field studies (Massmann et al., 2006) as well as laboratory studies (Zuehlke et al., 2006; Massmann et al., 2008), suggested that biodegradation was the predominant removal mechanism of phenazone and aerobic conditions were necessary for this removal. However, the level of HA contained in the feed water and the temperature can also affect the removal of phenazone. In particular, complete removal of phenazone is expected only under aerobic conditions and in the presence of a limited amount of HA (TOC < 10 mg/L). A high level of HA in the feed water (TOC ~ 20mg/L) may inhibit the biodegradation of phenazone, leading toward no to limited removal even in the presence of aerobic conditions. Results obtained using the sandbox confirmed this overall trend. During the first spike in the presence of reduced levels of oxygen (< 2mg/L) no removal of phenazone was observed in either side of the sandbox. On the other hand, during the second spike in the presence of higher levels of oxygen (4 mg/L) partial removal (~ 42%) of phenazone occurred. A higher level of oxygen (~ 8 mg/L) was probably needed to further reduce the concentration of phenazone in LW. During the second spike in LW- HA, in the presence of higher level of oxygen (4 mg/L), no removal of phenazone was observed due to inhibition by HA.

Caffeine

During the first spike, in the presence of lake water alone, a slow movement of caffeine was observed. The C_i/C_0 ratio slowly increased and reached one at the first two sampling ports days and 0.8 and 0.4 after the third and fourth sampling port after 6 (Fig. 30a). A progressive decrease of the C_i/C_0 ratio was observed from day 8 at the first four sampling ports (10 to 40 cm below the water-sand interface). The C_i/C_0 ratio ranged between 0 and 0.1 between day 15 and day 20, and then it progressively increased between day 20 and day 30 (Fig. 30a). After that the C_i/C_0 ratio started to decrease, and at the end of the spike was below 0.2 in the entire unit (Fig. 30a). In the presence of HA (LW-HA), the movement of caffeine mimics the movement observed in LW, however the extent of the

fluctuation of the C_i/C_0 ratio was limited (Fig. 30b). The C_i/C_0 ratio, after reaching a maximum of 1, 0.9, 0.7 and 0.4 at the first four sampling ports by day 6, started to decrease (Fig. 30b). The C_i/C_0 ratio reached a minimum, approximately 0.2, between day 15 and day 17 and then started to increase again.

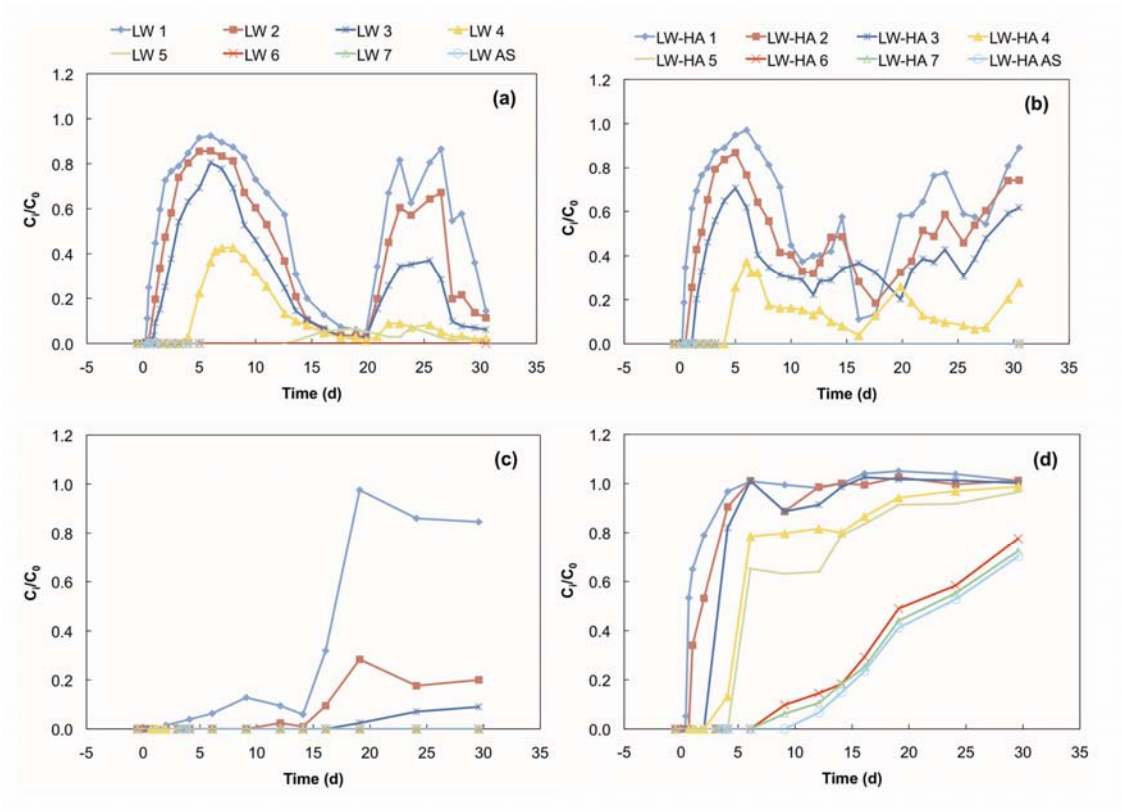


Figure 30. BTCs of caffeine during the first spike in the presence of lake water alone (a), and lake water with humic acid (b); BTCs during the second spike in the presence of lake water alone (c), and lake water with humic acid (d).

During the second spike in LW caffeine did not appear in port 1, located 10 cm below the water-sand interface, until day 14 (Fig. 30c). Then the C_i/C_0 ratio increased to 1 and a slight decrease occurred at day 19 (Fig. 30c). At port 2, located 5 cm below port 1, caffeine appeared on day 16, reached a maximum of the C_i/C_0 ratio of 0.3 by day 13, and it remained constant after that (Fig. 30c). At the beginning of the second spike in LW-HA caffeine increased in the upper portion and the C_i/C_0 ratio reached 1 by day 5 in port 1, 2, and 3 (Fig. 30d). After 7 days, at port 4 and 5 the C_i/C_0 ratio reached 0.8 and 0.6. From

day 6, caffeine was also observed in the lower section of LW-HA at port 6, port 7, and in the effluent (Fig. 30d). Caffeine started to progressively increase throughout LW-HA. The slow movement of caffeine observed during both spikes may be due to the presence of bacteria able to degrade caffeine. However the bacteria may change over time (as shown in chapter 3) and this can lead to the fluctuation observed in C_i/C_0 during the first spike. During the second spike, the higher level of oxygen present in the sandbox (4 mg/L) compared to the first spike (2mg/L) may have enhanced the presence of bacteria able to remove caffeine. In addition, during the second spike, the packing material had already been exposed to feed water containing caffeine, as well as to unfiltered lake water for a longer period than during the first spike, leading to the development of a microbial community able to remove caffeine. Results obtained in chapter 3 suggested that the possible presence of bacteria able to degrade caffeine, such as *pseudomonas*, in the biolayer may lead to a progressive removal of caffeine, which slows and limits the movement of caffeine throughout the filtration unit. Longer exposure of the packing material as well as of the biolayer led to a more pronounced removal of caffeine (spike 3 vs. spike 1 – chapter 3). The presence of HA in the feed water may inhibit the ability of the bacteria to remove caffeine. During the second spike in LW-HA limited removal of caffeine occurred in the upper half of the sandbox and consequently high concentrations of caffeine were also observed in the effluent.

E2

During the first spike, in LW fed with lake water alone, early appearance of E2 occurred, and the C_i/C_0 ratio rapidly increased during the first 5 days primarily in the upper half of the sandbox (Fig. 31a). Between day 6 and day 12 the C_i/C_0 ratio was relatively stable throughout LW (Fig. 31a) and slowly decreased to a plateau at approximately 0.55 in the upper half and 0.3 in the lower half of Lake Water. After that it slowly decreased and plateaued at approximately 0.55 and in the upper half and 0.3 in the lower half of LW (Fig. 31a).

During the second spike, in LW, an early appearance of E2 and a rapid increase in the C_i/C_0 ratio was observed throughout the sandbox. A maximum C_i/C_0 ratio of approximately 0.8 was achieved in the top 20 cm of LW at sampling ports 1, 2, and 3,

and a progressively lower maximum was seen in the remaining sampling ports. After that the C_i/C_0 ratio started to decrease and reached a minimum at approximately 0.2 throughout LW after day 20. No further removal was observed at the end of the spike (Fig. 31c).

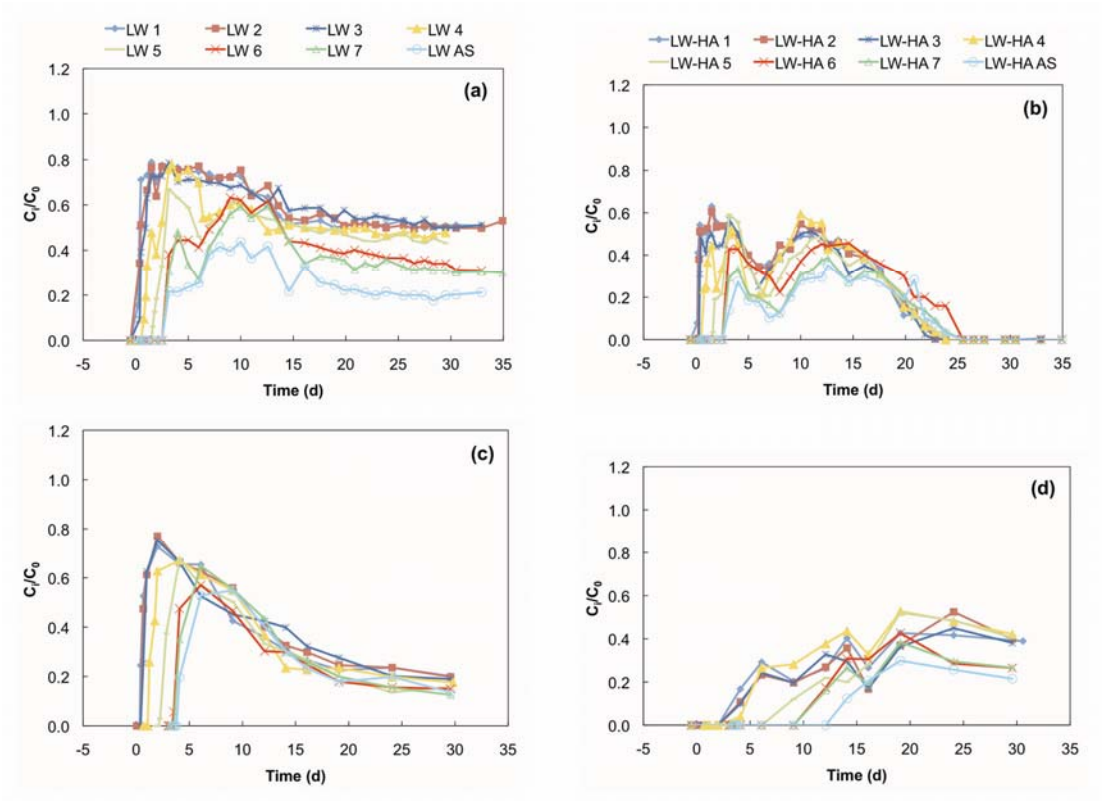


Figure 31. BTCs of E2 during the first spike in the presence of lake water alone (a), and lake water with humic acid (b); BTCs during the second spike in the presence of lake water alone (c), and lake water with humic acid (d).

Early appearance of E2 was also observed during the first few days of spike 1 in LW-HA, which received lake water with HA. The C_i/C_0 ratio of E2 rapidly increased during the first 5 days; after which the C_i/C_0 ratio was stable (Fig. 31b). A plateau at approximately 0.6 and 0.4 was achieved after the first sampling port and in the outflow, respectively. The plateau was maintained for approximately 10 days; following which the C_i/C_0 ratio began to decrease (Fig. 31b). Complete removal of E2 occurred by day 25.

During the second spike, in LW-HA late appearance of E2 was observed throughout the sandbox. E2 started to appear after 5, 10, and 13 days in the top, middle and lower section of LW-HA (Fig. 31d). The C_i/C_0 ratio increased gradually until reaching 0.6 in the upper section of the LW-HA after 25 days (Fig. 31d).

In LW, results from the first spike suggested that partial removal of E2 occurred within the first few centimeters below the water-sand interface, while further but limited removal occurred in the lower half of the sandbox. During the second spike enhanced removal of E2 was achieved. Results obtained in chapter 4 suggested that in waters without HA biodegradation was the main mechanism involved in the removal of E2. The different removal achieved during the two spikes was probably related to the different level of oxygen present in the sandbox. During the second spike, in the presence of higher level of oxygen, enhanced biodegradation of E2 was observed.

During the first spike in LW-HA, there was a partial removal of E2 with complete removal on day 25. During the second spike, complete removal of E2 occurred during the first 5 days, followed by a slow increase in the C_i/C_0 ratio to 0.6. The previous column studies suggested that in the presence of HA, removal of E2 appeared to be due to adsorption to HA. The results also suggested that HA can inhibit biodegradation of E2, if biodegradation occur. During spike 1 and 2 in LW-HA, removal of E2 may be due to a combination of both biodegradation and adsorption by HA. As seen previously, the aerobic condition appears to increase the removal of E2.

<u>E1</u>

In LW, at the beginning of the first spike, E1, the breakdown product of E2, showed a behavior similar to E2. Early appearance of E1 was observed during the first 5 days of the spike (Fig. 32a). The C_i/C_0 ratio rapidly increased and reached a plateau at approximately 1.1 (Fig. 32a). After day 8, the C_i/C_0 ratio started to decrease slowly, and at the end of the spike the C_i/C_0 ratio ranged between 0.8 and 0.9 throughout LW (Fig. 32a). Slightly higher values of the C_i/C_0 ratio were achieved in the lower section compared to the upper section of LW, suggesting production of E1 may have occurred in the lower section of LW.

At the beginning of the second spike, in LW, a rapid increase in E1 was observed. After 8 days the C_i/C_0 ratio reached a maximum of approximately 1 in the first 4 sampling ports of LW, while a maximum of 0.9 was achieved after 10 days in the remaining sampling ports (Fig. 32c). The C_i/C_0 ratio decreased throughout LW and reached a minimum of approximately 0.25 at the end of the spike (Fig. 32c).

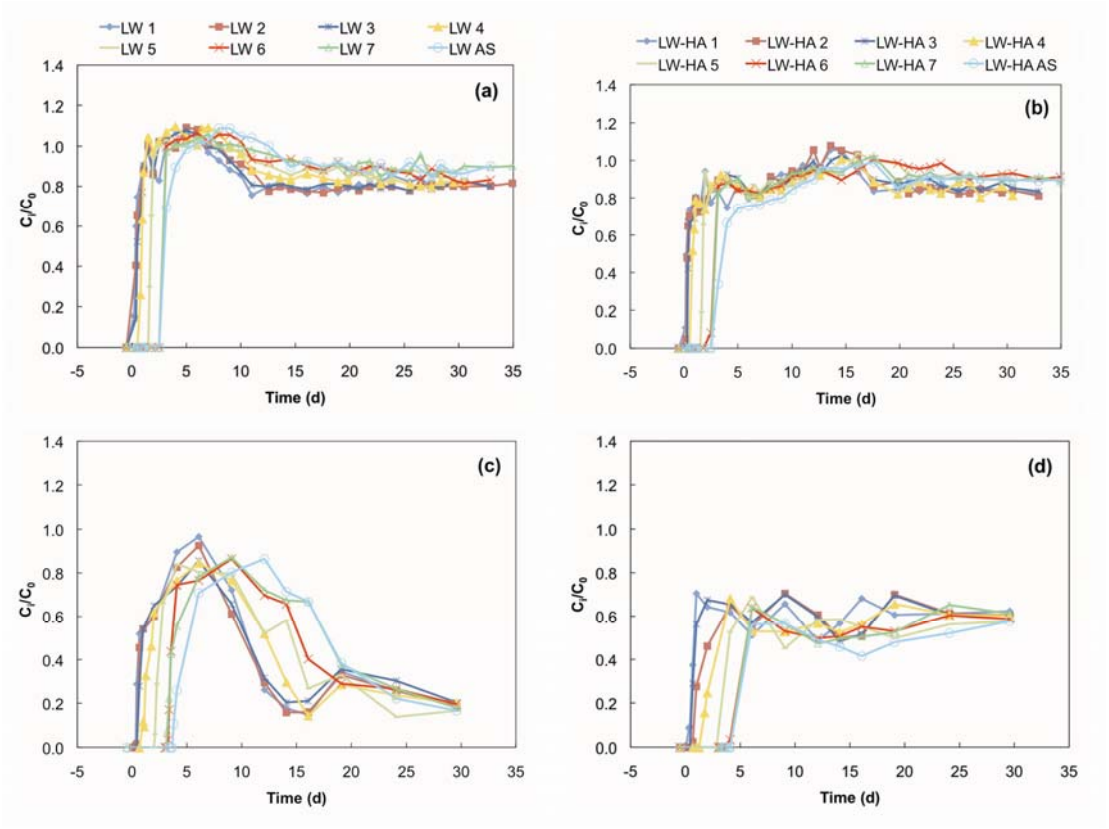


Figure 32. BTCs of E1 during the first spike in the presence of lake water alone (a), and lake water with humic acid (b); BTCs during the second spike in the presence of lake water alone (c), and lake water with humic acid (d).

During the first spike in LW-HA early appearance of E1 and rapid increase of the C_i/C_0 ratio was observed throughout the sandbox (Fig. 32b). The C_i/C_0 ratio ranged between 0.8 and 1 during the entire spike, suggesting no to limited removal of E1, as well as no to limited production of E1 from E2 occurred during the spike.

At the beginning of the second spike, in LW-HA, E1 increased and reached a plateau at approximately 0.6. No significant change in E1 was observed after 5 days (Fig. 32d).

In lake water alone, any removal of E1 was probably related to biodegradation. During the first spike a limited removal, < 10%, of E1 was observed but during the second spike higher removal, approximately, 80% was observed. The difference in the levels of oxygen between the two spikes (2mg/L vs. 4mg/L) suggested that enhanced removal of E1 was probably due to enhanced biodegradation. Oxygen levels greater than 4 mg/L or longer exposure time of E1 may be required for complete removal of E1.

Due to the high level of HA present in the feed water, partial removal of E1 is expected during both spikes. Even if sorption onto HA would be the predominant removal mechanisms of E1, biodegradation may also occur. The biodegradation is primarily impacted by the level of oxygen, suggesting a higher removal of E1 during the second spike compared to the first spike.

CONCLUSIONS

- RBF can be effectively used to remove PhACs present in surface waters. However the geochemistry of the RBF site is expected to play a key role in their removal.

- The occurrence of air beneath the clogging layer may create aerobic areas with enhanced removal of PhACs.
- No to limited removal (< 10%) of selected PhACs occurred under environmental conditions characterized by low levels (< 2 mg/L) of oxygen.
- Carbamazepine and gemfirbrozil were persistent regardless of the different environmental conditions simulated.
- Phenazone was highly impacted by presence of oxygen as well as by the level of HA contained in the feed water. No removal occurred in the presence of limited amount of oxygen and in the presence of high level of HA (TOC ~ 20 mg/L). Partial removal (~ 50%) of phenazone occurred in the presence of higher levels of oxygen.

- Removal of caffeine was impacted by the level of oxygen as well as by the presence of HA. Biodegradation was probably the predominant removal mechanism involved. Under aerobic and in the presence of lake water alone, favorable conditions for biodegradation, high removal of caffeine occurred in the top 10-20 cm. The presence of HA may inhibit the biodegradation of caffeine.
- Limited removal of E1 occurred throughout the study, while higher removal of E2 was observed.
- Removal of E1 was impacted by the level of oxygen as well as by the presence of HA. No to limited removal occurred under reduced conditions (oxygen < 2 mg/L). In the presence of moderate levels of oxygen (4 mg/L), higher removal occurred in the presence of lake water alone (80%) compared to lake water and HA (40%). The presence of HA may inhibit the removal of E1.

CHAPTER 6. CONCLUSIONS

Natural filtration can be used as sustainable technology to enhance the quality of the feed water during water treatment. Among the different natural filtration processes, slow sand filtration (SSF) and riverbank filtration (RBF) were investigated.

- SSF proved to be a valuable technology to enhance the quality of the raw water in the aftermath of a natural disaster, as well as in underdeveloped countries and rural areas. Results obtained from this study suggested that SSF can also be used in the presence of high concentrations of PhACs that may be the results of a wastewater treatment plant spills or pharmaceutical industry spills. However, the nature and concentrations of the PhACs, the duration of the spill, and the age of the SSF may significantly impact the overall performance of the filtration unit.

- Regardless of the configuration, the hydraulic loading rate, the starting turbidity of the feed water and the presence or absence of PhACs, turbidity removal ranging from 40% to 80% was observed and removal of total coliforms and *E. coli* greater than 95% was consistently achieved. However, the presence of a post treatment unit, such as UV unit, is desirable to further enhance the quality of the feed water, especially in the presence of bacterial loads greater than 10^6 MPN/100 mL.

- The effective lifetime of the SSF unit is primarily related to the hydraulic loading rate. At high hydraulic loading rates, fast development of clogging conditions and consequent decrease of the output is expected. Scraping the top of the SSF unit is required in the presence of clogging conditions.

- The bacterial removal efficiency of the SSF unit is primarily related to the quality of the biolayer and to the maturity of the filter unit. Higher removal can be achieved by a more biologically mature unit. The type of feed water used to develop the biolayer plays a limited role in terms of bacterial removal. The time required to produce the biolayer is a function of the amount and quality of water passed through the SSF.

- Among the different PhACs used during this thesis, 17-β estradiol (E2) had the highest impact on the biolayer of the SSF unit. However, in the presence of a "biologically mature" SSF unit, removal greater than 99% of total coliforms and *E. coli* can still be achieved even in the presence of a compromised biolayer.

- Changes in the microbial community composition were significant over time. In general, Bacteroidetes were replaced by Proteobacteria in the biolayer.

- SSF showed potential ability to remove PhACs. Complete removal of caffeine, partial removal (11–92%) of E2 and estrone (E1), but no to limited removal (< 10%) of carbamazepine, gemfibrozil, and phenazone were achieved by the SSF unit.

- RBF can be effectively used to remove some of the PhACs present in surface waters. However the geochemistry of the RBF site is expected to play a key role in their removal. Depending on the compound, removal of PhACs may predominantly occur due to biodegradation; however environmental variables, such as oxygen and temperature may enhance or limit biodegradation. In the presence of humic acid (HA), adsorption of some PhACs may occur. Limited and slower removal of selected PhACs may occur during the winter temperature conditions.

- The occurrence of air beneath the riverbed can enhance the development of locally present aerobic conditions which can lead to an enhanced removal of redox sensitive PhACs.
- Among the different PhACs investigated, carbamazepine and gemfibrozil showed no to limited removal regardless of the different environmental conditions; removal of caffeine, E1 and phenazone was highly impacted by the environmental conditions; E2 was removed under all conditions.
- Phenazone was highly impacted by the different environmental conditions according to the following order: level of oxygen > presence of HA > temperature. Removal of phenazone was primarily due to biodegradation and complete removal occurred only under aerobic conditions and in the presence of a low to moderate amount

of HA (TOC < 10 mg/L). No to limited removal occurred during under anaerobic conditions as well as in the presence of high level of HA (TOC > 10 mg/L) regardless of the temperature. Under aerobic conditions and low level of HA (TOC < 10 mg/L) faster removal (4 x) occurred during the summer than during the winter.

- Caffeine was also impacted by the different environmental conditions according to the following order: presence of HA > temperature > level of oxygen. Biodegradation and sorption are the main mechanisms impacting the removal of caffeine. Caffeine was completely removed in the presence of HA regardless of the different environmental conditions. However, faster removal occurred during the summer under aerobic conditions (2x) than winter. In the presence of lake water alone (no HA), caffeine was completely removed only during the summer under aerobic conditions.
- E1 was impacted by the different environmental conditions according to the following order: level of oxygen > presence of HA > temperature. E1 was completely removed only under aerobic conditions. In particular, during the summer, E1 was removed regardless of the presence or absence of HA, while during the winter E1 was completely removed only in the presence of a low level of HA (TOC < 10 mg/L).

FUTURE RESEARCH

- Natural filtration (SSF and RBF) is not capable of removing all PhACs. It is important to select the most appropriate post treatment.
- Detailed studies on possible direct uptake of PhACs by microorganisms in the presence of different carbon sources are required to better understand the removal of PhACs.
- Investigate the impact of global climate change on the performance of natural filtration. Particularly, the impact of higher temperature and the impact of higher intensity precipitation should be investigated.
- 2-D and 3-D computer simulations to investigate the impact of geochemistry at RBF sites should be used, even if it would be extremely hard to have accurate measurements for the simulations.

APPENDIX A. Experimental set-up and sampling locations

Fig. A1: Sampling location: Manoa Stream (21°18’35.04” N, 157°48’37.22W), (top) and Lake Wilson (21º29'41" N, 158º01'29" W) (bottom)

Fig. A2: Representation of the two slow filtration (SSF) units used in chapter 2 and chapter 3. The two units were placed in series (top) and in parallel (bottom).

Fig. A3. Dynamics of the biolayer in a slow sand filtration (SSF) unit.

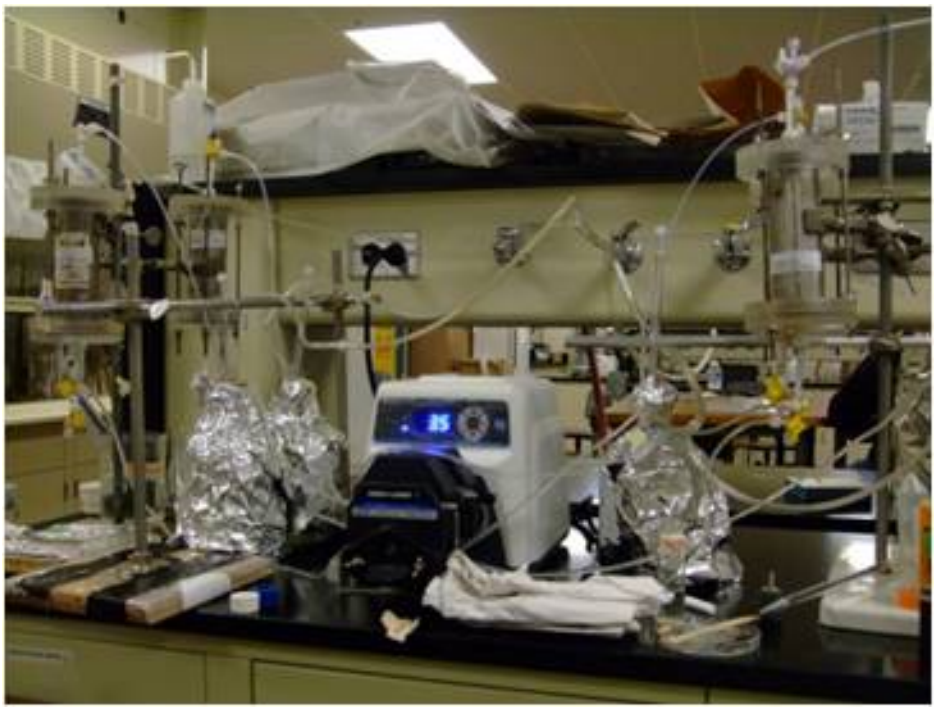


Fig. A4: Experimental set-up used in chapter 4 (column experiments conducted under re-circulated conditions).

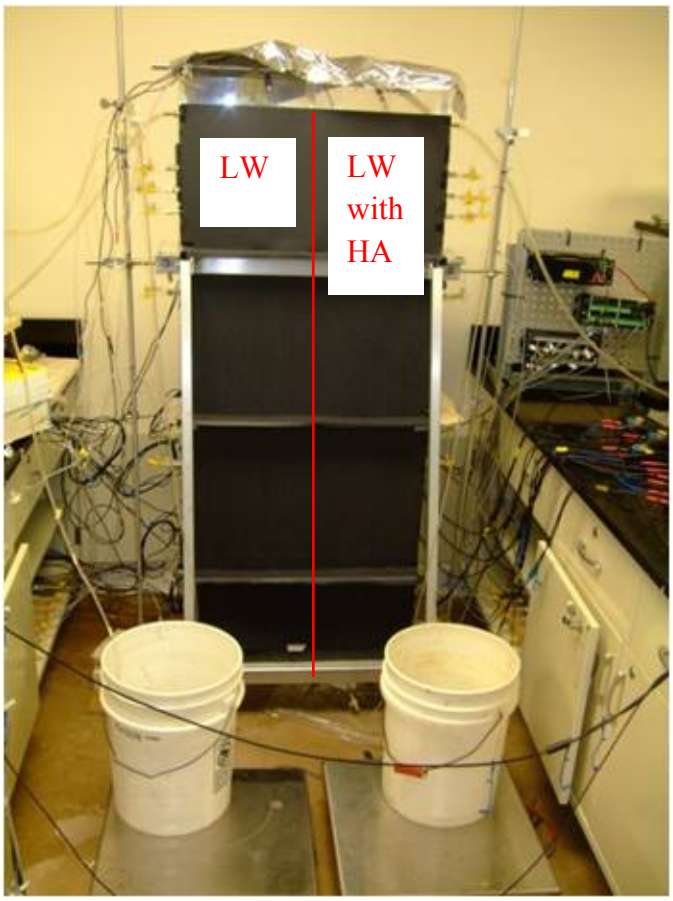


Fig. 5: Experimental set-up used in chapter 5 (two side-by-side sandbox). LW = lake Wilson alone (TOC = 3 mg/L); LW with HA = Lake Wilson with humic acid (TOC = 20 mg/L).

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