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Evaluating Pilot Scale Slow Sand Filtration Columns to Effectively Remove Emerging Contaminants in Recycled Water

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Chapter 1

Introduction

A slow sand filtration (SSF) system is a filtration process which contaminated water percolates through a sand medium and through various physical, chemical, and biological processes, the contaminants are removed. The first known slow sand filtration system was made in 1804 by John Gibb in Scotland to produce drinking water (Zearley & Summers, 2012). Since then, this technique has been widely used not only for drinking water production (Bichai et al., 2014), but also for improving the quality of wastewater before being reused (Lee & Oki, 2013; Nyberg et al., 2014) or discharged into the environment (Kader Yettefti et al., 2013).

Slow sand filtration is a type of biofiltration. Biofiltration generally encompasses any type of filtration of contaminated water through sand, soil, or other various media that contains biomass to aid degradation and removal. Several types of biofiltration have been extensively studied in literature: bioswales, trickling filters, constructed wetlands and natural wetlands, treatment ponds, riparian zones, bank filtration, and slow sand filtration. An effective filter is the result of biological degradation and physical/chemical processes such as adsorption and straining of contaminants on the biofilter media. Both of these processes can be effective as a result of the slow flow rates and long hydraulic residence times that allow the formation of a biological active layer composed of alga, protozoa, bacterium, fungus, actinomycetes, plankton, diatoms, and rotifer population (Nyberg et al., 2014). This layer, called the schmutzdecke, develops within the top centimeters of the filter as a result of the accumulation of the organic matter, microbes, and other particulates that settle from the fluid (Ellis, 1985; Nyberg et al., 2014). Thus, as leachate water is passed through, pathogens and contaminants are trapped and broken down by these microbes as a food source, aiding to the physical and biological processes required for filtration.

Depending on the raw and target effluent water quality, a slow sand filtration system can be used by itself or in series to other additional treatments, like pretreatment to protect sensible processes such as reverse osmosis or membrane filtrations (Derlon et al., 2014), or as a polishing process to eliminate disinfection by-products after ozonation or chlorination (Lautenschlager et al., 2014).

Benefits to use slow sand filtration

The benefits of SSF combines a high efficiency system in reducing cloudiness and harmful bacteria and viruses along with an economical edge. SSF uses minimal power input and no chemical requirements, does not require close operator supervision, uses locally available materials and labor, and does not produce unwanted by-products (Casas & Bester, 2015). This cost-effective technique that was once used in big cities like London, now has special application in the treatment of water at smaller scales such as isolated households in rural areas, in developing countries, or in small businesses with high water consumption, like plant nurseries (Lee & Oki, 2013; Nyberg et al., 2014).

Design and operation of a slow sand filtration system

Filter size and depth/flow rate/water head: Slow sand filtration has specific features that differentiate it from other types of biofiltration. In SSF, the media is uniform to promote consistency of removal throughout the column (uniformity coefficient lower than 3) and consists of fine size (0.15 - 0.30 mm), non-calcareous sand that contains no more than 10% by weight of grains smaller than 0.2 mm and no more than 10% greater than 1.0 mm (Horicultural Development Council, 2005).

Some other media used in biofiltration are biochar (Kaetzl et al., 2014), compost (Antonious, 2012), woodchips (Ilhan & Ong, 2012), activated carbon (Farre et al., 2011), pressmud (Viswanathan et al., 2012), anthracite (Lee & Oki, 2013), agricultural wastes (Sniegowski et al., 2012), etc. In a study by Nyberg et al. (2014), various substrates were researched as the best effective SSF medium to remove zoospores of *P. nicotianae* from nursery production effluent. Substrates included sand, crushed brick, calcined clay, Kaldes medium, and polyethylene beads. They discovered that within 21 days, all substrate treatments removed more zoospores than day 0. Of all the substrate treatments evaluated, the columns with 10 cm of sand removed the most zoospores on day 21. By their research, sand was the most effective medium using physical filtration alone at depths of 40 cm and 60 cm. The growth and development of microbes in the sand column increased removal of zoospores by 20%, to the extent that 99.5% of zoospores were filtered from the recirculating water. This research on a laboratory scale has solidified some of the concerns of using sand as the medium of choice for SSF; sand acts both as an effective biological and physical filtration medium.

The filter size (cross sectional area) depends on the target capacity, but its depth should be about 0.5 m minimum, whilst the optimum is between 0.8 and 1.0 m, which would allow for some clean-up operations (Horicultural Development Council, 2005). A water head ranging between 0.4 and 2 m should be also kept constant (Horicultural Development Council, 2005). Also the flow rate should be steady and comparably slow, usually between 0.1 and 0.3 m h^{-1}.

Physical and chemical processes: The slow flow rate is what allows the physical and chemical processes to occur. At first, the sand media will act as a strainer where the pollutants, both biological and chemical, will get slowed down or trapped within the filter media due to size exclusion and/or adsorption.

Biological processes: Then, after a few hours, days or weeks of ripening, depending on the raw water content, a biofilm or zoogloeal, also called schmutzdecke, is formed on the surfaces of the sand grains (Horicultural Development Council, 2005), especially within the top few centimeters of the filter. At this point, the SSF is working at its maximum capacity. The top "jelly" is not only able to strain smaller particles in size, but also increases the filter adsorption capacity and increases the abundance of the bacteria, viruses and protozoa that make it up and helps break down some of the components of the trapped particles and even eventually mineralizes them (Horicultural Development Council, 2005).

Temperature effects/declogging: For satisfactory biochemical degradation of pollutants, water temperature should not fall too low and sufficient contact time within the filter bed should be assured. In addition, bacterial populations are adapted to the type and amount of food supplied by the passing water. Sudden changes in filtration rate or raw water quality should be avoided (Huisman & Wood, 1974). On the other hand, as the schmutzdecke gets denser, the filter gets slower. To prevent clogging, some maintenance has to be carried out. Raw water pre-filtration is sometimes necessary to remove suspended fine particulates that otherwise would clog the sand surface more rapidly.

Mechanisms: Various types and concentrations of bacteria, responsible for different types and rates of degradation, are normally found at different filter depths (Calvo-Bado et al., 2003). Some other modalities of biofiltration inoculate or seed the filter with certain bacteria to try to foster specific compound degradations (Sniegowski et al., 2012; Ho et al., 2011).

Seeding biofiltration with substrates: The addition of electron donors like alcohol, sugars or acetic acid has been common in some other biofiltration approaches (Aslan & Cakici, 2007). However,

in a purist SSF setting, the raw water alone should provide with the necessary food for the decontaminating bacteria to develop.

Most of the documented studies about SSF to date has focused on its efficiency on biological (Bichai et al., 2014; Kaetzl et al., 2014; Lee & Oki, 2013) and physical pollutant removal (Corral et al., 2014). Chemical pollution has been mainly addressed as macro magnitudes (Kader Yettefti et al., 2013; Aslan, 2008).

Application of SSF to reduce emerging contaminants in the environment

Slow sand filtration has been researched more frequently as reuse of water has been increasing (Nyberg et al., 2014). Nurseries and greenhouses have looked to slow sand filtration to increase water use efficiency by reducing the amount of irrigation water needed (Nyberg et al., 2014). In recirculating nursery and greenhouse systems, the irrigation runoff water can be collected, treated, and recycled back to the original system. In multiple studies, slow sand filtration has been useful to remove and eliminate propagules of plant pathogens: many species of *phytophthora – P. cinnamomi* (van Os et al. 1998), *P. cryptogea* (Garibaldi et al., 2003), *P. nicotianae* (Nyberg et al., 2014), and species of *Pythium* (Garibaldi et al., 2003), *Fusarium* (Lee & Oki, 2013), *E. coli* (Unger and Collins, 2008), and nematodes including *Radopholus similis* (van Os et al., 1998). These zoosporic fungi, viruses, and nematodes cause crop damage including severe root rot (Garibaldi et al., 2003) and if not treated in a recirculating nursery and greenhouse system, widespread epidemic may impact all the crops within the facilities.

While SSF has been shown to remove plant pathogens, its efficacy to remove other compounds has yet to be explored in depth. Contaminants of emerging concern (CECs), also named “emerging contaminants”, are a group of contaminants that consist of pharmaceutically

active compounds, endocrine disrupting compounds, personal care products, plasticizers, pesticides and herbicides, and flame retardants that are found in trace amounts (μg L^{-1} to ng L^{-1}) in the environments, primarily discharged from wastewater treatment plants that insufficiently treat these contaminants through secondary and tertiary treatment (Maeng et al., 2011). Nonpoint sources, such as overland flow during rainfall or land drainage in agricultural areas deliver veterinary medicines and pesticide runoff to surface water or groundwater (Maeng et al., 2011).

The existence of emerging contaminants is unknown in the environment due to the lack of monitoring and due to their low concentrations in surface waters. Many of these compounds vary due to their application and consumption from region to region and will depend on the efficiency of removal by wastewater treatment plants (Cunningham, 2004; Maeng et al. 2011).

Technologies already proven effective in removing contaminants are activated carbon (Snyder et al., 2007; Ternes et al., 2002; Yu et al., 2008), ozonation and advanced oxidation processes (Ternes et al., 2003), and membrane filtration (Snyder et al., 2007). However, these processes are costly and require large amounts of resources to operate. In turn, biofiltration systems are simple to operate, relatively low in cost and maintenance, removes both turbidity and propagules of pathogens, and overall improves the quality of the water (Ufer et al., 2008, Nyberg et al., 2014). Thus, biofiltration like SSF may offer a low cost alternative for the treatment of contaminants in wastewater (Ho et al., 2011).

River Bank filtration involving CECS

The most commonly researched biofiltration systems to treat emerging contaminants are managed aquifer recharge processes. Managed aquifer recharge (MAR) processes are robust and cost-effective systems and include a variety of applications such as aquifer storage and recovery,

infiltration ponds, percolation tanks, soil aquifer treatment, and sand dams (Dillon, 2005). MAR systems like bank filtration and artificial recharge are adopted by wastewater treatment plants to reduce the cost of using more costly advanced treatment systems like nanofiltration and reverse osmosis (Maeng et al., 2011). MAR systems are typically adopted if surface water quality is inadequate or if the amount of raw water like in groundwater is not sufficient (Maeng et al., 2011); MAR systems can replenish these natural systems.

However, in comparison to SSF, biodegradation in MAR systems are very important mechanisms, much more than sorption as sorption sites can become exhausted or desportion can occur (Maeng et al., 2011). This practice may not be as easy to maintain like slow sand filters, as sand can be backwashed and the supernatant of the disturbed schmutzdecke can be drained. Also, it is suggested that MAR such as bank filtration may not be optimal for smaller operating facilities that have less land space. There have been many studies that focus on biofilm reactors such as bank filtration and underground dams that can remove organic micropollutants but these are not usually designed, only grown in natural conditions (Baumgarten et al., 2011; Grunheid et al., 2005; Heberer et al., 2008; Onesios and Bouwer, 2012, Patterson et al., 2010; Rauch-Williams et al., 2010). Therefore these systems are very limited and may need to overcome obstacles of releasing WWTP effluent water directly to natural waters. However, given substantial research on MAR systems, pharmaceuticals and endocrine disrupting compounds, which are usually main contaminants of interest due to their potentially adverse effects on human health and aquatic life even at low concentrations (Maeng, et al., 2011), have shown positive results for their removal. Most of these studies were laboratory scaled and field studies.

Some contaminants researched in MAR systems are endocrine disrupting compounds (bisphenol A, 17β-estradiol, 17α-ethynylestradiol, and iodipamide), antiseptics (Biosol,

biphenylol, p-chloro-m-cresol, p-chloro-m-xylenol, chlorophene, and triclosan), pharmaceuticals (carbamazepine, acetaminophen, diclofenac, 5-fluorouracil, gabapentin, gemfibrozil, ibuprofen, ketoprofen, naproxen, phenytoin, valproic acid, and oxazepam), and disinfection by-products (N-nitrosodimethylamine, N-nitrosomorpholine) (Patterson et al., 2010; Onesios and Bouwer, 2012). Baumgarten et al. (2011) investigated the removal of poorly degradable antibiotic sulfamethoxazole in laboratory columns for its removal in bank filtration. Results showed that 60% of sulfamethoxazole was removed within 14 days of column passage in aerobic conditions while no removal occurred under anoxic conditions in a 2 year long system operation. The degradation of poorly degradable compounds in aerobic conditions may give bank filtration its benefits in the top layers of sand, but shows complications with anoxic conditions, which can be representative of some MAR and SSF. Adaptation of the system may require long operation time as would be realistic in an actual bank filtration site. Thus, more biofilter systems with MAR and SSF should be studied for their processes that provide both oxic and anoxic conditions. Typically, varying redox conditions are effective for removing redox-sensitive organic micropollutants during MAR (Maeng et al., 2011).

Maeng et al. (2011) summarized the literature in a review regarding removal efficiencies of CECs using bank filtration and aquifer recharge column and full scale studies. To briefly summarize that work, there are CECs that are promising for removal by filtration and others which are more recalcitrant such as carbamazepine are ineffective. Antibiotics in a study by Heberer et al. (2008) investigated 19 targeted antibiotics at a lake bank filtration site in Berlin, Germany for 2.5 years. They detected 7 out of 19 target antibiotics. All antibiotics were completely removed after 2-4 months of travel time except for sulfamethoxazole, which in previous studies was discovered to be redox-dependent that degrades more effectively in anoxic

conditions (Grunheid et al., 2005; Heberer et al., 2008). Depending on the residence time, removal of antibiotics can increase. It can be concluded MAR is an effective treatment step for removing antibiotics, giving way for other filtration studies to filter veterinary antibiotics that may potentially be found in runoff from dairy farms.

For non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics, many have been removed at rates greater than 50% during bank filtration and aquifer recharge systems. Field and laboratory scale studies have shown significant removals of diclofenac, ibuprofen, naproxen, and phenazone during soil passage (Heberer and Adam, 2004; Massmann et al., 2006, 2008). Diclofenac, ibuprofen, and naproxen have moderately high octanol-water partition coefficients ($\log K_{ow} > 2.5$), suggesting sorption would be the main mechanism of removal. Phenazone, however, is more redox-dependent and can be removed under oxic conditions than anoxic conditions. Therefore, it is necessary to monitor the pH during soil passage as these NSAIDs may remain as ionic species in the aquatic environment, with more potential to be sorbed.

Anticonvulsant pharmaceuticals have been shown numerous times their persistency in degradation in multiple treatment methods. Carbamazepine is one of the notorious poorly degradable compounds and has low removal (<10%) in wastewater treatment plants (Ternes, 1998). Drewes et al. (2002) showed no change in carbamazepine and primidone concentrations in soil aquifer treatment for estimated travel times up to six years. The extended research on this contaminant concluded bank filtration and aquifer recharge are not effective for anticonvulsant removal.

Antidepressants removal has still yet to be studied. A study by Snyder et al. (2007) investigated three antidepressants and their fate during a pilot scale bank filtration. Fluoxetine

was removed significantly at 99% and meprobamate was only 66%. There could be more research developed in understanding the fate of more antidepressants since they are commonly used drugs in the pharmaceutical industry.

Lipid regulators are similar to NSAIDs, where they remain in ionic species. Thus, pH plays an important role in the removal mechanism. One of these lipid regulators, clofibric acid, is a common metabolite of clofibrate, and is detected frequently in the aquatic environment. Interestingly, research suggests clofibric acid concentrations increased at bank filtration sites in Germany due to the high consumption of liquid regulators during the 1990s. They discovered clofibric acid present in deeper layers of the aquifer (Heberer et al., 2004). Lipid regulators and NSAIDs can be included in a joint research to test pH conditions for their removal.

Steroid hormones are also a very particular group of CECs because they can produce potentially adverse effect on human health and aquatic life even at very low concentrations (Maeng et al., 2011). However, laboratory scale and field studies using bank filtration in Berlin, Germany showed positive results (Heberer et al., 2004). 17β-estradiol and 17α-ethinylestradiol were not detected in surface water from Berlin and estrone was removed greater than 80%. Snyder et al. (2004) used batch experiments and field studies with bank filtration and demonstrated estrone, 17β-estradiol, and 17α-ethinylestradiol were removed by biodegradation and sorption. There is suggestion that these removal processes may be aided by aerobic bacteria. Given conditions of certain MAR, steroid hormones and potentially a wide range of endocrine disrupting compounds can be reliably treated.

For pesticides/herbicides, most studies have been focused on atrazine. Atrazine is a member of the S-triazine group herbicides and is a probable human carcinogen (Nasseri et al.,

2014). Atrazine is resistant in the environment and penetrates through the surface and subsurface due to its high mobility, persistence, low vapor pressure, and massive application since it has been in use since 1959. Ho et al. (2011) and Zhang et al. (2014) showed triazine herbicides like atrazine were poorly removed by biofilters, but some studies have shown that atrazine can be readily biodegradable in aquatic environments, with reported removal rates ranging from weeks to years (Ho et al., 2011).

Limited studies on CECS and SSFs

Based on the previous studies, it is apparent that emerging contaminants can be removed and have shown adequate removals with a variety of conditions; aerobic and anaerobic conditions, biological substrate feeds, bacterial community development, filter mediums, variety of physicochemical properties of contaminants, and type of leachate water filtered using a SSF system. However, despite the variance in studies, most research agrees that the general contaminant removal increases over time as filter matures (Calvo-Bado et al. 2003; Deniel et al. 2004; Ellis 1985; Lee & Oki 2013; Weber-Shirk & Dick 1997).

Now that available freshwater resources are continuously limited and increases in world population have raised the pressure on natural resources (Zimmerman et al., 2008), water resource management have turned to water reclamation and reuse to sustain agricultural activities. Reclaimed water use is not only limited to agriculture but widely used in other purposes such as irrigating landscapes, nurseries and greenhouses, flushing toilets, and replenishing groundwater aquifers (Pedersen et al., 2003; Levine and Asano, 2004; Miller, 2006, Xu et al., 2009). In 2006, an estimated 9.8×10^6 m^3 d^{-1} of treated municipal wastewater was used in the United States (Miller, 2006, Xu et al., 2009). Studies have documented the presence of many microcontaminants such as pharmaceuticals and personal care products, pesticides, phenolic

estrogens, surfactants, biocides, and disinfection by-products in reclaimed water (Kolpin et al. 2002, Calderon-Preciado et al., 2011, Calderon-Preciado et al., 2013). These contaminants may accumulate in the area irrigated by reclaimed water and may result in contamination of the soil and plants. Along with the combined use of pesticides in greenhouses, it is possible for receiving waters to receive a wide range of pollutants. This is especially true in rural areas where contaminated runoff from farmland contributes a significant proportion of the pesticide load (Antonious, 2012).

The overall objective of this study was to promote slow sand filtration columns as a cost effective engineered solution to treat emerging contaminants. Specifically we simulated a greenhouse irrigation system that contains emerging contaminant concentrations of 400 ng L^{-1} based on the range of literature values for concentrations of emerging contaminants found in reclaimed nonpotable wastewater (Loraine & Pettigrove, 2006), a concentration higher than the average to be able to adequately detect the compounds in our study.

This pilot scale study is a preliminary study to see how viable SSF columns can be to remove emerging contaminants. Results of the present study can be combined with previous studies of using SSF columns to remove both pathogens and contaminants provided by reclaimed water and pesticide use. The removal efficiencies of 14 selected pharmaceuticals and personal care products (PPCPs), 7 pesticides, 3 plasticizers, and 2 detergents/emulsifers, and the trends after the project's initial start and declogging maintenance removal rates thereafter were examined.

Chapter 2

Introduction to CECs and typical analytical methods

Chemical residues have been ubiquitous in the environment as they are found in many environmental matrices, from sewage water, effluent water from wastewater treatment plants, river water, to even drinking water. These compounds can come from sources such as households, nurseries, wastewater treatment plants, factories, hospitals, and any other facility dispensing chemical waste such as pharmaceuticals, personal care products, antibiotics, plasticizers, pesticides, etc (Fatta et al., 2007). This group of compounds, known collectively as "emerging compounds" or "compounds of emerging concern" (CECs), is notorious for their occurrence in the environment and their complexity existing in particular environmental matrices (Kostopoulou & Nikolaou, 2008). Even at their low concentrations they can affect human health and environmental health.

Analytical techniques using Gas Chromatography-Mass Spectrometry (GC-MS) or Liquid Chromatography-Mass Spectrometry (LC-MS) have paved the way for determining the concentrations of CECs even at their trace amount. GC-MS was first used to determine pharmaceuticals and personal care products in the environment in 1976 (Hao et al., 2007). Advances to the quantification of CECs in environmental samples have increased detection sensitivity and reliability. CECs come in a wide variability in their concentrations, polarities, and thermal labilities (Hao et al., 2007). Tandem with a host of myriad of environmental matrices to affect the matrix effect, quantification of CECs is challenging both with GC-MS and LC-MS.

Matrix effect exists due to the co-eluted, interfering compounds in the sample extract that have similar ions in the Mass Spectrometry (MS) or MS-MS segment (Hao et al., 2007). It may also arise from the interaction between the target analytes and those co-extracted matrix

components (organic or inorganic) during sample preparation and in the ionization chamber (Hao et al., 2007). The former is more common in GC-MS and GC-MS-MS analysis, and might be encountered in LC-MS and LC-MS-MS analysis. GC-MS and GC-MS-MS are still the commonly used techniques because of their wide availability in environmental laboratories (Hao et al., 2007). GC-MS or GC-MS-MS also suffers less from matrix effect that is more commonly observed in electrospray ionization (ESI)-based LC-MS or LC-MS-MS (Hao et al., 2007).

Sample preparation

Environmental concentrations of CECs exist in the ng L^{-1} or µg L^{-1} ranges. Extraction is a necessary step to concentrate the analytes prior to instrumental analysis. Solid Phase Extraction (SPE) is the most common technique applied sample preparation and purification in the analysis of CECs (Kostopoulou & Nikolaou, 2008). SPE separation depends on the kind of solid stationary phase through which the sample is passed, and on the types of target compound. The target compounds adhere to the stationary phase, while impurities in the sample are washed away, obtaining a clear extract (Kostopoulou & Nikolaou, 2008). This procedure uses a vacuum manifold and has the advantage that 12 or 24 solid phase extraction cartridges can be prepared simultaneously, thus minimizing time and effort for sample preparation (Kostopoulou & Nikolaou, 2008).

The target compounds are finally eluted from the stationary phase using an appropriate solvent (Kostopoulou & Nikolaou, 2008). The effectiveness of solid phase extraction cartridges have been widely researched; the best being ENV+, Oasis HLB, Oasis MAXSPE, Oasis MCX, Strata-X, Lichrolut C18 and LiChrolut EN for pre-concentration in aqueous samples (Kostopoulou & Nikolaou, 2008).

Since most pharmaceuticals and personal care products are polar, non-volatile, and thermally labile compounds unsuitable for GC separation, derivatization is necessary after extraction and elution from the aqueous sample and prior to GC-MS analysis of polar compounds. Various derivatization agents have been applied to various CECs. However, this comes with inaccuracy of the method and it can affect the losses of analytes that cannot be fully derivatized (Kostopoulou & Nikolaou, 2008). Also, derivatization uses highly toxic and carcinogenic diazomethane, or less frequently, acid anhydrides, benzyl halides, and aklylchloroformates (Hao et al., 2007). Derivatization can be incomplete, inhibiting completely the analysis of some compounds (for example, Atenolol which cannot be analyzed by GC-MS). Some compounds are also thermolabile and decompose during GC analysis (Kostopoulou & Nikolaou, 2008). However, after derivatization, compounds improve in both volatility and thermal stability.

The final step of sample preparation before elution is the clean up of the extract. This step is usually added to enhance the accuracy and reproducibility of the results by eliminating matrix effects and generally any impurities occurring in the final extract that can interfere with the analysis (Kostopoulou & Nikolaou, 2008). The clean up step is usually performed with SPE cartridges, as described in (Kostopoulou & Nikolaou, 2008). SPE is a step with a double goal: sample concentration and cleanup, and takes place before the derivatization.

However, while sample clean up may help remove those interfering compounds, it is time consuming and runs the risk of losing analytes of interest, especially those that were polar to begin with. Allowing better chromatographic separation allows the analytes to be eluted in an appropriate time interval, avoiding coeluting with matrix components (Kostopoulou and Nikolaou, 2008). Nevertheless, matrix effect can hardly ever be eliminated. Initial method

validation can help document and qualify the performance of the GC-MS to analyze the test compounds, as well as the pretreatment steps to concentrate and provide for injection into GC-MS. Initial method validation provides method performance parameters such as method recoveries, precision, and matrix effect to deliver consistent estimation of the analyte concentrations.

Objectives

It is becoming crucial to properly assess the risk posed by the presence of CECs in the environment. This research has aimed to develop a multi-residue analytical method for GC-MS that has allowed for simultaneous monitoring of CECs. This provides the ease of evaluating different physical-chemical varieties of CECs simultaneously without having to undergo different processes for certain types of trace organic compounds. Since GC-MS has wide availability around the world, the multi-residue analytical method allows many researchers to gain a larger understanding of the derivatization and extraction processes possible for a multitude of contaminants. Thus, the occurrence, distribution, and fate of CECs will be better monitored and more efficiently regulated. In this study, we used N-(tertbutyldimethylsilyl)-N-methyltriflouroacetamide (MTBSTFA) to initially derivatize 50 compounds in GC-MS. This analytical method was developed using the approach by Yu and Wu (2012) such that 14 compounds in his study were derivatized and analyzed in GC-MS. In addition to his 14 compounds, this study has successfully included 1 additional anti-inflammatory drug, 2 cardiovascular drugs/beta blockers, 1 estrogen, 1 personal care product, 7 pesticides, and 4 plasticizers using MTBSTFA and GC-MS.

The work presented here consists of a meticulous and successful development of a method for 29 emerging compounds in tertiary treated greenhouse runoff water.

Analytical methodology development

Chemicals

All analytes and internal standards were of high purity grade (>90%) and are listed in the Appendix A (Table A-1).

High purity solvents such as Optima-LC/MS-grade MeOH, Optima-grade ethyl acetate (EA), HPLC grade acetone and 37% hydrochloric acid (HCl) were supplied by Fisher Scientific (Waltham, MA, USA). Ethylenediaminetetraacetic acid disodium salt dehydrate (Na_2EDTA) was 99.7% from J.T. Baker Chemical Co. (Phillipsburg, NJ, USA). N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide, purity >97%, (MTBSTFA), was obtained from Sigma Aldrich (St. Louis, MO, USA). Pesticide grade glass wool was purchased from Supelco (Bellefonte, PA, USA). Deionized water was in-house produced. Nitrogen 99.97% (N_2) and helium 99.999% (He) gases were purchased from Airgas (Riverside, CA, USA).

Both individual stock standard and isotopically labeled internal standard solutions were prepared on a weight basis in methanol (MeOH). After preparation, standards were stored at -20 °C in darkness. A mixture of all contaminants was then prepared by appropriate dilution of individual stock solutions in MeOH in volumetric flasks. For calculations of labeled diluted standards and internal standards see Supplementary Data. A 2-L aqueous solution at 400 µg L^{-1}, named as “spiking solution”, was freshly prepared in a volumetric flask every week during the project performance. A separate mixture of isotopically labeled internal standards and further dilutions, used for internal standard calibration, was similarly prepared in MeOH (see Supplementary Data).

Initial method protocol

After reviewing the scientific literature available (Gros et al., 2006; Yu & Wu, 2012) and considering the analytes' physical-chemical features (see Supplementary Data) and the type of target samples, the following extraction method protocol was used as a starting point. 1) One-hundred mL of deionized water was fortified at 200 ng L^{-1} of the target CECs in a volumetric flask. 2) In this study, we have chosen Waters Oasis HLB (Hydrophillic-lipophillic Balanced) cartridge to pretreat polar and nonpolar compounds using the same extraction conditions. The resulting solution was then concentrated by SPE in a Waters Oasis HLB 60 mg, 3 mL cartridge (Milford, MA, USA), which was previously activated with 4 mL of methanol (MeOH) and then conditioned with 4 mL of deionized water. 3) Once the extraction was finished, the cartridge was dried under vacuum for 30 min to remove excess of water, and unless eluted immediately, samples were stored at -20 °C wrapped in aluminum foil. 4) The cartridge elution was carried out in 2x2 mL of MeOH. 5) Extract was then evaporated to dryness under a gentle nitrogen stream (Organomation N-evap nitrogen evaporator) at room temperature and reconstituted in a 2 mL GC glass vial in a mixture of 900 µL of ethyl acetate (EA) and 100 µL of the derivatization agent MTBSTFA; and finally, 6) The resulting solution underwent 60 min at 70 °C to foster the derivatization reaction, and after, the extract was vortexed, cooled off, and then analyzed by GC-MS.

Several parameters, such as concentration rate, sample size, and type of SPE cartridge were optimized. Sample pH adjustment and addition of chelating agents were also assessed for optimization. Each feature was tested in triplicate in the order described below. Once a parameter was optimized, it was incorporated in the method protocol for the optimization of the subsequent parameters. Sensitivity and accuracy were the criteria followed to select each parameter.

1). Impact of glass wool: Water samples were not filtrated before SPE to take into consideration the whole content of CECs, both in solution and attached to particulate matter. In order to avoid or alleviate cartridge-clogging issues during the SPE, a plug of glass wool inside the cartridge, on top of the cartridge filling was considered. Compared to original extraction conditions, no significant difference between samples with and without glass wool were observed. Hence, glass wool was not used as it was time consuming to insert the plug in the sample cartridges.

2). Derivatization agent vs. solvent ratio: This step was tested directly in microsolutions at 20, 40, 50, 80, 100 and 200 μg L^{-1} of the target compounds, simulating the final extracts ready to be analyzed by the instrumental method. Three different MTBSTFA:EA ratios were tested, i.e., 1:10, 1:5 and 1:2.5. In all cases, the amount of derivatization agent remained at 100 μL. It was observed that the lower the final volume, the better the sensitivity. This was attributed to a better performance of the derivatization reaction when the MTBSTFA was in a higher concentration in the solution. Therefore, 250 μL was selected as the final extract volume with 100 μL derivatization agent and 150 μL of extract in EA. Three-hundred-μL inserts were used to lift the level of the extracts inside the GC vials.

3). Sample size: In order to improve the method sensitivity, three sample sizes of 100, 250, and 500 mL were tested. Higher volumes were not considered, as they would be inappropriate for analytical laboratories with limited storage capacity, as well as involve lengthy extraction times. Moreover, they could potentially surpass the SPE cartridge threshold breakthrough volume. It was observed that sensitivity clearly improved with the higher volumes. In contrast, the recovery rate got worse. This was thought to be due to the cartridge size. Therefore, cartridges with a larger amount of filling were tested as described below section

4). SPE cartridge type: Waters Oasis HLB 500 mg, 6 mL cartridges (Milford, MA, USA), were tested in the extractions of the same sample volumes as in the section above (100, 250 and 500 mL). Recovery rates were generally found to be better for all volumes in comparison with the ones obtained with the smaller 60 mg, 3 mL cartridges. In addition, no significant differences were observed among sample sizes. An improvement in the sensitivity was observed when the biggest volumes were extracted. Therefore, the largest cartridges (Oasis HLB 500 mg) were selected to extract the largest sample size (500 mL). This implied a 2000-fold concentration ratio during the SPE step. However, in this process, no matrix effects were observed between the different sample volumes as the matrix was DI water. In the sample matrix, there showed a trade-off between increasing sample size to increase sensitivity, and increasing matrix effect.

4). Sample pH: Some of the worst recovery rates (<50%) were found for acidic compounds like acetylsalicylic acid, clofibric acid, acetaminophen, and carbamazepine. Usually in the case of pharmaceuticals containing acidic groups in their structure and existing largely in their ionized form at neutral pH, acidification of water samples is necessary. Sample solutions were adjusted to pH 2 before the SPE extraction. This way, these substances would be present mainly as neutral molecules, improving their sorption on the cartridge, and consequently their recovery. This was verified with dramatic improvements for acetylsalicylic acid (from 3 to 60%), and in clofibric acid (from 25 to 71%). Some other acid compounds like diclofenac, ibuprofen, gemfibrozil, naproxen, and ketoprofen improved their recovery rates as well. The pH adjustment had no substantial impact with the extraction of the rest of compounds, which in general the recoveries were above 50%. Therefore, for better results, samples were adjusted at pH 2 before the SPE in the optimized protocol.

5). Addition of chelating agents: The addition of Na_2EDTA has been proved to considerably improve the extraction efficiency of tetracycline and macrolide antibiotics. This is explained by the fact that these compounds can potentially bind residual metals present in the sample matrix and glassware, reducing their extraction recoveries (Hernández et al., 2007). By adding Na_2EDTA, soluble metals would bind to the chelating agent, increasing the extraction efficiency of antibiotics (Hernández et al., 2007; Gros et al., 2009; López-Serna, 2010). In order to test the impact in the antibiotics as well as in the rest of the target analytes, 10 mL of a 5% Na_2EDTA aqueous solution was included in the 500 mL water samples before the SPE. It was observed that in general, the recovery rates slightly improved. However, some of the solution presented cloudiness after the addition of the chelating agent. This could be explained because its solubility decreases at acid pH (Sigma-Aldrich). Therefore, the addition of this agent was disregarded.

Finalized sample pre-treatment

Once the samples were collected, the samples were kept in the dark and transported in a cooler with ice to the laboratory. Once there, the samples were immediately processed. Otherwise, the samples were kept in a cold room at 34 º F for only a day before its extraction. No filtration was carried out to consider the CECs attached to suspended solids that would otherwise be discarded.

Sample pre-treatment protocol: This sample pre-treatment protocol is based on prior experiments to optimize several parameters, such as the concentration rate, sample size, and type of SPE cartridge, and also the pH adjustment and addition of chelating agents that would affect the accuracy and sensitivity of our analysis. See Chapter 2: Initial method protocol. Thus, this is

the final optimization protocol for the method pretreatment process involving all tertiary treated wastewater (TTW) leachate samples collected in the field.

A volumetric flask of 500 mL volume was loaded with 200 µL of a 0.5 mg L^{-1} standard mixture containing 4 internal standards (ibuprofen-d3, triclosan-d3, N-(4-hydroxyphenyl) acetamide-2,2,2-d3, bisphenol A-d6) (See Chapter 3: Surrogates). Afterwards, pH was adjusted to bring the average pH of the TTW leachate water of 9.0 pH to 2.0 pH. The mixture was then topped off with TTW sample water and shaken to mix.

Extraction: The resulting solution underwent a pre-concentration/cleanup process through solid phase extraction (SPE) in an Alltech Vacuum manifold (Deerfield, IL, USA). The solutions were loaded onto polymeric Oasis HLB cartridges (500 mg, 6 mL) from Waters Corporation (Milford, MA, USA) and extracted via vacuum. Activation with MeOH in the cartridges was conditioned with gravity driven filtration of 2 passes of 5 mL of MeOH and deionized water each. After pre-conditioning the cartridges, extraction was started by opening vacuum to the SPE manifold. The extraction waste was deposited in a 6 L pyrex container.

The flow rate for extraction was controlled by opening the SPE manifold valves to a slow drip rate of 5 mL min^{-1}. Once the extraction was finished and all sample water had passed through their respective cartridges, each cartridge was still under vacuum and continued to dry for 30 minutes to remove the excess of water. Unless they were eluted immediately, samples were wrapped in aluminum foil and stored at -20 °C in a freezer.

SPE manifold and SPE tubing cleaning process: Prior to the extractions, SPE manifold and tubing were cleaned to ensure no contamination and carry over of contaminants. SPE tubing was placed in a cleaned 500 mL graduated cylinder filled with deionized water. Old cartridges

previously used and no longer needed were used for cleaning. Old cartridges were attached to the SPE manifold ports and attached to SPE Teflon tubing placed in a graduated cylinder with deionized water. The valves on the manifold and vacuum were opened to suction deionized water to clean the tubing. Once deionized water was drained, acetone was poured in the graduated flask and vacuumed until tubing was dried.

All SPE manifold valves were previously cleaned with two passes of deionized water, two passes of methanol, and two passes of acetone and vacuumed until dried.

Elution: After extraction, elution process was prepared. Cleaning of the manifold and tubing occurred prior to elution (See Chapter 2: SPE manifold and SPE tubing cleaning process). Eluted samples were collected in labeled borosilicate tubes placed in the manifold rack. After placing its respective labeled cartridge with its tube, cartridge elution was carried out in 2 consecutive volumes of 5 mL of MeOH each and collected at gravity driven rate. If samples were difficult to start eluting via gravity driven rate, the manifold was connected to the vacuum to help accelerate dripping. Once dripping began, vacuum was turned off and manifold pressure acclimated to ambient pressure.

Evaporation: Extracts were immediately evaporated to dryness under a gentle nitrogen stream using an Organomation N-evap nitrogen evaporator and water bath of 35 °C. Nitrogen purity was 99.97%.

Reconstitution: The dried extract in tubes was reconstituted by redissolving methanol in the tubes and transferring to a 2 mL GC glass vial. This involved roughly 4 consecutive transfers of 500 µL of MeOH from tube to vial to ensure most contaminants had transferred to the GC vial. The GC vial was then evaporated to dryness under a gentle nitrogen stream as above. The extract

was then reconstituted in 150 µL of EA and with 100 µL of the derivatization agent MTBSTFA in the 2 mL glass vials.

Derivatization: The vials were vortexed to mix the solution. N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide, purity >97%, (MTBSTFA) was used to derivatized the solution (see Chapter 2: Sample pretreatment development). The vials were placed in the GC oven at 70 °C for 1 hour to derivatize them into compounds suitable for gas chromatography.

Microfiltration: In all samples after reconstitution, including validation vials, it appeared that insoluble matrix components started to form in the ethyl acetate, such as some salts, that were otherwise more soluble in MeOH when previously started. It is interesting to note that these salts were a result of the matrix using the tertiary treated water that had passed through the potted bell pepper plants and soil. Samples that were in deionized water did not produce the same insoluble matrix components. Tertiary treated water before passing the potted bell pepper plants and soil did not produce those insoluble matrix components either. To prevent matrix from damaging the GC injection port, extracts were filtered through EMD Millipore 0.22 µm PVDF centrifugal filters (Temecula, CA, USA) in a Beckman Coulter Microfuge 18 Centrifuge (Miami, FL, USA) at 13,500 RPM for 5 minutes.

The eluates were then transferred to 300 µL glass vial inserts in GC vials to make sure extract level was reachable for the GC injector needle.

Instrumental Analysis

Detection with GC-MS

For the development of the instrumental method used in this present study, the protocol developed by Yu and Wu (2012) was used as a starting point. However, the protocol only differed in the GC leg, where our GC leg reached a higher final temperature of 300 °C instead of 280 °C and for a longer time (5 minutes versus 3 minutes) to ensure optimal GC column clean-up between injections.

The instrumental analysis of the sample extracts was carried out in an Agilent 6890N Network GC system coupled to a 5975C inert MS, equipped with an Agilent 7683B series injector (Santa Clara, CA, USA). The capillary HP-5MS GC column (30 m, 0.25 mm i.d., 0.25 µm film thickness) (Santa Clara, CA, USA) was used for the chromatographic separation with He as carrier gas at a constant flow rate of 1.2 mL min^{-1}. Two consecutive injections with two different GC-MS methods (See Chapter 2: Methods for acquisition windows) were carried out per sample. Both methods were chromatographically identical. Hence, the following temperature ramp programs were identical. The injector temperature was 250 °C. The GC oven temperature was programmed from 70 °C (held for 1 min) to 120 °C at 20 °C min^{-1}, then to 250 °C at 10 °C min^{-1}, and finally to 300 °C (held for 5 min) at 5 °C min^{-1}. The total analysis time for each GC run was 31.5 min. The injection volume was 2 µL and injected in pulsed splitless mode. Most of the features in the MS leg were also common between Methods 1 and 2. Thus, mass spectra in both cases were obtained in electron impact ionization mode (70 eV) with selected ion monitoring (SIM) and a filament delay time of 11 min. The GC-MS interface, ion source and quadrupole temperatures were set at 280, 230 and 150 °C, respectively. Resolution at the quadrupole was set at low.

Methods for acquisition windows

In order to increase the method sensitivity, acquisition windows were established using the following criteria: 1) No more than 15 ions were monitored in each one; 2) The isotopically labeled internal standards were included in the same window as their corresponding analytes; and 3) The window had to be long enough to be trustworthy in case a change in the retention time took place. Having all this into consideration, two separate instrumental methods, Method 1 and Method 2, had to be created, both of them sharing the same chromatographic conditions. However, Method 1 and Method 2 differed in the acquisition windows as well as in the SIM ions monitored in each of them. Appendix A (Table A-2) shows the target compounds and their SIM ions monitored for each of them recorded by Method 1 (in white) and Method 2 (shaded) distributed in acquisition windows. One primary (in italics) and two secondary ions, used for quantification and confirmation, respectively, were monitored in all cases except for 17β-estradiol, which presented a poor fragmentation, so only one secondary ion was registered. Acquisition stopped at min 29 and 25 for Method 1 and Method 2, respectively, to prevent damage and pollution of the MS detector. Eleven minutes of solvent delay were set in both methods to prevent damage in the filament. Therefore, each sample extract was intended to undergo two consecutive injections, one for Method 1 and then Method 2.

Instrument control and data acquisition and evaluation, were performed with Agilent Technology MSD Productivity Chemstation E.01.00 software.

SIM method

As noted above, SIM ions were monitored in the acquisition windows. A SIM method was developed intending to include 50 CECS targeted in the project (See Appendix A, Table A-2 and Supplementary Data) but initial adaptations of the SIM methods and pretreatment methods

showed that 21 compounds are unable to be utilized in the analytical method. Retention times and ion fragments were obtained by injecting 10 mg L^{-1} individual standard solution of each analyte under full scans ranging from 50 to 1000 amu. After comparing the chromatographic baseline with an injection of 100μL of derivatization agent and 150 μL of EA, the characteristic peak was chosen as the analyte's peak. After observing the mass spectrum under the chromatographic peak obtained, the three most intense fragment masses were selected.

The most intense one, called the primary ion, is intended for quantification purposes as this would best represent what the analyte would undergo in ion fragmentation in the MS and would provide with the most intense response. The other two, the secondary ions, will help to confirm the presence of that analyte if found in the chromatograph at the same retention times with the retention time of the primary ion. The ratios, SIM2 / SIM1 and SIM3 / SIM1 rates, are also qualifying parameters. We did not use them, because if applied strictly, they lead to false negatives. But in theory we should have observed them. Eleven of the fifty analytes, including the pharmaceuticals atenolol (and its isotopically labeled identical, atenolol-d7), cyclophosphamide, nadolol, penciclovir, ranitidine, sulfamethoxazole, and tetracycline, 17α-ethynylestradiol as well as, the pesticides 1,2-dibromoethane, bentazon, and picloram showed no response whatsoever in the whole chromatographic run at full scans, even at varying individual standard solution concentrations. In addition, the pesticides chlorsulfuron and devrinol and the pharmaceuticals tylosin and 10,11-epoxy carbamazepine (carbamazepine's active metabolite) showed a very weak SIM signal even at high concentrated solutions like 400 μg L^{-1}, which is close to the maximum extract concentration expected in the samples collected during the pilot scale study. After validation, enalapril, meloxicam, hexachlorobenzene, tramadol, octyl-methoxycinnamate, and propranolol had sensitivity too low in tertiary treated wastewater.

As this method development is carried via GC, it is possible that those compounds mentioned are not able to derivatize in a sufficient extent and are unable to be volatilized for analysis. These 21 compounds were ruled out and not included in the method. Some of the other 29, such as triclosan (Kantiani et al., 2008), diazinon, chlorpyrifos (Navarro-Ortega et al., 2010), atrazine, metolachlor, octylphenol (Bono-Blay et al., 2012), bisphenol A, dibuyl phthalate, bis-2-ethylhexyl phthalate and nonylphenol (Guart et al., 2013) have been analyzed by GC without being derivatized in other studies. Some of them like triclosan, showed a higher response when derivatized. But in general most of these showed no difference between their signal before and after the derivatization step, suggesting that they did not undergo such reaction. Therefore, we derivatized all samples to avoid multiple varying sample pretreatment steps.

Despite having two sets of acquisition windows, certain compounds could not be quantified in the samples because retention time did shift slightly and limited the ions able to be detected in each acquisition window, generally 15 ions or less. However, this method was optimized to have the highest number of compounds analyzed while retaining sensitivity. Unexpectedly, Bis-(2-ethylhexyl)phthalate and terbutaline hemisulfate salt were unfortunately unable to be quantified in the samples as they were cut off due to their long chromatographic peak tails and slight shifts in retention time cut off as the project continued.

Surrogates

Five internal standards, ibuprofen-d3, N-(4-hydroxyphenyl)-acetamide, triclosan-d3, carbamazepine-d10, and bisphenol A-d6 were considered for this study based on the availability and cost of deuterated standards. However, the internal standard of carbamazepine-d10 used for the pretreatment of samples ran out prematurely in the study. A mixture containing 200 µL of the internal standards at 0.5 mg L^{-1} was added to all the samples in the pretreatment step to treat them

as surrogates (See Supplemental Data). Surrogates are added to samples prior to any sample extraction or analysis to undergo and experience the same treatment processes as the analyte of interest. This is to help correct for the errors introduced by sample pretreatment, extraction, evaporation, transfers, and the variability in GC-MS between runs that would decrease method recoveries. Typically, an analyte would have its own unique deuterated internal standard. Given the limitations of the cost and availability for all the compounds presented here, it is much more economical and widely accepted to use surrogates to match as closely as possible to the physical (volatility, molecular weight) and chemical characteristics (molecular structure, functional groups, polarity), and method restrictions (acquisition windows, retention times) that will also best correct for losses in the extraction recovery (Lopez-Serna et al., 2012).

Results

Quantification

The samples followed the above procedures for the sample pretreatment using the tertiary treated reclaimed nursery runoff as the environmental matrix to be extracted. An 8-point calibration curve covered the concentrations of the compound ranging from 1 to 800 ng L^{-1} in TTW. Solvent blanks in ethyl acetate were used to monitor the procedural and instrument background and possible carry over, as well as they helped clean the system between set of samples. See Appendix A, Table A-3 – for calculations on the validation parameters.

Validation parameters tested were accuracy, sensitivity, matrix effect, precision, and range of linearity.

Accuracy

Absolute recoveries for target compounds were determined by spiking samples, triplicates each, at two levels of concentration, 100 ng L^{-1} and 400 ng L^{-1}. These levels were chosen as typical low and high concentrations for most of compounds in these types of waters. For TTW matrix, recoveries were determined as percentages by comparing the peak areas obtained after the whole optimized method with the peak areas obtained from direct injection (2 µL) of equivalent amounts of standards in the GC column. Since the system used TTW, the matrix may have already contained the target compounds. Thus, TTW without spiking was analyzed and the peak areas were subtracted afterwards. Relative recoveries were determined as the ratio between the absolute recoveries for each compound versus the absolute recoveries for the corresponding surrogates. The corresponding surrogate for each compound is found in Appendix A, Table A-1.

Absolute recoveries mostly ranged from 50 to 135%. The lowest recoveries, under 50%, were acetylsalicylic acid, terbutaline hemisulfate salt, diazinon, Prowl™, diisobutyl phthalate, and 4-tert-octylphenol. Carbamazepine showed usually high absolute recovery most likely due to oversaturation of the direct vial containing carbamazepine at 800 µg L^{-1} and at 200 µg L^{-1} (correlating to a 400 ng L^{-1} 2000x concentration and 100 ng L^{-1} at 2000x concentration) in ethyl acetate, which showed domed peaks (not sharp) and is a clear sign of oversaturation in the GC-MS. Thus, analysis of carbamazepine is unreliable in this sense. Unfortunately, the solution containing the internal standard of carbamazepine used for pretreatment of samples ran out prematurely in the study, and therefore carbamazepine could not be quantified using its respectively IS, and no other IS used in this study was able to correct for carbamazepine's relative recovery.

Ketoprofen and dibutyl phthalate have also started to show oversaturation in their direct vials, but were corrected by their IS. The compounds that are under our considered threshold of relative recovery of 70% are: acetylsalicylic acid, acetaminophen, and Prowl ™. Unusually high relative recoveries were metolachlor and bis-(2-ethylhexyl) phthalate at 145% and 163%, respectively.

Nevertheless, aside from the 5 compounds mentioned above, all compounds shown a range from 70% to 111% relative recovery. Acetaminophen, acetylsalicylic acid, and Prowl ™ had relative recoveries lower than 75%. Acetylsalicylic acid is shown to have low absolute and relative recovery, suggesting that acetylsalicylic acid suffered loss in the extraction process due to its polarity to be retained in the Oasis HLB cartridges. In general, the range of recoveries still shows that our extraction process could be successfully applied to the compounds.

Table 2-1 Validation parameters for accuracy and matrix effect.

Target Group	Compound	% Absolute Recoveries	% Relative Recoveries	% Signal Suppression
Analgesics Anti-inflammatories				
	Acetylsalicylic acid	27.38	29.72	81.27
	Ibuprofen	55.90	87.88	65.75
	Salicylic acid	123.40	81.07	148.56
	Acetaminophen	58.29	38.29	61.73
	Naproxen	105.34	111.60	25.34
	Ketoprofen	169.25	84.22	38.24
	Diclofenac	135.80	92.52	87.82
Lipid regulators				
	Clofibric acid	97.91	106.29	65.57
	Gemfibrozil	96.64	75.20	27.05
Psychiatric drugs				
	Carbamazepine	78.82	96.02	160.09
Antibiotics				
	Triclosan	108.18	84.18	100.93

Cardiovascular drugs Beta blockers				
	Metoprolol	52.80	108.43	18.98
	Terbutaline	37.58	77.17	350.05
Estrogens				
	Estrone	52.68	78.52	25.26
	17beta-Estradiol	71.95	107.22	27.29
Personal Care Products (UV-Filters)				
	4-Methyl-benzylidene camphor	50.66	75.49	35.28
Pesticides Herbicides				
	Atrazine	56.92	84.13	243.82
	Diazinon	40.46	83.74	136.88
	2.4-D	66.35	104.31	54.25
	Metolachlor	68.02	145.99	56.80
	Chlorpyrifos	84.33	98.08	59.26
	Bromacil	79.68	92.67	45.41
	Prowl(TM)	38.38	60.33	30.36
Plasticizers				
	Diisobutyl phthalate	131.75	86.56	48.63
	Dibutyl phthalate	155.52	77.38	71.58
	Bis-(2-ethylhexyl) phthalate	109.49	163.18	49.55
	Bisphenol A	134.92	91.92	152.69
Detergents (Alkylphenols)				
	Octylphenol	21.90	70.23	135.29
	Nonylphenol	94.07	102.12	38.66

Matrix effect

The matrix effect was evaluated for each compound by comparing the peak area obtained for the TTW spiked with analytes at 100 ng L^{-1} and 400 ng L^{-1}, subtracting out the existing compounds in the TTW, with those obtained from deionized water (DI) solutions spiked at the same concentrations. In the absence of matrix effects, the analytes' peak areas should be similar

in both types of matrices. However, it is to be expected that with higher amounts of matrix in water, the matrix effect will be greater than deionized water matrix. Signal suppression was calculated as:

$$\text{Signal suppression (\%)} = \frac{Area(spiked) - Area(blank)}{Area(DI)} x\ 100 \qquad (2\text{-}1)$$

Where Area(spiked) is the analyte peak area in the spiked matrix sample, Area(blank) is the analyte peak in the nonspiked matrix, and Area(DI) is the analyte peak in the spiked DI water. Values over 100% signify signal enhancement, and values under 100% signify signal suppression.

Most of the compounds experienced signal suppression (21 compounds), where 6 compounds experienced enhancement, 3 did not experience suppression nor enhancement. Most of the analgesics/anti-inflammatory pharmaceuticals showed suppression – 6 out of 7 compounds showed signal suppression except for salicylic acid. This correlation between the polarity of the compounds and suppression effect was previously observed by Postigo et al. (2008). Estrogens showed some of the worst suppression effect at 25-27%. Pesticides and herbicides also showed signal suppression except for atrazine and diazinon. The compounds that showed enhancement were salicylic acid, terbutaline, atrazine, diazinon, bisphenol A, and 4-tert-octylphenol. Compounds like salicylic acid, bisphenol A, and 4-tert-octylphenol with the presence of matrix effect may enable them to show a higher sensitivity than presumed despite higher background noise. The calibration curve development also used the same matrix water which would already compensate for the matrix effect through the use of surrogates for quantification.

Table 2-2 Validation parameters for method and instrumental sensitivities.

Target Group	Compound	LOD instrumental (pg)	LOQ instrumental (pg)	LOD (ng/L)	LOQ(ng/L)
				TTW	TTW
Analgesics Anti-inflammatories					
	Acetylsalicylic acid	4.03	13.43	15.26	50.86
	Ibuprofen	1.13	3.76	0.34	1.14
	Salicylic acid	0.15	0.49	0.14	0.46
	Acetaminophen	1.60	5.32	1.61	5.38
	Naproxen	0.89	2.97	4.27	14.24
	Ketoprofen	1.03	3.44	2.25	7.49
	Diclofenac	1.46	4.87	2.91	9.69
Lipid regulators					
	Gemfibrozil	0.20	0.66	14.11	47.03
	Clofibric acid	1.72	5.74	7.33	24.44
Psychiatric drugs					
	Carbamazepine	2.63	8.77	0.19	0.64
Antibiotics					
	Triclosan	0.28	0.93	1.44	4.80
Cardiovascular drugs Beta blockers					
	Metoprolol	15.91	53.05	19.54	65.13
	Terbutaline	0.38	1.28	15.66	52.20
Estrogens					
	Estrone	5.69	18.96	1.15	3.85
	17beta-Estradiol	21.05	70.16	12.25	40.83
Personal Care Products (UV-Filters)					
	4-Methyl-benzylidene camphor	1.38	4.60	6.92	23.05
Pesticides Herbicides					
	Atrazine	28.13	93.78	18.19	60.62
	Diazinon	7.52	25.06	5.92	19.72
	2.4-D	3.12	10.39	16.35	54.50
	Metolachlor	2.48	8.27	2.62	8.74
	Chlorpyrifos	10.61	35.36	11.14	37.12
	Bromacil	1.29	4.29	6.27	20.90
	Prowl(TM)	1.07	3.56	27.94	93.15

Plasticizers					
	Diisobutyl phthalate	3.53	11.77	16.80	56.00
	Dibutyl phthalate	5.03	16.75	1.37	4.57
	Bis-(2-ethylhexyl) phthalate	1.47	4.89	2.13	7.09
	Bisphenol A	0.11	0.37	0.65	2.16
Detergents (Alkylphenols)					
	Octylphenol	0.33	1.10	1.26	4.21
	Nonylphenol	6.52	21.74	15.47	51.58

Sensitivity

The limits of detection (LODs) and limits of quantification (LOQs) of the method were experimentally determined as the concentration of analyte giving a signal to noise ratio of 3 and 10, respectively (Lopez-Serna et al., 2012). Table 2-2 shows the calculated LODs and LOQs for the target compounds. With TTW matrix, it can be expected that a greater matrix effect will create higher background noise of the chromatography. However, analyzing the concentrations of the analytes at 400 ng L^{-1} and 100 ng L^{-1}, the method sensitivity showed 14 out of 29 compounds were able to be detected at less than 5 ng L^{-1}; and 28 out of 29 compounds were able to show detection less than 20 ng L^{-1}. Thus this shows the multicomponent analysis is sufficient for a variety of compounds, including polar compounds like pharmaceuticals.

However, 8 compounds suffered LOQ, among them 7 compounds were able to be quantified at 50 – 65 ng L^{-1}, and 1 compound (Prowl ™) at 93 ng/L. Thus, this method requires further improvement in order to better quantify these compounds. However, we had hoped that the spiking of 400 ng L^{-1} in our samples would be sufficient to see the compounds even at that quantification limit, and it is suitable for detection of trace levels of these chemicals in a complex water matrix, possibly suitable for river water.

Precision

The overall method repeatability was calculated as the relative standard deviation (RSD) between the triplicates of the spiked TTW at 100 ng L^{-1} and 400 ng L^{-1} of each analyte. RSD values lower than 10% for intraday and 20% for interday sampling analyses are considered to be satisfactory. All compounds except for acetylsalicylic acid and ketoprofen showed Intraday RSD to be lower than 10%. All compounds except for acetylsalicylic acid, diclofenac, metoprolol, and bisphenol A had lower than 20% Interday RSD. Acetylsalicylic acid RSD is poor possibly due to the difference of conditioning the SPE cartridges to retain the compound.

Table 2-3 Validation parameters for precision and linearity.

Target Group	Compound	Repeatability (%RSD)		Linearity		
		Intraday	Interday	R^2	Range (ng/L)	Linear Regression Eqn
Analgesics Anti-inflammatories						
	Acetylsalicylic acid	11.07	22.88	0.9983	LOQ - 800	y = 38.553x + 10769
	Ibuprofen	9.04	14.39	0.9971	LOQ - 800	y = 4238.7x + 29316
	Salicylic acid	2.18	7.87	0.984	LOQ - 800	y = 10777x + 580075
	Acetaminophen	1.78	18.38	0.9916	LOQ - 800	y = 3080.5x - 1844.9
	Naproxen	4.53	20.07	0.9976	LOQ - 800	y = 2600.6x + 14.244
	Ketoprofen	11.60	15.60	0.9918	LOQ - 800	y = 1868.3x + 93568
	Diclofenac	4.55	33.09	0.9944	LOQ - 800	y = 334.81x - 6468.5
Lipid regulators						
	Clofibric acid	8.07	17.55	0.99	LOQ - 800	y = 1487.8x - 6564.5
	Gemfibrozil	4.11	10.24	0.999	LOQ - 800	y = 1265.1x + 53353
Psychiatric drugs						
	Carbamazepine	5.31	12.41	0.9148	LOQ - 800	y = 3846.1x + 1E+06
Antibiotics						
	Triclosan	6.22	13.96	0.9931	LOQ - 800	y = 1468x + 65276
Cardiovascular drugs Beta blockers						

	Metoprolol	5.16	55.59	0.999	LOQ - 800	y = 81.661x - 4466.3
	Terbutaline	2.55	15.00	0.891	LOQ - 800	y = 66.953x + 100344
Estrogens						
	Estrone	7.00	16.39	0.9936	LOQ - 800	y = 1126.3x + 29853
	17beta-Estradiol	9.46	17.45	0.9962	LOQ - 800	y = 90.174x + 6255.6
Personal Care Products (UV-Filters)						
	4-Methyl-benzylidene camphor	5.42	10.64	0.9902	LOQ - 800	y = 332.96x + 8049
Pesticides Herbicides						
	Atrazine	3.48	13.62	0.9937	LOQ - 800	y = 98.345x + 9022.9
	Diazinon	4.19	13.83	0.9816	LOQ - 800	y = 94.404x + 4265.2
	2.4-D	6.77	7.32	0.9978	LOQ - 800	y = 179.38x + 19239
	Metolachlor	4.13	11.32	0.9905	LOQ - 800	y = 1771.9x + 35042
	Chlorpyrifos	7.10	20.41	0.9923	LOQ - 800	y = 234.1x + 13255
	Bromacil	4.00	13.38	0.9914	LOQ - 800	y = 1715.5x + 45684
	Prowl(TM)	5.75	17.65	0.9947	LOQ - 800	y = 215.1x - 14855
Plasticizers						
	Diisobutyl phthalate	6.54	9.16	0.9909	LOQ - 800	y = 2515.6x + 567989
	Dibutyl phthalate	2.39	18.66	0.9857	LOQ - 800	y = 3993.6x + 694395
	Bis-(2-ethylhexyl) phthalate	5.41	15.95	0.9065	LOQ - 800	y = 1144.5x + 2E+06
	Bisphenol A	7.22	22.41	0.9193	LOQ - 800	y = 3218.2x + 190584
Detergents (Alkylphenols)						
	Octylphenol	3.77	9.07	0.9957	LOQ - 800	y = 1878.1x + 28829
	Nonylphenol	4.33	19.11	0.9707	LOQ - 800	y = 327.99x + 65256

Linearity

An 8-point calibration curve was constructed, using least-squares linear regression analysis, analyzing the linearity of the spiked TTW with analytes at concentrations ranging from 1 ng L^{-1} (or the limit of quantification) to 800 ng L^{-1}. Linearity is evaluated by measuring the coefficient

of determination (R^2) to quantify the goodness of fit of the linear regression (Yu & Wu, 2012). All compounds extended their range of linearity from their limits of quantification (noted in Table 2-3) to 800 ng L^{-1}. All calibration curves were linear with correlation coefficients (R^2) higher than 0.90; 25 compounds showed $R^2 > 0.97$, except terbutaline hemisulfate salt, bisphenol A, bis-(2-ethylhexyl)phthalate, and carbamazepine whose R^2 values were $0.90 < R^2 < 0.97$.

Chapter 3

Design and operation of slow sand filtration columns

Site description

The pilot setting was located at the South Coast Research and Extension Center (SCREC) in Irvine, CA. Five slow sand filtration columns were utilized for the experiment and were constructed and set up inside a greenhouse at SCREC (See Appendix B, Figure B-1). The tertiary treated wastewater (TTW) used in the present study was provided by the Irvine Ranch Water District (Irvine, CA) and was treated through primary, secondary (activated sludge and membrane bioreactors), and tertiary (disinfection by UV and chlorine) treatments.

The design of the slow sand columns for this experiment was based on optimizations of slow sand filtration columns by Harris (2006) and Lee and Oki (2013), with slight adjustments to include the injection of a spiked system of contaminants and a stabilized influent flow rate.

SSF design

Column design

Five slow sand filters were utilized for the experiment. The column dimensions and design was based on the design of the columns by Lee and Oki (2013) as follows (See Figure 3-1): Each column was created with 1 m sections of 10.16 cm (4 in) diameter PVC pipe, joined end to end with a flange. The bottom flange was layered with a size gradient of rocks and pebbles. In order of bottommost to upmost, the layers were: small rocks (ca. 3 cm), large aquarium pebbles (Kordon LLC, Hayward, CA), smaller pebbles (Kordon LLC, Hayward, CA), #2-/16 coarse sand (RMC Pacific Materials, Inc., Pleasanton, CA), #3 coarse sand (RMC Pacific Materials, Inc., Pleasanton, CA), and #60 sand lapis luster, quartz sand (RMC Pacific Materials, Inc., Pleasanton,

CA); the uppermost, fine sand was the same as the filtering media. Each layer was just thick enough to cover the layer below.

The bottom half of each SSf was filled with 1 m (39.37 in) depth of #60 mesh quartz luster sand (RMC Pacific Materials, Inc., Pleasanton, CA) over the gradient of pebbles and rocks. The sand was thoroughly rinsed with drinking water and the supernatant was decanted several times to remove fine particulate matter.

The top half of the filter was continuously filled to a depth of about 1 m above the sand surface with tertiary treated wastewater. A head of 1 m provides enough pressure to push the leachate through the system. The leachate was supplied by a peristaltic pump that maintained the flow rate at 120 mL min^{-1} in the system manifold (See Figure 3-1, Figure 3-2). It was intended that 120 mL min^{-1} was sufficient to fill the columns and ensure the columns would have sufficient water levels (of 1 m head) at all times if the column outlet rates were controlled at 20 mL min^{-1}. It would leave 20 mL min^{-1} excess to dispose into the overflow line. Outlet flow rates were set using Cole Palmer 50 mL rotameters and maintained each column flow at 20 mL min^{-1}. These rotameters controlled the flow at the very end of each column (see Figure 3-1, Figure 3-2). From each rotameter, 25 cm of 1.3 cm diameter irrigation tubing extends into the discharge line to release outlet flow. This discharge line contains all the treated wastewater from the outlets, along with the excess water in the overflow line.

Unsampled leachate in the discharged line is sent to a discharge barrel prior to sending it to into a line that irrigates plants outside of the greenhouse.

TTW plant leachate

Eighty-two bell pepper potted plants were irrigated with the greenhouse tertiary treated wastewater (See Appendix B, Figure B-3). Each plant was irrigated every two hours for 2 min through a drip irrigation nozzle at a rate of 8 L h^{-1}. This leachate will eventually provide the inoculation of the bacterial community known as the schmutzdecke. Leachate was collected in a 190 L plastic tank (Ronco Plastics, Tustin, CA), and was pumped by a Little Giant 2E-38N-WG pump (Hackensack, NJ, USA) and was forced strained through two 100 µm pipe thread screen filters in series on the leachate supply line. The irrigation regime ensured that the water level in the collection tanks remained at constant level and prevent any larger settled particulate matter from entering the pump. The filtered water on the leachate supply line joined the peristaltic pump where the flow rate was furthered controlled at 120 mL min^{-1} to a manifold distributing the leachate to the five replicate SSFs (See Figure 3-2, Figure B-4). The distribution manifold consisted of 5 cm diameter PVC pipe with 1.3 cm diameter irrigation tubing connecting it to each slow sand filter.

To relieve back pressure on the peristaltic pump, excess leachate in the leachate supply line was returned to the collection tank (See Figure 3-2).

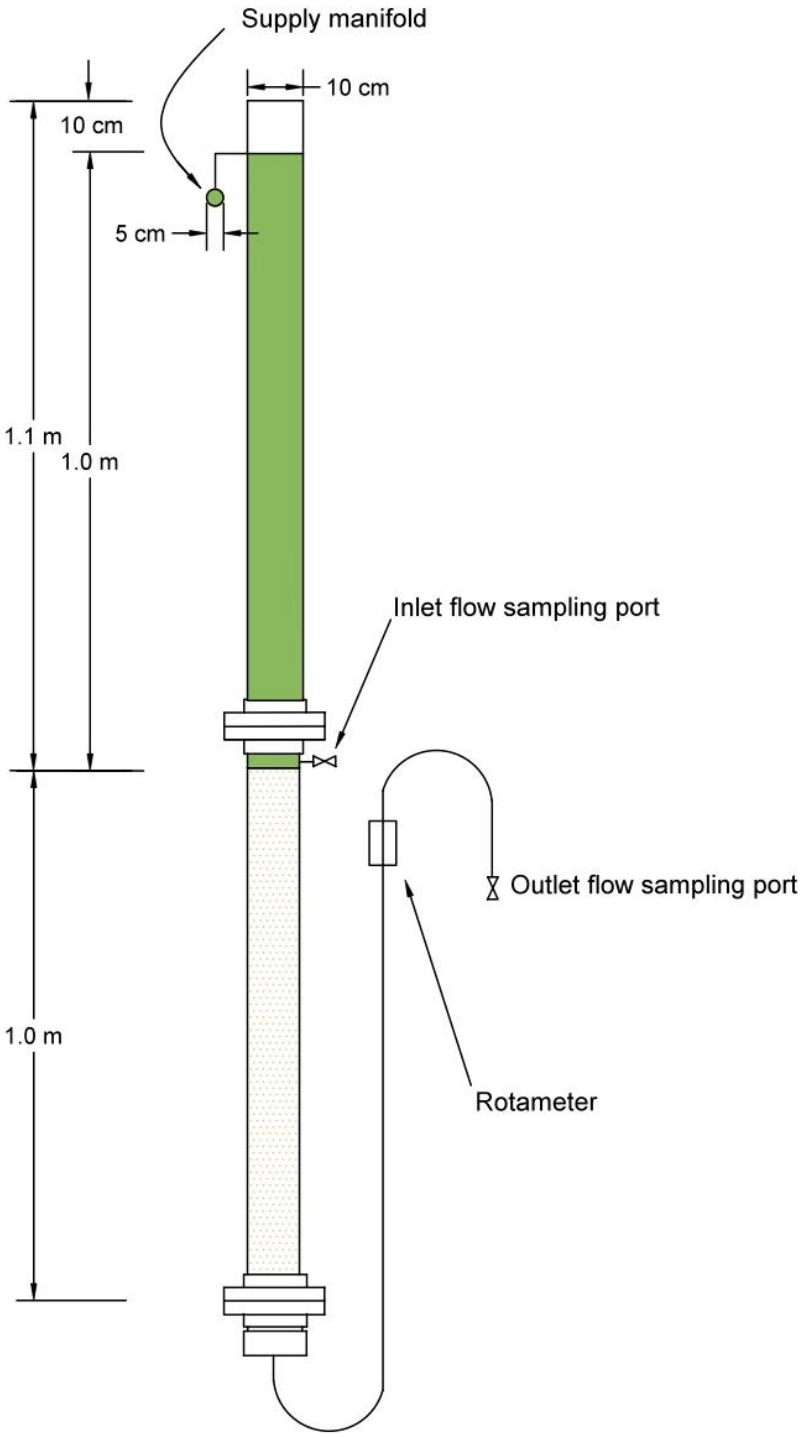

Figure 3-1 Diagram of a slow sand filter.

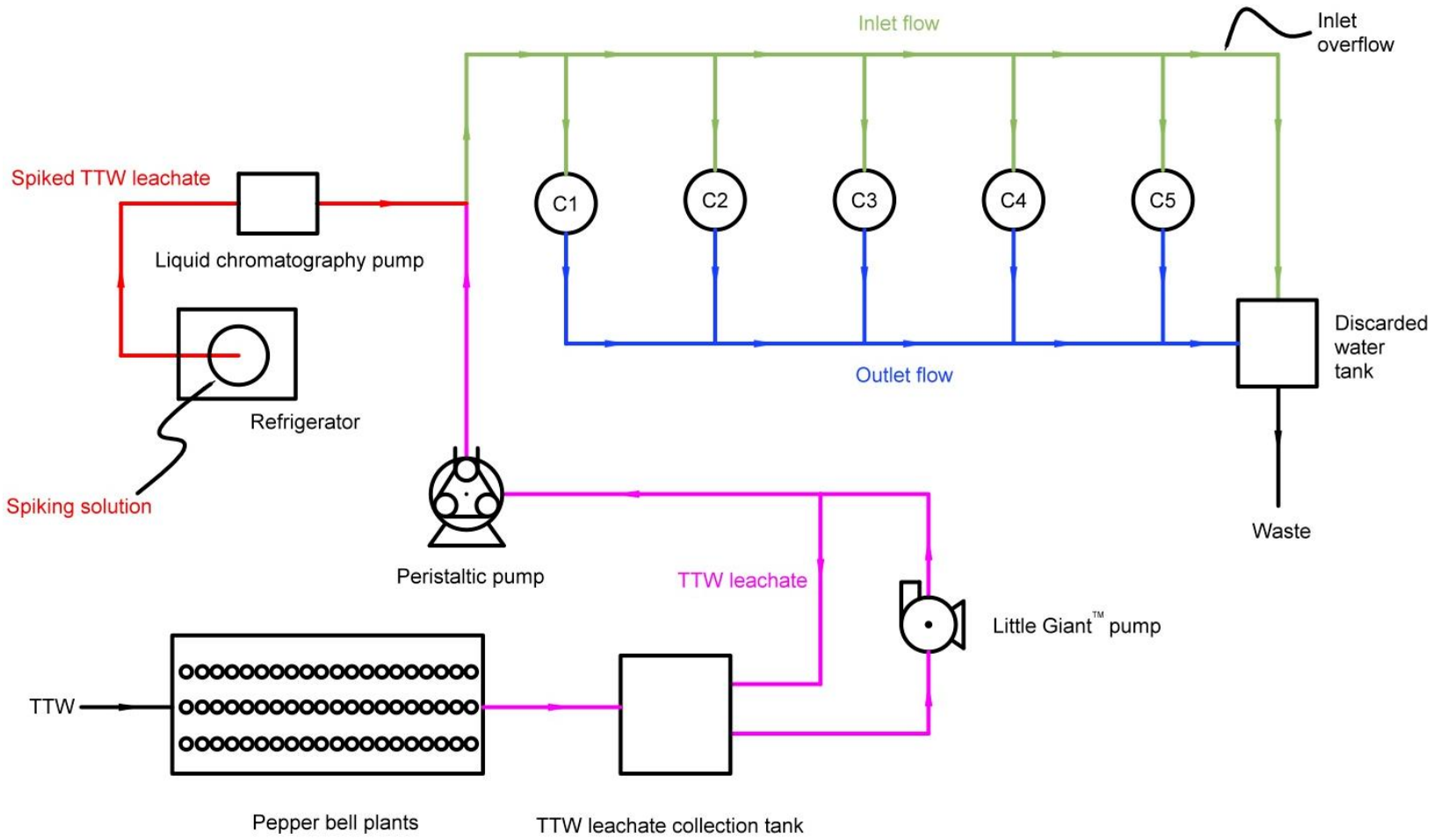

Figure 3-2 Schematic shows the layout and flow of TTW plant leachate throughout the greenhouse.

Online spiking design

In order to assure an observable concentration level and be able to reliably monitor the efficiency of the SSFs, the tertiary treated wastewater was online spiked at 400 ng L^{-1} with the 30 CECS studied in this project. The aqueous 400 µg L^{-1} "spiking solution" was pumped at a flow rate of 0.2 mL min^{-1} by a Perkin Elmer Series 10 Liquid Chromatography Pump (Waltham, MA, USA) into the leachate supply line just below the manifold. The spiking solution was kept in darkness at a mean temperature of 4 °C inside a portable refrigerator at all times.

Data monitoring

Campbell Scientific, Inc. Micrologger 21X (Logan, UT, USA) catalogued the ambient and refrigerator temperatures. Copper constantan thermocouple probes were used to measure temperature. Data collection included the average, maximum, and minimum temperature recordings of both ambient and refrigerator temperatures at hourly increments throughout the entire experiment.

Sample Collection and Pilot Scale Study Initiation and Maintenance:

Pretreatment

Before the study was initiated, SSFs were fed direct drinking water for two weeks to soak, settle, and compact the sand bed. This also helped remove the air bubbles trapped in the sand and removed air bubbles that formed in the rotameters. After preconditioning the SSFs, the drinking water was swapped to the tertiary treated wastewater leachate to establish biofilm layers in the columns. Once the drinking water was swapped to the tertiary treated wastewater leachate, the project officially began. The online spiking solution was also immediately initiated (See Chapter 3: Online system design).

Project timeline

The pilot scale project took place during November 2014 through January 2015. Sample collection began one day after the project kick off. Samples were taken twice or three times per week during the first month and then weekly until the end of the experiment. Hence, a total of 14 days were sampled; days 1, 2, 4, 8, 10, 15, 20, 25, 30, 36, 43, 51, 57, and 59.

Sample collection

At each sampling port, one liter (1 L) sample was collected. SSF inlets were simultaneously collected from the port above the sand surface for all five SSF replicates and totaled five inlet samples, with one sample per inlet port (See Appendix B, Figure B-2). SSF outlets were the filtered leachate that had passed through the SSFs and were collected over 50 min at approximately 20 mL min^{-1} to maintain the flow rate through the columns while sampling. Five outlet samples were taken, with one sample per outlet port. At the leachate supply line sampling port, one liter (1 L) sample was collected. This collected the plant leachate before being spiked. In total, eleven samples were collected for one day. All samples used one liter (1 L) amber glass bottles. Once sampled, bottles were immediately stored in darkness inside a cooler filled with ice and transported to the lab for processing.

Column maintenance

When the issue arose that the flow rates through the columns could not be kept at 20 mL min^{-1}, de-clogging maintenance was carried out. The sign of clogging was the rate of the outlet flow decreasing and that opening the rotameter further could not maintain the desired flow. As part of common SSF practices, de-clogging was necessary to help maintain flows and crucial to the understanding of post-maintenance filtration processes.

In this event, all pumps were stopped and water in the lines was drained. Water in the columns were drained by draining through the inlet sampling port or drained by opening the middle flange, leaving just an inch or so of water on top of the sand surface. Afterward, a copper rod connected to drinking water was inserted through each column to help loosen up sand aggregates and to skim off the schmutzdecke layer out of the column. This was kept at a low flow rate to ensure minimal loss of sand.

After each maintenance session, the system was filled up again with the TTW leachate and the experiment resumed. This happened three times during the whole project, in particular days 22, 33, and 44. Sampling was not reliable until 24 hours after each maintenance session until the spiked TTW leachate has reliably passed through the column. However, it may take several days to settle sand particles and disperse bubbles produced in the sand via maintenance. Thus, sampling dates were adjusted accordingly.

Temperature

The experiment ran through November 11, 2014 to January 8, 2015. The average daily ambient temperature ranged from roughly 12 °C to 20 °C on the days of sampling. The average temperature for the entire experiment was 16 °C. There were two weeks of the project, from December 27, 2014 to January 6, 2015, where the ambient daily average temperature was the coldest at 11 – 14 °C. From November 11, 2014 to December 26, 2014, and from January 7, 2015 to January 8, 2015, ambient temperatures ranged from 15 to 19 °C. Refrigerator temperature was kept to a maximum of 5 °C.

Physical and chemical parameters of TTW

See supplementary data for water quality laboratory analysis reports provided by Irvine Ranch Water District.

Results and Discussion of SSF

This chapter shows the results of the SSF columns removal rates and trends and provides a summarized literature review of other related biofiltration column studies' removal rates and processes for removal.

Percent removal rates were calculated from the equation for each column and averaged

$$\frac{(C_{inlet}-C_{outlet})}{C_{inlet}} x\ 100 \qquad (3\text{-}1)$$

where C_{inlet} is the concentration measured in the inlet and C_{outlet} is the concentration measured in the outlet of the column. C_{inlet} and C_{outlet} have already subtracted the concentrations of the existing compounds in the main inlet supply line.

Pharmaceutically Active Compounds (PhACs)

Analgesics/Anti-inflammatory drugs (NSAIDs)

Table 3-1 Mean removals in the entire project, highest mean removals, and lowest mean removals for analgesics/anti-inflammatory drugs.

Compound	Average Removal (%)	Highest Removal (%)	Day of Highest removal	Lowest Removal (%)	Day of Lowest removal
Ibuprofen	28.52	70.62	10	5.76	1
Salicylic acid	61.15	87.83	8	22.38	1
Acetaminophen	33.79	80.06	10	9.96	36
Naproxen	25.97	39.73	20	11.22	59
Ketoprofen	41.70	53.77	15	13.70	59
Diclofenac	30.49	49.12	43	18.48	8

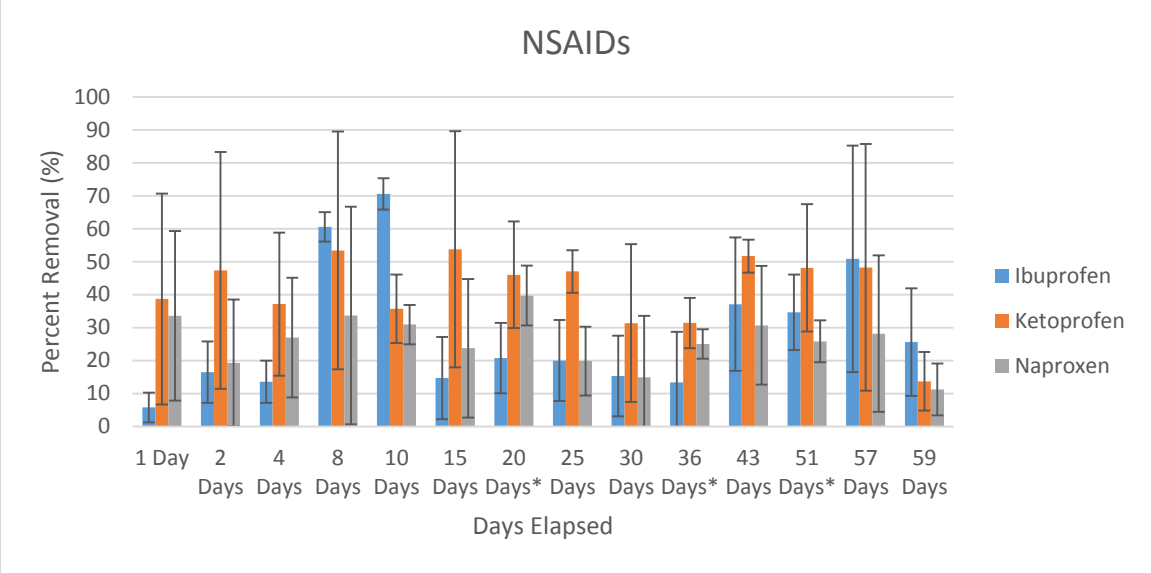

Figure 3-3 Mean percent removals of selected NSAIDs.. Asterisk (*) denotes a maintenance event has occurred. Maintenance events occurred on Days 20, 31, and 42. Error bars denote standard deviation between the five columns.

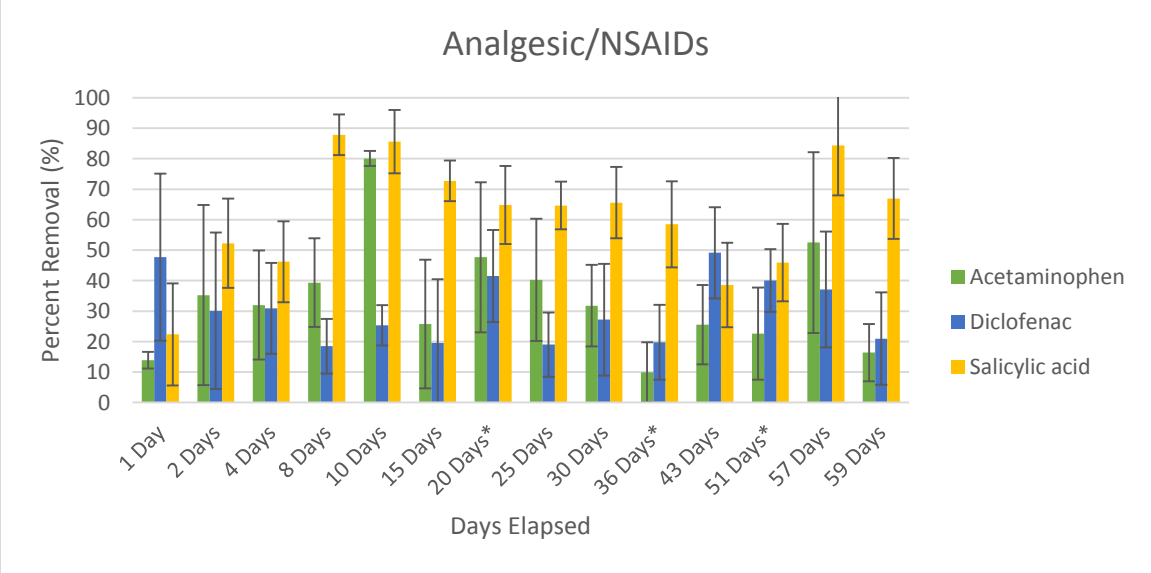

Figure 3-4 Mean percent removals of selected NSAIDs and analgesic (acetaminophen). Asterisk (*) denotes a maintenance event has occurred. Maintenance events occurred on Days 20, 31, and 42. Error bars denote standard deviation between the five columns.

In Table 3-3, mean removal rates of each pharmaceutically active compound are shown. Among analgesics and anti-inflammatory drugs, the mean percent removal ranged from 25-61%. Salicylic acid had the highest average removal (61.15 ± 12.2%) and up to 87% removal. Contradictory to other studies, NSAIDs like ibuprofen, ketoprofen, and naproxen were not greatly removed, with mean removal rates of 28 ± 12.5%, 41 ± 21.21%, and 25% ± 15.8%, respectively. However, the removal of ibuprofen reached 87% after 10 days. In Figure 3-3, ibuprofen has low removal but immediately increases from 4-8 days, a 47% increase and eventually plateaus on day 10, reaching 70%. Salicylic follows a similar trend and also eventually starts plateauing. Given more time, the result could be similar to other literature showing salicylic acid removal averaging 97% in other low cost treatment techniques (Camacho-Munoz et al., 2012). The general trend of these NSAIDs appear to reach their plateaus after the 8^{th} and 10^{th} day of the project (naproxen also experiences a 14% increase from 2-8 days as well), and/or declines or reaches a steady state from days 10 – 43 days. Afterwards, there appears to be an increase once again from 43 days – 57 days and a rapid decline afterwards. Ibuprofen, ketoprofen, naproxen, and salicylic acid are all part of the propionic acid class (carboxylic acid with chemical formula CH_3CH_2COOH), sharing similar chemical structures, functional groups, and molecular weight. It is not surprising to see that these compounds share the same removal trend and extent of reduction. However, it is very contradicting to other literature, which has found ibuprofen, naproxen, and ketoprofen to be removed in a range from 40-92% of various conventional and low-cost wastewater treatments such as activated sludge, lagooning, and constructed wetlands (Camacho-Munoz et al., 2012). However, worst removal rates were shown in lagooning systems, where typically the water matrix is poorly aerated and has lowest production of sludge for adsorption (Camacho-Munoz). Other studies have also reported that aerobic conditions result in higher PhAC removal efficiencies compared to anaerobic systems (Krkosek et al., 2014; Matamoros et al., 2009; Suarez

et al., 2010). Thus, it is possible that although our system was initially aerated, the decline shown by day 10 may have had microorganisms use up these compounds as substrates or other compounds, and may leave anaerobic conditions in the columns. Mersmann et al. (2002) also reported significant degradation for ibuprofen in a column experiment with aerobic conditions and nitrate conditions of 5.2 mg L-1. In a study to remove organic micropollutants in a drinking water biofilter (Zearley & Summers, 2012), ibuprofen and naproxen were removed greater than 95% and 72%, respectively, which they attributed the increases in removal to be from acclimation over 2-3 months of micropollutant exposure. They regarded the increasing removal with time to be an indication of secondary substrate utilization – microorganisms which were not originally present in the biofilter may have eventually adapted to utilizing these micropollutants. While our research has extended to two months, acidic PhACs such as ibuprofen, naproxen, ketoprofen, and salicylic acid have reached a steady state already by 10 days. Thus, it would appear that the microbial community has already adapted in this time period to this group of compounds, and removal could be more attributed to adsorption and secondary substrate utilization.

It is known that the ambient redox conditions and pH are regarded as key parameters for the removal of PhACs. While log K_{ow} can usually describe how hydrophobic or hydrophilic a compound is, it may not accurately describe the adsorption behavior between soil and water for acidic PhACs because of electrostatic interactions (Maeng et al., 2011). Many non-steroidal anti-inflammatory drugs (NSAIDs) and lipid regulators are acidic PhACs and remain in ionized forms at certain pH levels (Cunningham, 2008; Maeng et al., 2011). For acidic compounds, log D (distribution coefficient) is a better indicator of the hydrophobicity of a compound. A log D less than 1 is a hydrophilic compound and a compound with a log D value of equal or greater than 3 is a hydrophobic compound (Maeng et al., 2011). From Maeng et al. (2011) ibuprofen, ketoprofen, and naproxen have a log D of 1.44, 0.41, and 0.05 at pH 8, respectively which can explain why

these compounds may not sorb onto organic matter at all, and its failure for retention may not have allowed biodegradation to occur whatsoever. However, column studies by Maeng et al. (2011) simulating bank filtration, acidic PhACs such as ibuprofen, ketoprofen, and naproxen were removed with efficiencies greater than 88%. The higher removals for these compounds was suggested as a result of less potential competition of humic substances and PhACs for binding sites in the column media. In their column studies of variable time acclimated columns, their 60 day columns had higher removals than the 10 day columns, which they attributed to the organic carbon content acclimated to the sand, with 20% diclofenac removal in the 60 day acclimated column and 5% in the 10 day acclimated column. They found that removal efficiencies of acidic PhACs (such as diclofenac) increases as initial biodegradable organic carbon (BDOC) increases. Given that diclofenac in Figure 3-4 has shown to be relatively consistently averaging 30±15.7% removal and similar in removal by Maeng et al. (2011), it may confirm that low BDOC has a relationship in the low removals. This finding is consistent with diclofenac being poorly removed (0-28%) in WWTP and other biofiltration column studies (Reungoat et al., 2014; Camacho-Munoz et al., 2012; Zearley & Summers, 2012; Maeng et al., 2011; Casas & Bester, 2015;).
In the study by Maeng et al. (2011), removal efficiencies of diclofenac decreased to 10% when filtering with only non-chlorinated tap water. As non-chlorinated tap water has fewer humic substances and lower BDOC concentrations, they proposed diclofenac should have more favorable conditions of adsorption to sand. However, with the low removal of diclofenac with non-chlorinated tap water compared to the other matrices researched, they assumed diclofenac removal is dependent on BDOC available. This contradicts the finding by Onesios & Bouwer (2012) who discovered diclofenac had mean removals of <10% in all columns, regardless of the varying acetate concentration used to promote substrate utilization. Other studies have reported diclofenac with low to no sorption (Banzhaf et al., 2012) and others (Krkosek et al., 2014) have

reported diclofenac removal as high as (80 to 100%). The proposed main mechanism for the high removal (Krkosek et al., 2014) was due to adsorption as their biofilter columns highly removed diclofenac and then decreased after 7 days, which is consistent with having more binding sites available as a new biofilm was rapidly forming.

Our findings are in line with their column studies under abiotic conditions, which an average removal efficiency of 21% was observed for acidic PhACs and under biotic conditions, the efficiency increased to 59%. However, since salicylic acid (also an acidic PhAC) has such a higher average removal, in our supposed biotic conditions, removal would be 37±15.9% averaged for acidic PhACs. From these results, low removal efficiencies shown by acidic PhACs may be a result of low biodegradable carbon or a combination of competition with humic substances for binding sites. As our low removal rates correspond with that of abiotic column results by Maeng et al. (2011), it may suggest that these compounds are not biodegrading by microorganisms and are rather removed simply by poor sorption processes. However, low biodegradable organic content is most likely not a probable cause as the water was running through plants and soil rich in BDOC. As salicylic acid had increased significantly from 4 – 8 days (41% increase) and remained steadily constant (roughly 71% average), it could suggest that among the acidic PhACs, salicylic acid was the preferred substrate. Standard deviation between the columns show that salicylic acid was very strongly removed in all columns, indicating that BDOC content may have been consistently supplied rather than utilizing salicylic acid as the preferred substrate since it is unlikely salicylic acid is the preferred substrate across all columns.

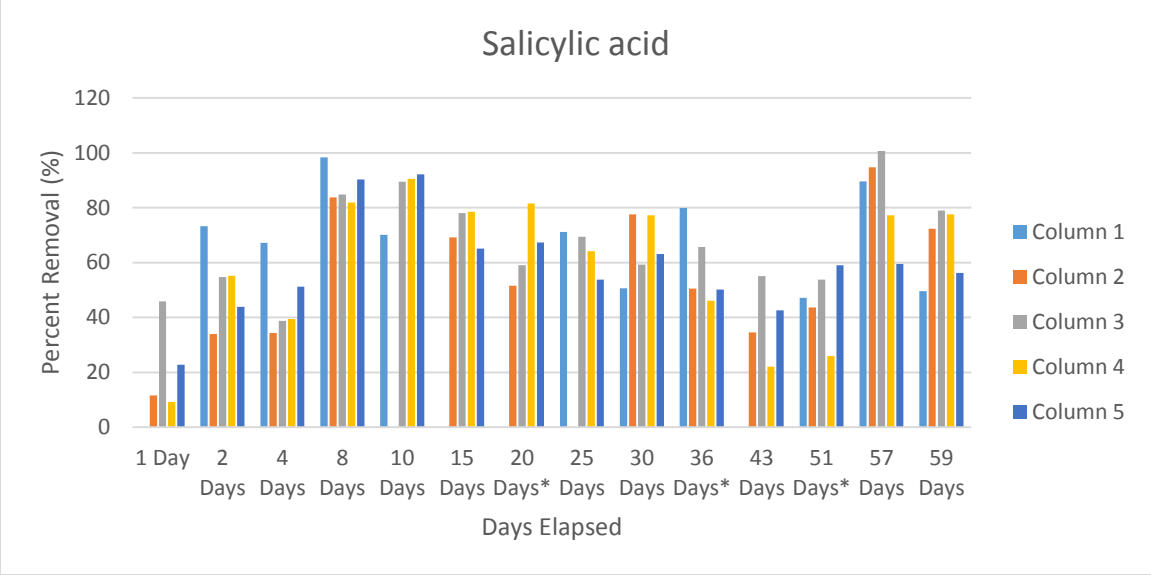

Figure 3-5 Mean percent removal by the five columns over the length of the project. Salicyclic acid shows little variation between columns in its removal.

With a more neutral PhAC like acetaminophen, the log K_{ow} was used to estimate hydrophobicity of the compound. Acetaminophen has a log K_{ow} of 0.27, a hydrophilic compound. Acetaminophen is predicted to not sorb onto the compounds. However, 30±15.9% removal was observed with the highest removal of 80% on day 10. Despite the hydrophilicity, higher removals can be observed on various days, with removals of 50% or more which suggests biodegradation is the main mechanism as sorption would not be possible. Acetaminophen has shown to have moderate to high removal (59%->95%) in multiple biofilter column studies (Bertelkamp et al., 2014; Maeng et al., 2011; Zearley & Summers, 2012; Yu-Chen Lin et al., 2010; Reungoat et al., 2011). Maeng et al. (2011) showed acetaminophen removed even under biodegradable carbon-limited conditions, up to 91%. In their abiotic column study, they showed acetaminophen to be highly affected, with only 6% average removal. This suggests microorganisms were capable of removing acetaminophen even under limited BDOC conditions. In a study by Reungoat et al.

(2014), acetaminophen was well removed (85%) with reclaimed water and filtered through sand. Although no direct conclusion was made, the results showed a significant removal with sand filtration with prior aeration. With the findings of our other NSAIDs, it is possible that a low BDOC was occurring and microorganisms were preferentially degrading compounds like salicylic acid and acetaminophen, even under lower aerobic conditions.

Lipid Regulators

Table 3-2 Mean removals in the entire project, highest mean removals, and lowest mean removals for lipid regulators.

Compound	Average Removal (%)	Highest Removal (%)	Day of Highest removal	Lowest Removal (%)	Day of Lowest Removal
Gemfibrozil	23.61	49.37	57.00	14.37	10
Clofibric acid	27.03	52.51	10.00	9.50	25

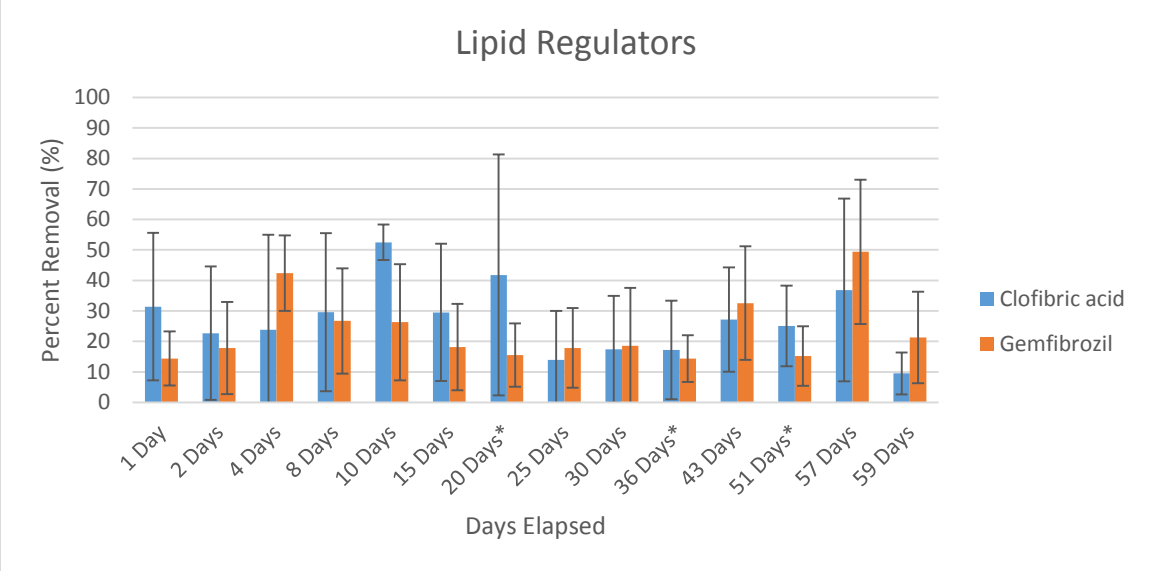

Figure 3-6 Mean percent removals of lipid regulators over the length of the project. Asterisk (*) denotes a maintenance event has occurred. Maintenance events occurred on Days 20, 31, and 42. Error bars denote standard deviation between the five columns.

Lipid regulators fared the worst mean removal rates among any group. In Table 3-2, Gemfibrozil and clofibric acid had a mean removal of 23±14.6% and 27±20.6%, respectively and the highest removal experienced by gemfibrozil and clofibric acid were 49 and 52%, respectively. Gemfibrozil and clofibric acid both share similar and consistent trends where removal never significantly changes (Figure 3-6). We expect biodegradation to occur as removal significantly increases at the beginning of the project due to the biofilm growing and increasing the binding sites. This is not happening here as shown in Figure 3-6. From this, the low removal can be interpreted as adsorption based mechanism. Gemfibrozil and clofibric acid can be considered acidic PhACs, with log D of 2.22 and clofibric acid of -1.08; Gemfibrozil is classified as ionic and clofibric acid hydrophilic at pH of 8 (Maeng et al., 2011). Our results are consistent with poor removal rates of gemfibrozil and clofibric acid in most of the literature (Camacho-Munoz et al., 2012; Maeng et al., 2011; Onesios & Bouwer, 2012). However, contradicting studies have shown higher removals of gemfibrozil. Zearley and Summers (2012) found 70-94% removal of gemfibrozil, and Reungoat et al. (2011) found roughly 50% removal of gemfibrozil. There are conflicting results that support whether the presence of carbon substrates supplying more BDOC increased removal (Onesios & Bouwer, 2012; Maeng et al., 2011; Rauch-Williams et al., 2010). In biofiltration studies actively supplying acetate as carbon substrates, gemfibrozil showed better removals in columns with no acetate and 50 μg L^{-1} acetate than the column receiving 1000 μg L^{-1} acetate (Onesios & Bouwer, 2012). However, Rauch-Williams et al. (2010) studied the effects of various WWTP effluent-derived carbon substrates on trace organic chemical removals in biologically active laboratory columns and found that gemfibrozil was better removed in the presence of carbon substrates. Maeng et al. (2011) performed batch reactor studies and found both compound removal to be low, which they attributed to the biodiversity of microorganisms,

BDOC, or adsorption competition between humic substances and selected PhACs. They compared batch reactors that were supplied with non-chlorinated tap water and found a significant decrease to 28% and 0% mean removal of gemfibrozil and clofibric acid, respectively. Therefore, the supply of BDOC and cometabolism may play an important role in the removal. However, in their biofilter column studies, columns with nonchlorinated tap water compared with MAR supplied water was 7% and 36% mean removal for gemfibrozil, and 20 and 11% for clofibric acid, respectively. The complex matric of MAR did not seem to increase the mean removal for clofibric acid. This study and Maeng et al. (2011) are in agreement with that of Onesios and Bouwer (2012). In fact, in abiotic column studies, gemfibrozil and clofibric acid experienced a mean removal of 29% and 46%, respectively (Maeng et al., 2011). This is comparable to the removal that gemfibrozil achieved in MAR acclimated media water, and produced even higher removal for clofibric acid; which signifies carbon substrates do not enhance removal. In time acclimated columns in 10 and 60 days, they discovered removal increased from 0 to 35% for gemfibrozil and increased from 5 to 8% for clofibric acid. From this data, we interpret the low removal in our results are not affected by BDOC.

Estrogenic compounds

Table 3-3 Mean removals in the entire project, highest mean removals, and lowest mean removals for estrogenic compounds.

Compound	Average Removal (%)	Highest Removal (%)	Day of Highest removal	Lowest Removal (%)	Day of Lowest removal
Estrone	39.24	66.16	43	10.79	10
17β-Estradiol	35.49	65.19	57	10.56	25

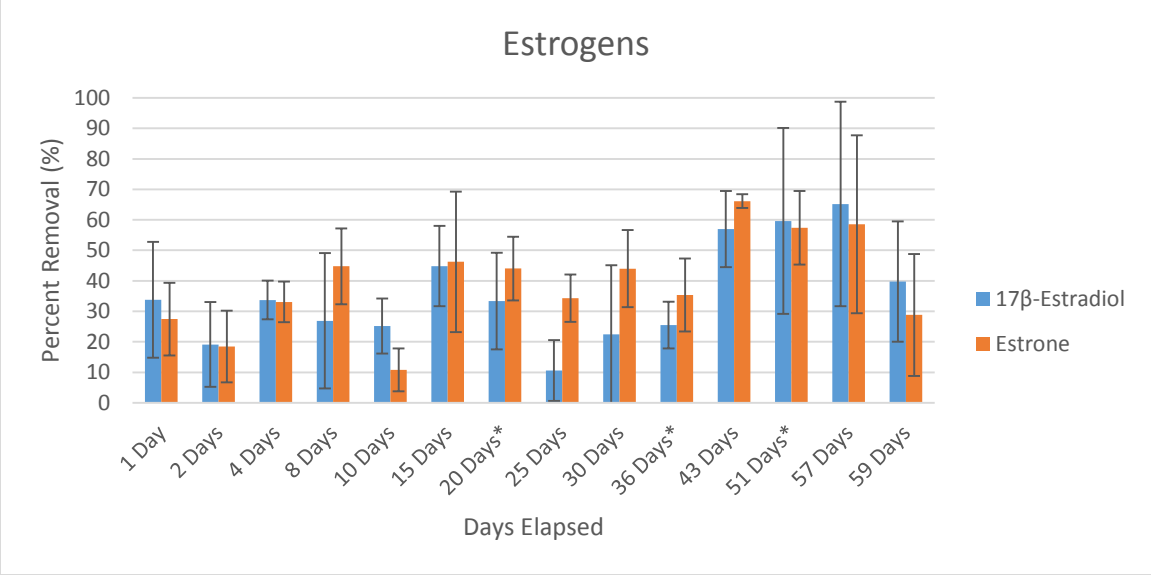

Figure 3-7 Mean percent removals of estrogenic compounds over the length of the project. Asterisk (*) denotes a maintenance event has occurred. Maintenance events occurred on Days 20, 31, and 42. Error bars denote standard deviation between the five columns.

Table 3-3 shows the mean removal of estrogenic compounds, estrone and 17β-estradiol (39% and 35%, respectively). While the estrogenic compounds show moderately low removal, towards the end of the project both compounds were reaching up to >60% removal by 43 days. Studies have shown that conventional techniques using activated sludge can remove estrone from 25% to greater than 99% (Nakada et al., 2007; Camacho-Munoz et al., 2012). Estrone has shown reduction efficiencies of 66% to >99% when ozone was used, and chlorine reduced estrone from 70% to >99%. However, there are limited biofilter column studies to show removal of estrone and 17β-estradiol. In biosand filter studies, removal efficiencies for estrone was 14.4±12% (Kennedy et al., 2013), which is even lower than our results. In previous SSF column studies using tertiary treated wastewater as the water matrix, results showed little to no removal of estrone within 150 days as well (Ho et al., 2011). Thus, it is possible that with more time for the column to acclimate, more positive removal can occur. Between days 150 and 316 of the study of

Ho et al. (2011), they showed estrone removal was 96±10%. From our increasing removal results towards the end of our project, it is possible that the organisms involved in degradation were still present in the biofilm eventually acclimating to the presence of estrogens (Muller et al., 2010; Ho et al., 2011) and if continued with the project, more degradation may occur. In this study, certain columns showed 80-100% removal of estrone and 17β-estradiol by 43 – 51 days. Ho et al. (2011) explained the abundance of estrogen-degrading organisms may need to reach a critical limit before measurable removal can occur – this observation has been observed in other biological studies. Ho et al. (2011) ran sterilized controls to determine any losses due to non-biological processes. While the biotic column experienced 98% removal, the sterile column experienced little removal, which supports SSF contains organisms responsible for degradation of estrone. Octanol-water partition coefficient (log K_{ow}) of estrone and 17β-estradiol are 3.13 and 3.9, respectively which are moderately hydrophobic. In anaerobic MAR biofiltration column studies, the retardation coefficients for the trace organics they studied generally reflected their octanol-water partitioning coefficient (Patterson et al., 2010). 17β-estradiol showed a retardation factor of R of 52 (contaminant would travel 52 times slower than the calculated filter water velocity), showing substantially retarding in the sediment. Their column studies showed a lag time of 1-2 months for 17β-estradiol to be degraded from 150 μg L^{-1} to near 10 μg L^{-1} (a near 90% reduction). While our studies did not show high removals, the removal of estrogenic compounds by biodegradation is promising. The study by Patterson et al. (2010) suggested the potential removal for organic removal would be through anaerobic reductive degradation or co-metabolism. Studies have shown that natural estrogens are generally readily biodegradable by bacteria such as E. coli (Kennedy et al., 2013). Specific ammonia monooxygenase enzymes have been postulated to be responsible for the degradation of estrone (Racz and Goel, 2010, Ho et al., 2011). While this study has not advanced to inoculating the columns with specific bacteria, it is possible for the

columns to contain estrogenic degradable microorganisms. Haig et al. (2014) have discovered dominant phyla in their sand filter media such as *Proteobacteria, Bacteriodetes, Acidobacteria, Actinobacteria, Planctomycetes, Verrucomicrobia,* and *Gemmatimonadetes*.

Herbicides and Pesticides

Table 3-4 Mean removals in the entire project, highest mean removals, and lowest mean removals for herbicides and pesticides.

Compound	Average Removal (%)	Highest Removal (%)	Day of Highest removal	Lowest Removal (%)	Day of Lowest removal
Atrazine	40.84	89.22	8	8.70	59
Diazinon	58.58	81.27	51	37.18	36
2.4-D	51.69	92.89	4	22.70	59
Metolachlor	38.26	69.50	51	17.56	4
Chlorpyrifos	44.78	75.13	43	13.05	10
Bromacil	41.20	81.22	51	17.55	36

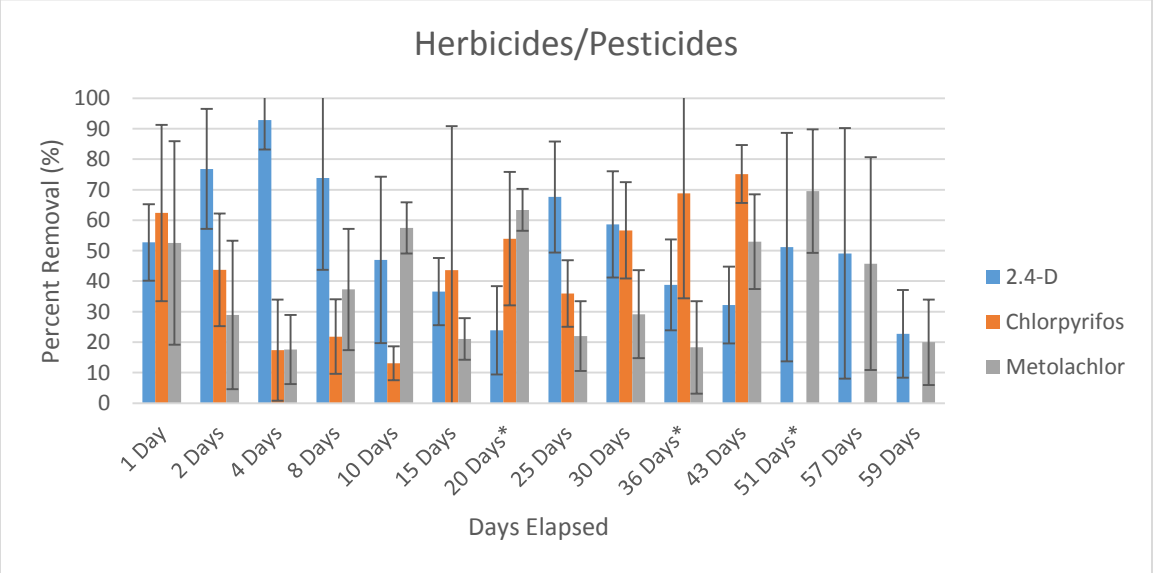

Figure 3-8 Mean percent removals of selected herbicides (2.4-D, metolachlor) and pesticide (chlorpyrifos) over the length of the project. Asterisk (*) denotes a maintenance event has occurred. Maintenance events occurred on Days 20, 31, and 42. Error bars denote standard deviation between the five columns.

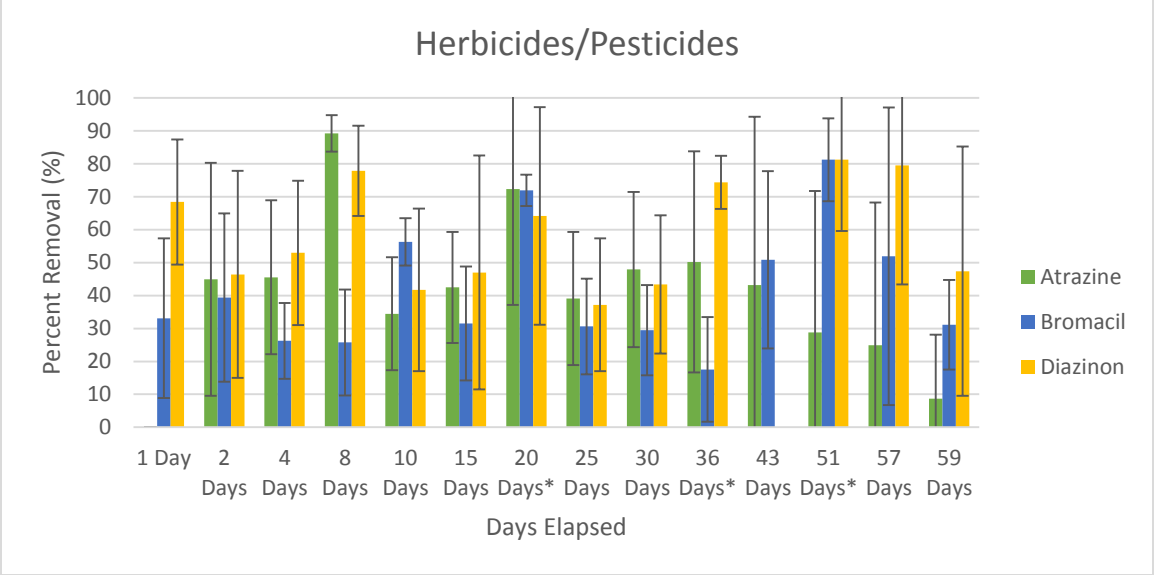

Figure 3-9 Mean percent removals of selected herbicides (atrazine, bromacil) and pesticide (diazinon) over the length of the project. Asterisk (*) denotes a maintenance event has occurred. Maintenance events occurred on Days 20, 31, and 42. Error bars denote standard deviation between the five columns.

Table 3-4 shows the percent mean removals for herbicides and pesticides. Mean removals for this group were classified as moderate removals with a small range of removal from 38 to 58%. Diazinon has the highest removal at 58% and metolachlor the lowest at 38%. The trends of the compounds are split into two graphs for better representation, see Figure 3-8 and Figure 3-9. To the author's knowledge, there are limited studies evaluating the removal of pesticides and herbicides in biofilter systems. Most to date have been limited to atrazine, a popular herbicide. In Figure 3-8, 2.4-D and metolachlor have variable trends. 2.4-D increases in removal for the first 4 days and then declines afterwards. Chlorpyrifos and metolachlor follow a similar trend in the first 4 days where removal is declining from day 1. Chlorpyrifos begins increasing removal from day 10 to 43, which is a sign of biodegradation occurring. With the initial decline of metolaclor, this could suggest saturation of binding sites in the first couple of days, which is typical of slow

sand filters. Metolachlor also experiences some increases in removal by the last couple weeks of the project, but the removals between the columns have high variability. This is also consistent with the log P values of chlorpyrifos and metolachlor, which have log P values of 5.0 and 3.0, hydrophobic and moderately hydrophobic. Log P is used as an indication of the hydrophobicity of non-ionizable compounds. Thus, we can anticipate adsorption in the beginning of the project. The fact that there is an initial decrease may signify saturation, but also preference of substrate of the microbial community. While chlorpyrifos and metolachlor were declining in days 1-4, 2.4-D seems to be the preferred substrate among the group. 2.4-D begins a decreasing removal trend from days 4 – 20 which could imply that other microbial communities were evolving to be more generalists and possibly preferring chlorpyrifos and metolachlor. In a study by Zearley and Summers (2012) using drinking water biofilters, they found 2.4-D mean removal rates ranged from 68-77%, metolachlor 6.6-8.7%, and chlorpyrifos 63-85%. 2.4-D removal was found to plateau at steady state within 3 months of exposure (Zearley & Summers, 2012). Microorganisms acclimated to 2,4-D in batch reactors has been observed (Celis et al., 2008). 2,4-D showed increasing removal with time in the study by Zearley and Summers (2012), an indication of secondary substrate utilization. Our results were not that quite similar, as mean removals of 2.4-D and chlorpyrifos were lower and metolachlor removal was significantly higher than removals in that study. However, differences could arise from that study using biofilters that had been in full scale for several years prior to the laboratory study. In our case, the SSFs have started from scratch and adsorption and microbial growth will play a larger part in the beginning of the removal. Increasing removal trend of up to 75% for chlorpyrifos is a good indication that more removal can occur and the SSF can effectively remove chlorpyrifos. However, the variability of removal for 2.4-D and metolachlor is still uncertain but possible for at least moderate rates of removal.

Diazinon, atrazine, and bromacil all show steady state from the beginning of the project to the end, with some variable high removals in between but no relationship could be determined. The trends would suggest adsorption was the main mechanism of removal, but their log P values suggest biodegradation should be the main mechanism. Log P values of diazinon, atrazine, and bromacil are 3.8, 2.6, and 2.1 respectively, which are moderate to low hydrophobicity. Our results are not in agreement with other sand/biofilter studies. Atrazine showed very poor removal in other soil column studies (Benotti et al., 2012; Zearley & Summers, 2012; Ho et al., 2011; Ilhan & Ong, 2012). In the study by Zearley and Summers (2012), diazinon and atrazine mean removals were 12-40% and 0.2-3%, respectively. Ho et al. (2011) conducted laboratory sand filter studies and found negligible atrazine removal despite nearly 250 days of exposure. Although no atrazine removal was found, they conducted biological tests and found presence of the *atzA* gene, which is responsible for the initial step of atrazine degradation (Ho et al., 2011). Previous studies have shown biodegradation of atrazine can be limited by presence of other nitrogenous compounds (Hunter and Shaner, 2009; Ho et al., 2011). The tertiary treated effluent that Ho et al. (2011) used did contain nitrogenous compounds at a concentration much greater than that of atrazine. The lack of crucial inorganic compounds required by atrazine degrading bacteria or lack of time for acclimation could explain lack of atrazine degradation (Ho et al., 2011). In another stormwater biofilter study (Zhang et al., 2014), removal of atrazine was variable in their series of tests (13.8 and 70.5% removal was observed). Zhang et al. (2014) noted the importance of ambient temperatures for degradation. The differences in temperature may have allowed for the large disparity in removal. Winter conditions allowed atrazine removal at 70.5% and summer conditions were 13.8%. As our study was conducted in a winter-time setting, it is possible that lower temperature conditions favored microbial development towards degrading atrazine.

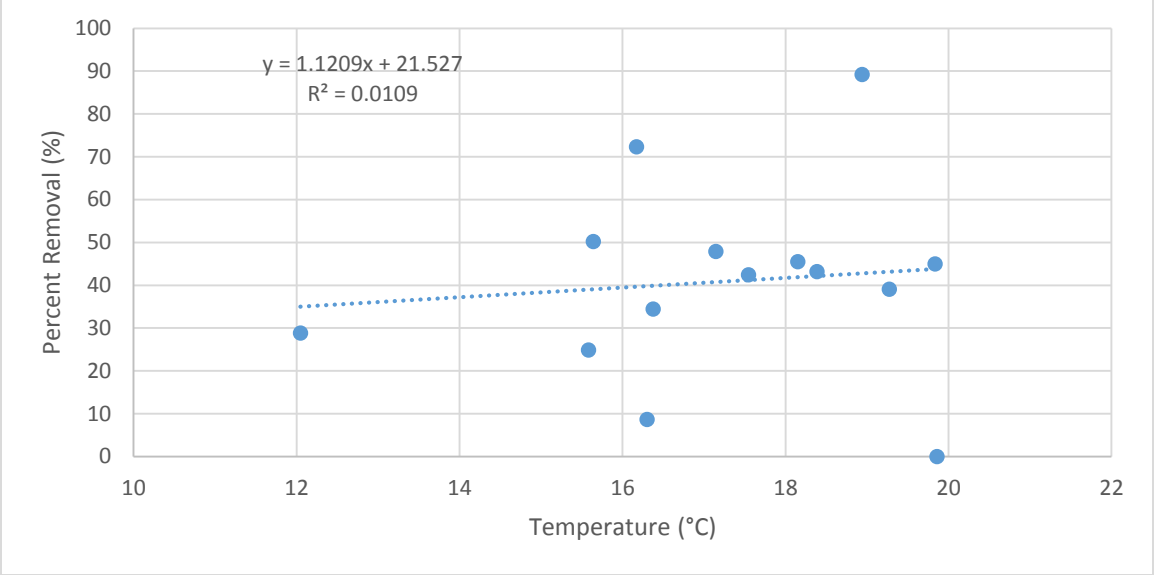

Figure 3-10 Linear regression shows percent removal of atrazine and temperature are not correlated. R^2 is 0.01.

In a linear regression analysis with temperature and percent removal, no significant correlation, with R^2=0.01. The ambient temperature was not as variable as it could be in a daily temperature cycle in summer; our ambient temperature maintained between 12°C to 20°C in the Fall-Winter months. Although most of these compounds' removal rates show no correlation with temperature, it can be noted that the range of temperature is regulated with the greenhouse and there is little extreme temperature variation to differentiate any trends. This project would be better to monitor in a summertime setting where temperatures are consistently higher.

Atrazine removal between columns was one of the most variable, with 0% removal up to 95% removal observed between columns, especially within the last few weeks of the project which may lead to no atrazine removal for most columns (See Figure 3-11). This is most likely due to microbial variation between columns. While we used five columns to be replicates of each other, they are not exact replicates due to the environmental conditions and substrates for

microorganisms to grow. Averaging the removals between the columns may not be an accurate representation of the removal rate. The consistent supply of TTW plant leachate used in the study should allow adaptation over time. However, the TTW plant leachate may have changed as a result of the maturity in the bell pepper plants during the project. Studies have reported greater atrazine removal with the use of natural water as influent causing dramatic increases in atrazine degradation since microorganisms indigenous to natural waters are more adapted to the column environment (Goux et al., 2000).

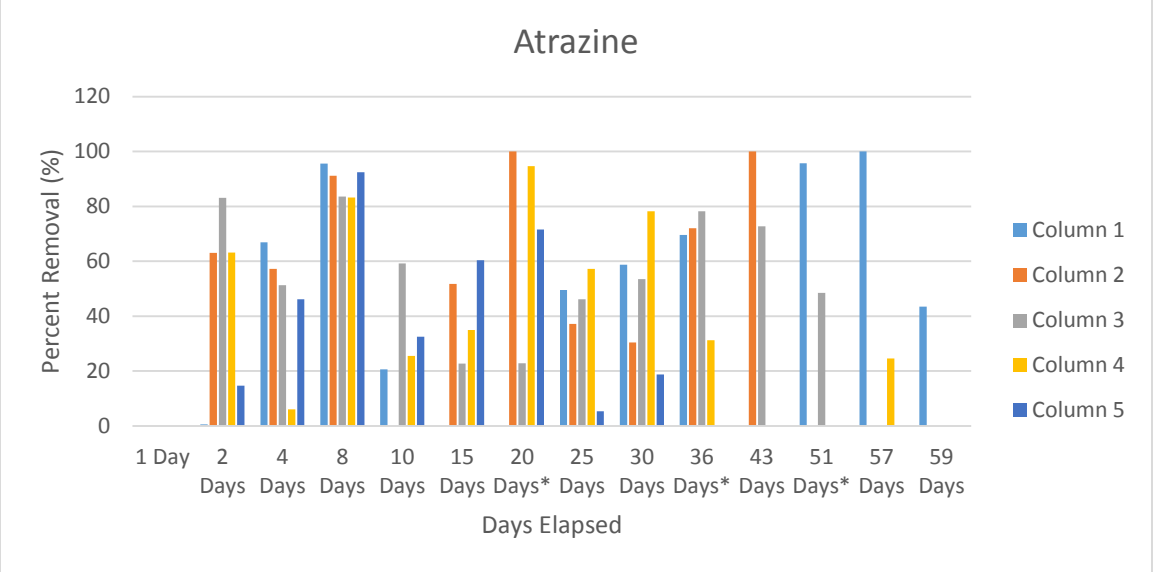

Figure 3-11 Mean percent removal of atrazine in the five columns over the length of the project. Compared to Salicylic acid in Figure 3-5-, Atrazine has widely variable removal rates between the columns, especially from 43 days to 59 days.

Plasticizers, Alkylphenols, Antibacterial, and UV-Filter

Plasticizers

Table 3-5 Mean removals in the entire project, highest mean removals, and lowest mean removals for plasticizers.

Compound	Average Removal (%)	Highest Removal (%)	Day of Highest removal	Lowest Removal (%)	Day of Lowest removal
Diisobutyl phthalate	47.06	57.93	8	32.28	43
Dibutyl phthalate	56.22	72.62	36	41.70	4
Bisphenol A	23.47	63.62	10	3.96	4

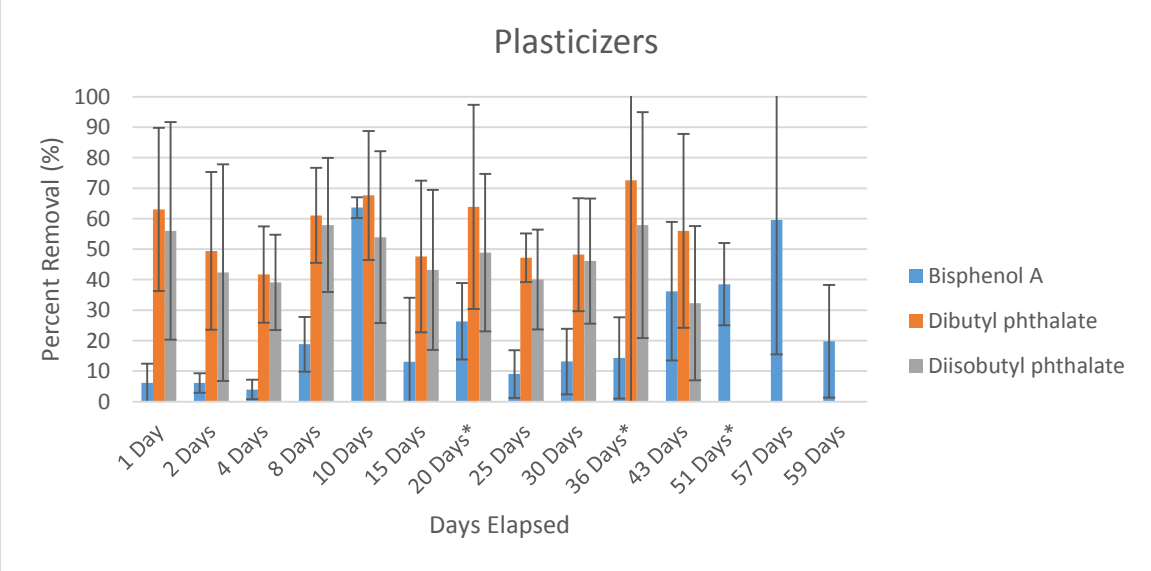

Figure 3-12 Mean percent removals of plasticizers over the length of the project. Asterisk (*) denotes a maintenance event has occurred. Maintenance events occurred on Days 20, 31, and 42. Error bars denote standard deviation between the five columns.

Bisphenol A was classified as one of the worst removal in the study, with an average removal of 23±13.5%. Mean removals as low as 3-6% were experienced in the first 4 days, although there seemed to be a substantial removal increase on day 10 and day 57, averaging

63±3.4% and 59±44% removal, indicating that the sudden increase and rapid decrease in removal is attributed to sorption saturation rather than a more consistent utilization of BPA as a substrate. It is also possible that BPA eventually begins to increase in removal as a result of acclimation of microorganisms to degrade BPA. In most cases, we begin to see <0% removal among columns, showing that BPA was beginning to oversaturate the columns in days 10 – 36 with the outlet having higher concentrations than the inlet. The oversaturation may allow a higher toxicity to persist in the columns, potentially adversely affecting the microbial community. BPA has a literature log K_{ow} of 3.6, which shows high hydrophobicity. In anaerobic MAR biofilter column studies, Patterson et al. (2010) figured the experimental retardation coefficient to be R=18, which is substantially retarded. Ying et al. (2003) determined R value of 8 for BPA in sorption batch experiments using deep anaerobic limestone sediment. These findings would suggest that the high hydrophobicity and retardation would make BPA partition to the sand media. Patterson et al. (2010) estimated a lag time of 1-2 months for BPA, with removals decreasing from 500 µg L^{-1} to complete removal in less than 30 days. In comparison, their sterile columns took nearly 400 days to decrease from 500 µg L^{-1} to roughly 180 µg L^{-1}. This shows that biological degradation is a major mechanism for removal. Thus, as we can see in Figure 3-12, as the removal is increasing on day 57, it is possible that the BPA accumulated and sorbed onto the sand is being biodegraded, which allows for a higher positive removal. The combination of saturation of BPA has also probably led to more unavailable sites for other compounds to sorb onto. Degradation of BPA and other bisphenols by slow sand filtration studied by Katayama-Hirayama et al. (2010) was only 20% average, consistent with our findings. There may not also be enough time to degrade BPA, as it took 180 days to remove BPA at a >80% removal rate in a study by Zearley & Summers (2012).

The pththalates fared better in removal. Diisobutyl phthalate and dibutyl phthalate had moderately good removals of 47±26.3% and 56±27.4%, respectively. From day 1, the phthalates showed consistent steady removals. The last few days of the project could not detect any phthalates in the inlet and this could be a result of poor injection of phthalates from the HPLC pump spiking into the system, or degradation of the compounds prior to sampling. Log K_{ow} of dibutyl and diisobutyl phthalate are 4.11- 4.57 which shows high hydrophobicity, even higher than BPA. Thus, we do expect higher removals from these phthalates, the primary mechanism as adsorption. The relatively similar phthalate removal suggests that the shared similar chemical structures or physical properties played an important part in the removal. This also suggests that microbial community is unbiased towards removal of one or the other phthalate, or that sorption favored the removal of both compounds nearly equally. To the author's knowledge, there are very limited studies on the removal of dibutyl phthalate and none for diisobutyl phthalates for biofiltration column studies. Zhang et al. (2014) studied the use of stormwater biofilters to remove micropollutants, among them was dibutyl phthalate. Dibutyl phthalate was classified as good removal, 71-92% removal in a series of tests. They attributed removal by the ability of dibutyl phthalate to strong adsorb (log K_{oc} >4.0). There was no conclusion on the biodegradation of dibutyl phthalate in that particular study. Regarding the trends of the phthalates, there seems to be no strong increase in removal, which does not suggest heavy biological activity to occur, even if adsorption and biodegradation were occurring simultaneously.

Alkylphenols

Table 3-6 Mean removals in the entire project, highest mean removals, and lowest mean removals for alkylphenols.

Compound	Average Removal (%)	Highest Removal (%)	Day of Highest removal	Lowest Removal (%)	Day of Lowest removal
4-(tert-octyl)phenol	35.63	53.09	1	12.81	43
4-Nonylphenol	43.78	91.75	1	14.08	30

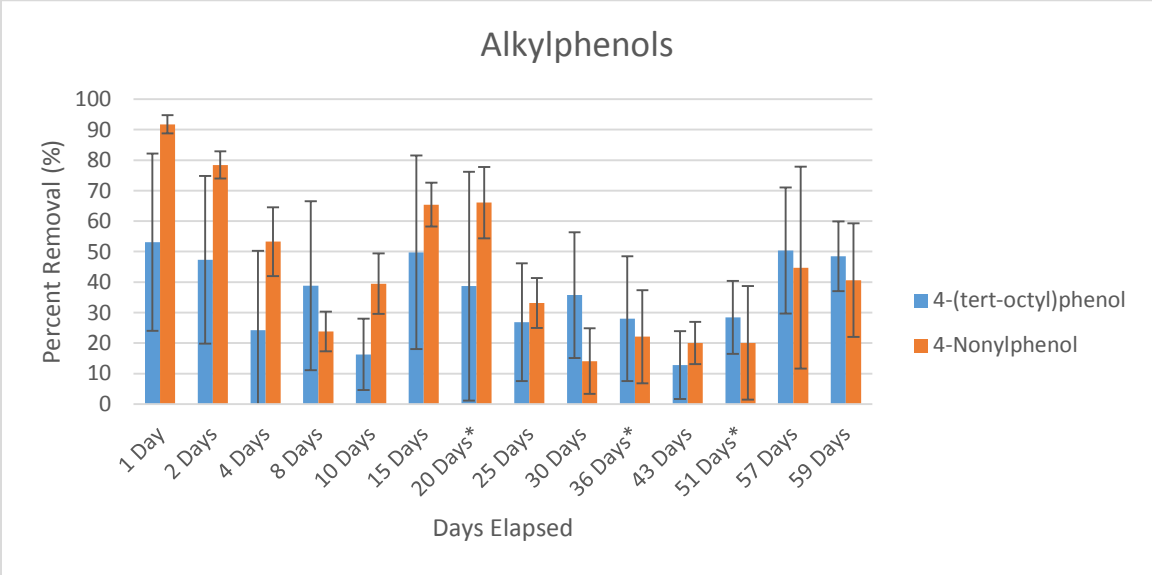

Figure 3-13 Mean percent removals of alkylphenols over the length of the project. Asterisk (*) denotes a maintenance event has occurred. Maintenance events occurred on Days 20, 31, and 42. Error bars denote standard deviation between the five columns.

Alkylphenolic compounds like 4-(tert-octyl)phenol and 4-nonylphenol have not been researched in biofilter column studies to the author's knowledge. By the log P values of 4-(tert-octyl)phenol and 4-nonylphenol (log P values of 15.0 and 6.14, respectively), we expect adsorption to be the primary mechanism of removal since these compounds are highly hydrophobic. 4-nonylphenol experienced the highest removal of 91% on day 1, and a mean

removal of 43%. 4-(tert-octyl)phenol had a consistent removal, mean 35%. 4-(tert-octyl)phenol had more consistent removal throughout the project, which is most likely an indicator of sorption. 4-nonylphenol has shown a declining trend throughout the project. From day 1 – 8, we see a rapid decrease from 91% to 23%, which could signify oversaturation of the binding sites or toxicity. However, 4-nonylphenol removal picks up again from day 10 – 20 and declines. This increasing and decreasing trend may indicate while sorption was the main mechanism on day 1, a microbial community may have evolved in combination with sorption to degrade 4-nonylphenol. The trend of 4-nonylphenol appeared to be increasing by day 20, but since a maintenance event occurred, it is hard to predict if higher removals could occur. The low removals from day 25 and onwards could suggest the maintenance events that occurred eliminated the microorganisms capable of degrading 4-nonylphenol. Other reasons could be that sorption sites were highly saturated at this point and the existing microorganisms had developed preference for other compounds.

As maintenance was proposed to not vastly affect the percent removals, higher standard deviation between the columns were observed for 4-methyl-benzylidene camphor, estrone, 17β-estradiol, 4-nonylphenol and 2.4-D were observed (See Figures 3-7, 3-8, 3-13, 3-15) after maintenance occurred.

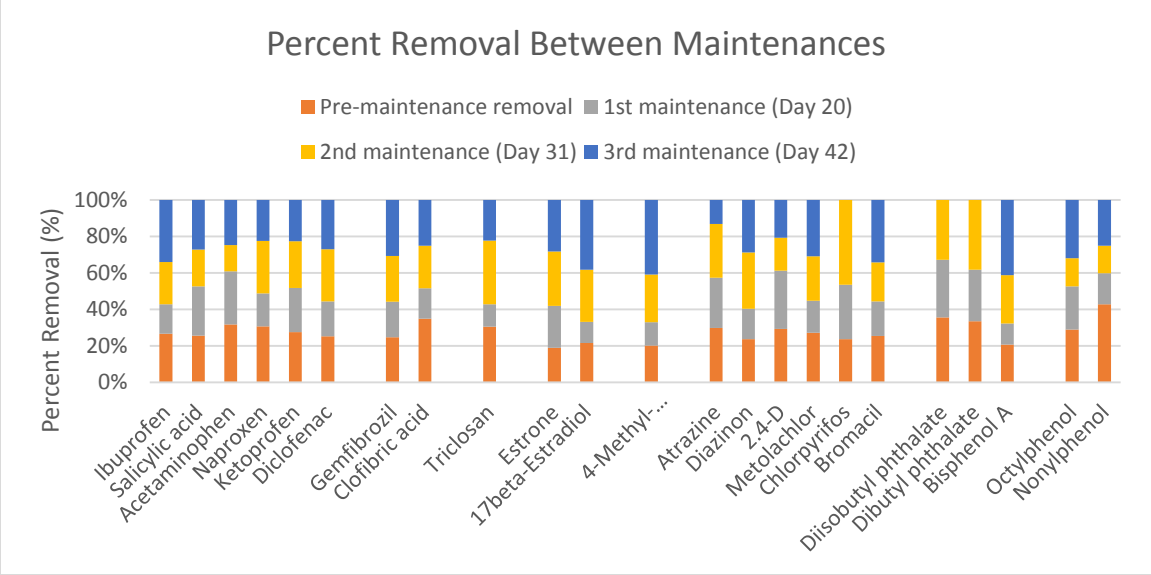

Figure 3-14 Mean percent removal of each compound during each maintenance period relative to each other.

We had noticed that the biofilm was changing its color and texture as the experiment progressed. After the first initial maintenance day (Day 20), sand texture changed to a reddish gelantinous film at the top of the sand layer (See Appendix B, Figure B-5, Figure B-6). This texture was observed in the second maintenance day (Day 31). But to the end of the project, after all columns were drained of water, a very thick gelatinous green film (See Appendix B, Figure B-5), about 2 mm thick formed on top of the sand layer and was observed in all columns. The changing of the color and texture of the biofilm can indicate the transitioning microbial communities from the first maintenance event to the end of the project. However, most of the compounds have shown a negligible change in mean removal after declogging or have shown an increasing change (See Figure 3-14). Figure 3-14 shows the percent removal of each maintenance period relative to each other. Maintenance is believed to not have an adverse effect on

microorganisms, given that most of the sampling occurred days after maintenance which should have allowed sufficient acclimation between the columns.

Antibiotics and Personal Care Products (UV-Filter)

Table 3-7 Mean removals in the entire project, highest mean removals, and lowest mean removals for Triclosan (an antibiotic) and 4-methyl-benzylidene camphor (a UV filter).

Compound	Average Removal (%)	Highest Removal (%)	Day of Highest removal	Lowest Removal (%)	Day of Lowest removal
Triclosan	38.09	74.69	15	13.33	10
4-Methyl-benzylidene camphor	44.53	90.07	59	11.42	4

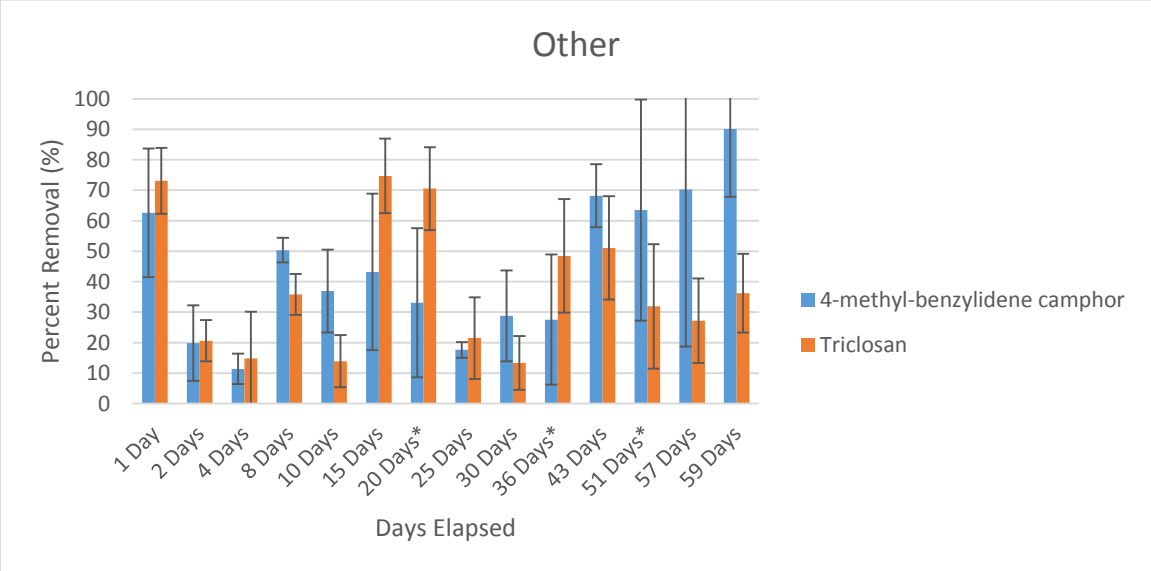

Figure 3-15 Mean percent removals of the antibiotic Triclosan and UV-filter 4-methyl-benzylidene camphor over the length of the project. Asterisk (*) denotes a maintenance event has occurred. Maintenance events occurred on Days 20, 31, and 42. Error bars denote standard deviation between the five columns.

Triclosan seemed to have the most variable trend among all the compounds, with mean removals ranging from 13% to 74% throughout the project. From Table 3-7, mean removal was 38±12% for triclosan. Triclosan initially started with a very high removal which is most likely attributed to adsorption. However, after the first day, removal rapidly declined. This may be a sign of saturation of binding sites in the column after the first day. There are two unusually high removal days, day 15 and day 20 (>70% removal). This could be due to experimental error, but it is possible that during the time period from 1-15 days, a biofilm layer rapidly grew and allowed for biodegradation to occur. Especially since there is a continuously high removal from 15-20 days, it is possible the microbial community that was degrading triclosan was at its maximum capacity. However, results are ambiguous as maintenance of the columns took place shortly after and removals were low once again. Our findings contradict most of the literature found. Triclosan has been shown to be biodegraded >80% in several studies (Onesios et al., 2012, Onesios-Barry et al., 2014, Zearley & Summers, 2012). Triclosan was readily transformed in a variety of batch and flow-through systems summarized by Onesios et al. (2012). Onesios et al. (2012) found in their acetate substrate amended biofiltration column studies, that removals of 81-87% were found in their biotic columns. In a similar study, Onesios-Barry et al. (2014) found that triclosan was biotransformed in their active column studies but was not removed to significantly different extents with various initial micropollutant concentrations. This would mean that having higher concentrations of specific micropollutants does not guarantee increasing removal. In fact, in the study by Onesios-Barry et al. (2014), they found that the compounds most likely responsible for suppressing biofilm growth at higher concentrations shown by protein assay results were the antiseptics. Synergistic toxicity involving multiple low concentration antiseptics could be responsible for inhibiting biomass in the columns (Onesios-Barry et al., 2014). The variability of removal for triclosan in our study could indicate toxicity if variable concentrations pumped to the

system occurred. However, even the highest concentrations of the micropollutants studied by Onesios-Barry et al. (2014) did not inhibit biotransformation. Their reasoning was that biofilms allowed the physical protection, favorable microenvironments, and proximity of cells aiding in horizontal transferring of genes to help tolerate exposure to antiseptics as they mentioned in a different study, bacterial strains exposed to a particular antiseptic was more tolerant in biofilms than when grown and exposed in planktonic form. Onesios-Barry et al. (2014) identified *Sphingomonas sp.*, a triclosan degrader, in their columns. So despite variable results, it is promising for triclosan to be readily degraded if particular bacterial strains known to degrade triclosan exist in our columns. Bacterial analysis of our columns have yet to be assessed.

4-methyl-benzylidene camphor (4-MBC abbreviated) shows moderately high average removals of 44±19%, with the highest average removal experienced by day 59 (90%). Initially 4-MBC has relatively high removal which is most likely attributed to adsorption of widely available binding sites. Based on the K_{ow} value of 4.95, 4-MBC is considered moderately hydrophobic which may explain the saturation of the compound to the binding sites and decreasing thereafter. However, we do see an increasing trend throughout the experiment, as complete removals are experienced by day 51. To the author's knowledge, there is very limited studies focusing on 4-MBC removal in biofiltration systems. In a vertical flow soil filter study to remove micropollutant from storm and wastewater, 4-MBC retardation factor was found to be 4.65, which is a relatively high retardation factor, indicating high sorption affinity of 4-MBC (Janzen et al., 2009). Under different filter media of peat, sand and gravel, 4-MBC was eliminated 73% in the peat layer (which eventually was discovered to contribute only a small fraction of removal), and after water percolation through peat, sand and gravel layers, elimination rate was >96% which is consistent with our results showing that 4-MBC as a moderate hydrophobic compound is able to adsorb effectively. It is unknown whether biological degradation is occurring, but since 4-

MBC has reached an initial saturation point early in the study and has slowly increased throughout the study, it is possible the overall hydrophobicity allowed separation of 4-MBC from the TTW matrix, enough so that its high retardation through the column allowed microbial degradation to occur simultaneously.

Prowl ™, metoprolol, and acetylsalicylic acid were unable to be detected greater than LOQ in the influent and effluent which may suggest degradation prior to reaching the columns or losses from the extraction process in the analytical step.

Chapter 4

Conclusion and Future Recommendations

Based on the results obtained in this study, the following conclusions and limitations can be drawn and suggestions given for further improvement:

Analytical Methodology

We developed a sensitive and reliable analytical protocol to determine a variety of organic micropollutants present in a complicated tertiary treated wastewater plant leachate, potentially equivalent to river matrix. Utilizing SPE, derivatization with MTBSTFA, followed by GC-MS allowed for satisfactory results in our validation parameters.

While using two-method acquisition windows allowed for more compounds to be analyzed to increase method sensitivity, it was also more time consuming as two sequences of injections had to occur for the same sample and can allow for degradation to occur while samples are waiting for their queue. The four internal standards (IS) used was limited in quantity and while internal standards should be utilized for their methods respectively, the large retention time differences and physical-chemical properties of the compounds made it difficult to attribute certain IS with the large variety of compounds. Thus, while it may not be ideal to utilize all four IS in different methods in accounting for the losses experienced by the compounds, satisfactory recovery was still observed.

Twelve of the fifty analytes showed no response whatsoever in the whole chromatographic run at full scans, even at varying individual standard solution concentrations, signifying no derivatization was possible. In addition, four analytes showed a very weak SIM

signal even at high concentrated solutions like 400 µg L^{-1}, which is close to the maximum extract concentration expected in the samples collected during the pilot scale study. After validation, six analytes had sensitivity too low in tertiary treated wastewater.

Satisfactory relative recoveries were observed for most of the twenty-nine target compounds, ranging from 75 to 135%. The lowest recoveries, under 75%, were acetylsalicylic acid and acetaminophen, most likely they suffered loss in the extraction process due to their polarity to be retained in the Oasis HLB cartridges.

Most analgesics/anti-inflammatory pharmaceuticals showed signal suppression except for salicylic acid. Twenty-one compounds experienced signal suppression and six experienced enhancement.

Fourteen (14) out of 29 compounds were able to be detected at less than 5 ng L^{-1}; and 28 out of 29 compounds were able to show detection less than 20 ng L^{-1}. Thus this shows the multicomponent analysis is sufficient for a variety of compounds, including polar compounds like pharmaceuticals. Seven compounds were not able to be quantified at lower than 50 ng L^{-1}, which the method may require further improvement to better quantify those compounds. The spiking of 400 ng L^{-1} in our samples was sufficient to detect those compounds.

All compounds except for acetylsalicylic acid and ketoprofen showed Intraday RSD to be lower than 10%. All compounds were lower than 20% Interday RSD except for acetylsalicylic acid, diclofenac, metoprolol, and bisphenol A.

All compounds extended their range of linearity from their limits of quantification to 800 ng L^{-1}. All calibration curves were linear with correlation coefficients (R^2) higher than 0.90; 25 compounds showed $R^2 > 0.97$, except 5 compounds whose R^2 values were $0.90 < R^2 < 0.97$.

The method developed can allow many laboratories using GCMS to study the occurrence, behavior and fate of emerging contaminants in the environment.

SSF removal efficiencies

PhACs

Analgesics and anti-inflammatory (NSAIDs) drugs had mean percent removal ranging 25-61%. NSAIDs, except for salicyclic acid, had low-moderate removals. NSAIDs like ibuprofen, ketoprofen, and naproxen were not greatly removed, with mean removal rates of 28%, 41%, and 25%, respectively, which has contradicted many studies showing high removals. Salicylic acid had the highest average removal (61%) and up to 87% removal. Our results correlate with low removal of acidic PhACs in abiotic studies, suggesting these compounds are not being utilized by microorganisms in our SSF and are rather removed by poor sorption processes.

Mean removal of 30% was observed for acetaminophen, with the highest removal of 80% experienced in 10 days. Biodegradation was the main mechanism for removal as sorption was unlikely.

Gemfibrozil and clofibric acid had poor mean removals of 23% and 27%, respectively and the highest removal experienced by gemfibrozil and clofibric acid were 49 and 52%, respectively. Low removals were attributed to adsorption.

While the estrogenic compounds estrone and 17β-estradiol showed moderately low removal, 39% and 35%, respectively, towards the end of the project both compounds were reaching up to >60% removal by 43 days. Biological degradation was an important mechanism

for estrogenic compounds and may require significant amount of time (>40 days) for high removals to occur.

Pesticides

Mean removals for this group were classified as moderate removals from 38 to 58%. Diazinon has the highest removal at 58% and metolachlor the lowest at 38%. Increasing removal of up to 75% for chlorpyrifos was a good indication that more removal could occur and the SSF may effectively remove chlorpyrifos via biodegradation. Other compounds such as metolachlor and 2.4-D were variable in removal; the main mechanisms were proposed to be adsorption and biodegradation, respectively.

Diazinon, atrazine, and bromacil all show steady state from the beginning of the project to the end, with some variable high removals in between but no relationship could be determined. The trends would suggest adsorption was the main mechanism of removal, but their log P values suggest biodegradation should be the main mechanism.

Higher mean removal of atrazine (40%) contradicted low removal reported in other literature. Atrazine removal between columns was one of the most variable, with 0% removal up to 95% removal observed between the columns, a sign that biological activity varied among the columns.

Plasticizers and others

Bisphenol A was classified as one of the worst removal in the study, with an average removal of 23±13.5%. Mean removals as low as 3-6% were experienced in the first 4 days. In most cases, <0% removal among columns was observed. Toxicity or saturation of BPA was likely responsible for the low removals.

Diisobutyl phthalate and dibutyl phthalate had moderately good removals of 47% and 56%, respectively. Main mechanism was most likely to sorption.

4-nonylphenol had a moderate removal of 43%. The declining trends suggest oversaturation of the binding sites or toxicity. The mean removal for 4-(tert-octyl)phenol was 35%. 4-(tert-octyl)phenol had more consistent removal throughout the project, which is most likely an indicator of sorption.

Triclosan mean removal was 38%. Our results contradict high removals of triclosan (>80%) observed by other studies. The low removals could be a form of poor sorption or toxicity.

4-methyl-benzylidene camphor showed moderately high average removals of 44%, with the removals increasing greater than >90% by day 59. The consistently increasing trend indicated biodegradation was the main mechanism of removal.

Mechanisms and conditions of SSF to consider

Physical/chemical structures and properties: Similar chemical and physical properties like NSAIDs and estrogenic compounds have experienced similar removals and also their trends in degradation. Microorganisms responsible for their degradation may not have a preference for either compounds. Similar compounds like phthalates also showed similar trends in adsorption.

Aerobic vs anaerobic conditions: Most literature have reported higher removals with aerobic conditions. As our system was an open system to the tank of supplied leachate and a head of air above in the slow sand filter columns – aerobic conditions are assumed. However, low removals can be due to the utilization of BDOC or the contaminants, and/or depletion of oxygen in the columns. Redox conditions may have adversely affected removal for certain acidic PhACs, such as ibuprofen, naproxen, diclofenac, gemfibrozil, and clofibric acid; all have removals <30%. To

enhance this study, columns can be separated into several groups, aerobic and anaerobic, and biotic and abiotic conditions.

Length of project: The length of the project was relatively short in comparison to most biofilter column studies, particularly since most of them are bank filtration studies which require low flow rates. However, while some studies have shown complete removals in little than 30 days, such as 17β-estradiol and BPA, it has also taken up to 180 days to remove BPA. This project has just given a preliminary look into the extent of SSF column efficacy. Even under 60 days, certain compounds, like 17β-estradiol, estrone, 4-methyl-benzylidene camphor, diazinon, chlorpyrifos, and salicylic acid have all shown promise to be highly degraded. These results have shown SSF is capable of removing these contaminants. Although mean removals have been moderate, they do not show the extent of removal. Longer studies are obviously needed to conclude these results.

Filter media and water matrix: The filter media type varies in biofilter columns such as bank filtration column studies, which would typically use the soil coinciding the river banks. Other slow sand filtration have granular activated carbon to bolster the removal rate. Others have seeded their media with activated sludge from wastewater treatment plants or they have seeded the growth of the biofilm with acetate. In our project, we see that removal rates were moderate, although given if activated carbon was utilized, removal rates could be higher. Our inoculation with the plants was enough to create biofilm development and obviously from the removal rates, some microbial communities had to play an important removal. The water matrix provided also had plenty of BDOC which was essential to the growth of biofilm, as some compounds experienced a rapid removal at their maximum in typically 8-15 days. Increases in removal was a likely indicator of biodegradation, as utilization of BDOC or the organic micropollutants as substrates would increase linearly over time. These trends were observed for 17β-estradiol,

estrone, 4-methyl-benzylidene camphor, diazinon, chlorpyrifos, salicylic acid, and acetaminophen.

Organisms to degrade: While we do not know for certain the bacteria in our biofilm to degrade the compounds, it will be an interesting study for the future. Studies have inoculated their laboratory columns with specific bacteria with highly various results.

Hydraulic residence time: Our column volume was 0.032 m^3 and flow rate was 20 mL min^{-1}. It was determined that the hydraulic retention time is approximately 26 hours. Changing the flow rate will ultimately affect the growth of the biofilm layer, as greater retention time allows the growth of the biofilm layer and consumption of BDOC and organic micropollutants, and allow for retention of the contaminants to sorb onto binding sites. As a future recommendation, more duplicates are required to measure removal differences between flow rates.

Temperature: Temperature of our columns did not have any correlation with the removal rates of any of the compounds. R^2 was 0 to 0.3. Although wintertime was proposed to have higher removals for atrazine, colder temperatures is believed to slow down the growth of the biofilm layer. More trials will need to be held in summer time months where temperatures will vary.

Saturation and toxicity: A variety of compounds had rapidly declining removals which could be a result of the saturation of the contaminant on the biofilm. This pattern was observed for the first couple of days which is typical of a fresh slow sand filter. However, afterwards, oversaturation of the binding sites led to some compounds having higher concentrations in the outlet than inlet. This was observed for BPA, 4-nonylphenol, and triclosan. Initial losses by adsorption was also observed for chlorpyrifos and metolachlor. This was most likely a result as the competition for adsorption onto binding sites with other contaminants or humic substances in the water media.

It is unknown the extent of the toxicity of our spiking solution (400 ng L^{-1}), as there were days when compounds may have reached 2000 ng L^{-1}, which can be due to the inconsistencies in the flow rate supplied to the SSF in the first days of the project. This could have led to toxicity to the biofilm, especially since other studies have reported biofilm suppression with higher antibiotics concentrations. The multitude of contaminants is very diverse with large differences in physical-chemical properties. It is also uncertain how many contaminants begin to overwhelm the biofilm communities, especially if particular communities are not well tolerant of other ones. Despite the variety, the study still showed microorganisms were able to degrade in as little as eight days, typical of a rapid biofilm growth. Most compound removals reach their plateau at eight to fifteen days, which could suggest the biofilm maximum capacity. Also, despite the variety of contaminants, adsorption sites still allowed several contaminants to sorb effectively, such as the phthalates and pesticides.

Column maintenance: While we screened for larger particulates in the supply line to the columns, it was not enough to prevent clogging. Especially because the growth of the biofilm itself was gradually clogging the columns too. Maintenance may have disturbed the microbial community. However, removals in the maintenance periods were averaged and were not found to be highly affected. The intervals between each period was very brief, and the acclimation was not ideal. Column 1 experienced the most clogging as heavier matrix settled into that column first. A way to have relieved this was to decrease the amount of bell pepper plants used, but consistency was kept. It may also be necessary to have two sets of five replicate columns ensuring that while one set is maintained, the other one is running.

Physical and chemical parameters of TTW: The matrix was originally believed to be consistent as we started our operation in late summer. Initial studies of the matrix showed relatively stable

physical and chemical qualities. However, the changing ambient conditions over the five month period and maturity of the plants may have altered the complexity of the water matrix. This was exemplified in the texture and color of our schmutzdecke over time either as a result of the adaptation of the biofilm to the contaminants or as a result of the variability in the water. This was unforeseen. For more accurate measurements of how the physical and chemical properties of the water are affecting removal, daily quantification of pH, temperature, salinity, nitrate, and etc. should be monitored.

Removal between columns: The removal between columns varied. Low standard deviation was observed for salicylic acid (Std Dev was 12.2%) while high standard deviations like atrazine had up to 43% standard deviation. This shows that although we averaged our five columns for a compound's removal, the columns are not exact replicates of each other due to the environmental conditions and substrates for microorganisms to grow. Averaging the removals between columns may not be an accurate representation and we should look closely at the trends of removal.

Summary

We utilized slow sand filter columns because they are a high efficiency system proposed to reduce a multitude of physical and chemical contaminants while remaining cost effective and not land intensive. We have tried to conclude using SSF is a low cost alternative for the treatment of contaminants in recycled water, in particular a greenhouse utilizing reclaimed water with a variety of emerging contaminants.

While slow sand filter is not a new concept, its research on removing emerging contaminants has yet to be discussed. Much of the literature has focused on bank filtration systems to manage aquifer systems, or biofilters that have some combination of chemical

treatment or various granular media that could bolster the effectiveness of the biofilters such as activated carbon. However, with only utilizing the inoculation supplied by the tertiary treated waterwater plant leachate in our greenhouse, biofilm was able to grow in these conditions. In certain cases, utilization of the biological degradable organic carbon supplied with the plant container leachate could have been the reason of biological degradation as biofilm layer is growing and using the micropollutants as a secondary source.

The redox conditions have been known to be crucial in the sorption of pharmaceutically active compounds, since they tend to exist in ionic forms under certain pH conditions. Although removals have occurred, it is difficult to distinguish whether removal is occurring based on adsorption or biodegradation. There were many factors to consider, as this is a preliminary study, more tests concerning the following issues should be addressed. Adsorption is primarily based on the hydrophobicity of the compound to adhere to binding sites, usually an indication of larger log K_{ow} values. However, we have seen signs of oversaturation of compounds with large K_{ow} values and decreases in removal. Retardation as studied in other literature have regarded some compounds to be highly retarded in the soils but retardation does not always mean adsorption or degradation will occur.

In most literature, given enough time, high removals of nearly all compounds of our interest have been found. Given the short amount of time that our study was conducted, it is insufficient to know the extent of the compounds' removal and thus further studies should be conducted. Given the limits of our preliminary study and analytical method, we have successfully analyzed 29 compounds able to undergo derivatization and maintain quality recoveries, and have used SSF to remove a mean 20-60%. This project has shown that columns have developed unique microbial communities and averaging removals between columns may not be an accurate

representation. To understand a compound's removal, it is crucial to observe trends over time. Even under 60 days, certain compounds, like 17β-estradiol, estrone, 4-methyl-benzylidene camphor, diazinon, chlorpyrifos, and salicylic acid have all shown capability to be highly degraded. The highest removals experienced were >90% for some days of the project. There is promise that the conditions provided by our greenhouse system and design of the SSF allows for sufficient, at least moderate, removals of our emerging contaminants.

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Appendix A

Table A-1 List of contaminants and their chemical name, brand acquired, and chemical CAS number.

Analyte		Chemical_Name	Brand	Chemical CAS number
Analgesics/antiinflammatories				
Acetylsalicylic acid	Pharmaceutical (NSAID)	Acetylsalicylic acid	Fisher Science	50-78-2
Ibuprofen	Pharmaceutical (NSAID)	Ibuprofen	Acros Organics	15687-27-1
Salicylic acid	Pharmaceutical (NSAID)	Salicylic acid	Aldrich	69-72-7
Acetaminophen	Pharmaceutical (Analgesic)	4-Acetamidophenol	Sigma-Aldrich	103-90-2
Naproxen	Pharmaceutical (NSAID)	Naproxen	MP Biomedicals, LLC	22204-53-1
Ketoprofen	Pharmaceutical (NSAID)	Ketoprofen	MP Biomedicals, LLC	22071-15-4
Diclofenac	Pharmaceutical (NSAID)	Diclofenac sodium salt	MP Biomedicals, LLC	15307-79-6
Lipid regulators				
Gemfibrozil	Pharmaceutical (Lipid regulator)	Gemfibrozil	Sigma	25812-30-0
Clofibric acid	Pharmaceutical (Lipid regulator)	Clofibric acid	MP Biomedicals, LLC	882-09-7
Psychiatric drugs				
Carbamazepine	Pharmaceutical (Psychiatric drug)	Carbamazepine	MP Biomedicals, LLC	298-46-4
Antibiotics				

Triclosan (Also called Irgasan)	Personal care product (Antibacterial/antifungal)	Irgasan	Fluka	3380-34-5
Cardiovascular drugs and beta blockers				
Metoprolol	Pharmaceutical (Cardiovascular drug (Beta-blocker))	Metoprolol (tartrate)	LKT Laboratories Inc.	56392-17-7
Terbutaline	Pharmaceutical (Brochodilator)	Terbutaline hemisulfate salt	MP Biomedicals, LLC	23031-32-5
Estrogens				
Estrone	Estrogen	Estrone	Acros Organics	53-16-7
17beta-Estradiol	Estrogen	17beta-Estradiol	Calbiochem	50-28-2
Personal Care Products (UV-Filters)				
4-Methyl-benzylidene camphor	Personal care product (UV-Filter)	4-Methyl-benzylidene camphor	Accu Standard	36861-47-9
Pesticides and Herbicides				
Atrazine	Pesticide (Herbicide)	Atrazine	Chem Service	1912-24-9
Diazinon	Pesticide (Insecticide) (Organophosphate)	Diazinon	Chem Service	333-41-5
2,4-Dichlorophenoxyacetic acid (2.4-D)	Pesticide (Herbicide)	2.4-D	Chem service	94-75-7
Metolachlor	Pesticide (Herbicide)	Metolachlor	Chem Service	51218-45-2
Chlorpyrifos	Pesticide (Insecticide) (Organophosphate)	Chlorpyrifos	Chem Service	2921-88-2
Bromacil	Pesticide (Herbicide)	Bromacil	Chem Service	314-40-9
Prowl(TM)	Pesticide (Herbicide)	Pendimethalin	Chem Service	40487-42-1

Plasticizers				
Diisobutyl phthalate	Plasticizer	Diisobutyl phthalate	Acros Organics	84-69-5
Dibutyl phthalate	Plasticizer	Dibutyl phthalate pestanal	Riedel-de-Haën	84-74-2
Bis-(2-ethylhexyl) phthalate	Plasticizer	Bis-(2-ethylhexyl) phthalate	Riedel-de-Haën	117-81-7
Bisphenol A	Plasticizer	Bisphenol A	Aldrich	80-05-7
Detergent/emulsifier/antioxidant				
Octylphenol	Detergent/emulsifier/antioxidant	4-(tert-octyl)phenol	Aldrich	140-66-9
Nonylphenol	Detergent/emulsifier/antioxidant	4-Nonylphenol, mixtre of isomers	Acros Organics	84852-15-3

Table A-2 List of contaminants and their chemical formula, molecular weight, chemical purity, chemical weighted mass, and corresponding surrogate.

Analyte	Chemical Formula	Chemical MW	Chemical Purity (%)	Chemical Weighed mass (mg)	Corresponding Surrogate
Analgesics/antiinflammatories					
Acetylsalicylic acid	$C_9H_8O_4$	180.16	99	5.5	(+-)-Ibuprofen-d3 (alpha-methyl-d3)
Ibuprofen	$C_{13}H_{18}O_2$	206.27	99	5.7	(+-)-Ibuprofen-d3 (alpha-methyl-d3)
Salicylic acid	$C_7H_6O_3$	138.12	99	5.2	Triclosan-d3
Acetaminophen	$C_8H_9NO_2$	151.16	99	5.8	N-(4-Hydroxyphenyl)-acetamide-2,2,2-d3
Naproxen	$C_{14}H_{14}O_3$	230.3	99	5.4	Bisphenol A-d6
Ketoprofen	$C_{16}H_{14}O_3$	254.3	98	5.5	Triclosan-d3

Diclofenac	$C_{14}H_{10}Cl_2NNaO_2$	318.1	99	5.1	Bisphenol A-d6
Lipid regulators					
Gemfibrozil	$C_{15}H_{22}O_3$	250.3	98.5	5.1	Triclosan-d3
Clofibric acid	$C_{10}H_{11}ClO_3$	214.6	98	5	(+-)-Ibuprofen-d3 (alpha-methyl-d3)
Psychiatric drugs					
Carbamazepine	$C_{15}H_{12}N_2O$	236.27	98	5.1	Carbamazepine-d10
Antibiotics					
Triclosan (Also called Irgasan)	$C_{12}H_7Cl_3O_2$	289.54	97	5.2	Triclosan-d3
Cardiovascular drugs and beta blockers					
Metoprolol	$C_{15}H_{25}NO_3$	684.82	98	6.5	Triclosan-d3
Terbutaline	$C_{24}H_{40}N_2O_{10}S$	274.3		5.2	Triclosan-d3
Estrogens					
Estrone	$C_{18}H_{22}O_2$	270.36	99	5.4	Bisphenol A-d6
17beta-Estradiol	$C_{18}H_{24}O_2$	272.4	97	5.6	Bisphenol A-d6
Personal Care Products (UV-Filters)					
4-Methyl-benzylidene camphor	$C_{18}H_{22}O$	254.37	98	5.7	Bisphenol A-d6
Pesticides and Herbicides					
Atrazine	$C_8H_{14}ClN_5$	215.69	98	8.1	(+-)-Ibuprofen-d3 (alpha-methyl-d3)
Diazinon	$C_{12}H_{21}N_2O_3PS$	304.35	98	9.3	(+-)-Ibuprofen-d3 (alpha-methyl-d3)

2,4-Dichlorophenoxyacetic acid (2.4-D)	$C_8H_6Cl_2O_3$	221.04	98	10.8	(+-)-Ibuprofen-d3 (alpha-methyl-d3)
Metolachlor	$C_{15}H_{22}ClNO_2$	283.8	96.1	14.9	N-(4-Hydroxyphenyl)-acetamide-2,2,2-d3
Chlorpyrifos	$C_9H_{11}Cl_3NO_3PS$	350.59	98	10	N-(4-Hydroxyphenyl)-acetamide-2,2,2-d3
Bromacil	$C_9H_{13}BrN_2O_2$	261.1157	98.5	6.8	N-(4-Hydroxyphenyl)-acetamide-2,2,2-d3
Prowl™	$C_{13}H_{19}N_3O_4$	281.31	99.2	13.3	(+-)-Ibuprofen-d3 (alpha-methyl-d3)
Plasticizers					
Diisobutyl phthalate	$C_{16}H_{22}O_4$	278.34	99	12	N-(4-Hydroxyphenyl)-acetamide-2,2,2-d3
Dibutyl phthalate	$C_{16}H_{22}O_4$	278.35	98.7	11.6	N-(4-Hydroxyphenyl)-acetamide-2,2,2-d3
Bis-(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.56	99.5	6.1	(+-)-Ibuprofen-d3 (alpha-methyl-d3)
Bisphenol A	$C_{15}H_{16}O_2$	228.29	99	5.7	Bisphenol A-d6
Detergent/emulsifier/antioxidant					
Octylphenol	$C_{14}H_{22}O$	206.33	97	6	(+-)-Ibuprofen-d3 (alpha-methyl-d3)
Nonylphenol	$C_{15}H_{24}O$	220.35	99	5.3	Triclosan-d3

Table A-3 Method 1 and Method 2 acquisition windows, compound characteristic ions, and retention times.

				Method 1	Method 2	Characteristic ions, m/z		
IS	**Compounds**	**Group**	**Compound**	**Retention Time (min)**	**Retention Time (min)**	**Primary Ion**	**Secondary Ions**	
	1	Pharmaceutical (NSAID)	Acetylsalicylic acid	11.77		195	237	135
	2	Pesticide (Herbicide)	Atrazine		11.77	215.1	200.1	75
	3		Diazinon		12.24	137.1	179.1	199.1
	4	Pesticide (Insecticide) (Organophosphate)	Clofibric acid		12.28	143	271	273
1			(+-)-Ibuprofen-d3 (alpha-methyl-d3)	12.66		266	267	268
	5	Pharmaceutical (NSAID)	Ibuprofen	12.68		263	264	161
	6	Detergent/emulsifier/antioxidant	4-(tert-octyl)phenol	12.99		249	250	320
	7	Plasticizer	Diisobutyl phthalate	13.19		149	104	57.1
	8	Plasticizer	Salicylic acid		13.78	309.1	310.1	209
	9	Pharmaceutical (NSAID)	4-Nonylphenol		13.91	263.1	305.1	264.1
	10	Detergent/emulsifier/antioxidant	Dibutyl phthalate pestanal		13.92	149	76	104
	11	Pesticide (Herbicide)	2.4-D	14.06		277	278.9	213
	12	Pesticide (Herbicide)	Metolachlor	14.15		162.1	238.1	146.1
	13	Pesticide (Insecticide)	Chlorpyrifos	14.19		196.9	198.9	313.9
	14	Pesticide (Herbicide)	Prowl(TM)		14.82	252.1	162.1	281.1
	15	Personal care product (UV-Filter)	Bromacil		14.88	263	261	319
	16	Pesticide (Herbicide)	4-Methyl-benzylidene camphor		14.88	254.2	128	115.1
	17		Acetaminophen	15.08		322	379	248

2		Pharmaceutical (NSAID)	N-(4-Hydroxyphenyl)-acetamide-2,2,2-d3	15.10		325	251	326
	18	Pharmaceutical (Lipid regulator)	Gemfibrozil	15.85		243	179	307
	19	Pharmaceutical (Cardiovascular drug (Beta-blocker))	Metoprolol (tartrate)		16.93	152	223	324
	20	Pharmaceutical (NSAID)	Naproxen		17.09	287	185	288
3		Personal care product (Antibacterial and antifungal)	Triclosan-d3		17.58	350	348	200
	21		Triclosan		17.59	347	200	345
	23	Pharmaceutical (NSAID)	Ketoprofen	18.00		311	295	312
	24	Pharmaceutical (Psychiatric drug)	Carbamazepine		18.93	193	194	293
	25	Pharmaceutical (brochodilator)	Terbutaline hemisulfate salt		18.94	482	483	484
4			Carbamazepine-d10		19.01	203.1	204	202
	26	Plasticizer	Bis-(2-ethylhexyl) phthalate	19.24		149	167	279.1
	27	Pharmaceutical (NSAID)	Diclofenac sodium salt	19.41		352	214	409
5			Bisphenol A-d6		20.90	444.3	213	445.3
	28	Plasticizer	Bisphenol A		20.94	441	207	442
	29	Estrogen	Estrone	23.45		327	328	384
	30	Estrogen	17beta-Estradiol	23.45		329	386	

Table A-4 Equations used in determining method validation parameters.

Method accuracy				
Absolute recovery (%)	=	(Area CAL 400 ppt - Area Blank) / Area Direct Vial 800 ppb	x	100
	=	(Area CAL 100 ppt - Area Blank) / Area Direct Vial 200 ppb	x	100
Relative recovery (%)	=	Absolute recovery analyte / Absolute recovery IS	x	100
Method precision				
Intraday				
Relative standard deviation RSD (%)	=	Standev triplicate area CAL 400 ppt / Average triplicate area CAL 400 ppt	x	100
	=	Standev triplicate area CAL 100 ppt / Average triplicate area CAL 100 ppt	x	100
Interday				
Relative standard deviation RSD (%)	=	Standev sixtuplicate area CAL 400 ppt / Average sixtuplicate area CAL 400 ppt	x	100
	=	Standev sixtuplicate area CAL 100 ppt / Average sixtuplicate area CAL 100 ppt	x	100
Sensitivity				
Method sensitivity		3 * Real Conc. CAL 100 ppt (substract blank)		

LOD	=	3 * Real Conc. CAL 100 ppt (subtract blank) --- S/N		
LOQ	=	10 * Real Conc. CAL 100 ppt (subtract blank) --- S/N		
Instrumental sensitivity				
LOD	=	3 * Real Conc. Direct vial 200 ppb in pg/µL --- S/N	x	V inj (µL)
LOQ	=	10 * Real Conc. Direct Vial 200 ppb in pg/µL --- S/N	x	V inj (µL)
Matrix effect				
ME	=	(Area CAL 400 ppt - Area Blank) --- Area HPLC 400 ppt	x	100
	=	(Area CAL 100 ppt - Area Blank) --- Area HPLC 100 ppt	x	100
Dynamic range				
Range of linearity	LOQ - Highest real concentration (considering blank) in the linear part of calibration curve			
R^2 in linear range				
Linear curve eqn	Y = a X + b			

Appendix B

Figure B-1 Slow sand filtration column system

Figure B-2 Inlet samples were collected at the Inlet ports. Outlet rotameters were set at 20 mL min^{-1}.

Figure B-3 Bell pepper plants used for inoculation to the SSF columns. Drip irrigation lines supply tertiary treated wastewater to 82 bell pepper plants.

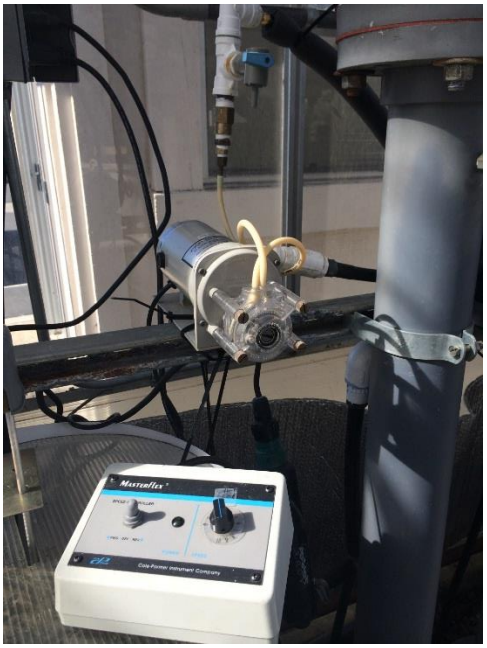

Figure B-4 Peristaltic pump controller limited the flow rate to the system at 120 mL min^{-1}.

Figure B-5 Red biofilm layer formed on top of the sand columns after the first maintenance event (Day 20). Thick green gelatinous biofilm layer developed after 60 days of the project.

Figure B-6 Red crusty biofilm layer developed after first maintenance day.