

**Removal of Trace Organic Compounds in Domestic Wastewater
using Recirculating Packed-Bed Media Filters**

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Master of Science
Degree

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Brittani Nikole Perez

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ABSTRACT

Pharmaceuticals and personal care products (PPCPs) are commonly detected in the environment resulting from their survival from conventional wastewater treatment systems. More information is needed about the fate and transfer of these trace organic compounds in domestic wastewater and their associated risks so that efficient strategies for their removal can be developed for both large/small scale treatment systems. This study aimed to determine whether onsite wastewater treatment systems were capable of providing PPCP removal, in addition to quantifying different forms of removal (biodegradation/sorption). A column study was constructed to determine the removal efficiencies of 3 target PPCPs, endocrine disrupting compound triclosan (TRI) and non-steroidal anti-inflammatory drugs ibuprofen (IBU) and naproxen (NAP), in a small-scale recirculating media filter. To ensure bioreactor productivity the pH, chemical oxygen demand (COD), total organic carbon (TOC), and total nitrogen (TN) of the influent and effluent were analyzed. All columns showed consistent neutralization of pH, coupled with a large removal of COD (>90%) and TOC (>95%). Nitrifying/denitrifying conditions were attained, presenting removal of TN between 35% and 85% in all columns. Spiked experimental columns (0.1 ppm) with the target PPCPs were compared to one controlled column. Mean total removal of the trace organics were moderately high (>80%). Sorption of the PPCPs onto biofilm was quantified; TRI experienced the highest sorption ($2.5 \pm 0.2\%$), followed by IBU and NAP (0.3 ± 0.1 and $0.4 \pm 0.3\%$). Therefore, estimated degradation percentages of parent compounds for IBU, NAP, and TRI were $85 \pm 8.2\%$, $88 \pm 4.6\%$, and $86 \pm 2.2\%$, respectively. Negative mass balances of PPCP removal occurred within experimental columns only, suggesting possible desorption or change in degradation kinetics attributed to compound addition.

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LIST OF ABBREVIATIONS

AOA	Ammonia Oxidizing Archaea
AOB	Ammonia Oxidizing Bacteria
AMO	Ammonia Monooxygenase
ANOVA	Analysis of Variance
BOD	Biochemical Oxygen Demand
IBU	Ibuprofen
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
EDC	Endocrine Disrupting Compound
GAC	Granular Activated Carbon
GC-MS	Gas Chromatography-Mass Spectrometry
HLR	Hydraulic Loading Rate
HRT	Hydraulic Retention Time
LC-MS	Liquid Chromatography-Mass Spectrometry
NAP	Naproxen
NOB	Nitrite Oxidizing Bacteria
NSAID	Non-Steroidal Anti-Inflammatory Drug
OLR	Organic Loading Rate
OWC	Organic Waste Contaminant
OWTS	Onsite Wastewater Treatment System
PPCP	Pharmaceuticals and Personal Care Product
RMF	Recirculating Media Filter
SPE	Solid Phase Extraction
SRT	Solids Retention Time
TN	Total Nitrogen
TOC	Total Organic Carbon
TRI	Triclosan
WWTP	Waste Water Treatment Plant

CHAPTER I: Introduction and Literature Review

Background

In the world today many human and animal maladies can be alleviated or cured through the usage of pharmaceuticals. According to an ongoing study since 2007, over 50% of Americans use at least one or two prescription drugs per month (CDC, 2013). While there are people who do question the potential overuse of pharmaceuticals, the concern is more focused on personal safety rather than environmental. Some pharmaceuticals present a real danger towards the environment; however they are not the only concern. Active ingredients found in personal care products such as soaps, detergents, lip balm, deodorant, and fragrances, are also being detected in environmental samples. Further, there are groups of these compounds that have the ability to mimic hormones in the endocrine system, known as endocrine-disrupting compounds (EDCs), and have been documented as having adverse effects on both humans and animals (Snyder et al., 2003). Together, these trace organics are termed pharmaceuticals and personal care products (PPCPs) and they are listed as “emerging contaminants” due to their recent detection in the environment. It should be noted, however, that ‘emerging’ is a misleading title because these compounds have been present in our nation’s waters for as long as they have been developed, but their detection has only become possible until recently.

Source, Occurrence, and Threat of PPCPs

There is a multitude of ways that trace organic compounds enter into the environment. Figure 1 (Halford, 2008) accurately depicts many sources, including wastewater treatment effluent, septic systems, stormwater and agricultural runoff, leaching from landfills, and illegal or improper disposal.



Figure 1. Sources of PPCPs and their transfer into the environment.

Most compounds are carried into the environment after being dissolved in water, but some have the ability to adsorb onto other materials, such as sludge or soil. The most significant source is from wastewater treatment plant effluent (Halford, 2008). While conventional wastewater treatment plants (WWTPs) provide an excellent job of removing many contaminants from domestic and municipal wastewater, they were not designed with PPCP removal in mind. There are almost no regulations that limit the release of these compounds into receiving streams (Pasquini et al., 2014), however, many projects are underway to update current treatment plants.

It is a discouraging fact that no single treatment is able to completely removal all compounds down to non-detectable concentrations (Kummerer, 2009). It is well documented that WWTPs are only effective at removing trace compounds that are readily biodegradable. Most treatment processes are able to degrade many trace compounds down to nearly non-detectable concentrations; although, there are some compounds that remain persistent. A few compounds that have been known to survive municipal wastewater treatment systems include, but are not limited to, carbamazepine, fluoxetine, clofibric acid, mefenamic acid, phenazone, diclofenac, and

dimethylaminophenazone (Lubliner et al., 2010). There are over 10,500 different chemicals used in personal care products, but unfortunately only 11% have been tested for human health safety, while even less for environmental safety (Lubliner et al., 2010). Pharmaceutical occurrences in the environment are largely becoming a concern to many scientists because of their frequent detection in surface water, groundwater, drinking water, and sediment samples, as shown in table 1 (Daughton and Jones-Lepp, 2001). Numerous trace organics have been detected at concentrations as low as in the ng/L range. (Xia et al., 2005).

The frequent detection of PPCPs are a rising concern because of the potential adverse effects on the environment. Side effects on aquatic life have already been documented in multiple studies. For example, Vajda et al. (2008) analyzed effluent coming from a WWTP outfall and found high concentrations of alkylphenols, bisphenol-A, and reproductive steroids. Collecting fish from both upstream and downstream of the outfall, they found noticeable differences in the male to female ratios: upstream was roughly equal, while downstream was 90% female and 10% male. Further, most of the remaining males had severely abnormal reproductive organs. The study concluded that the released compounds from the discharged effluent were causing sexual disruption and reproductive failure among the native fish.

Table 1. Detection frequency of PPCPs in streams.

PPCPs (mean conc., ppm)	Category	Detection Frequency
Triclosan (0.03) Phenol (0.04)	Disinfectant	>90%
Cholesterol (0.83) Coprostanol (0.88)	Steroids	89%
Acetaminophen (0.11) Caffeine (0.081) Ibuprofen (0.2) Cotinine (0.05)	Nonprescription Drugs	81%
DEET (0.06)	Insect Repellent	66%
Erythromycin (0.1) Ciproflaxin (0.02) Sulfamethoxazole (0.15)	Antibiotics	48%
17 α -ethinly estradiol (0.073) Estrone (0.027)	Reproductive hormones	37%
Codeine (0.012) Diltiazem (0.021) Fluoxetine (0.012)	Other prescription drugs	32%
Acetophenone (0.15)	Fragrances	27%

PPCPs and Wastewater Treatment

Large-Scale Treatment

Treating wastewater is important because it is not only a matter of caring for our environment, but public health as well. The purpose of a WWTP is to reduce the amount of pollutants before release back into the environment. These pollutants can be anything from suspended solids, biodegradable organics, pathogens, and nutrients. This is done by implementing three different levels of treatment called primary, secondary, and tertiary treatment (World Bank Group, 2015).

Primary treatment is where suspended and floating solids are removed either through screening or sedimentation by gravity. The level has also been called physical treatment because there is a mechanical system that catches floating solids, however sometimes chemicals are added instead. Once the floating solids have removed, sludge is collected at the bottom and sent to a digestion tank where it can be properly managed and disposed.



Figure 2. Primary clarifier tank at Hill Canyon WWTP in Ventura County, California. Photo credit: www.greenstockphotos.com

While primary treatment is able to remove trash and larger solids from wastewater, it is only successful at removing about 50% to 60% of suspended solids and is not able to remove the dissolve organic material. Therefore, secondary treatment is required to remove most of these constituents that pass through primary treatment. Using biological processes, secondary treatment utilizes active microorganisms within the wastewater to appropriately degrade the biodegradable organic matter. These microorganisms break down the organic compounds as a food/energy source and converts them into water, CO₂, and new cells. The organic matter that is broken down promote the growth of more microbial life, so aeration within the tanks is needed to satisfy the growing oxygen demand. In addition, this growth generates the formation of biological flocs, called activated sludge, which is collected during clarification and sent for further treatment. This level of treatment is very successful and is able to degrade over 80% of the suspended solids and organic matter within the wastewater. Two common methods of utilizing microorganisms for wastewater treatment are suspended growth and fixed film systems.

Suspended growth is a method of treatment where the microorganisms are suspended by turbulence within a system. In contrast, fixed-film systems, or sometimes called attached-growth systems, use a medium (gravel, sand, synthetic material) for the microorganisms to bind and grow on rather than freely moving. One example of this is called a trickling filter. A trickling filter consists of a fixed bed of media where the wastewater trickles through, and forms a biological film on the media. As wastewater passes through this film the active bacteria are continually breaking down the dissolved organic material, allowing treated wastewater to exit at the bottom. There are pros and cons to each type of treatment, but generally suspended growth systems are for urban facilities that process large wastewater volumes on limited land resources, whereas fixed film systems are for smaller communities, as land is more available.



Figure 3. Aeration tank promoting the growth of activated sludge.
Photo credit: DCM Process Controls



Figure 4. A trickling filter that is evenly discharging wastewater over medium.
Photo credit: Utility Compliance INC.

Although primary and secondary treatment perform exceptionally well at removing most of the pollutants from wastewater, there are some compounds that are more persistent and require further treatment. The tertiary treatment of wastewater, often called an “effluent polishing”, is focused on further improving the effluent quality through filtration, disinfection or nutrient removal, and is able to remove almost 99% of impurities. This allows the production of an effluent that is almost drinking water quality (Malik, 2014). While successful, these systems are very expensive and require a large amount of energy and management to ensure proper treatment. Final treatment can be accomplished through a number of different methods such as filtration, reverse osmosis, and extended secondary treatment (nutrient removal).



Figure 5. The world’s largest reverse osmosis desalination plant, located in Hadera, Israel. Photo credit: Slate Magazine.

Unfortunately, even with the wide variety of treatment processes that are applied, trace amounts of PPCPs are still able to survive WWTPs and exit with the treated effluent. It is argued that one of the main reasons for their survival is that PPCPs were designed to be biologically active

at low concentrations (Grenni et al., 2013). Different treatments have been shown to break down certain PPCPs, but as it was mentioned before, there is no single process that has been successful in removing all. Many researchers have experimented with different styles of treatment, to evaluate what works best or not at all.

Lee et al. (2009) evaluated over 20 research articles and summarized their findings as shown in table 2. This report lists granular activated carbon (GAC), nanofiltration, and reverse osmosis as the three treatment processes that experienced the highest PPCP removal rates. It is important to note that GAC is an excellent adsorbent that is used to remove many dissolved compounds, meaning that there is little degradation that is occurring in this type of process because the compounds are simply being transferred from one source to another. On another note, nanofiltration and reverse osmosis are typically types of potable water treatment processes, not necessarily wastewater treatment processes. But as the table shows, these can be applied to wastewater treatment as well and do show promise for contaminant removal.

Research is underway to help update current treatment plants so that they may be able to better remove PPCPs from wastewater influent and effluent. Many European studies, such as Germany, Switzerland, and the Netherlands, have tried to find different pharmaceutical “cocktails”, which is a mix of different advanced treatment systems to determine what will provide the best elimination. It is probable that WWTPs with multiple advanced treatments will increase costs substantially, and that is why they are not fully implemented to other facilities (Hernandez, 2010). However, it remains hopeful that through continued research a solution can be found to help minimize PPCP release from WWTPs, while also minimizing treatment expenditures.

Table 2. List of removal effectiveness on PPCPs through selected wastewater treatment processes.

Process	Studies	Compounds	Average Removal of PPCPs		
			No Removal	Below 50%	Above 90%
Membrane Bioreactor	12	49	14	33	39
Activated Sludge	12	33	9	64	27
Nanofiltration	15	57	-	17	82
Reverse Osmosis	15	60	-	12	82
Granular Activated Carbon	10	29	-	-	97
Powdered Activated Carbon	10	71	6	31	41

Small-Scale Treatment

Although different options on PPCP removal have been extensively researched, most studies tend to focus on large-scale treatment plants, while very few exist on decentralized small-scale treatment systems. This is important to note, because almost 25% of the estimated 115 million occupied homes in the United States are served by some type of onsite wastewater treatment systems (OWTSs). For example, New Hampshire and Maine both report that almost half of all their homes are served by individual treatment systems (Wayland and Opellet, 2002). OWTSs are used to treat wastewater discharged from individual homes and/or small communities, typically treating no more than 100,000 gallons per day of influent. Figure 6 shows a single-origin septic system that collects wastewater through a series of underground plumbing that eventually will extend to a pretreatment component before release into the environment (Lesikar et al., 2014).

A single septic system should not be confused with a decentralized system. A decentralized system is an OWST that collects wastewater originating from a small group of homes/businesses to one common system, where it will then be treated and released. The pretreatment components of OWTSs can provide secondary treatment and remove many contaminants that reside in wastewater or are at least able to remove them down to a low enough concentration that is acceptable for environmental treatment. These types of treatment systems are a good option for low population areas because they are low-cost and are able to provide relatively comparable results to that of a large-scale treatment facility.

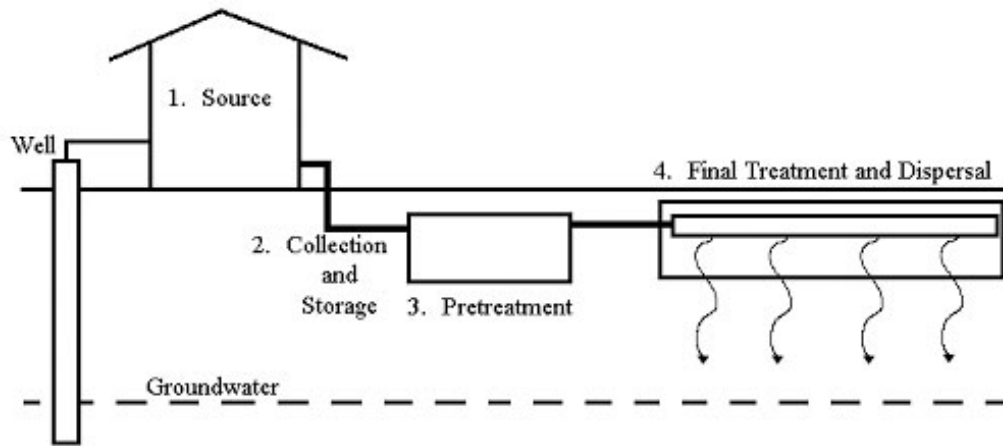


Figure 6. Components of an onsite wastewater treatment system.

Recirculating Media Filters (RMFs)

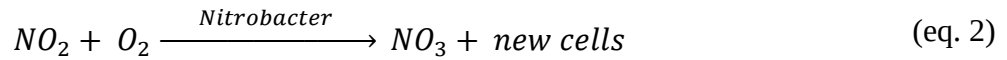
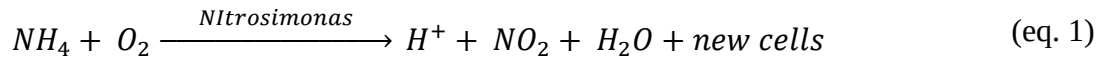
Scientists and engineers have been able to develop diverse OWTSS with their own unique way of pollutant removal that can be applied anywhere. Septic systems are generally the more common OWTSS to be found, however the use of recirculating media filters have been slowly growing in popularity. Much like a municipal trickling filter, recirculating media filters are aerobic, fixed-film bioreactors that provide advanced secondary treatment of the wastewater. Media filters are some of the oldest onsite wastewater treatment technologies known. If properly designed, constructed, and maintained, a RMF can produce a very high quality effluent (Gerlich, 2013), providing removal values as shown in table 3 (Hantzsch, 2007).

An added bonus of these treatment systems are that they are able to provide both nitrification and denitrification. Nitrification is a two-step process that involves the biological oxidation of ammonia or ammonium to nitrite, performed by ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA), and then followed by the oxidation of nitrite to nitrate, performed by

nitrite-oxidizing bacteria (NOB). The first step of nitrification is generally represented by the *Nitrosimonas* species, while the second is represented by the *Nitrobacter* species. Unbalanced chemical equations of both steps are shown below in equations 1 and 2.

Table 3. Typical effluent quality values from properly designed RMFs.

Tested Parameter	Average Removal	Typical Effluent Conc. (ppm)	
		Summer	Winter
Biochemical Oxygen Demand (BOD)	96%	2 – 10	3 – 15
Total Suspended Solids (TSS)	96%	2 – 10	2 – 10
Ammonia (NH ₃)	87%	ND – 5	1 – 20
Phosphorus (P)	50%	3 – 5	3 – 5
Dissolved Oxygen (DO)	n/a	3 – 5	6 – 12



Likewise, RMFs are also capable of providing denitrification, however this is typically not as easily achieved as nitrification. Denitrification depends on nitrogen present in the form of nitrates, a relative amount of organic carbon for an energy source, and an anaerobic environment because this process is mainly driven by large groups of heterotrophic facultative anaerobic bacteria (Yang and Zhang, 1996). Denitrification can be tricky to achieve within these systems because there must be anoxic conditions present. The concentration of the dissolved oxygen (DO) of the wastewater will control whether the denitrifying bacteria use nitrate or oxygen as the electron acceptor; if DO concentrations are too high the bacteria will instead oxidize the organic matter present, rather than the nitrate (Buchanan, 2011). The need for a proper anaerobic environment, coupled with the fact that nitrification is environmentally sensitive (pH, temperature, dissolved oxygen, loading rates, ect.), nitrogen removal percentages from RMFs are variable, typically providing between 40% to 80% removal.



Figure 7. A recirculating media filter located at Murfreesboro, Tennessee, which treats an average 30,000 GPD.

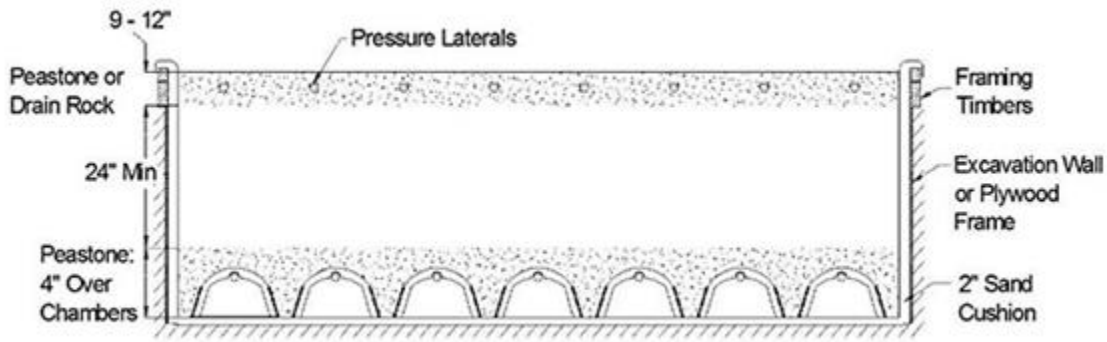


Figure 8. Cross-sectional area of the RSF shown in Figure 7.

In a RMF, wastewater trickles downwards through the media and is collected at the bottom where a large fraction of the flow is recycled back (recirculation) to the media filter (Wayland III and Opellet, 2002). Dosing frequencies onto these filter beds vary, but typical treatment is around 1 to 3 times per hour (Krkosek et al., 2014). Another factor, the recirculation ratio, is the amount of wastewater that flows through the media filter divided by the amount of wastewater that is sent to the final treatment and dispersal component. Recirculation ratios are usually between 3:1 and 5:1 and can be changed depending on the desired level of treatment. Typical design criteria for these type of treatment systems are that the media depth is at least 60 cm (24 in) in depth, contains some sort of durable, inert media, and has a hydraulic loading rate less than 0.203 m/day (5.00 gpd/ft²) and an organic loading rate of 0.026 to 0.107 g/m²/day (0.002 to 0.0080 lb BOD₅/ft²/day) (Solomon et al. 1998).

PPCP Removal Studies from OWTs

As mentioned before, not much focus has been placed on the capabilities of OWTs removing PPCPs from wastewater, but there are a select few that have performed preliminary research. For example, one study compared the effluent from several different OWTs after one day of treatment

to determine the removal of selected organic wastewater contaminants (OWCs) and PPCPs (Zimmerman and Heufelder, 2006). Seven different OWTs were studied: a single-pass media filter bed, a standard septic system, an aerobic treatment unit, a peat treatment system, an absorbent synthetic porous foam media bed, a sulfur denitrification system, and a recirculating media filter. Though concentrations were not provided, there were 13 confirmed OWCs and PPCPs in the untreated wastewater influent. After treatment, it was found that the standard septic systems had the highest removal (3 confirmed OWCs and PPCPs), and the sulfur denitrification system had the lowest removal (10 confirmed OWCs and PPCPs). Likewise, final concentrations were not provided.

Another study compared 13 different small-scale systems, mainly biological sand filters, compact biofilters, and vertical/horizontal flow constructed wetlands (Matamoros et al., 2008). While all systems were able to degrade over 80% of the identified PPCPs within the untreated wastewater, vertical flow constructed wetlands showed to consistently perform better for PPCP removal, hypothesized to be from the unsaturated flow (better oxygenation). Although, they did conclude that while the vertical flow systems performed better, all options were deemed feasible technologies for the removal of a wide variety of PPCPs.

PPCP Fate and Transfer

Fate in WWTPs

WWTP discharge is the primary route for PPCP introduction into the environment. Because WWTPs were not designed with PPCP removal in mind, most of these trace compounds are removed in unintentional methods, resulting in variable elimination. The topic of how PPCPs survive/degrade and move through the wastewater treatment process and environment is not

simple, mainly because no two compounds are alike. The majority of PPCPs react differently once they enter into the wastewater system; some are degraded easily, some remain completely unchanged, and others can be transformed prior to human excretion, only to be re-transformed back into the parent compound during treatment (Karnjanapiboonwong et al., 2010).

PPCP Sorption

Sorption is an important, and sometimes unspecified, pathway of PPCP removal within wastewater systems. Sewage sludge is frequently separated during wastewater treatment, and after further treatment on the sludge, the resulting biosolids are EPA-approved for land application. Many WWTPs allow portions of the biosolids to be sent to farmers as a source of nutrition for their non-food crops. Unfortunately, many PPCPs adsorb onto sewage sludge, survive treatment and released with the “treated” biosolids. This uninterrupted pathway into the environment allows PPCPs to potentially travel through agricultural runoff into receiving streams or leaching into the groundwater. The binding of these compounds to the solids typically causes a loss in detectability, which only further makes it more difficult to analyze.

To understand a compound’s ability to adsorb, it is important to know that each class of PPCPs are extremely complex with their own chemical properties. For example, one property is the octanol-water partition coefficient (K_{ow}) on pH. Smith et al. (1988) defined K_{ow} as “the ratio of the compound's concentration in a known volume of n-octanol to its concentration in a known volume of water after the octanol and water have reached equilibrium.” This concept is important because many PPCPs are known to have low to moderately low K_{ow} values and studies have shown that the likeliness of compound sorption onto sewage sludge from secondary treatment correlated positively with their log K_{ow} . This said relationship was documented (Dobbs et al., 1989) and is shown in figure 9.

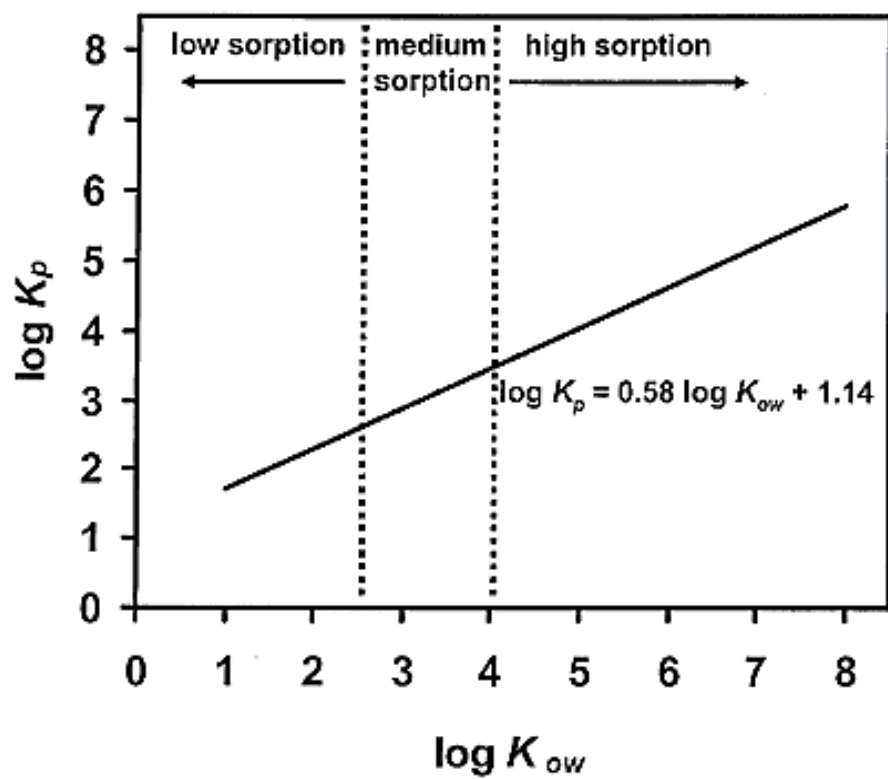


Figure 9. Experimental data showing a positive correlation between $\log K_{ow}$ coefficient of trace organic compounds and their sorption onto sewage sludge.

PPCP Degradation

Evidence has shown that many PPCPs are able to be broken down during various stages of wastewater treatment, whether it be through physical, chemical, or biological operations. While PPCP removal through degradation during primary treatment does occur, the amount removed is relatively insignificant, and therefore not studied in detail. It has been frequently noted that a significant fraction of most trace organic compounds can be removed through secondary treatment (Larsson et al., 2014). The microorganisms that flourish during this level of treatment can be a powerful ally when removing PPCPs from wastewater, such as with easily degraded acidic pharmaceuticals like ibuprofen or naproxen. Biological degradation has been said to potentially be one of the more promising “clean-up” technologies for PPCP removal because of its low cost and ability to remove a large amount of pollutants (Tran et al., 2013).

The biodegradation of PPCPs varies significantly between different WWTPs, and the main reason for this is because of both the treatment systems in use and the quality of influent they are dealing with. Although the topic of PPCP within wastewater has been widely documented over the past decade, researchers are still undecided on what the controlling factor is in understanding the biodegradation of PPCPs. For example, some researchers claim that biodegradation is dependent on PPCP physiochemical properties and chemical structures, while some put more focus on WWTP operation parameters (pH, hydraulics/solids retention time, and temperature), and others believe that it falls more on the nature of the microorganisms and their enzymes involved in biodegradation (Tran et al., 2013).

The biodegradation of a compound is known as converting large molecular weight compounds into those with a lower complexity. With organic compounds, generally they are broken down into

simple inorganic molecules such as water or carbon dioxide. Biodegradation pathways of organic compounds can occur in two ways, either through metabolism, where the organic compounds are completely broken down and used for cell growth, and/or co-metabolism, where the organic compounds are not the sole carbon or energy source to maintain growth, meaning the presence of another growth substrate is needed (Tran et al., 2013). The fact that many PPCPs are often present in wastewater effluent at trace concentrations shows that co-metabolism does occur – this pathway generally results only in the modification and transformation of organic compounds, and does not end with complete destruction. Still today, it remains unclear which biodegradation pathway is predominant in PPCP removal, however co-metabolism is suspected.

For trace organics to be removed through metabolism, it is required that the compound should not be in anyway toxic or harmful to the microbial growth and that its presence is at a high enough concentration that will allow the biomass to be sustained. Although, it is unknown which PPCPs are able to initiate these metabolic activities. It is known that microorganisms that participate in such removal methods are heterotrophic bacteria. Quintana et al. (2005) studied possible metabolic degradation pathways of five different PPCPs by ensuring that each compound was the sole carbon source under aerobic conditions. The results from this experiment showed that only ketoprofen, a non-steroidal anti-inflammatory drug, showed removal through degradation while the other pharmaceuticals did not. The study was not able to conclude ketoprofen as being the sole carbon source to induce degradation, saying that the oxidative enzymes could have used dead cells instead.

Unlike typical organic compounds, many PPCPs are either toxic or resistant to microorganisms, for example triclosan, which is an anti-bacterial. Because of this, the energy that the microorganisms take from these trace organics is usually not enough to support microbial growth and induce the necessary enzymes involved in biodegradation; therefore, the presence of

another growth substrate is necessary. Unfortunately, co-metabolism is a common pathway for many PPCPs and this has been shown to be more detrimental than the original presence of the compounds. Many times, especially with EDCs, the incomplete breakdown of a compound produces a new compound that can show properties more toxic than the parent (Haiss and Kummerer, 2006). Co-metabolism degradation can take place under both aerobic and anaerobic conditions, although most studies show that aerobic co-metabolism is more predominant.

Co-metabolism of PPCPs are more noted with autotrophic bacteria, such as ammonia oxidizers through non-specific enzymes, such as ammonia monooxygenase (AMO), which is a key enzyme in nitrification. Literature reviews have suggested that the presence of ammonia oxidizing bacteria (AOB) during nitrifying conditions can promote certain PPCP degradation. One often researched AOB is *Nitrosomonas euopea* (*N.euopea*) because of its ability to degrade estrogens and some antibiotics (Shi et al., 2004). Due to the AMO enzyme, *N.euopea* has also been documented to catalyze the oxidation of many different aromatic compounds, such as benzene and phenol. It is also speculated that other dominant AOBs present specifically in activated sludge, such as *N.mobilis*, *N.eutropha*, and *N.halophila*, may play a role in other PPCP degradation (Juretschko et al., 1998).

Although co-metabolism has been speculated to be a major removal mechanisms of PPCPs (Hai et al., 2011), both ammonia oxidizing and heterotrophic bacteria can participate in degradation. While AOBs are generally associated with co-metabolisms, heterotrophs can perform both co-metabolism and metabolism, depending on the concentration of the PPCPs present and their level of toxicity to the microorganism (Tran et al., 2013). There have also been studies that have shown AOBs and heterotrophs working cooperatively together to degrade a compound. Khunjar et al. (2011) documented that while studying the degradation of EE2, an EDC found in

birth control, AOBs were able to quickly break down the compound, while the heterotrophs mineralized the PPCP independently of the AOB activity and breakdown the corresponding metabolites created from the AOBs. Despite the information provided, the question of autotrophs vs. heterotrophs for PPCP degradation cannot be answered currently. More insight is needed into the involvement of both of these microorganisms in the biodegradation of PPCPs.

Quantifying PPCPs in Wastewater Samples

Extraction Methods

There are multiple methods of extraction for PPCPs residing within wastewater because one method cannot identify all types of organic compounds. Typically, one type of process will extract only a certain group of compounds with similar physiochemical structures and properties. Because multiple methods of extraction and detection of PPCPs, analysis can be costly and time consuming. For the extraction of PPCPs from a water sample, processes such as liquid-phase microextraction, solid-phase extraction, solid-phase microextraction, and polar organic chemical integrative samplers are more successful methods (Samaras et al., 2010). Solid-phase extraction (SPE) is favored highly amongst researchers because of its high extraction efficiency for a wide range of analytes.

SPE is an extraction procedure that allows the separation of dissolved compounds (analytes) from a liquid matrix. Cartridges with different based packing are available so that the highest extraction efficiency can be achieved. For example, reversed-phase cartridges are for analytes that are non-polar to moderately polar compounds, normal-phase cartridges are for higher polar compounds, and ion-exchange cartridges are for compounds that have strong/weak cation/anion exchanges.



Figure 10. Twelve-port vacuum system for solid phase extraction; GL Sciences.

Researchers that want to extract acidic pharmaceuticals, such as naproxen and ibuprofen, typically use the commercial Oasis[®] HLB, while the reversed phase of c18 cartridges are used for moderately polar compounds, such as triclosan. Few studies have investigated the simultaneous extraction and detection of different PPCPs, however, Samaras et al. (2010) looked into extracting a somewhat-board range of PPCPs with different cartridges and found similar extraction efficiencies, as shown in table 4.

Table 4. Comparison of mean extraction efficiencies of Oasis HLF and c18 cartridges for selected acidic and phenolic PPCPs.

Compound	C18, %	Oasis HLB, %
Ibuprofen	77±1	76±2
Naproxen	85±1	84±2
Bisphenol A	74±3	77±14
Triclosan	67±1	65±11

Detection Methods

There are multiple methods that can be used for the detection of PPCPs once they have been appropriately extracted, some examples being pressurized liquid extraction, microwave assisted extraction, and ultrasound sonification (Samaras et al., 2010). However, the most commonly used detection methods are gas chromatograph coupled to mass spectrometry (GC-MS) or liquid chromatography coupled to mass spectrometry (LC-MS). Sometimes tandem mass spectrometry (GC-MS/MS, LC-MS/MS) is needed for the detection of low-concentrated compounds (ng/L range) that are present in a highly complex water sample, such as untreated wastewater. Both methods of detection have their own advantages, for example, GC-MS has been noted to be reliable in the detection of neutral and acidic pharmaceuticals, while LC-MS is used for highly polar compounds (Gomez et al., 2007). While both popular, these detection methods are also not without faults; they are susceptible to background noise and signal suppression/enhancement, especially with older MS instruments, which can both affect sensitivity and proper compound detection (Samaras et al., 2010). Generally, GC-MS is favored over LC-MS because of built-in databases of known compounds and the cost is substantially less. However, LC-MS does not require an additional step known as derivatization, which will be discussed next, and therefore can analyze a much wider range of analytes than GC-MS. LC-MS is usually best suited for discovery-based approaches when researching unknown analytes (Danielson and Gallagher, 2000).

When using GC-MS for the detection of trace organics, samples must go through derivatization immediately after extraction. This step is important because most PPCPs are not stable or volatile enough for GC-MS detection. This procedure allows the elimination of hydroxyl, amine, and thiol groups while targeting oxygen, sulfate, nitrogen, and phosphorous groups, thus increasing volatility, stability, detectability, and sensitivity. There are a number of different types

of derivatization, however the most prevalent approach is through silylation, where active hydrogens are replaced with a trimethylsilyl (TMS) group. The solvents required to perform silylation must be as pure as possible to ensure the elimination of excessive peaks. More studies that focus on PPCP extraction and detection use either BSTFA+1%TMCS or MSTFA+1%TMCS as silylating reagents, and pyridine or acetonitrile as solvents.

Literature Review for PPCP Removal from Wastewater

PPCP removal from WWTPs has recently become an exciting area of research for many scientists within the past decade. The physiochemical diversity of PPCPs and their fate in the environmental has made their complete removal from wastewater challenging. Microbial degradation is a known and partially effective method of pharmaceutical removal, however in depth research on this is limited, many resulting with more questions than answers. For example, Burke et al. (2013) studied the performance of microorganisms and their ability to degrade PPCPs under both aerobic and anaerobic conditions. This study was able to prove that organic pollutants are redox-sensitive, and the results confirmed that their biodegradation was influenced directly from the redox environment. In a separate study (Lubliner et al., 2010), it was noted that operating the WWTPs with a longer solids retention time, allowing a longer biological contact time, increased PPCP removal rates. After a series of experiments, they documented that pH changes within a treatment system had a large impact and would either increase or decrease the rate of antibiotic removals. The most effective processes that have been documented so far are biological treatments such as conventional activated sludge and membrane bioreactors coupled with nitrification (Meige et al., 2008).

There are other methods applied to help in the removal of pharmaceuticals. For example, one study analyzed the removal efficiency of PPCPs from urban wastewater in both a vertical and

horizontal flow constructed wetlands, comparing these results against a typical sand filter (Matamoros et al., 2007). The main reason for this comparison was to evaluate the influence of vegetation on PPCP removal. Along with determining the concentration of the compounds both prior and after testing, dissolved oxygen, temperature, and pH of the influent and effluent measurements were also collected. It was found that on average a vertical flow constructed wetland was more efficient at PPCP removal (73% to 98% removal) than a horizontal flow (16% to 96% removal), presumed to be from a more oxygenated bed, and comparable to the results received from the sand filter (82% to 98% removal). Studies that focused on using media filters as a way to remove organic pollutants from water samples have been published as well. For example, one study focused on the biodegradation of mononitrophenols, commonly used in the agriculture industry, in a packed-bed aerobic reactor (Halecky et al., 2013). They discovered that while operating under a low hydraulic retention time removal efficiencies were above 85%, and removal efficiencies were above 98% under a high hydraulic retention time.

One pathway into the environment that PPCPs have known to take is through sorption onto other materials such as biological film, activated sludge (untreated and treated) and soil. Literature reviews that focused on the quantification of PPCP concentrations after sorption are limited, most showing different methods and results. Mohapatra et al. (2011) were trying to determine the occurrence of bisphenol A in both wastewater and sludge samples, particularly looking at the partitioning of the compounds in both soil and liquid fractions. Bisphenol A was found to be present in all samples (influent, effluent, mixed sludge) within the range of 0.07 to 1.68 $\mu\text{g/L}$, but they were particularly surprised with the concentrations (0.104 to 0.312 $\mu\text{g/L}$) that were detected in dewatered sludge. They concluded their study by questioning the reuse of “treated” sludge for environmental purposes, such as landfill and crop nutrition.

Another literature review was documenting the transport of numerous PPCPs, namely atenolol, carbamazepine, cotinine, caffeine, gemfibrozil, naproxen, ibuprofen, acetaminophen, sulfamethoxazole, triclosan and triclocarban, in runoff from plots that received liquid and dewatered municipal solids for crop growth (Sabourin et al., 2009). They found that trace organic compounds with a larger K_{ow} were found to have little transport potential. Likewise, the compounds with lower K_{ow} values were detected in the runoff from the plots, with an average concentrations range of 0.0034 to 0.1097 $\mu\text{g/L}$.

Literature Review for PPCP Microbial Degradation

Little is known about how specific bacterial communities interact in the fate of pharmaceuticals and personal care products through the wastewater treatment process. While it is known that aerobic conditions are more successful than anaerobic, it is difficult to pinpoint the specific microbes responsible for this degradation. It is important to identify such bacteria because it will allow scientists to potentially spike incoming wastewater with these microbial communities and promote important degradation pathways.

One study was interested in identifying microorganisms related to PPCP degradation and found that the white rot fungus, *Phanerochaete chrysosporium*, was able to almost completely degrade both NSAIDs ibuprofen and diclofenac in fed-batch bioreactors (Langenhoff et al., 2012). Denaturing gradient gel electrophoresis (DGGE) analysis of the samples collected from this study showed evidence of a highly enriched bacterial culture that originated from the inoculum from the wastewater treatment plant. By the end of the study, all 250 mg/L of the added ibuprofen and 75% of the added 300 mg/L of diclofenac was degraded. This study was able to conclude that specific ibuprofen and diclofenac degrading bacteria was present in original inoculum and, while not being

able to identify specifically, concluded through DGGE that at least two different bacterial species were responsible.

As mentioned before, the presence of nitrifying and denitrifying bacteria have shown to aid in the degradation of certain PPCPs. Research into this has suggested that for many PPCPs, different metabolites will form under aerobic or anoxic conditions, indicating that there are different degradation pathways for different processes. For example, many research articles (Vader et al. 2000; Andersen et al., 2003) have stated that the estrogen 17 α -ethinylestradiol, when under nitrifying conditions, will transform into metabolites lacking estrogenic activity. Suarez et al. (2010) studied the degradation of 16 different PPCPs under both nitrification and denitrification conditions in separate continually stirred tank reactors. They were able to conclude that biotransformation of the trace organics was the primary source of PPCP removal, reporting that acidic pharmaceuticals, estrogens, and musk fragrances were the compounds that showed the highest degradation when the tank reactors enriched with both nitrifying and denitrifying bacteria.

Research Hypothesis and Goals

This research is intended to answer the following question: Are OWTs (specifically packed-bed recirculating media filters) able to provide adequate, or even comparable, PPCP removal rates to that of large-scale WWTPs? In addition, this study intends to investigate specific aims such as:

- 1) Can different methods of removal (adsorption onto biofilm or biodegradation) be quantified through simple mass balance equations?
- 2) Can nitrifying and denitrifying bacterial populations be quantified at different depths of the RMF and can these be attributed to PPCP degradation?

- 3) Do RMFs experience changes in effluent quality or microbial abundance when exposed to higher concentrations of different PPCPs, especially an anti-bacterial?

It is hypothesized that over 80% of the investigated trace organic compounds will be removed through biodegradation or adsorption, such as observed in large-scale treatment facilities. With the conclusion of this project, it is anticipated that similarly constructed OWTs will allow small communities to remove most trace organic compounds from their domestic wastewater and allow the renovated water to be safely discharged back into the environment.

CHAPTER II: Target Compounds

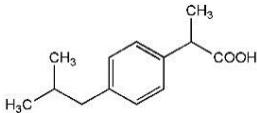
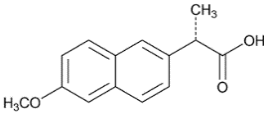
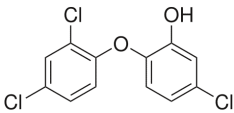
Compound Introductions

The three target compounds of this study are the acidic pharmaceuticals ibuprofen (IBU) and naproxen (NAP), and the phenolic EDC triclosan (TRI). These compounds are known for their worldwide high use and toxic effects on the environment. These compounds are frequently detected in many environmental samples and have continually shown that a large percentage survives typical WWTPs, as can be observed in table 5 (Guerra et al., 2014). While different in regards to their toxicity, compound makeup, and metabolite formation, these compounds are physiochemically similar when comparing molecular weight, Log K_{ow} , water solubility, and pK_a . These values are given in table 6.

Table 5. Detection frequency and expected concentration of PPCPs in influent and effluent from six Canadian WWTPs (secondary treatment).

Compound	Detection Frequency, %	Mean Influent, ppb	Mean Effluent, ppb
Ibuprofen	99	45	4.7
Naproxen	92	25	3.5
Triclosan	100	2.9	0.4

Table 6. Various chemical properties of the target compounds.

Compound	Formula	Molecular Weight	Log K_{ow}	Water Solubility, mg/L @ 25°C	pK _a	Compound Structure
Ibuprofen (IBU)	C ₁₃ H ₁₈ O ₂	206.28	3.97	21.0	4.91	
Naproxen (NAP)	C ₁₄ H ₁₄ O ₃	230.26	3.18	15.9	4.15	
Triclosan (TRI)	C ₁₂ H ₇ Cl ₂ O ₂	220.36	4.48	10.0	7.9	

Non-Steroidal Anti-Inflammatory Drugs

Non-steroidal anti-inflammatory drugs, or NSAIDs, are one of the most prescribed type of drugs in the world for pain relief. While they are given for mild to moderate pain relief, they are also used for the reducing inflammation and fever within the human body, as well as the prevention of blood clotting (OrthoInfo, 2009). These drugs work by preventing an enzyme named cyclooxygenase (COX) from triggering changes within the body from doing what they would do naturally. Most NSAIDs are weak acids, typically having a pK_a value around 3 to 5. While there are many different forms that NSAIDs take on, table 7 lists a few of the most popular and their commonly delivered doses (OrthoInfo, 2009).

Table 7. Commonly used NSAIDs and their delivered dosing.

NSAID	Brand Names	Typical Dose (mg)
Aspirin	Several companies	650-925
Ibuprofen	Motrin®, Advil®	400-800
Naproxen	Naprosen®, Aleve®	250
Nabumetone	Relafen®	500-1000

2-(4-isobutylphenyl)propanoic acid

2-(4-isobutylphenyl)propanoic acid, or more commonly known as ibuprofen, is a common fever reducer that moderates the hormones that cause inflammation and pain in our bodies. This compound was listed as the 3rd most popular drug in the world and is normally distributed in a solid pill form within a dose range of 600 to 1200 mg/day (Buser et al., 1999).

IBU is classified as a propanoic acid derivative, because of the attached carboxyl group (C(O)OH), along with a number of other compounds. As an NSAID, it works by inhibiting COX enzymes within the human body, but in a different way than most NSAIDs such as aspirin. Rather than forming a covalent bond with the enzyme, IBU works through reverse inhibition by binding non-covalently and competing with the enzyme's natural substrate (Flower, 2003).

IBU is normally administered orally, however there are some reports of topical and intravenous administration. This allows the compound to be partly metabolized within the human body; research showing that about 44% of the drug is passed through urine and feces within 24hs and 80% of that being 2-hydroxy and carboxy IBU, which are two major corresponding metabolites. 1-hydroxy and 3-hydroxy IBU have also been recorded in WWTP effluent, but in much smaller

concentrations (Davies, 1998). IBU and its major metabolites are shown in figure 11 (Kummerer, 2008). Although a large amount of IBU is degraded during the wastewater treatment process, trace amount of the compound are still released into the environment. Because of the physiochemical properties of IBU, this compound has been shown to have a high mobility and low volatility in the aquatic environment, giving a reason as to why it frequently survives treatment. While not one of the most dangerous drugs in regard to environmental health, there have been reports on potential links to fish health and aquatic plant growth (University of Exeter, 2014).

(2S)-2-(6-methoxynaphthalen-2-yl)propanoic acid

Naproxen, like IBU, is a NSAID that focuses on alleviating pain in the body through hormone regulation. While not as frequently used worldwide as IBU, NAP is still listed as one of the more commonly used drugs and is typically administered around 250 mg/day. It also joins IBU as another propanoic acid derivative. In many ways, NAP and IBU are identical, however they differ based on reaction speed and targeted enzymes (Curiosity Aroused, 2015). IBU works as a quick pain relief and is a non-selective NSAID, inhibiting both COX-1 and COX-2 enzymes. NAP has a slower reaction time than IBU, but provides a longer pain relief. As a selective NSAID, focusing on only COX-2; because of the lower dose range and more specific enzyme targeting, NAP is easier on the human heart than IBU. Also unlike IBU, the breakdown of NAP is quick and nearly complete; less than 1% of the parent compound found in urine samples. Therefore, almost all of the drug is excreted as corresponding conjugates (51% as NAP-acylglucuronide, 14% as 6- O-desmethyl-NAP acylglucuronide, and 6% to 7% as their respective isoglucuronides) (Davies and Anderson, 1997).

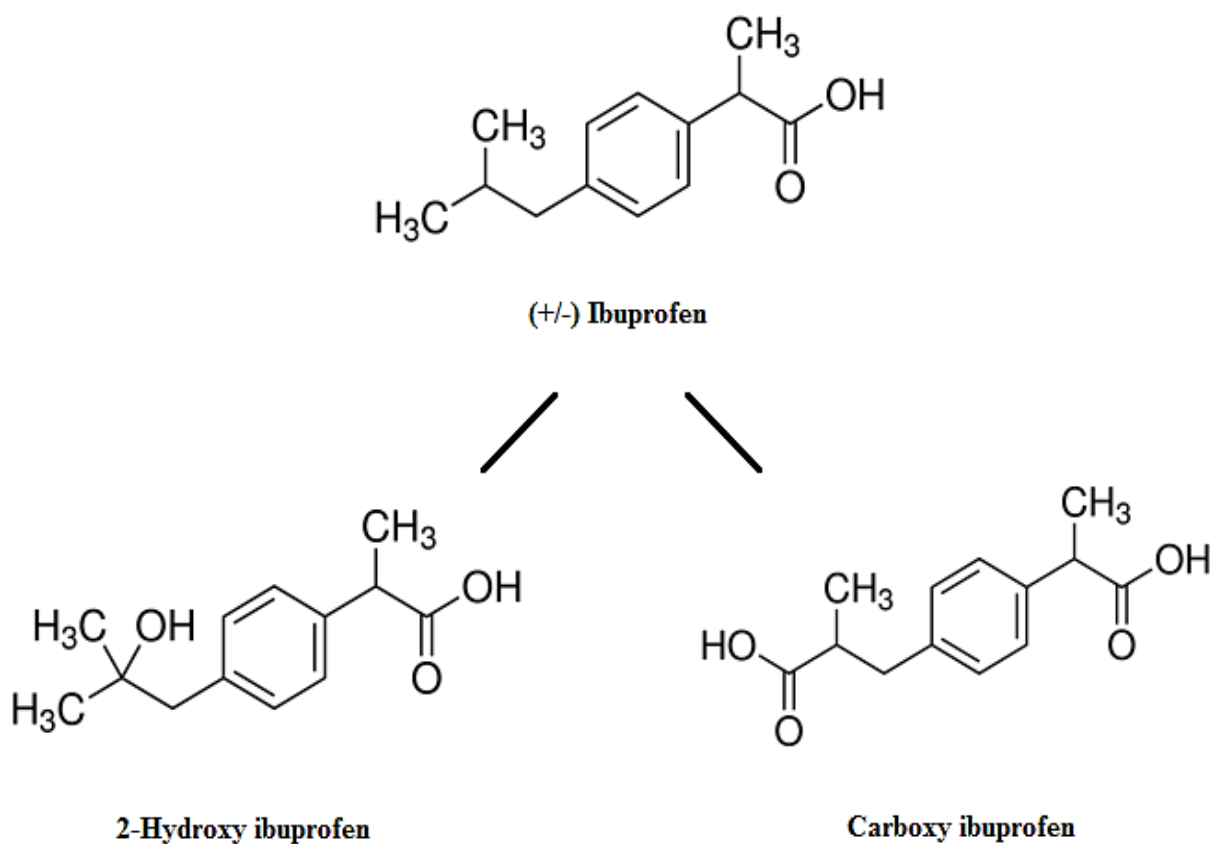


Figure 11. Chemical structures of parent compound ibuprofen and major metabolites hydroxy ibuprofen and carboxy ibuprofen.

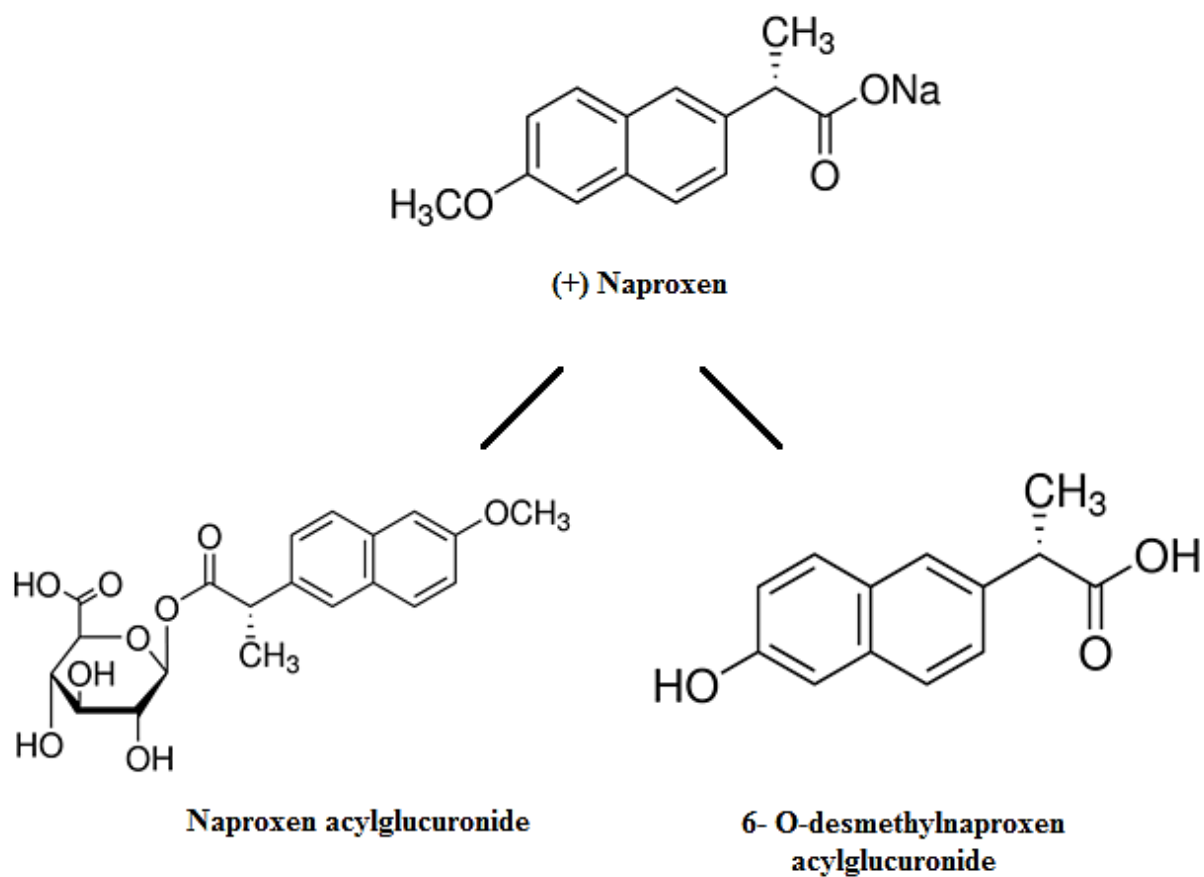


Figure 12. Chemical structures of parent compound naproxen and metabolites naproxen acylglucuronide and 6-O-desmethylnaproxen acylglucuronide.

Although a large amount of NAP is excreted through urine and bio-transformed into corresponding conjugates, a small percentage of the compound remains unchanged and untreated as it passed through wastewater treatment, not unlike its NSAID counterpart IBU. While a weak acid just as IBU, NAP is easily degraded and weakly adsorbed onto other substances (Yu et al., 2011).

Endocrine Disrupting Compounds

According to the National Institute of Environmental Health Sciences, endocrine disrupting compounds are chemicals that may interfere with the body's endocrine system and produce adverse developmental, reproductive, neurological, and immune effects in both humans and wildlife (NIH, 2015). These chemicals can be found in a variety of products such as pharmaceuticals, plastics, cleaning products, and cosmetics because of their useful properties. EDCs work by disrupting or mimicking certain endocrine pathways through direct hormone interaction. These interactions can cause chemical changes in the body anywhere from sexual development, metabolism, and brain development. The chemical structure of EDCs are commonly different from one another, making it very difficult to determine if a compound is an EDC by simply looking at the structure. Because of this variability and unpredictability, they are sometimes synthesized unintentionally. Table 8 includes a list of a few common EDCs and their applications (Hess-Fischl, 2015).

Table 8. Commonly found EDCs and corresponding applications.

EDC	Applications
Bisphenol A (BPA)	Toys, plastics, food containers, receipts
Dichlorodiphenyltrichloroethane (DDT)	Pesticides (now banned)
Polychlorinated biphenyls (PCBs)	Electronics, building materials
Triclosan	Antibacterial products

5-chloro-2-(2,4dichlorophenoxy)phenol

As one of the more frequently detected PPCPs in environmental samples, 5-chloro-2-(2,4dichlorophenoxy)phenol, triclosan, it is a compound that can be found in almost every household. TRI is an ingredient that is added to many consumer products such as soaps, detergents, and cosmetics to help prevent or reduce bacterial contamination. While it has not yet been established to be directly harmful to humans, it has been widely researched to be extremely toxic to aquatic life and algae species from altering hormone regulation. In addition, researchers who specialize in bacterial studies have suggested that TRI is making certain bacteria resistant to many antibiotics (FDA, 2013).

TRI is a chlorinated aromatic compound that is highly soluble in water and has functional groups made of both ethers and phenols, which often show anti-bacterial properties. This compound works by effectively stopping the fatty acid chain growth through the inhibition of a bacterial enzyme, thus stopping the growth of the cell. This makes TRI an extremely powerful agent and is only needed in very low concentrations (>0.3%) (Angkadjaja, 2007).

TRI was not intended for oral consumption, and therefore is not metabolized within the human body. It does not easily undergo biodegradation during wastewater treatment from being a stable lipophilic compound with a relatively high octanol water partitioning coefficient (K_{ow}). In actuality, TRI is a large cause of concern in many WWTPs because its presence has been linked to the destabilization of important microbial communities that help treat sewage. Research has shown that TRI is susceptible to photodegradation, but not without the production of certain byproducts which have been proven to be even more toxic than the parent compound (Ricart et al., 2010). Other byproducts of TRI are listed in table 9 (Sanchez-Prado et al., 2006).

Table 9. A list of toxic byproducts of triclosan after undergoing photodegradation.

Byproduct	Description
2,8-Dichlorodibenzo-p-dioxin(2,8-DCDD)	Type of dioxin
2,4-Dichlorophenol(2,4-DCP)	Endocrine disrupting compound
2,4,6-trichlorophenol (2,4,6-TCP)	Endocrine disrupting compound
Chloroform	A carcinogen
Methyl triclosan	A toxic metabolite more bioaccumulative than TRI

TRI is largely an environmental concern because of the almost non-existent elimination after wastewater treatment processes. This compound has a high sorption rate and typically binds to solids such as sediment and sludge (Walker and Watson, 2010). The sewage sludge that originated from treatment plants are often re-used and sent to many agricultural sites as a source of nutrients and from there TRI leaches through the soil, potentially contaminating both surface and ground waters.

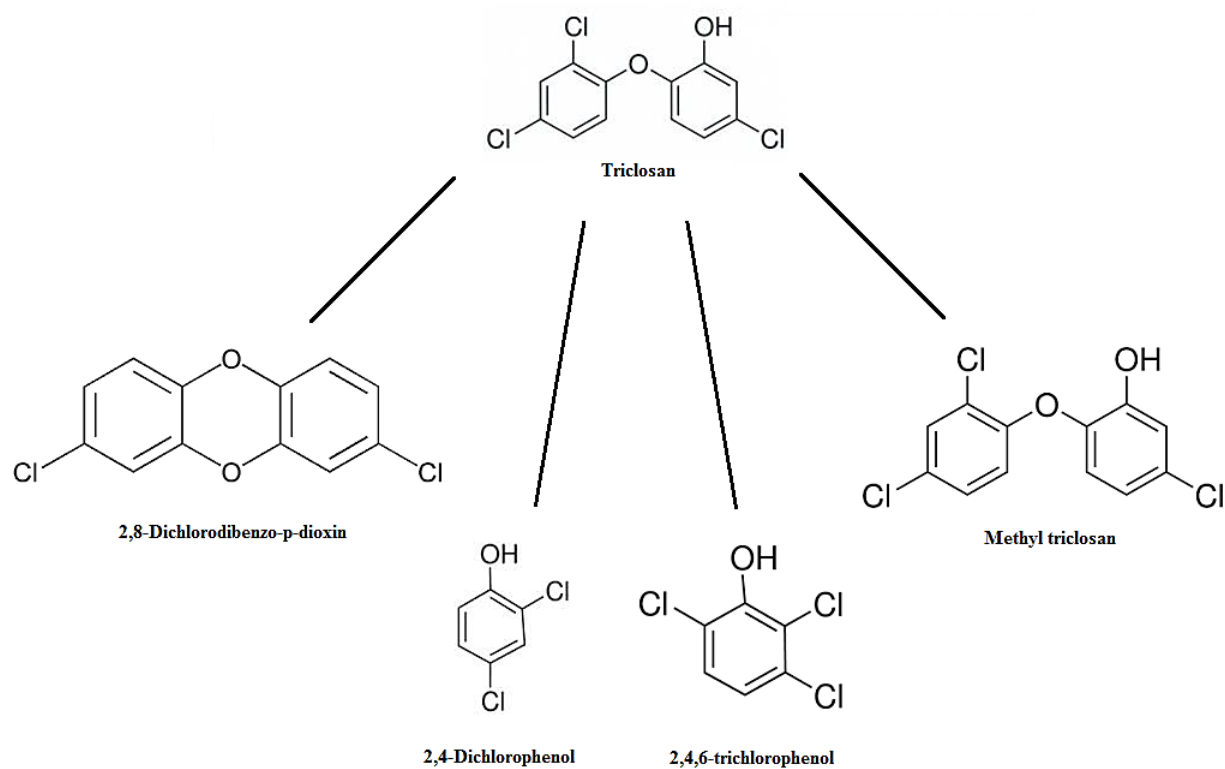


Figure 13. Chemical structures of parent compound triclosan and metabolites 2,8-dichlorodibenzo-p-dioxin, 2,4-dichlorophenol, 2,4,6-trichlorophenol and methyl triclosan.

There is a great deal that still remains unknown about the fate and transfer of this compound in the environment. There are a few known metabolic pathways of TRI; most can only be speculated because it has been difficult for researches to give a definite answer (Fang et al., 2010).

Metabolite Research

The more PPCPs are studied, the more it is discovered that not only are the parent compounds important to understand, but their metabolic transformations are as well. Many PPCPs are biologically transformed by organisms such as bacteria and fungi once they are consumed or absorbed in the human body, introduced into WWTPs, or from various environmental factors (such as sunlight). Although a good percentage of the parent compound are removed in typical wastewater treatment processes, their metabolites are still present, sometimes at higher concentrations than the parent compounds (Lee et al., 2013). It is important to identify these transformed compounds because it has been shown that they are able to form brand new molecules with different physicochemical properties than originally was with their parent compound, thus resulting in an altogether different fate and transfer. While not specifically addressed in this research, metabolic pathways and transformations should still be taken into consideration.

CHAPTER III: Materials and Methods

Chemicals and Working Standards

The three compounds of interest were each purchased at their highest purity (>98%) from Sigma Aldrich (St. Louis, Missouri, USA), Campbell Science (Rockford, Illinois, USA) and Fischer Scientific (Waltham, Massachusetts, USA). Working standards of each of the chosen compounds were prepared separately at 150 ppm in 99% HPLC grade methanol and diluted for analytical procedures. HPLC grade chemicals for analytical procedures such as ethyl-acetate, MSTFA + 1% TMCS, and acetonitrile were also purchased from Fischer Scientific and Campbell Science.

Wastewater Source

Domestic wastewater samples were collected from The University of Tennessee's Little River Animal and Environmental Unit of East Tennessee Research & Education Center (ETREC) in Walland, Tennessee. This site's primary use is a dairy farm and was opened in 2011 to research animal and environmental best management practices, supporting a herd of 200 to 250 holstein cattle. The wastewater collected was primarily sourced from four on-site homes, as shown in figure 14; this wastewater is purely from domestic use, not agricultural, and has only undergone primary treatment.



Figure 14. Collection site of wastewater and contributing homes.

Project Description and Design

Materials and Construction

Four experimental stainless steel columns 0.6 m (24 in) depth, 0.09 m (3.5 in) width) were placed parallel on a constructed wooden stand. Figure 15 shows the layout for one of the experimental columns for this study. The media column (A), was filled with a small gravel that was sieved to ensure a consistent 3 to 5 mm particle size. The bottom of the column was filled with #55 crushed limestone rock to prevent the smaller media from clogging the system. To compare the amount of free volume space allowed for biological growth, water flow, and air to passively travel through both types of media, particle and bulk densities were calculated and it was found that the upper media has an estimated 5% porosity while the crushed limestone has 20%.

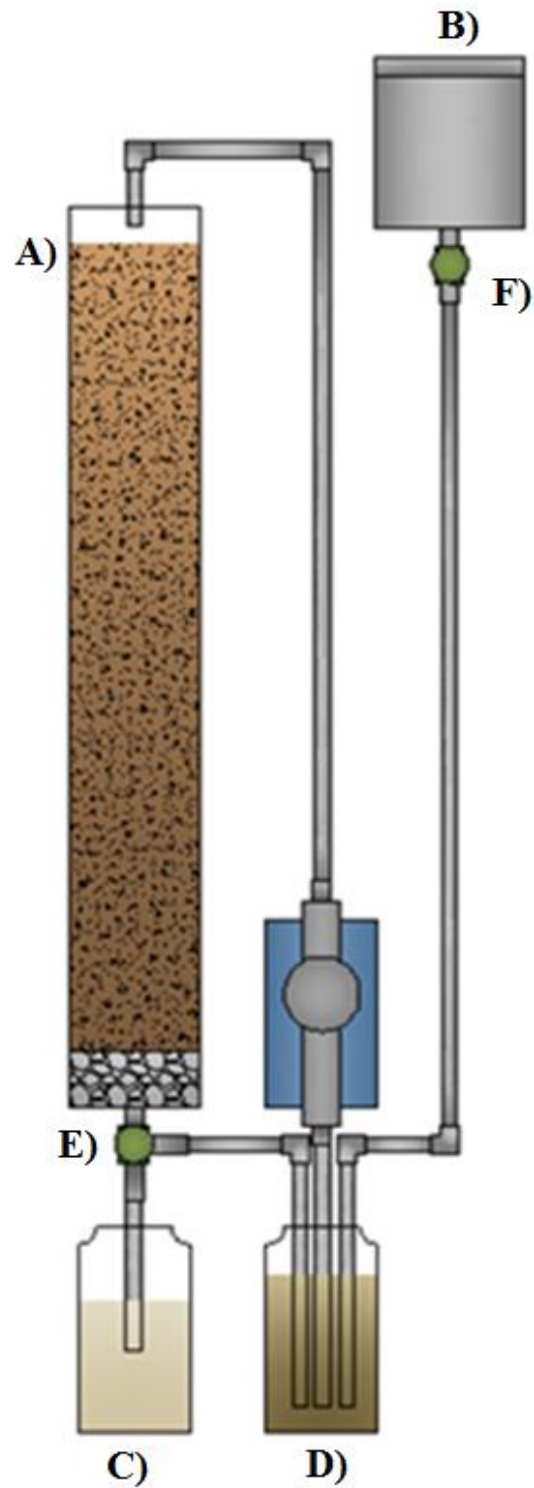


Figure 15. RSF configuration with (a) media column, (b) 5 L supply tank, (c) effluent collection, (d) recirculated collection, (e) three-way valve, and (f) two-way valve.

System Operation

Wastewater was introduced into the system from the 5 L supply tank (B) located on the top right of the column. When the float switch within the recirculated collection (D) was triggered, the two-way valve (F) beneath the supply tank opened to allow the wastewater to be discharged. When at least 2 L had been discharged, the float switch then triggered the two-way valve to close until needed again. A polytetrafluoroethylene coated diaphragm pump was also programmed to deliver a 50 mL dose of wastewater every hour from the recirculated collection. This system was on a 5:1 recirculation ratio, therefore every 5th pass through the system was diverted using a three-way valve (E) to the final effluent collection (C), while all other passes were brought back to recirculation. After 0.5 L has been pumped from the recirculated collection, the float switch will once again trigger and open the two way valve underneath the supply tank to allow wastewater to refill once again.

Four experimental units were set up in parallel as shown in figure 16. The first unit was designated as the control column, while the others were designated experimental columns. A methanol standard was added to each of the experimental column's supply tanks to provide a 0.1 ppm concentration of the specific trace organic compound to that column: the IBU supply tank was administered an IBU methanol standard, NAP with a NAP methanol standard, and TRI with TRI methanol standard. To remain consistent in any changes to the influent, the control column supply tank received an equal amount of methanol standard, but with the absence of the compounds.

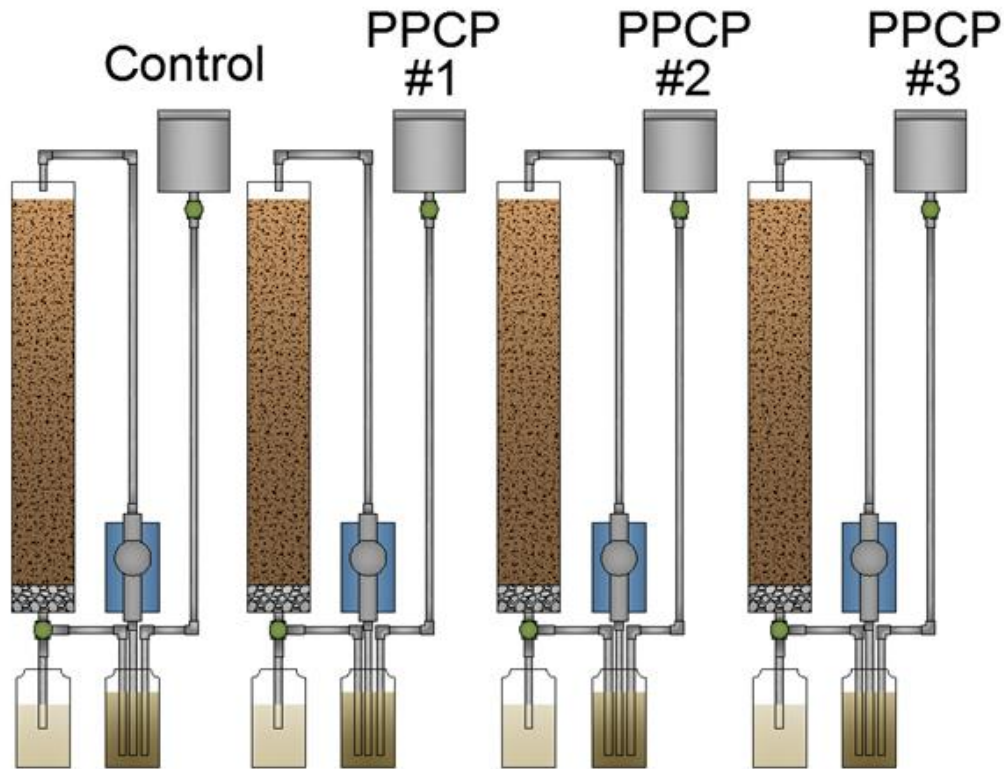


Figure 16. Schematic of experimental station.

Hydraulic/Organic Loading Rates

Two important calculations that must be made to determine the design of a RMF are the hydraulic and organic loading rates. They are critical design factors, and if not appropriately set, they could result in the improper treatment of wastewater. The hydraulic loading rate (HLR) is determined from the volume of wastewater that is applied to a surface area (Zhou and Mancl, 2007). Simply put, it is the amount of water applied over an area within a certain time, as shown in eq. 3. While there are many different design criteria for the HLR, it is typical to ensure a loading rate of 0.085 to 0.142 L/m²/day (3 to 5 gal/ft²/day) (Solomon et al., 1998). For this research, the HLR was calculated to be 0.135 L/m²/day (4.76 gal/ft²/day), which is within design specification.

$$\text{Hydraulic Loading Rate} = \frac{\text{Design Flow} \left(\frac{\text{gal}}{\text{day}} \right)}{\text{Area} \left(\text{ft}^2 \right)} \quad (\text{eq. 3})$$

Similar to the HLR, the organic loading rate (OLR) is the amount of organic matter that is applied to a specific area over a certain time, and this is typically dependent on the biochemical oxygen demand (BOD). BOD is defined as the amount of oxygen that must be present within a water sample for microorganisms to break down any organic material present. BOD₅ is a measure of this oxygen to degrade organic material within a 5 day time period. This is a common method for determining organic material present within wastewater. Once a BOD₅ test has been performed, a simple equation can be applied to determine the organic material present. To determine the OLR, the volume of water and area of application needs be considered. Typical BOD₅ values of domestic wastewater are within the range of 150 to 300 mg/L, however there is always the possibility of higher strength areas depending on location, population, and source. It is extremely important that the designed RSF does not exceed design criteria for OLRs because a system will soon become “clogged”, resulting in a shorter filter life. It is recommended that a system operate within the range of 0.005 to 0.039 kg BOD₅/m² (0.001 to 0.008 lb BOD₅/ft²-day).

$$\text{Organic Matter} = \frac{\text{BOD}_5 \left(\frac{\text{mg}}{\text{L}} \right) * 3.785 \left(\frac{\text{L}}{\text{gal}} \right)}{453,600 \left(\frac{\text{mg}}{\text{lb}} \right)} \quad (\text{eq. 4})$$

Organic Loading Rate

$$= \frac{\text{Organic Matter} \left(\frac{\text{lbs BOD}_5}{\text{gal}} \right) * \text{Design Flow} \left(\frac{\text{gal}}{\text{day}} \right)}{\text{Area} \left(\text{ft}^2 \right)} \quad (\text{eq. 5})$$

This study did not measure BOD₅, but instead measured the chemical oxygen demand (COD). COD is a way of indirectly measuring the amount of organic compounds within the water. It does not require to wait the 5-day incubation period to determine a measurement, and that is why it was chosen. Since there was no equation found to calculate organic matter using COD rather than BOD₅, a separate study was discovered that modelled the relationship between the two (Dubber and Gray, 2009). It discussed that a COD:BOD ratio was typically noted to be from 1.2 to 2.5, however it tended to be numerically constant when targeting specific wastewater. Therefore, a generic model was discovered for domestic wastewater:

$$COD = 1.64 * BOD_5 + 11.36 \quad (\text{eq. 6})$$

Using this model, the average COD measurement for the added wastewater into the system after spiking with the standards was used to determine the BOD₅ variable needed. Thus, the OLR was calculated to be 0.003 lb BOD₅/ft²-day, which was within reasonable design criteria.

Sampling Procedures

Collection

Domestic wastewater samples were collected from The University of Tennessee's Little River Animal and Environmental Unit. Wastewater was pumped from the unit's housing septic tank, which has only undergone primary treatment. The collection was pumped into three plastic containers and shaded from sunlight. After the wastewater had been administered into the system, samples of the supply tanks before and after standard additions were collected. Effluent samples were collected daily, with each treatment series lasting one week. At the end of the experiment,

the media within the columns was collected for biofilm analysis and separated based on location (top, middle, and bottom of column).

Storage

5 L samples were dispensed into each of the stainless steel supply tanks weekly with the corresponding standard added. The recirculated and final effluent collections were both wrapped in aluminum foil to prevent any photodegradation from occurring before the collection. The influent, effluent, and media samples were stored at 4°C (39.2°F). If analysis could not begin immediately after collection, samples were kept in the dark to prevent photodegradation.

Preparation

It was found mid-way through the research that the standards, even though refrigerated and kept in the dark, were still exhibiting some compound degradation. Therefore standards of each of the compound were made fresh every other month. High purity stock solution standards were prepared with a 500 mL Erlenmeyer flask in methanol at 150 ppm. To achieve a 0.1 ppm concentration, 0.665 mL of the standard were mixed with each liter of wastewater added to the supply tanks. Daily effluent samples were collected, passed through glass fiber filters, and separated: 300 mL was saved for solid phase extraction (SPE), while the rest remained for water quality analysis. Those samples sent for SPE were acidified to 2 to 3 pH by the addition of 50 µL of sulfuric acid to minimize microbial activity and degradation.

Effluent Analysis

Water Quality

Multiple parameters were tested for overall water quality: pH, COD, total organic carbon (TOC), and total nitrogen (TN). A Hach (Loveland, Colorado) HQ_{40d} dual probe multi-parameter meter was used to measure pH values after calibrating the probe with pH 2, pH 7, and pH 10 buffers. COD standards were created in lab; potassium dichromate, K₂Cr₂O₇, and sulfuric acid, H₂SO₄, standards were made when needed. TOC and TN samples were analyzed with a Shimadzu Analyzer (Colombia, MD) equipped with a TOC-V_{CPH} and TNM-1 measuring units.

Quantifying PPCP Release

Analytical methods developed by Samaras et al. (2011) were used for the simultaneous determination of the selected PPCPs within the water samples. While there were different extraction materials to choose from, C18 cartridges (6 mL, 500mg) were used because they are ideal for nonpolar to moderately polar compounds and contain a hydrophobic reverse phase material for compounds residing within a liquid matrix. The SPE process requires four critical steps: conditioning, loading, washing, and elution. The cartridges were conditioned with 3x2 mL (meaning three separate sets of 2 mL loadings within the cartridges) ethyl acetate, 3x2 mL methanol, and finally 3x2 mL of distilled water after allowing the cartridges to soak for 2 min while not under vacuum. The conditioning was performed under low vacuum settings. 4 mL of acidified distilled water (pH 2 to 3) were then added for additional conditioning. The acidified wastewater samples collected (300 mL) were pulled through the cartridge under a low vacuum. After loading, the cartridges were washed with another 2 mL of acidified distilled water and left to dry under vacuum for 1 h. Finally, the elution of the target compounds was performed by

collecting 3x2 mL of ethyl acetate by gravity into a 10 mL glass tube. The samples were then evaporated to 1 mL under a gentle stream of nitrogen, transferred into 2 mL analytical vials for GC-MS and evaporated once again, this time to dryness. Newly evaporated samples from SPE were reconstituted with 0.25 mL of bis(trimethylsilyl)trifluoroacetamide and 1% trimethyl chlorosilane (BSTFA+1%TMCS) and 0.25 mL of acetonitrile. After being vortex mixed for 1 min (about 15 pulses each), they were brought to 70°C for 75 min. After cooling, they were placed in a desiccator overnight and subjected to GC-MS analysis within a 3-day period of time.

To ensure an accurate analysis of the target compounds, a full scale mode was used. The Gas chromatographic analysis was carried out through a Shimadzu (GCMS-Q2010) with a Shimadzu SHR5xLB (30m x 25mm x 0.25µm film) capillary column. 1 µL samples were performed at splitless mode at 280°C with helium being the carrier gas at a constant flow mode of 0.9 mL/min. Other conditions include: the electron impact (EI) spectra mode will be at 70 eV, the transfer line will be at 280°C, and an ion source temperature of 180°C. It is expected that the m/z ion ratio will be 50 to 400. The temperature program for the GC-MS was set to 80°C for 1 min, 80°C to 248°C at 15°C/min, 248°C for 1 min, and finally from 248°C to 280°C at 3°C/min also held for 1 min.

Biofilm Analysis

Quantifying PPCP Sorption

Modified analytical methods developed by Samaras et al. (2011) were used for the simultaneous determination of the selected PPCPs from the media biofilm within each of the columns. The media was separated by location (top, middle, and bottom layer). A portion of the collected samples were analyzed for the estimated sorption of the compound into the biofilm on the media. For each location, three replicates were taken using centrifuge tubes. Each tube was

filled with media to 25 mL with the addition of 2.5 mL methanol and distilled water, and placed on an orbital mixer for 24 hrs. Once thoroughly mixed, the media was removed and the remaining mixture was centrifuged at 6000 rpm for 10 min. The supernatant was collected, combined into one sample, and dried under nitrogen gas until 1 mL had been reached. The 1 mL solution was added to 200 mL of acidified distilled water (pH 2 to 3). After this, normal SPE procedures were performed on the newly reconstituted samples.

DNA Extraction of Biofilm

Methods for the extraction of biofilm for DNA analysis were reproduced from Krkosek et al. (2014). The biofilm on the media was extracted using a MoBio (Carlsbad, California) PowerSoil DNA Extraction Kit. This analysis was done to quantify *amoA* and *nirS* genes using qPCR, which code for the specific enzymes responsible for nitrification and denitrification, respectively. This analysis was able to provide an estimation of the population sizes of potential AOB and denitrifying bacteria within the biofilm. Multiple centrifuge tubes were filled to the 25 mL volumetric mark with media and rinsed with 10 mL of sterile 10 mM phosphate buffer (PBS, pH 7.4) to dislodge organic matter. The buffer was decanted, and then 25 mL of fresh buffer was re-added. The samples were placed on a lateral displacement platform shaker at maximum speed for 15 min. 15 mL of the supernatant was transferred to a 50 mL conical tube and centrifuged at 5000 rpm for 30 min. The supernatant was removed, and then the pellet was re-suspended in 1.5 mL of the phosphate buffer, and then transferred to a 2 mL centrifuge tube. The sample was centrifuged again at 10000xg for 10 min. The supernatant was removed and the pellet was kept frozen at -20°C prior to extraction.

qPCR Analysis

Methods developed by Harms et al. (2003) were used for the quantification of the *amoA* genes, and Throbäck et al. (2004) for the *nirS* genes. Quantitative PCR assays were developed for the quantification of bacterial 16S rRNA, *N. oligotropha*-like *amoA*, and *nirS* gene. SYBR Green qPCR assays were used for amplification of the *amoA* and *nirS* genes instead of the more commonly used TaqMan assays. The primers amoNO550D2f and amoNO754r were used to target the *amoA* genes of AOB found in the biofilm on the media samples, while the *nirS* primers and standards were made in lab based on the reference material. 5 µL of the samples collected from the extracted biofilm were combined with the SYBR Green Supermix (Hercules, California) and primers and then analyzed. PCR amplifications consisted of 3 min at 50°C, 10 min at 95°C, 55 cycles at 95°C for 30 s, and 56°C for 60 s. All real-time PCR assays were performed using three replicates per sample. Gene copies were calculated by the comparison of the threshold cycle obtained in the PCR runs from known standard DNA concentrations. Standard curves for bacterial 16S rRNA provided a PCR efficiency of 101% and $r^2=0.99$, *N. oligotropha amoA* a PCR efficiency of 88.7% and $r^2=0.99$, and *nirS* a PCR efficiency of 91% and $r^2=0.97$. The linear range of detection for the bacterial 16S rRNA was 4 orders of magnitude, from 4.5×10^4 to 4.5×10^8 copies per PCR, and a detection limit of 4.5×10^3 target DNA copies. For the chosen *N. oligotropha amoA* PCR assay, the linear range of detection was at least 6 orders of magnitude, from 30 to 3.0×10^7 .

Facilities and Equipment

All equipment and facilities used were located at The University of Tennessee in Knoxville, Tennessee. All experimental procedures were performed primarily at the Biosystems Engineering & Soil Science building located on the Agricultural Campus.

CHAPTER IV: Results

Preliminary Testing of Experimental Apparatus

Prior to initiating the study, some preliminary tests were performed to validate the experimental apparatus. Each of the columns were inoculated with untreated domestic wastewater for two weeks. Bacterial inoculation was extremely important within the RMFs to ensure that an appropriate population of microorganisms were present. During this period, the effluent was continually monitored for basic water quality parameters: pH, COD, and TOC concentrations. Once appropriate concentrations were reached, 1 mL of methanol was added to each of the supply tanks to represent the addition of the PPCP standards. This was monitored for another two weeks to determine if the sharp increase in organic content interfered with the system. Table 10 represents the measurement of each of the parameters of the wastewater influent before the addition of methanol.

Figure 17 depicts the pH of the first two weeks of this monitoring. It was apparent that a neutralization of pH occurred early on and maintained throughout the weeks, which is appropriate of a RMF system. Week 2 shows much less variability in the data when compared to Week 1, suggesting that the columns became more stabilized over time.

Table 10. WQ results of untreated domestic wastewater before the addition of methanol standard.

Week Sampled	WQ Parameters		
	pH	COD (mg/L)	TOC (mg/L)
1	7.18	87.80	41.63
1	7.29	98.57	38.25
1	7.36	110.47	39.19
2	7.28	122.30	48.53
2	7.33	111.83	46.80
2	7.27	105.64	45.08
Mean WW Conc.	7.29±0.1	106.10±12	43.24±4.1

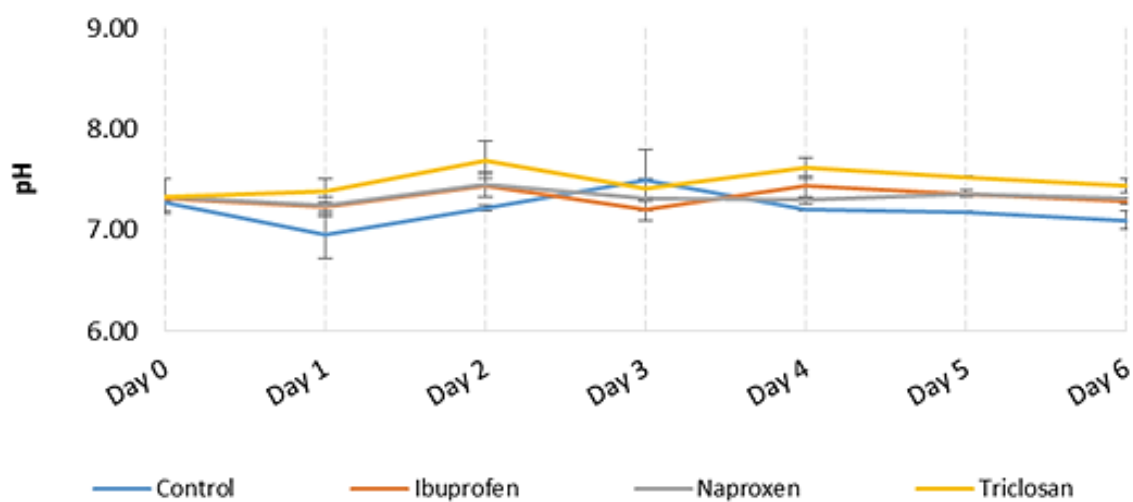


Figure 17. pH during preliminary weeks of monitoring; n = 2 weeks. Day 0 represents the sampling of the supply tanks after the methanol addition. Day 1, 2, 3, respectively represent sampling from the effluent collected after column treatment. Two-way ANOVA analysis showed no significant differences between columns and weeks ($P=0.433$, $\alpha=0.05$).

While no changes were expected from the pH after the addition of methanol, this was not the case for both COD and TOC concentrations. With this addition, the oxygen demand should rise in turn as well as the amount of organic compounds present. Figure 18 shows that after the addition of methanol, the COD measurement nearly doubled in comparison to the initial wastewater shown in table 10. A large reduction in the oxygen demand can be observed after one day of treatment. After the first week, total COD reductions for each column were calculated (85.2% control column, 85.7% IBU column, 90.4% NAP column, and 84.3% TRI column). Likewise, reductions were calculated for the second week (86.1% control column, 92.7% IBU column, 84.8% NAP column, and 90.2% TRI column). As what was noted previously, the reduction rates increased with the second week (2% to 7% increase), suggesting that the system promoted more treatment after longer operation.

Similar results were expected with TOC concentrations as with COD. Figure 19 shows an appropriately large increase in organic content from the original concentrations of the wastewater after the methanol standard. While a sharp reduction was not noticed immediately, a large decrease in organic matter was observed throughout the entirety of the week. After the first week, the total TOC reductions for each column were calculated (85.3% control column, 73.4% IBU column, 73.2% NAP column, and 73.9% TRI column). Reductions were calculated for the second week as well (72.7% control column, 78.8% IBU column, 74.1% NAP column, and 73.9% TRI column). Differences in the changes of reduction rates over the two weeks were observed when compared to COD values. TOC values remained constant around 75% reduction, showing little if any change. The lower reduction rates in TOC represent that although the oxygen demand of the wastewater decreased exceptionally (>90% reduction), organic compounds still remained within the system and were not able to be completely removed (>75% reduction).

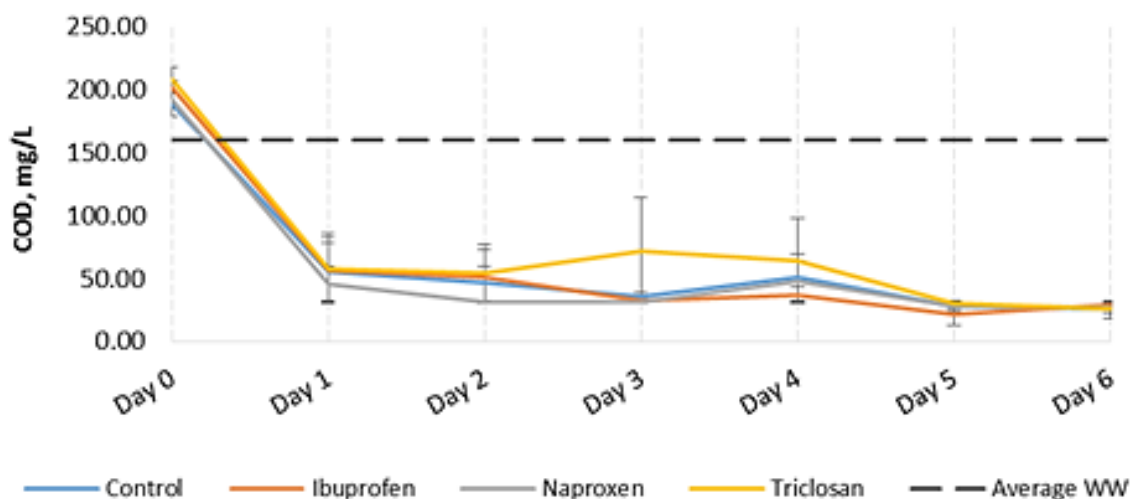


Figure 18. COD during preliminary weeks of monitoring; n = 2 weeks. Day 0 represents the sampling of the supply tanks after the methanol addition. Day 1, 2, 3, respectively represent sampling from the effluent collected after column treatment. Two-way ANOVA analysis showed no significant differences between columns and weeks ($P=0.988$, $\alpha=0.05$).

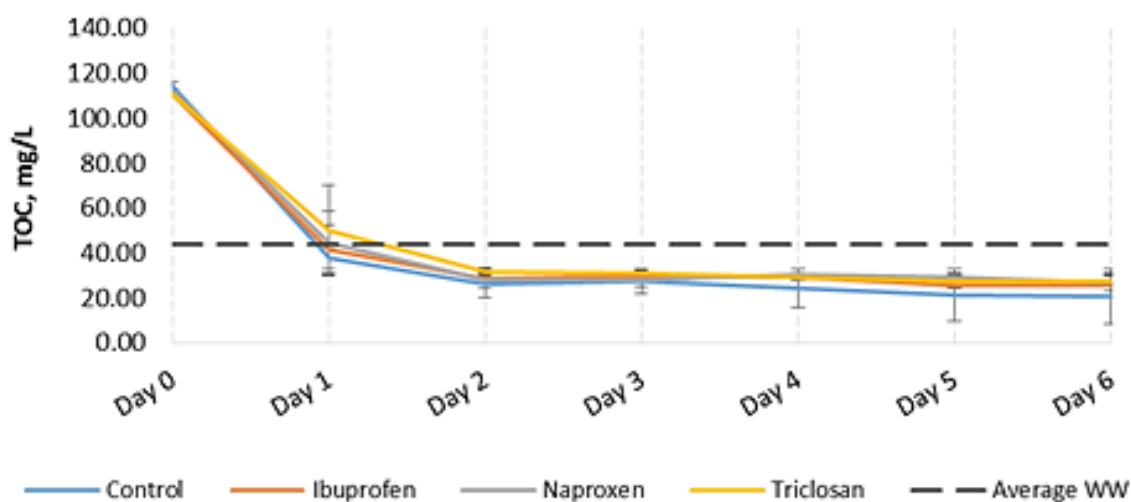


Figure 19. TOC during preliminary weeks of monitoring; n = 2 weeks. Day 0 represents the sampling of the supply tanks after the methanol addition. Day 1, 2, 3, respectively represent sampling from the effluent collected after column treatment. Two-way ANOVA analysis showed no significant differences between columns and weeks ($P=0.861$, $\alpha=0.05$).

In addition to monitoring the influent and effluent concentrations of the system, it was also important to ensure that changes in water quality were not occurring before entering the system. pH, COD, and TOC values were measured in both the system's stainless steel supply tanks and supplemental water supply outside the project that had not been added yet. These measurements were also monitored over a two-week period. The unused wastewater supply that had not been added yet sat beside the system in plastic containers that were partially exposed to light, as shown in the figure below. Because of these factors, it was extremely important to monitor the water on a daily basis.



Figure 20. External wastewater supply containers.

The results in figures 21-23 show no significant changes in pH, COD, and TOC were observed within the supply tanks between both the columns and days. Samples were collected for five days after methanol addition. It was important to ensure that with the increase in organic content that the natural microorganisms within the wastewater did not begin degradation until the influent had a chance to be treated through the system. Likewise, figures 24-26 showed no significant changes of the target parameters occurred between columns over a week's time period within the external supply containers. The test was repeated a second time, with similar results. The final conclusion for this experiment was that no concern would be needed from any biological changes to the wastewater before its addition into the system.

PPCP Compound Extraction and Detection

Alongside proper inoculation of the system, it was also important to be able to appropriately determine each of the target compounds through GC-MS analysis at varying concentrations. Four standards of each of the analytes were created at concentrations of 0.1, 0.5, 1.0, and 2.0 ppm. They were dissolved in a methanol solution. Three procedures were required: solid phase extraction (SPE), derivatization, and GC-MS analysis.

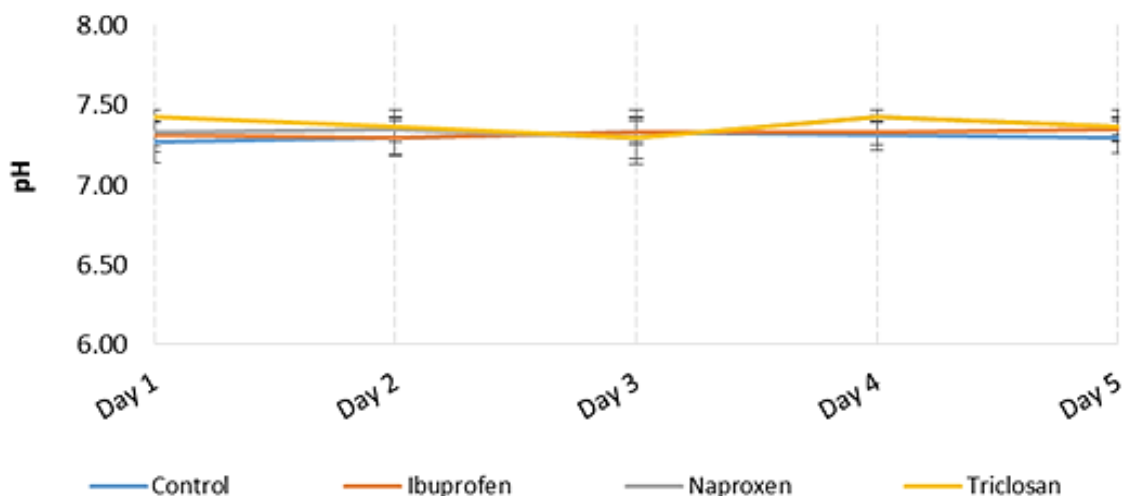


Figure 21. pH within the RMF supply over a 5 day period after methanol solution addition; n = 2 weeks. Two-way ANOVA analysis showed no significant changes in pH ($P=0.77$, $\alpha=0.05$), maintaining a mean pH of 7.32 ± 0.07 .

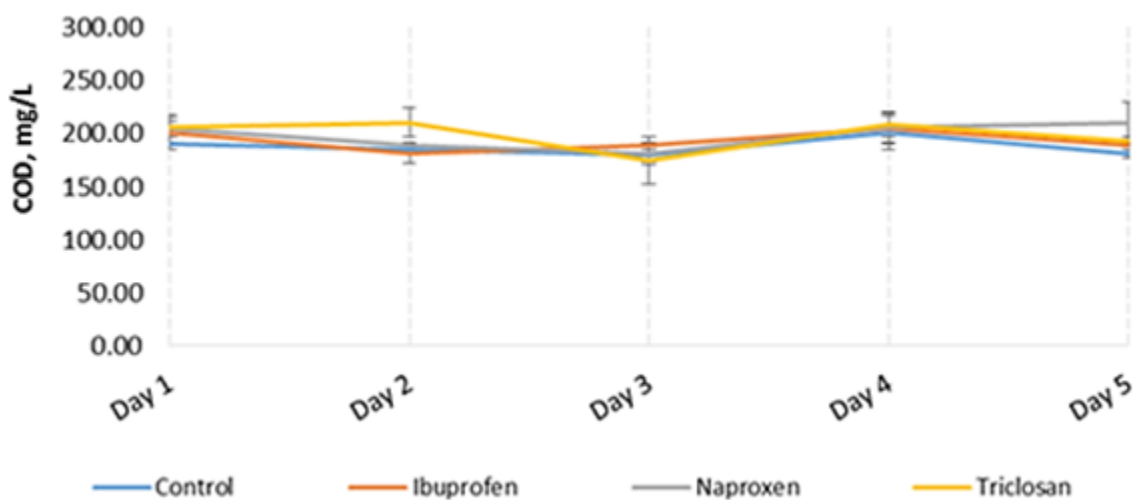


Figure 22. COD within the RMF supply over a 5 day period after methanol solution addition; n = 2 weeks. Two-way ANOVA analysis showed no significant changes in COD ($P=0.45$, $\alpha=0.05$), maintaining a mean COD of 191 ± 18.1 .

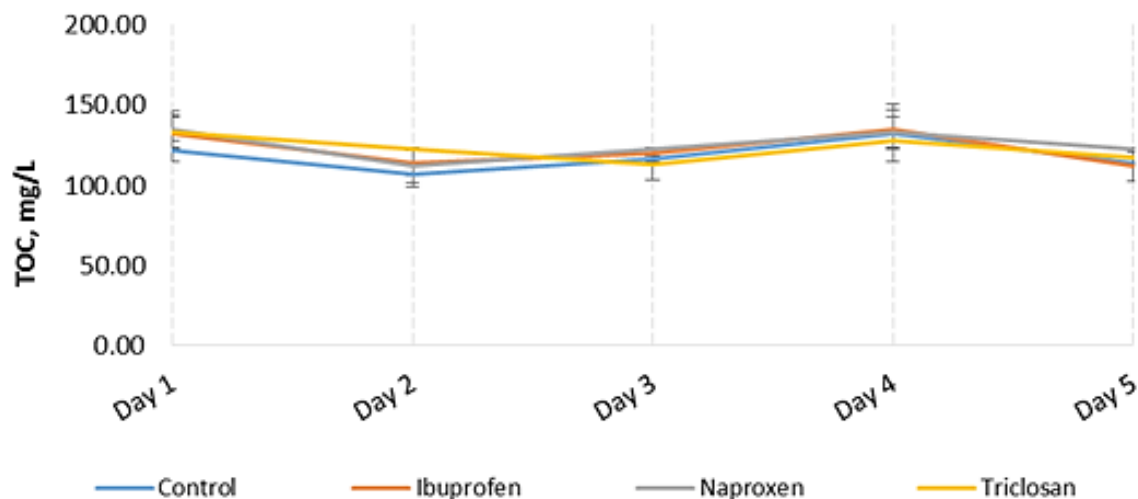


Figure 23. TOC within the RMF supply over a 5 day period after methanol solution addition; n = 2 weeks. Two-way ANOVA analysis showed no significant changes in TOC ($P=0.85$, $\alpha=0.05$), maintaining a mean TOC of 120 ± 11.7 .

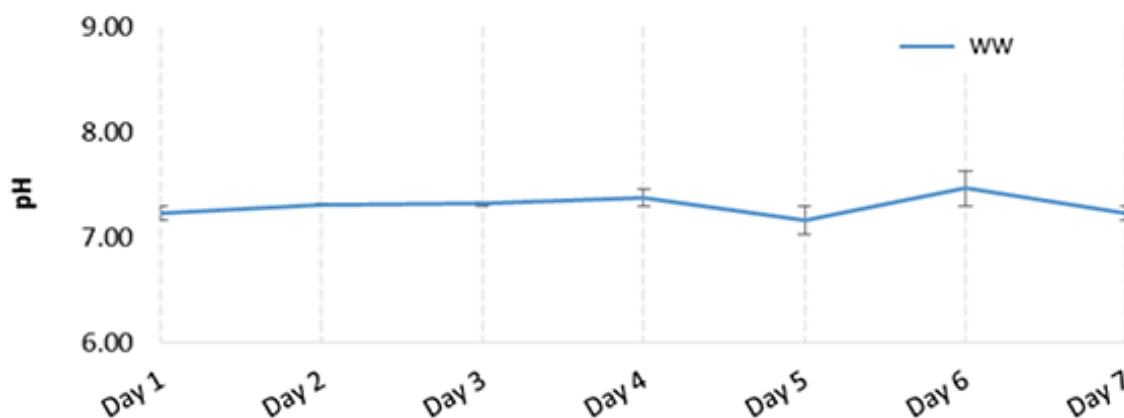


Figure 24. pH within the external wastewater supply over a 7 day period; n = 2 weeks. Two-way ANOVA analysis showed no significant changes in pH ($P=0.78$, $\alpha=0.05$), maintaining a mean pH 7.21 ± 0.11 .

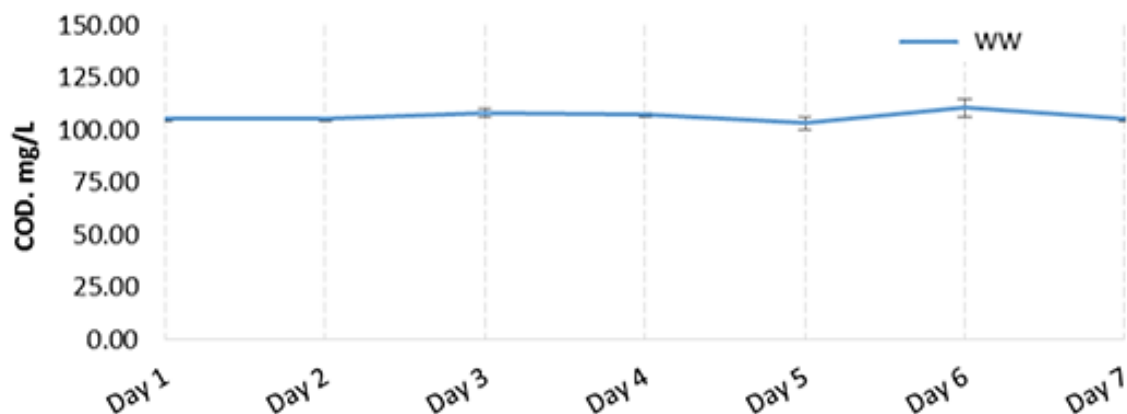


Figure 25. COD within the external wastewater supply over a 7 day period; n = 2 weeks. Two-way ANOVA analysis showed no significant changes in COD ($P=0.99$, $\alpha=0.05$), maintaining a mean COD of 106 ± 9.31 .

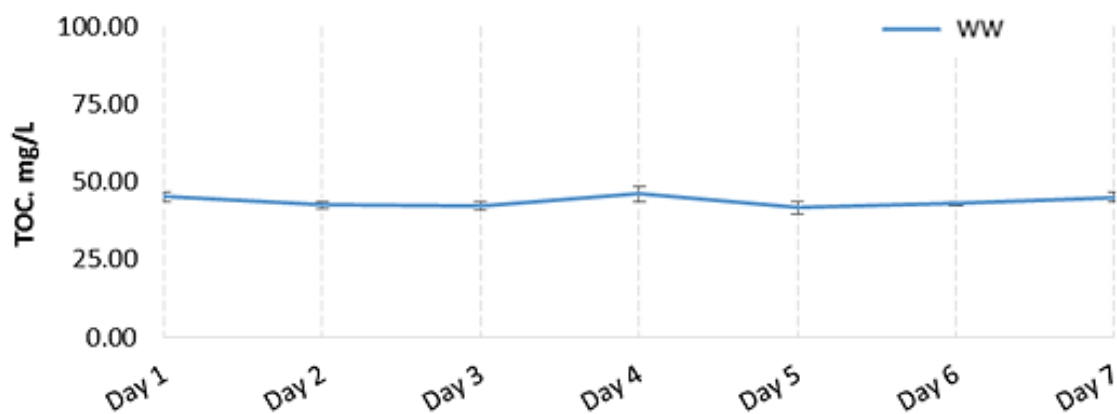


Figure 26. TOC within the external wastewater supply over a 7 day period; n = 2 weeks. Two-way ANOVA analysis showed no significant changes in TOC ($P=0.54$, $\alpha=0.05$), maintaining a mean TOC of 43.6 ± 1.77 .

Qualitative Methods of SPE and GC-MS Analysis

Standard samples were prepared differently during SPE and derivatization to help determine what outcome provided the best retention time and compound identification of all three compounds during GC-MS. These differences included experimenting with different cartridge base packing, elution material, and derivation solutions. To ensure that the SPE procedures were performed appropriately, different options were considered through literature review and lab testing. Focusing on only acidic and phenolic compounds for this project, reverse phase type cartridges were selected, and C-8 and C-18 base packages were studied. C-18 cartridges were found to not only provide a higher extraction efficiency, but were able to appropriately extract all target compounds. The SPE procedure mentioned in the previous chapter is what was decided upon as best for detecting all three target compounds. This procedure was heavily influenced from Samaras et al. (2010).

When performing SPE, it is impossible to extract 100% of the target compounds from the water sample. Therefore, it is necessary to determine the overall extraction efficiency of the procedure. To do this, two sample sets were created: one that underwent SPE and one that would not. The samples that were to not go through SPE were instead directly sent through derivatization and then GC-MS, so that no compounds would be lost. For the other sample set, distilled water samples were spiked to varying concentrations and SPE performed. The extraction efficiency of each of the samples, when compared to those that were not extracted, are shown below in table 11. One-way ANOVA analysis showed a significant difference between expected concentrations and extracted concentrations ($P > 0.01$, $\alpha = 0.5$). However, these efficiencies are comparable to the ones obtained from the reference material, shown previously in table 4, and therefore were acceptable.

Table 11. Mean extraction efficiency of SPE procedures at varying concentrations of standard additions.

Expected Concentration	Extraction Efficiency, %		
	Ibuprofen	Naproxen	Triclosan
0.1 ppm	85.1±0.02	74.9±0.01	70.7±0.01
0.5 ppm	78.8±0.01	75.6±0.01	76.6±0.02
1.0 ppm	77.7±0.04	73.5±0.03	72.6±0.01
2.0 ppm	83.8±0.12	80.4±0.08	75.1±0.07
Mean Efficiency	81.3±0.05	76.1±0.03	73.7±0.03

Different combinations of silyating reagents at different volumes were experimented with, and it was decided that 0.25 mL of BSTFA+1%TMCS and acetonitrile supplied the best results. After derivatization samples were heated for 1 h and placed in a desiccator overnight, they were analyzed through GC-MS. This analytical procedure was copied from Samaras et al. (2010) and not changed. Calibration of the system was important to determine if different concentrations of the analytes could be linearly graphed, as shown in figure 27. Initially, the varying concentrations were set to 0.4, 0.8, 2.0 and 4.0 ppm, and later checked again with lower concentrations down to 0.1 ppm. All coefficient of determination values were >0.99 .

With the calibration of each compound, the next step was to determine appropriate retention times. As each compound was pulled through the GC oven, they were done so at different speeds because of their differences in molecular make-up. Therefore, all compounds have their own unique retention time. The GC-MS instrument was able to take each compound's retention time, calculate the area under the curve of the intensity of the compound, measure, and read recorded as a corresponding concentration. Figure 28 appropriately shows the intensity of each of the compounds detected at concentrations of 0.5 and 1.0 ppm at its own retention time. Therefore, knowing the retention times, as shown in table 12, would allow the researcher to identify the compound in an unknown sample.

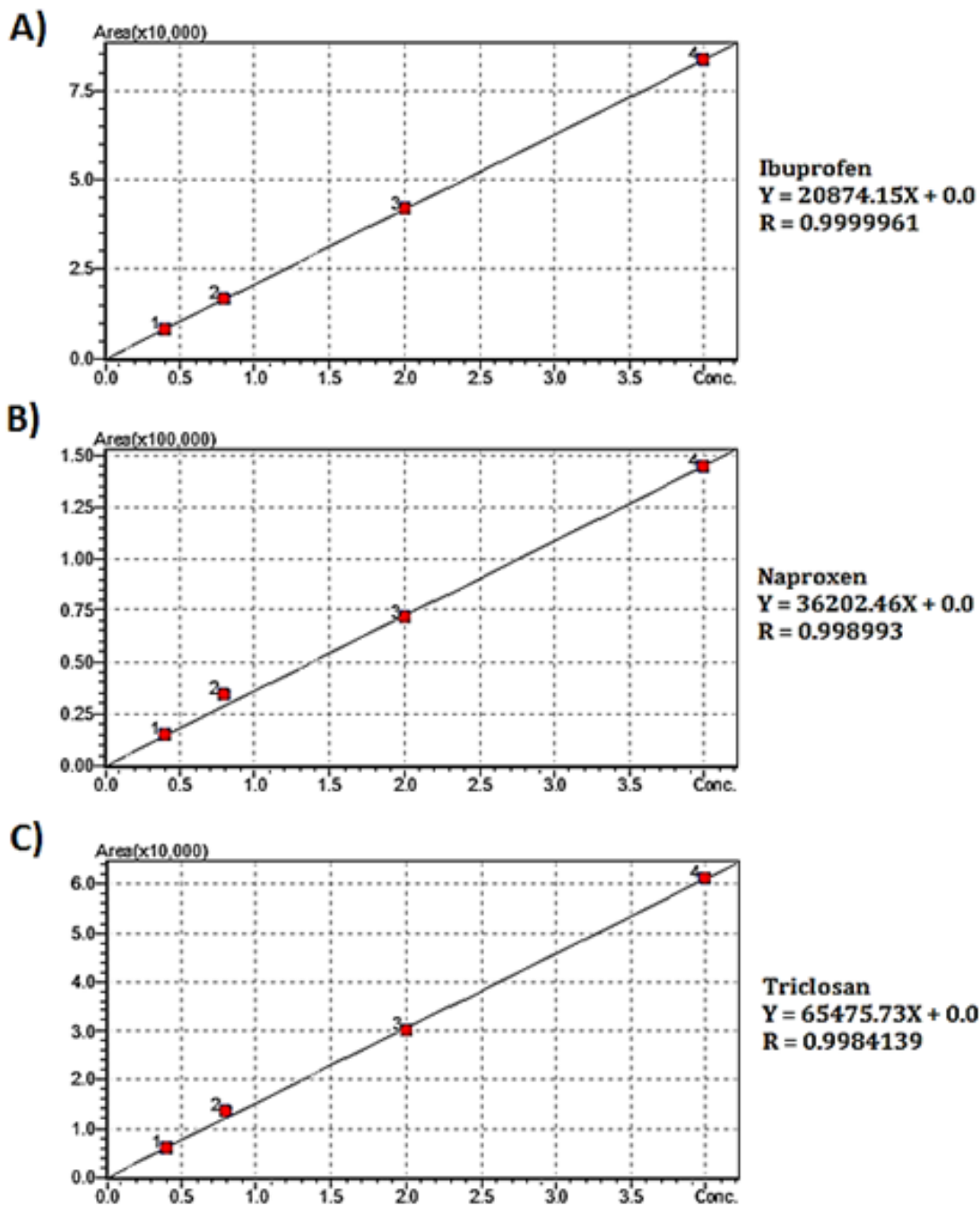


Figure 27. GC-MS analysis calibration curves of target compounds IBU (a), NAP (b), and TRI (c); points 1, 2, 3, and 4 are concentrations at 0.4, 0.8, 2.0, and 4.0 respectively.

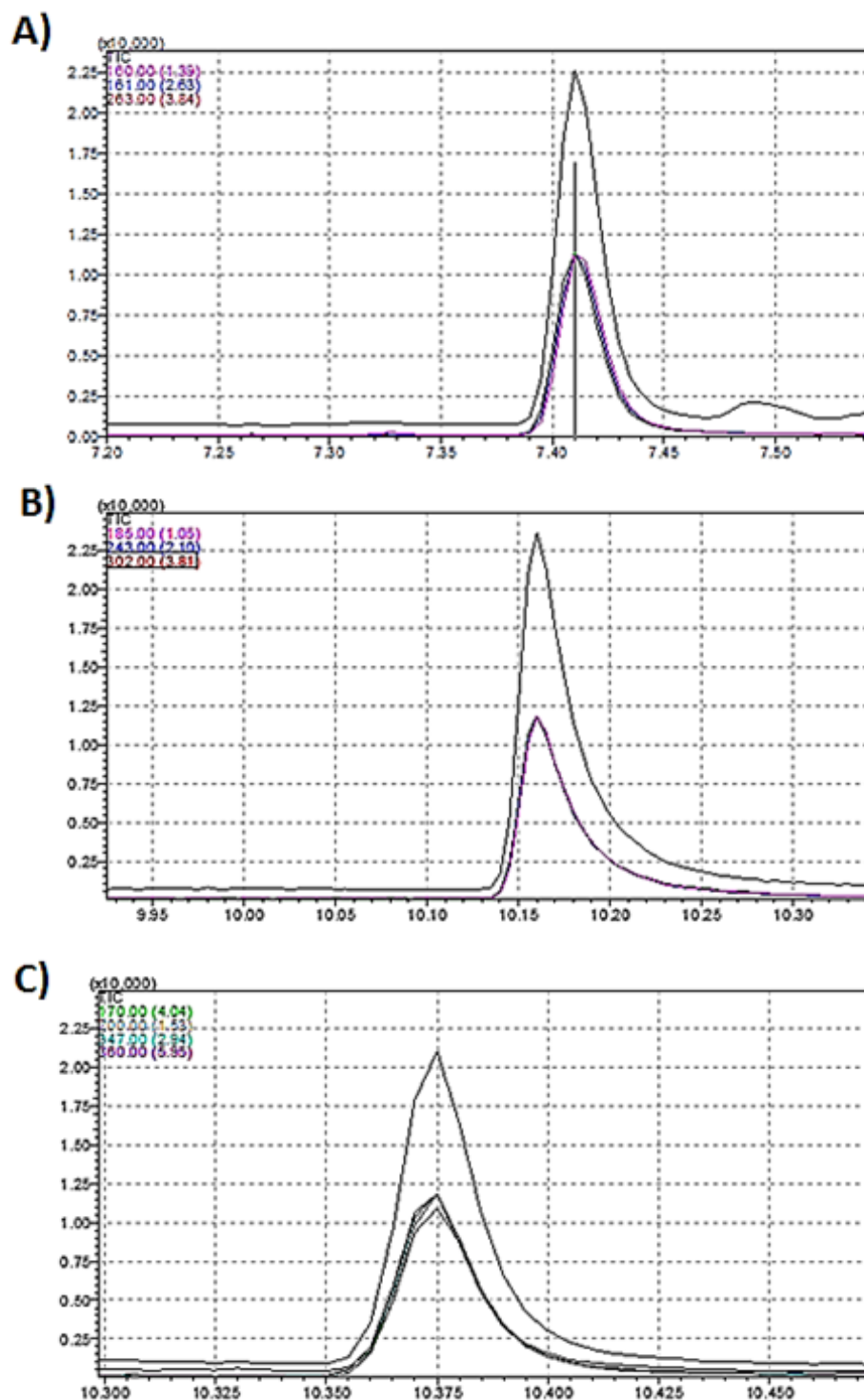


Figure 28. Corresponding retention times of the target PPCPs, IBU (a), NAP (b), and TRI (c), at 1.0 ppm and 2.0 ppm.

Table 12. Corresponding retention times of the target compounds.

Target Compound	Retention time (min)
Ibuprofen	7.4
Naproxen	10.2
Triclosan	10.4

Traditional Water Quality Analysis

RMFs are intended for the treatment of domestic wastewater. These systems are not monitored around the clock as typical of large-scale treatment plants, so it is important that these systems are designed to stand-alone and handle reasonable concentrations of wastewater strength. Before the effluent from these systems is released back into the environment, it is important that RMFs are tested early on for basic water quality treatment. Although this was a lab-scale study and treating a small amount of water, these parameters were still equally as important to monitor.

pH Stabilization

Over the entire course of the study, the pH stayed relatively neutral within 6.3 to 7.9, as can be shown in figures 29 and 30. Two-way ANOVA analysis showed no significant changes of pH means between columns and days ($P = 0.99$, $\alpha = 0.05$), as well as the effluent between weeks ($P=0.97$, $\alpha=0.05$). The overall mean pH of the effluent was 7.03 ± 0.21 . These are adequate for nitrification. If pH were to drop or increase significantly, nitrification could be inhibited. Results have shown that there is little to no variability between columns when it comes to monitoring pH, and likewise when comparing initial values to final effluent values.

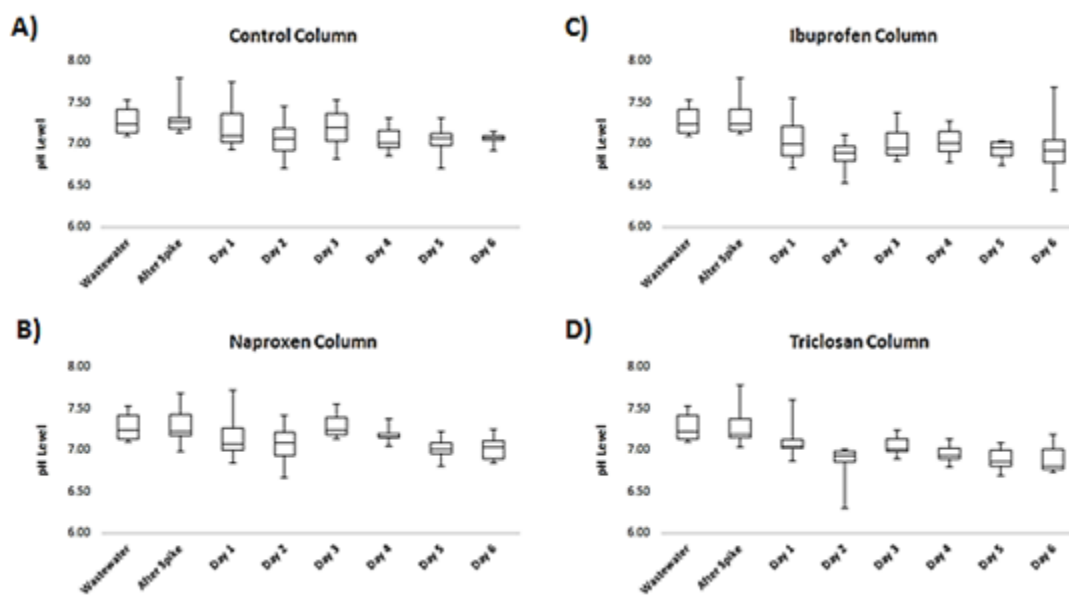


Figure 29. pH in each column throughout study; Control (a), IBU (b), NAP (c), and TRI (d). Minimum, maximum, median, and 1st - 3rd quartiles shown; sample size n = 8 weeks. No significant changes were observed between the columns and days (P=0.99).

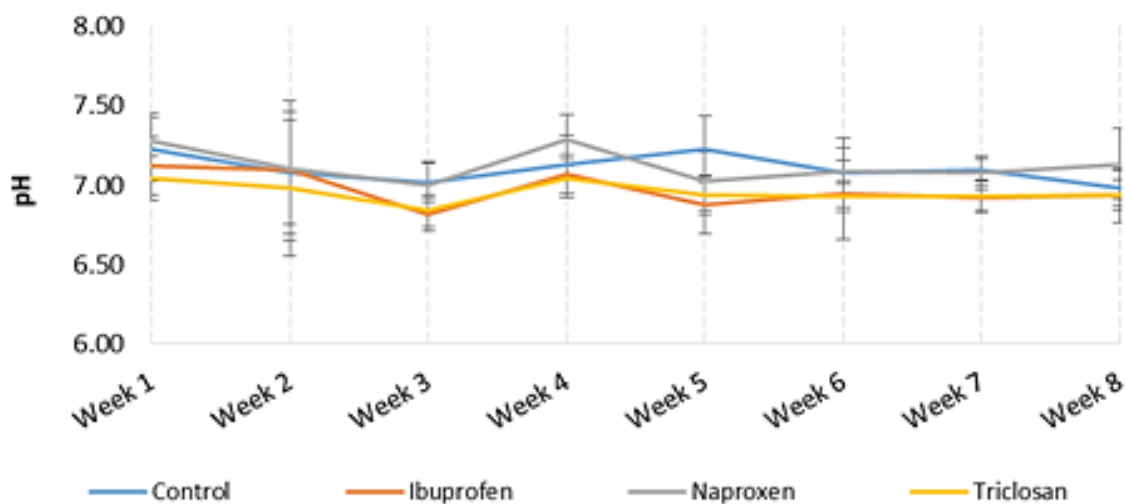


Figure 30. Mean pH of the effluent from each column for each week; n = 7 days. No significant changes were observed between columns and weeks (P=0.37, P=0.96) with a mean effluent pH of 7.03±0.21.

COD and TOC Reduction

For COD, two-way ANOVA analysis show a significant difference ($P > 0.01$, $\alpha = 0.05$) between original wastewater, after spike, and the days following, however there were no significant differences between columns ($P = 0.98$, $\alpha = 0.05$). There were also no significant differences of the mean effluent each week ($P = 0.06$, $\alpha = 0.05$). Overall, there was $>90\%$ removal of COD, with a mean effluent of 65.5 ± 36.4 mg/L. Likewise for TOC, ANOVA analysis showed a significant difference ($P > 0.01$, $\alpha = 0.05$) between wastewater, after spike, and the days following, and no significant changes between columns ($P = 0.33$, $\alpha = 0.05$). There were, however, significant differences between the mean effluent over the weeks ($P = 0.03$, $\alpha = 0.05$). Overall, there was $>95\%$ removal of TOC, with a mean effluent of 12.6 ± 8.38 mg/L.

TN Reduction

An important note is that TN was not monitored until halfway through the study, therefore the data only represents four weeks of collected data. The mean TN concentration of the original wastewater was approximately 35 ± 2.97 mg/L, which is considered average for domestic wastewater. Each column experiences a wide range of TN reduction, anywhere from 28.5% to 83.9% reduction from original concentration. Two-way ANOVA analysis showed that slightly significant differences were found between the original wastewater, after spike, and following days ($P = 0.04$, $\alpha = 0.05$), and there were also significant differences between the weeks ($P = 0.01$, $\alpha = 0.05$). There were also significant differences of the mean effluent of each columns ($P > 0.01$, $\alpha = 0.05$). The overall TN of the effluent was 15.2 ± 5.3 mg/L.

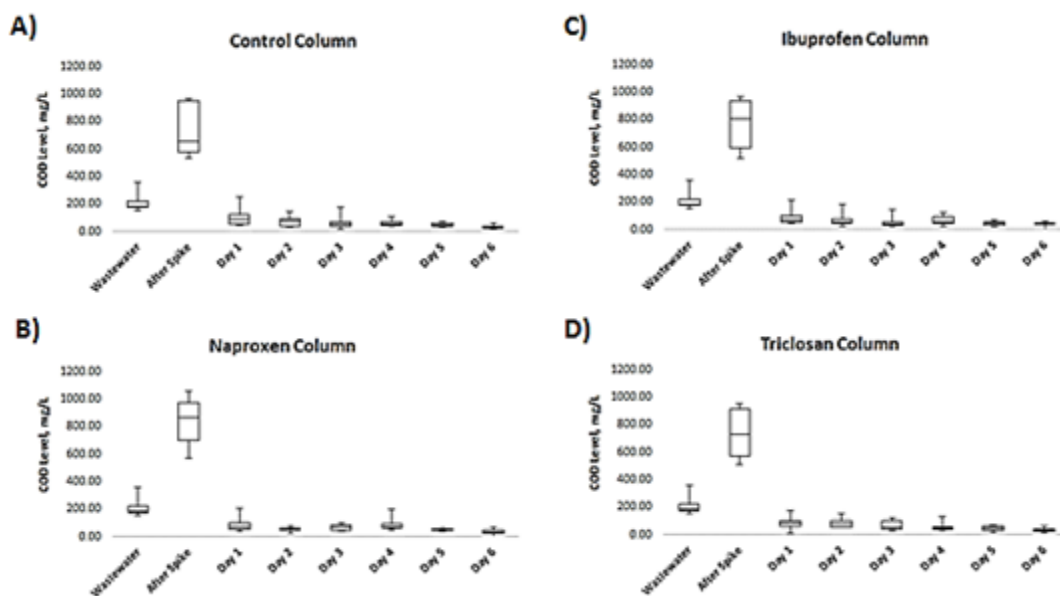


Figure 31. COD in each column throughout study; Control (a), IBU (b), NAP (c), and TRI (d). Minimum, maximum, median, and 1st-3rd quartiles shown; sample size n = 8 weeks. Significant changes were observed over the days ($P>0.01$), but none between columns ($P=0.98$).

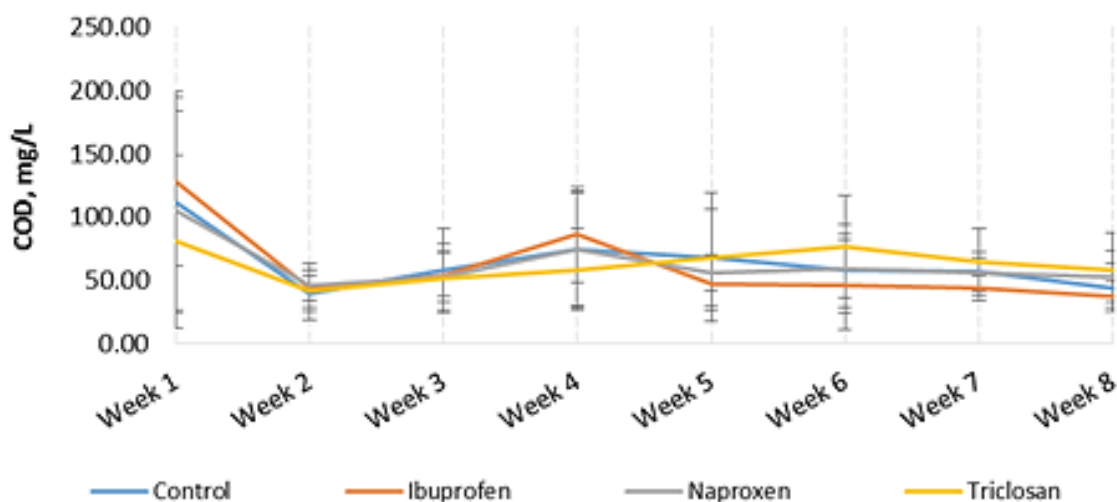


Figure 32. Mean COD of the effluent from each column for each week; n = 7 days. No significant changes were observed between columns ($P=0.98$), but were slightly significant between weeks ($P=0.06$). Mean effluent COD of 65.5 ± 36.4 mg/L.

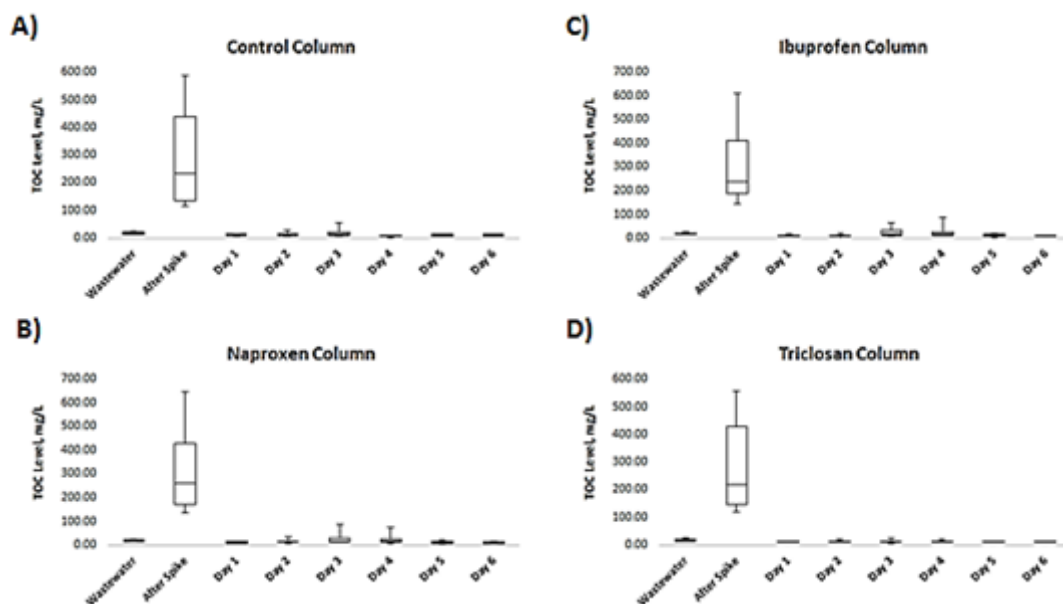


Figure 33. TOC in each column throughout study; Control (a), IBU (b), NAP (c), and TRI (d). Minimum, maximum, median, and 1st-3rd quartiles shown; sample size n = 8 weeks. Significant changes were observed over the days ($P>0.01$), but none between columns ($P=0.33$)

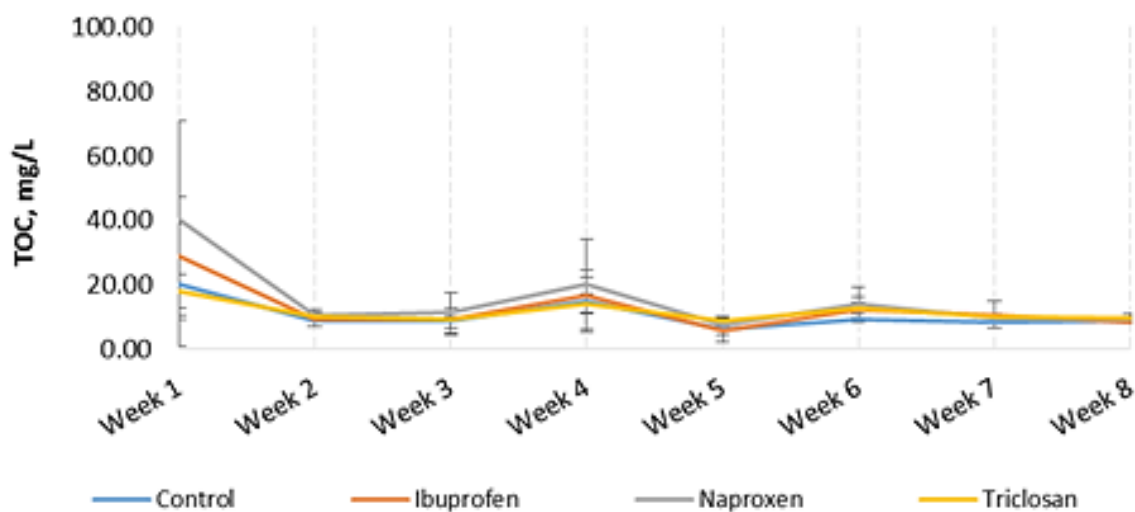


Figure 34. Mean TOC of the effluent from each column for each week; n = 7 days. No significant changes were observed between columns ($P=0.39$), and significant between weeks ($P=0.03$). Mean effluent TOC of 12.6 ± 8.38 mg/L.

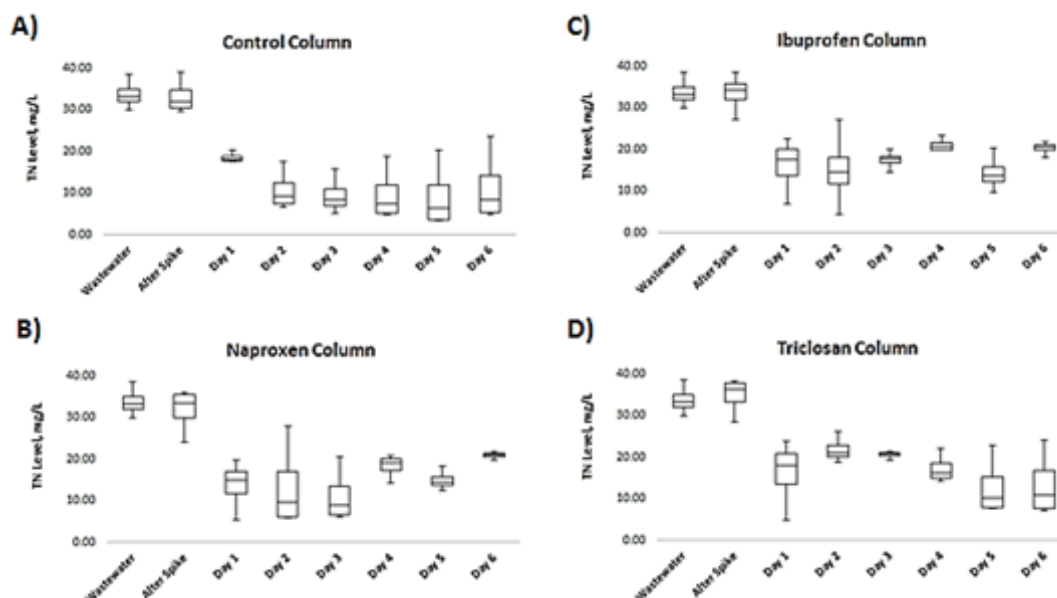


Figure 35. TN concentration in each column for the last four weeks of study. Minimum, maximum, median, and 1st, 2nd, and 3rd quartiles shown; sample size n = 4 weeks. Significant changes were observed over the days ($P>0.01$), as well as between weeks ($P=0.02$).

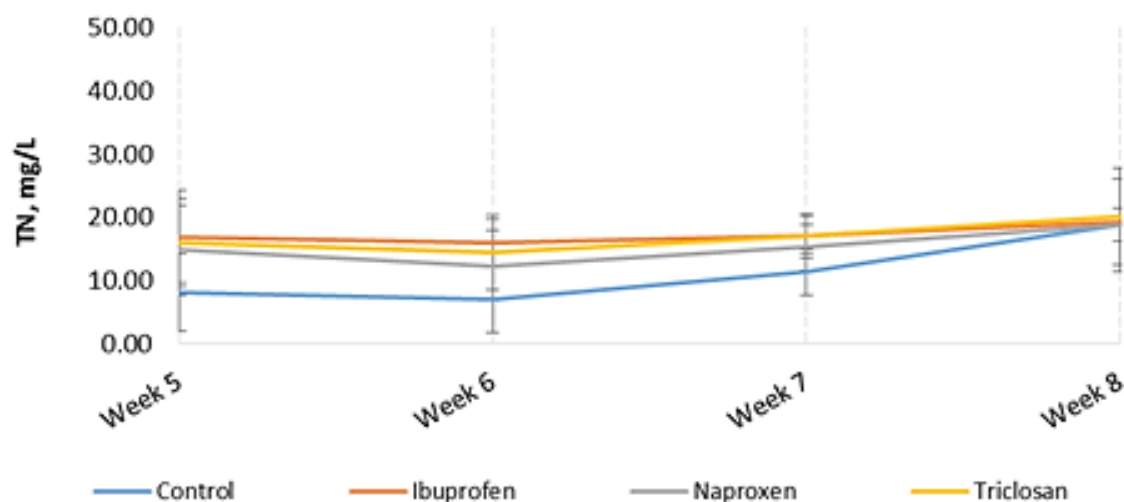


Figure 36. Mean TN of the effluent from each column for each week; n = 7 days. Significant changes were observed between columns and weeks ($P>0.01$). Mean effluent TN of 15.2 ± 5.93 mg/L.

What is interesting to note are the differences in reduction from column to column, which is unlike what was detected when monitoring pH, COD, and TOC. The control column, which received no additional compound, had an early drop in TN concentration after treatment and generally showed no large changes from that point out, settling around 5 to 10 mg/L until a shift in the final week. The TRI column showed a gradual decrease of nitrogen, finally ending around 8 to 10 mg/L, not unlike the control column. In comparison, the IBU and NAP columns showed the lowest removal rates, with an mean concentration of 20 mg/L by the end of each week.

PPCP Removal Analysis

The law of conservation states that matter cannot be created or destroyed, and therefore, anything that entered into the system ultimately was removed from the system. However, this removal occurs in different forms, such as effluent release, adsorption, and microbial degradation. A mass balance equation, eq. 7 and eq. 8, is shown below to help estimate these different removals.

$$\sum PPCPs_{in} = \sum PPCPs_{out} \quad (\text{eq. 7})$$

$$PPC S_{ww} + PPC S_{added} = \\ PPCPs_{released} + PPCPs_{sorption} + PPCPs_{degraded} \quad (\text{eq. 8})$$

When calculating removal rates, it is important to understand that there is a background concentration of PPCPs already present within the wastewater. By subtracting how much was added into the system, this initial concentration could be estimated. Total removal of PPCPs from the system was considered the “disappearance” of the organics, comparing the concentration of the effluent to the influent. This “disappearance” can be further evaluated by measuring the trace

organics that have become adsorbed into the collected sludge or biofilm within the system. Once this has been measured, the removal through degradation could be estimated, thus giving the percentage of removal attributed from either sorption or microbial degradation.

PPCP Effluent Release

PPCP removal was observed almost immediately after treatment began, as is shown in figure 37. The example graph represents data collected three weeks into treatment. The control column experienced the highest removal, with the complete disappearance of IBU and NAP, and >90% of TRI. The experimental columns experienced similar removal rates of IBU (89% to 93%), NAP (86% to 100%), and TRI (83% to 90%). This trend was common during the first half of the study. However, after 5 weeks of treatment there were noticeable changes in removal rates in all experimental columns, but not the control. An example of this change is shown in figure 38, which is showing seventh week of treatment.

During the second half of the study, although the control column showed a minute decrease in removal as compared to previous weeks, the experimental columns showed signs of PPCP concentration growth. Since it is very unlikely that trace organics were produced within the columns, it was assumed that any organics that have become adsorbed previously were now desorbing and released into the effluent. At the end of the study, reduction rates for the PPCPs within the experimental columns ranged from 21% to 55% (IBU), 1.5% to 62% (NAP), and 58% to 96% (TRI). The acidic pharmaceuticals interestingly has the largest decrease, while TRI was only slightly affected. By the end of the experiment, mean removal rates were calculated for each column (figure 39).

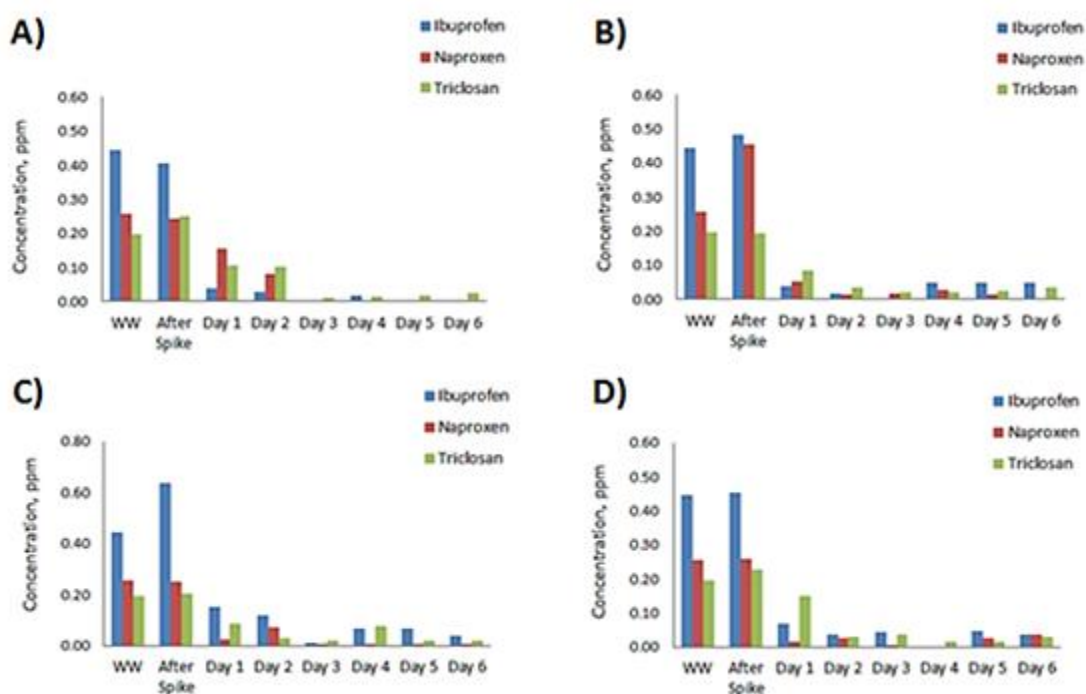


Figure 37. Third week of measured PPCP concentrations from collected effluent from each of the columns: A) control, B) ibuprofen, C) naproxen, and D) triclosan. X-axis represents original wastewater (WW), after standard addition (after spike), and days after treatment

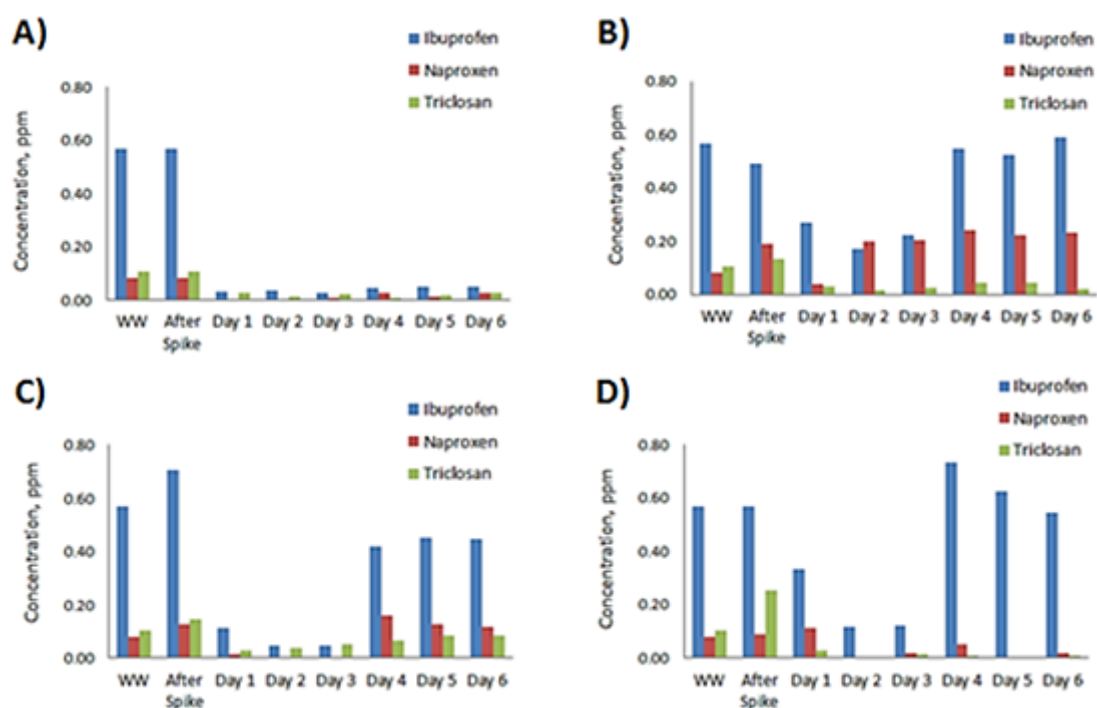


Figure 38. Seventh week of measured PPCP concentrations from collected effluent from each of the columns: A) control, B) ibuprofen, C) naproxen, and D) triclosan. X-axis represents original wastewater (WW), after standard addition (after spike), and days after treatment

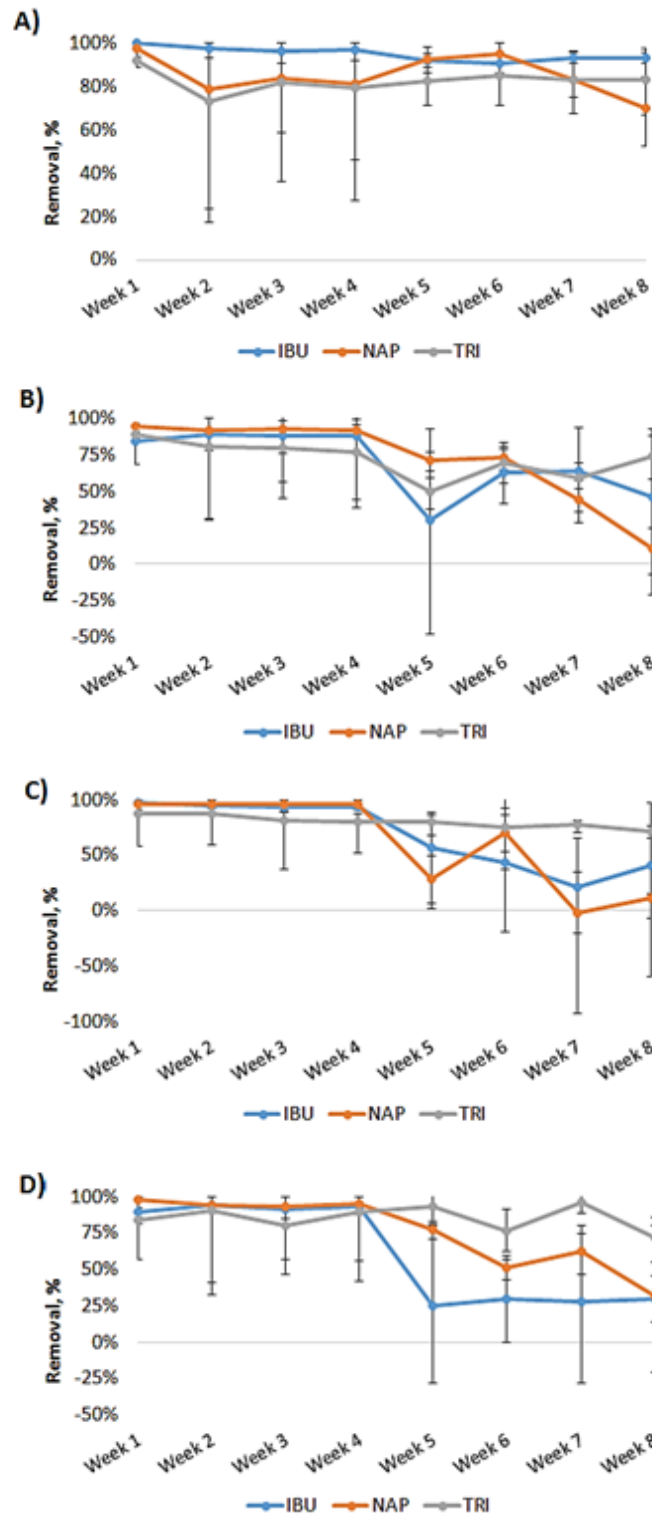


Figure 39. Total mean removal rates from the 8 weeks of treatment within each of the columns: A) control, B) ibuprofen, C) naproxen, and D) triclosan. n = 7 days.

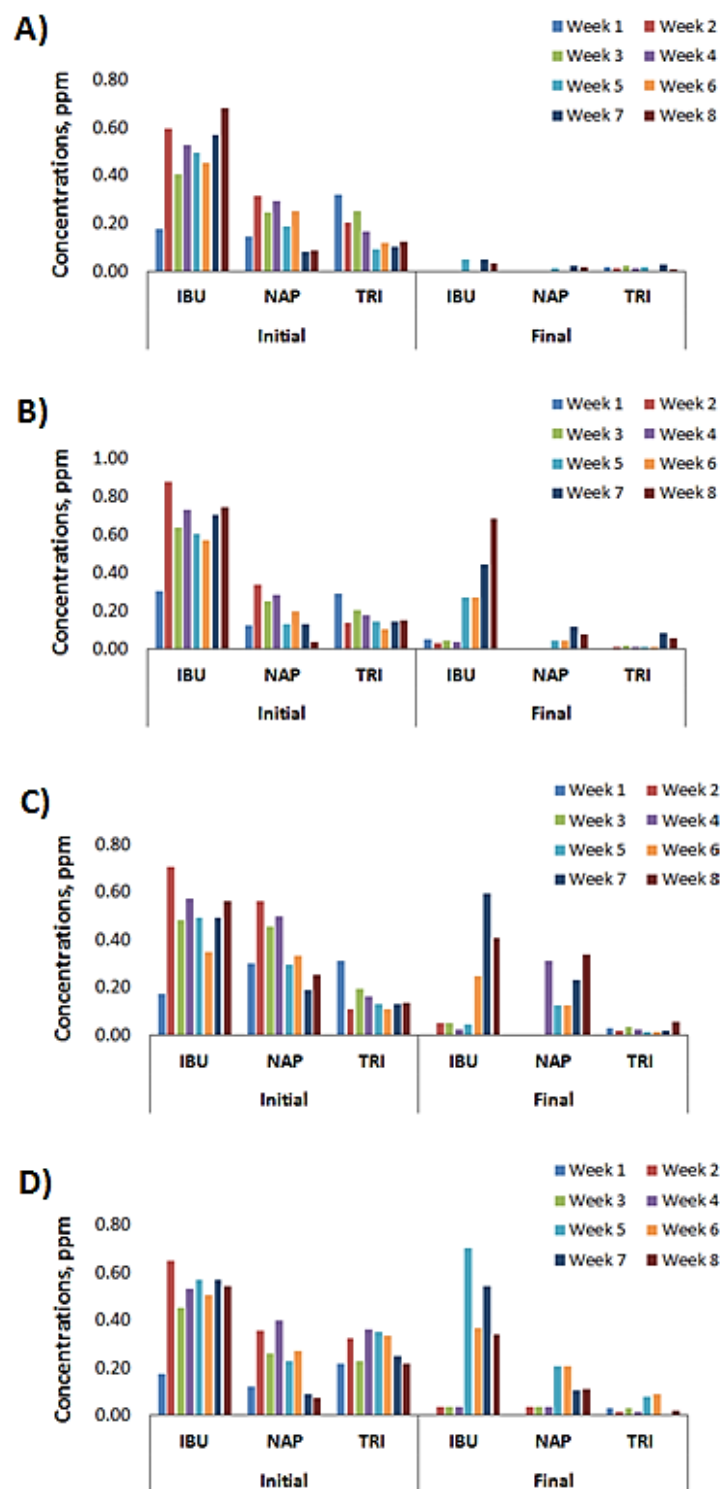


Figure 40. Comparative effluent PPCP concentrations from all columns: A) control, B) ibuprofen, C) naproxen, and D) triclosan. Initial represents concentrations measured after supply spike, and final represents concentrations measured at the end of the week.

Two-way ANOVA analysis showed that over the course of the study, the control column saw no significant differences in removal between the weeks ($P=0.14$, $\alpha=0.05$), but there were differences between the compounds ($P>0.01$, $\alpha=0.05$). In contrast, both the IBU and NAP columns saw significantly different removals over the weeks ($P>0.01$, $\alpha=0.05$), but no differences between the compounds. Likewise, the TRI columns saw significantly different removal rates over the weeks ($P=0.02$, $\alpha=0.05$), as well as significant differences in removal between the compounds as well ($P=0.04$, $\alpha=0.05$). These differences by week are shown graphically in the previous figures when the negative removal rates occurred. When separating the weeks where the change in removal appeared within the experimental columns, much different removals were observed: $91\pm2.1\%$ (Weeks 1-4) and $40\pm11\%$ (Weeks 5-8) of IBU, $95\pm3.2\%$ (Weeks 1-4) and $44\pm26\%$ (Weeks 5-8) of NAP, and $84\pm 5.8\%$ (Weeks 1-4) and $75\pm9.0\%$ (Weeks 5-8) of TRI. TRI, once again, was the trace organic that remained the most unaffected from the changes. A graphical representation of these changes are shown in figure 41.

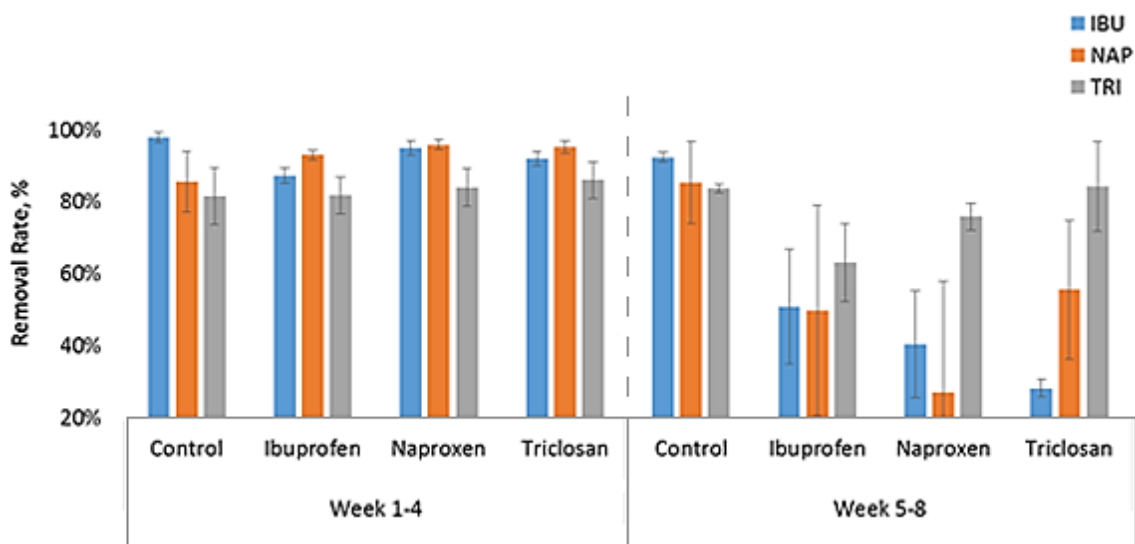


Figure 41. Mean differences of total removal for all of the columns between Weeks 1-4 and Weeks 5-8, n=4 weeks.

PPCP Adsorption to Biofilm

Media samples from each of the columns were collected from different depths (top, middle, and bottom layer) for analysis on the biofilm present. Initially, simple loss on ignitions (LOI) tests were performed to determine the amount of organics present between each of the layers. Two-way ANOVA analysis confirmed that there were statistical differences between the organics present within the layers ($P > 0.01$, $\alpha = 0.05$) and no significant differences between columns ($P = 0.68$, $\alpha = 0.05$). LOI measurements showed that the highest percentage of organics were consistently present in the top layers (3.4% to 3.8%) of the columns, with minimal shown in the middle and bottom (0.45% to 0.83%). Results like these are expected from RMFs, because biofilm typically forms within the first 6" of media. Therefore, it can be stated that most of the biological treatment within the system was occurring within the uppermost layers of the column.

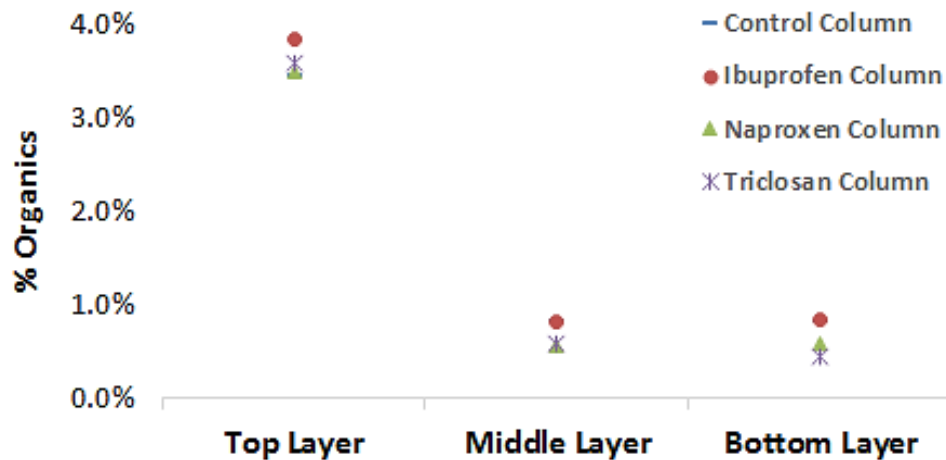


Figure 42. Percentage of organics present within each of the layers of each columns; $n = 3$. SD of columns are: 0.2% control, 0.5% ibuprofen, 0.07% naproxen, and 0.06% triclosan.

The reconstitution of the biofilm into a pure water sample was required so that SPE could be performed. This reconstitution was necessary to separate the PPCPs from the biofilm and into a liquid matrix for analysis. Two separate SPE procedures were performed on the media; triplicates was not possible because of the limited amount of media that was available for testing.

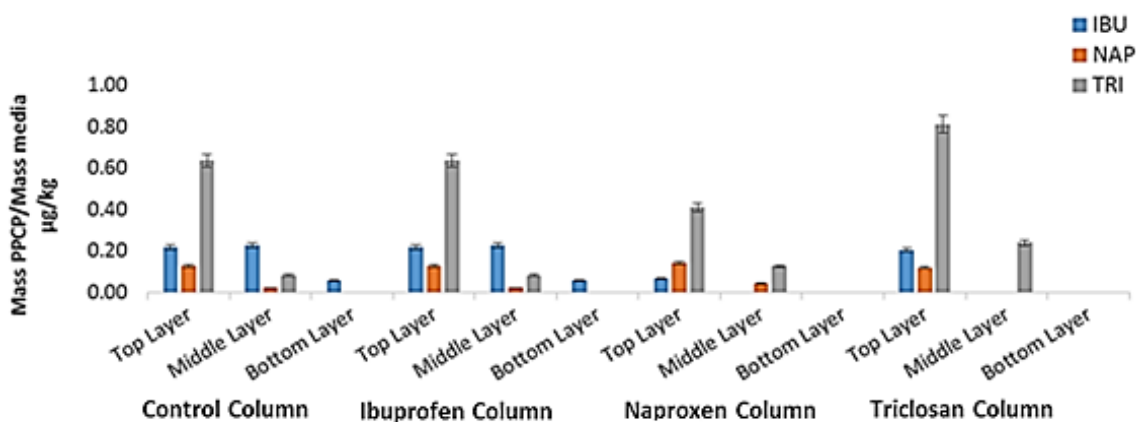


Figure 43. Mean PPCP concentrations per gram of media for each of the columns at varying locations (top, middle, bottom layers); n = 2.

Two-way ANOVA analysis showed significant differences of the amount of PPCP adsorbed in each of the layer ($P=0.04$, $\alpha=0.05$), but no significant differences between columns ($P=0.26$, $\alpha=0.05$). Most of the adsorbed trace organics were shown to be within the top layer of the columns, which correlated to the LOI data presented earlier. However, there was an adequate amount of PPCPs detected within the middle layer as well.

Totaling the concentrations of adsorbed PPCPs within each layer for each column, the control column was shown to have the highest amount ($0.52 \pm 0.01\%$, $0.73 \pm 0.01\%$, and $4.31 \pm 0.02\%$ of IBU, NAP, and TRI), with the NAP column showing minimal sorption of IBU only ($0.15 \pm 0.00\%$). TRI was shown to participate in sorption more than IBU and NAP, which corresponds to previous

research, and had the highest sorption percentages were within the control and TRI column ($4.31 \pm 0.03\%$ and $5.13 \pm 0.03\%$). It was interesting to note that TRI only did so within those two columns; there was no sorption of TRI within the NAP columns, and minimal in the IBU column. IBU had half as much sorption occurring within the IBU column when compared to the control column ($0.35 \pm 0.00\%$), with even less occurring within the NAP and TRI columns. NAP experienced low sorption in all columns except within the NAP column, where no sorption was detected.

PPCP Microbial Degradation

Using the adsorption values collected, degradation percentages were calculated using the law of conservation. Two-way ANOVA showed that there were no statistical differences in degradation rates between columns. The control column showed the highest calculated amount of microbial degradation: $96.8 \pm 6.67\%$ IBU, $92.0 \pm 4.88\%$ NAP, and $86.4 \pm 3.69\%$ TRI. The experimental columns all had similar degradation rates, with mean PPCP degradation percentages of $86 \pm 4.1\%$ IBU, $85 \pm 4.5\%$ NAP, and $84 \pm 5.6\%$ TRI. It was unexpected to observe low IBU percentages within the TRI column; IBU has been documented to be one of the more degradable compounds, so a mean value of $78.0 \pm 7.01\%$ is uncommon, as is shown in Table 13.

Table 13. Mean removals (released, adsorbed, or degraded) of PPCPs within each column; n = 8 weeks.

PPCP	Released, %			
	Control Column	Ibuprofen Column	Naproxen Column	Triclosan Column
Ibuprofen	2.71±0.06	17.7±0.06	16.5±0.06	21.8±0.08
Naproxen	7.23±0.04	9.64±0.04	18.3±0.01	9.82±0.06
Triclosan	9.31±0.03	13.4±0.03	9.86±0.03	9.51±0.04
Compound	Adsorbed, %			
	Control Column	Ibuprofen Column	Naproxen Column	Triclosan Column
Ibuprofen	0.72±0.01	0.35±0.00	0.15±0.00	0.20±0.02
Naproxen	0.73±0.01	0.17±0.00	0.00±0.00	0.52±0.02
Triclosan	4.31±0.02	0.71±0.01	0.00±0.00	5.13±0.03
Compound	Biodegraded, %			
	Control Column	Ibuprofen Column	Naproxen Column	Triclosan Column
Ibuprofen	96.8±6.67	82.0±6.13	83.4±6.31	78.0±7.01
Naproxen	92.0±4.88	90.2±4.34	81.7±4.52	89.6±5.22
Triclosan	86.4±3.69	85.9±3.14	90.2±3.32	85.4±4.01

Biofilm qPCR Analysis

Quantification of *amoA* genes

The relative abundance of the targeted genes were calculated from the ratio of detected *N. oligotropha*-like *amoA* copies to the mean number of all bacteria present within the samples, which was found to be 1.87×10^9 16S rRNA copies/L. Therefore, the relative abundance of the *amoA* copies/L ranged from 0.01% to 0.012%. The number of cells per L of *N. oligotropha*-like *amoA* from the biofilm samples were calculated from copies/L using several assumptions with gene copies per cell, as performed by Harms et al. (2003). The assumptions were that the averaged 16S rRNA gene copies per genome in bacterial cells were 3.6 copies based on the average 16S rRNA gene copies found in cultured bacteria, and that one cell of *N. oligotropha* was assumed to contain 2 copies of *amoA*. Using these calculations, and after normalizing the data to represent the bacterial cells per gram of media within each of the layers, figure 45 was created.

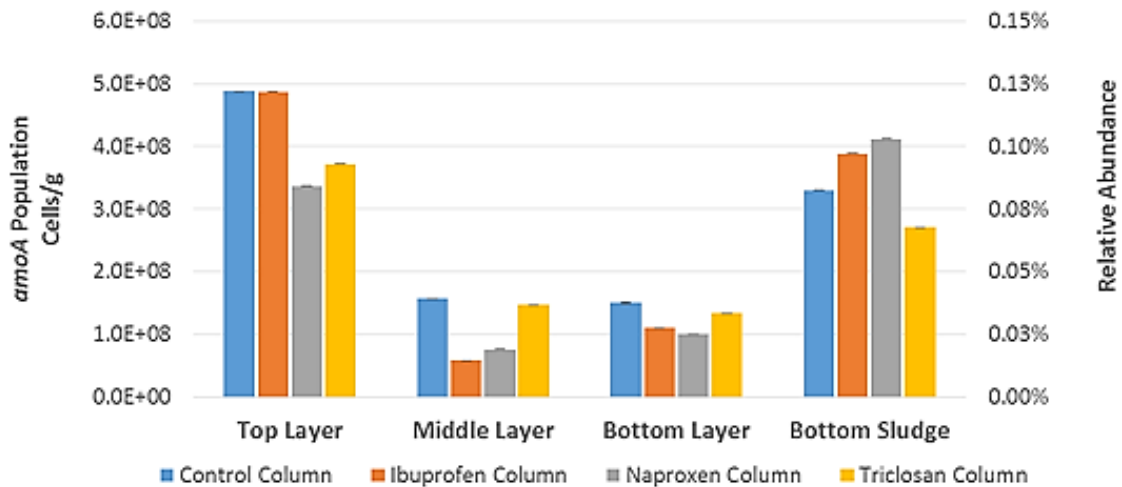


Figure 44. Mean normalized *amoA* populations (cells/g) and relative abundance (%) of each column, separated by location (top, middle, bottom layers)

The mean bacterial counts show that the highest AOB populations were present within the top layer and the sludge that was collected at the very bottom of the column. This was confirmed through two-way ANOVA analysis, showing that there were statistical differences of population and relative abundance between column layers ($P > 0.01$, $\alpha = 0.05$) and interestingly enough, that there were no statistical differences between columns ($P = 0.76$, $\alpha = 0.05$). The counts were averaged together to represent a total expected count of an RMF by layer. From the entire system, the top layer consisted of a mean $4.3 \times 10^8 \pm 7.8 \times 10^7$ cells/g of *N. oligotropha*-like *amoA*, followed by $1.1 \times 10^8 \pm 5.0 \times 10^7$, $1.2 \times 10^8 \pm 2.2 \times 10^7$, and $3.6 \times 10^8 \pm 6.3 \times 10^7$ cells/g for the middle & bottom layers, and collected sludge, respectively.

Quantification of nirS genes

In addition to the quantification of the nitrifying bacteria, denitrifying bacteria were measured to determine the potential for denitrification within the columns as well. The relative abundance was determined from the ratio of the *nirS* copies/L to the 16S rRNA copies/L, just as what was done with the *amoA* genes. The relative abundance of the *nirS* copies/L ranged from $>0.01\%$ to 0.04% . The number of cells per L of *nirS* genes were calculated from assumptions with gene copies per cell, as performed by Ward et al. (2007). The assumptions were that *nirS* genes contained one copy per cell. Using these calculations, and after normalizing the data to represent the bacterial cells per gram of media within each of the layers, figure 45 was created.

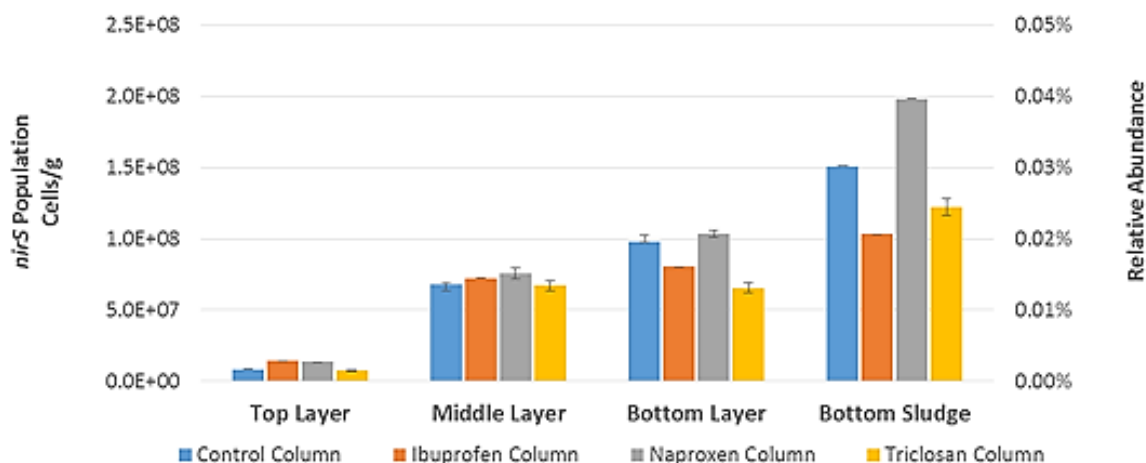


Figure 45. Mean normalized *nirS* populations (cells/g) and relative abundance (%) of each column, separated by location (top, middle, bottom layers)

Two-way ANOVA showed a significant difference of population counts between layers ($P > 0.01$, $\alpha = 0.05$) and unlike with the *amoA* genes, there were significant differences of populations between columns as well ($P > 0.01$, $\alpha = 0.01$). The mean bacterial counts showed that the highest *nirS* gene populations were present within the lower regions of the columns. The counts were then grouped together to represent a total expected count of an RMF by layer. From the entire system, the top layer consisted of a mean $1.1 \times 10^7 \pm 7.8 \times 10^7$ cells/g of *nirS* genes, followed by $7.1 \times 10^7 \pm 5.0 \times 10^7$, $8.7 \times 10^7 \pm 2.2 \times 10^7$, and $1.4 \times 10^8 \pm 6.3 \times 10^7$ cells/g for the middle & bottom layers, and collected sludge, respectively.

Figure 46 shows how the populations of the targeted *amoA* and *nirS* genes change throughout the layers of the averaged columns. The data shows that there is a larger amount of ammonia oxidizing bacteria than denitrifying bacteria. Excluding the top layer, which was predominantly *amoA* genes, there was on average twice as many AOBs within the middle, bottom, and sludge samples than denitrifying bacteria.

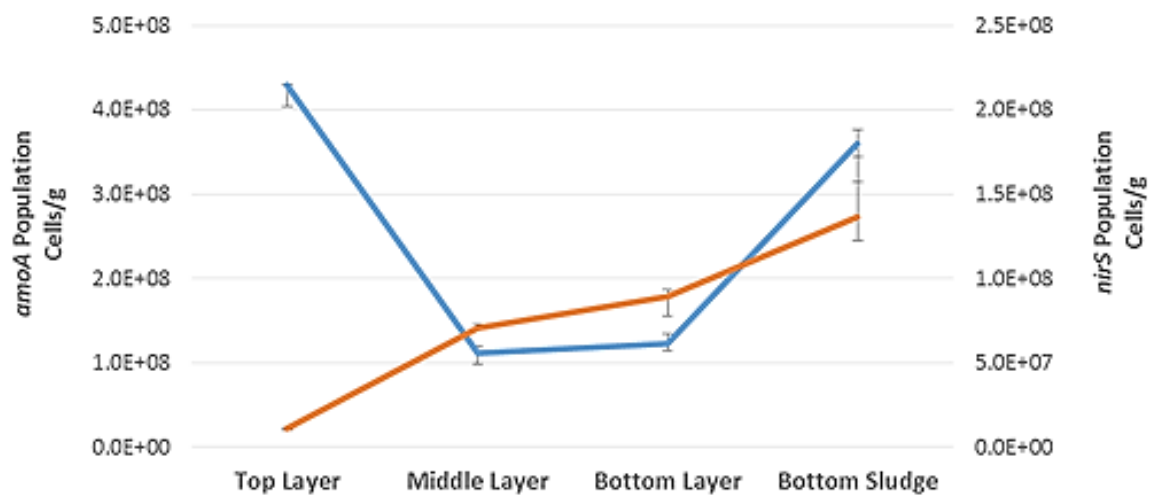


Figure 46. Comparison of mean population counts (cells/gram of media) between the targeted *amoA* and *nirS* genes, n=4 columns.

CHAPTER V: Discussion

RMF Effluent Quality

According to Tchobanoglous (2003) the mean values documented for COD and TOC from the untreated wastewater for this study is considered a high-strength, when comparing to typical organic concentrations found. Taking this into consideration, the reduction values observed for organics, paired with the shown pH neutralization, has proven that the designed RMF performed as should and produced a very high-quality effluent. Through the study, the mean effluent measurements were 7.03 ± 0.21 (pH), 65.5 ± 36.4 mg/L (COD), and 12.6 ± 8.38 mg/L (TOC), resulting in a >90% and >95% removal of COD and TOC.

Likewise, the overall reduction of nitrogen concentration within the effluent showed that nitrification and denitrification did occur, dropping from a mean 35 ± 3.0 mg/L to a mean of 15 ± 5.9 mg/L. However, the drop in nitrogen was much less consistent than the drop in COD and TOC, showing anywhere from a 28-84% decrease, but this is expected within a RMF. Nitrification is especially known to occur within the top layer because it remains anaerobic and there is an abundance of microbial life. While the upper layer of the RMF remains aerobic, the lower half tends to be anoxic, and although denitrification is occurring, the treated water is re-introduced into aerobic conditions shortly after exposure to oxygen when the lower valve is opened. It is from this back-forth cycling of aerobic to anaerobic conditions that lower TN reductions were expected, typically with a 40% to 80% reduction.

PPCP Fate in RMF Columns

PPCP Effluent Release

The designed system showed to be quite capable of providing a high percentage of PPCP removal within all of the columns. Looking at the four columns as a whole, the average amount of compounds that were added into the system were 18, 7.3, and 5.7 mg of IBU, NAP, and TRI, thus resulting in an average removal of 85.4% of IBU, 88.8% of NAP, and 89.5% of TRI. These removal percentages are somewhat comparable to what many studies report from WWTPs, however are a bit low for IBU and NAP.

While it was not found to be statistically significant, there were differences in total removal between the control column and the experimental columns at the end of the study. On its own, the control column provided a 97.3% removal of IBU, 92.8% of NAP, and 90.7% of TRI, which is more comparable to many studies. It was interesting to observe differences in the total removal of IBU between all of the columns. Overall, IBU showed to have the largest decrease in removal (~16%) within the experimental columns than NAP or TRI (~5% and ~2%); this decrease was most prominent within the TRI column, with only 78.2% of removal. High removal rates of NAP and TRI were observed within the TRI columns (~90%), so it is up to speculation if the added TRI to the system was directly affecting the removal of IBU, whether through adsorption or degradation.

PPCP Removal from Adsorption

The extraction of PPCPs from the biofilm within each of the columns revealed that while sorption did play a part in PPCP removal from the wastewater, it was a small amount. The system as a whole found an averaged 0.31% of IBU, 0.33% of NAP, and 2.86% of TRI was adsorbed over

the entire course of the study. It was not surprising that TRI was the PPCP that experienced the highest sorption, as has been shown in many previous studies. However, as before, there were notable differences in sorption percentages between the control column and experimental columns. The control column displayed the overall highest sorption values (0.52% IBU, 0.73% NAP, and 4.31% TRI) followed by the TRI column (0.20% IBU, 0.52% NAP, and 5.13% TRI). Interestingly, the NAP column showed almost no sorption occurring, especially with the highly adsorbed PPCP TRI. It is still up to debate as to what occurred within the NAP column that inhibited any sorption of PPCPs from occurring, but it could possibly be attributed to poor extraction efficiencies. Research has shown that the extraction of PPCPs from solids (soil, sludge, biofilm) can be quite variable (30-80% efficiency). This study was not able to determine the efficiency of the extraction procedure for the biofilm on the media within the columns, therefore sorption percentages can only be speculated and not validated.

PPCP Removal from Degradation

Compound degradation showed to play the largest part in PPCP removal from wastewater. As a whole, the system was able to degrade 85% of IBU, 88% NAP, and 87% of TRI. It should be noted that these reported values could only be obtained through the law of conservations (eq. 8), which requires comparing the influent to the effluent (PPCP release, sorption, and degradation); any error in the sorption percentages are passed onto the degradation percentages. While degradation values could not be considered true, they still offer an estimated explanation to what was occurring.

As before, the control column observed the largest PPCP degradation (96% IBU, 92% NAP, and 86% TRI), while the experimental columns ranged from 78% to 90%. Since the sorption values

for IBU were almost insignificant towards the total removal within each of the experimental columns, it can be stated that IBU was most affected on a microbial level, especially within the presence of TRI; TRI has anti-bacterial properties so it was not suspicious that this was occurring. It was interesting to note that NAP, which is an acidic pharmaceutical and NSAID like IBU, did not experience any large changes in degradation within each of the columns, except for the NAP column; it seemed as though the large concentration of NAP negatively reacted with its own degradation.

The changes in degradation within the experimental columns, which received a large concentration of a certain PPCP, has been observed before in other studies. Xu et al. (2009) investigated the sorption and degradation of PPCPs within agricultural soils and their influences from the addition of different concentrations. Comparing natural and sterilized soil that had each been administered reclaimed water spiked with varying concentrations of PPCPs (0.0, 0.05, 1.0, 2.5, 5.0, and 10 mg/L), they discovered that the sterilized samples exhibited a significantly lower percentage of compound removal. From this, they were able to conclude that the microbial activity within the soil and reclaimed water had a large influence on PPCP removal. Likewise, a decrease was observed within the non-sterilized samples that were given high concentrations of PPCPs. They concluded that PPCP degradation also decreased when a large concentration of PPCPs were present, and possibly were inhibiting microbial activity.

Negative Mass Balances

The high concentration of the added PPCPs into the experimental columns lead to another area of discussion, not about the overall removal rates, but more so the drastic change in removal that occurred half way through. Both IBU and NAP experienced a >70% difference in removal starting

the fifth week of treatment. This change remained the same for the remainder of the study. TRI experienced a similar drop, however, it was not nearly as significant, only an 11% change. The main questions to ask are: what prompted this change and why was TRI not nearly as affected?

Over the course of the study, the control column did not show any change within the data, showing a relatively high removal rate of all compounds (>90%). Therefore, the changes within the experimental columns could only be attributed to the addition of the trace organics to the experimental supply tanks. For the first half of the study, high removal rates were observed in all experimental columns, almost equal to the control column, for all compounds (>85%). However, an increase in IBU and NAP concentrations within the effluent began to show after the fourth week. This potentially was attributed to desorption occurring within the experimental columns, meaning all compounds that were initially adsorbed onto the biofilm were being released from or no longer adsorbing. Another argument could be that the experimental RMFs were not able to handle the large flux of PPCPs that they were receiving and were becoming “clogged”.

Negative mass balances have become a frequent issue with many experimental studies, and reasons for this negative removal has been: improperly addressing the fluid dynamics of the system, compounds previously not detected in the influent are becoming retransformed into the original due to biological processes, desorption, and the potential release of PPCPs from fecal particles as the feces is broken down by microorganisms (Blair et al., 2015). While the release of PPCPs from enclosed fecal particles raised an interesting point, other reasons were not agreed upon, such as the retransformation back into the parent compound. While retransformation such as this is entirely possible, it does not seem likely that the energy for such a retransformation, along with all other biological processes that were occurring, was available.

As mentioned previously, TRI did not experience a large decrease in total removal as did with IBU and NAP. Some possibilities for this is that TRI, as an anti-bacterial, has been known to cause problems for active bacteria that are utilized for organic breakdown. While TRI is usually persistent to degradation, IBU and NAP are not. If TRI was affecting the microbial communities that were degrading IBU and NAP, then that could explain the shift in removal rates. Another interesting note is that within the IBU and NAP columns there were no significant changes in removal between the two NSAIDs, however, there were significant differences within the TRI column, with IBU showing the largest decrease. Therefore, it can be stated that the larger concentration of TRI added to the TRI column did affect both IBU and NAP differently, in contrast to how the increase of IBU and NAP within the other two columns did not. It was also mentioned that perhaps RMF performance was in question, but no changes in pH, COD, TOC were observed during this period of time. The only inferences that could be made about the change in removal is that it was related to the added concentrations of trace organics and potentially from desorption and changes in microbial activity specific to the acidic pharmaceuticals.

Overall RMF Performance in PPCP Removal

The experimental columns were able to provide an understanding on what would potentially occur within a RMF if a frequent, high concentrations of PPCPs were to enter into the system. However, the administered concentrations (0.1 ppm) are extremely high and not typically recorded within WWTP influent or effluent. Therefore, it is important to note that the data collected within these columns is not what would be expected naturally. From this thinking, the control column, which received no additional concentrations of the trace organics, should be recorded as the “real world” result. The control column was able to remove over 90% of the targeted PPCPs from the

domestic wastewater; IBU ($97 \pm 6.7\%$ degraded, $0.72 \pm 0.01\%$ adsorbed), NAP ($93 \pm 4.9\%$ degraded, $0.73 \pm 0.01\%$ adsorbed), and TRI ($91 \pm 3.7\%$ degraded, $4.31 \pm 0.02\%$ adsorbed).

Biofilm qPCR Analysis

The highest populations of AOBs in all four units were in the top layer, and decreased as the depth increased. This showed that aerobic conditions (possible nitrification) were more present in the top layer, and anaerobic conditions (possible denitrification) were present as the depth increased. In addition, the sludge collected at the bottom was found to have higher concentrations of AOB present than the sludge-free media samples in the middle and bottom layer. There are a few speculations as to why this occurred, first being that the last in. of the columns was filled with larger media (#55 crushed limestone) than what was used for the upper layers of the columns. The larger media infers that there were larger volumes of pocketed oxygen, allowing the growth and continuation of ammonia oxidizing bacteria. Likewise, it also cannot be stated that the lowest areas of the columns remained completely anaerobic because of the periods where effluent samples were collected and required time to refill. While the effluent collections were empty, the bottom gravel was exposed to the atmosphere through the three way valve and stainless steel tubing. This, coupled with the large volumes of pocketed air, would allow the continuation and possible re-growth of ammonia oxidizing bacteria under anoxic conditions.

In addition, denitrifying bacteria were also quantified through qPCR analysis from the biofilm samples. The results showed a rapid increase in *nirS* genes from the top layer to the very bottom of the columns. Unlike with the *amoA* genes, there was not a significant change in population counts between the bottom layer and collected sludge. Besides the top layer, which was predominantly ammonia oxidizing bacteria, there was a mean of twice as many targeted nitrifying bacteria found within the middle & bottom layers and collected sludge than targeted denitrifying

bacteria. However, noticeable populations for both genes show that nitrification and denitrification were likely occurring at the same time within the lower regions of the columns.

Simultaneous nitrification-denitrification within wastewater treatment systems is a phenomena that has been, and still is, an important area of research because of its potential for simplifying treatment processes and reduction of energy consumptions. However, this process has also been reported to emit a significant amount of N₂O, which is considered a critical green-house gas (Jia et al., 2013). Studies focusing on media filters have documented that because the factors affecting simultaneous nitrification and denitrification can change depending on media depth, media filters are quite capable of achieving this process (Nakhla and Farooq, 2003).

Despite this, the results of this study show that although *amoA* and *nirS* genes are significant in most of the lower layers of the columns, there are more ammonia oxidizing bacteria present than denitrifying bacteria. It was questioned early in the project whether ammonia oxidizing bacteria could be attributed to playing a role in the degradation of the PPCPs. Unfortunately, the data collected cannot show if this is true because AOBs are able to co-metabolize, therefore growth cannot be attributed to degradation because they do not rely on the trace organics as a food source. If there were significant changes between the control column and experimental columns, perhaps a hypothesis could have been formed. All that can be stated is that both ammonia oxidizing and denitrifying bacteria are present within the systems, and are allowing the possibility of PPCP degradation, but so are other forms of bacteria, such as heterotrophs.

CHAPTER VI: Conclusions

Conclusions

The designed system was able to provide a high-quality effluent that meets basic EPA standards for environmental discharge, and also comparable PPCP removal rate to that documented from large-scale treatment facilities. In addition, this project was able to simultaneously detect two different types of trace organic compounds (acidic and phenolic PPCPs) using one common method. It would be beneficial for future work to determine other methods that would provide the simultaneous detection of even more compounds, as well as at lower concentrations.

This research was not able to conclude whether AOBs were the main source of degradation for the three target PPCPs, as was questioned. Future work in this research area, coupled with a continuous weekly monitoring of RMFs microbial community analysis, could provide an insight on what bacteria is flourishing or degrading while in the presence of TRI, which this study can conclude does have an impact on the effectiveness of a biological treatment system when in high concentrations. In addition, it would also be important to identify and quantify the concentrations of the major metabolite of TRI, methyl-TRI, because of its known toxicity and more aggressive bioaccumulation in the environment than its parent compound.

Finally, it was apparent that each of the experimental columns reacted differently when exposed to a high concentration of a specific compound, when compared to the control column, specifically showing lower estimated degradation percentages. This can only be attributed to the higher concentrations of PPCPs that were introduced into the IBU, NAP, and TRI columns, inferring that microbial activity was potentially inhibited. However, as mentioned before, these

affected microorganisms could not be related to AOB activity, and therefore more research is needed to determine specific bacteria that participate in PPCP degradation.

There were also noticeable interactions that occurred between specific compounds. IBU showed the most noticeable response when exposed to TRI. Within the TRI column, IBU had the lowest biodegradation rate, highest concentrations measured within the effluent, and also the largest weekly negative removal. Therefore, it can be inferred that high levels of TRI, an anti-bacterial, directly affects IBU, potentially through attacking vital microorganisms that play key in IBU breakdown.

Although much research has been performed on the fate and transfer of PPCPs before and after wastewater treatment, there are still many challenges that remain, whether it be for small- or large-scale systems. There had not been enough questions answered that will allow a proper risk management procedure for the disposal or degradation of these trace organic compounds. It is apparent that much more research is needed to determine a solution for all. However, this study was able to conclude that RMFs are capable of providing adequate PPCP removal from domestic wastewater, and with more research, has the potential for providing even further treatment.

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VITA

Brittani Nikole Perez is originally from Baltimore, Maryland. She attended Towson Catholic High School and completed her coursework in 2008. Following this, she moved to Dandridge, Tennessee to attend the University of Tennessee in Knoxville. She received her Bachelor of Science with a major in Biosystems Engineering in May 2013. During her years as an undergraduate she worked under Dr. John Buchanan in the wastewater lab of the Biosystems Engineering & Soil Science Department. After graduation, she was accepted into graduate school to achieve her Master's of Science in Biosystems Engineering & Soil Science along with a Watershed minor. As a graduate student, she was both a Research Assistant and a Teaching Assistant for Drs. John Buchanan and William Hart. She will be graduating in December 2015.