REMOVAL OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS BY THE SEWANEE WETLANDS

by

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(Under the Direction of Marsha Black)

ABSTRACT

Pharmaceuticals and personal care products (PPCP) are under investigation because of the potential to negatively affect human health and aquatic life. Secondary wastewater treatment processes do not effectively or efficiently remove these contaminants from wastewater, resulting in trace levels of PPCPs found in water sources around the world. Constructed wetlands have shown the potential to be a tertiary treatment to remove these contaminants while being cost effective to implement, maintain, and operate. We studied the Sewanee Wetlands to determine the efficacy for PPCP removal from partially treated wastewater. Of the 14 PPCPs studied, the wetland significantly removed atenolol ($p = 0.0002$), caffeine ($p = 0.0253$), and DEET ($p = 0.0367$). In a sediment mesocosm study, sorption to wetland sediments occurred for 7 of the 14 PPCPs studied. The Sewanee Wetland shows potential as a tertiary treatment to remove PPCPs.

INDEX WORDS: Constructed wetland, pharmaceuticals and personal care products, wastewater tertiary treatment, phytoremediation, sorption, photolysis, microbial degradation.

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DEDICATION

This is dedicated to my wife, Abigail, for your love and encouragement, to my son, William, for all the laughs and smiles, and to Charles, Catherine, Dave, and Deb, the grandparents, for the countless hours of babysitting.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1. Introduction

Contaminants of emerging concern (CEC) are a significant group of chemicals currently under investigation because of their potential to negatively affect human health and the environment. This contaminant group includes a diversity of chemicals such as salts, metals, metalloids, endocrine disrupting compounds, pesticides, and pharmaceuticals and personal care products (PPCP) (Koopaei and Abdollahi, 2017). PPCPs include prescription drugs, illegal drugs, antibiotics, hormones, over-the-counter medications, and veterinary medicines, which remain bioactive in multiple species (Cizmas *et al.*, 2015), and are of particular concern because, when found in water sources, they are a potential threat to human health and aquatic life (USEPA, 2018). Information about how PPCPs affect human health and the environment is limited to individual compound's effects and there is a lack of knowledge for effects from mixtures of compounds and interactions between compounds. Wastewater contains compounds that individually pose toxicological hazards when exposed to human and aquatic life such as acute and chronic toxicity, carcinogenicity, and reproductive, developmental, and neurotoxicity hazards (Koopaei and Abdollahi, 2017). However, there is a need to understand the effects of chronic, low dosage exposures to mixtures of PPCP parent compounds and metabolites commonly found in wastewater. Reliable, long-term

toxicological studies for these compounds do not exist (Koopaei and Abdollahi, 2017). As of 2012, less than 10% of prescribed medications have ecotoxicological data and fewer pharmaceuticals have had ecological risk assessments performed (Brausch *et al.*, 2012).

The two main sources of PPCPs in the environment are: 1) human and animal excretion of PPCPs in metabolized and non-metabolized forms and 2) disposal of unused and expired PPCPs into wastewater (Caldwell, 2015; Cizmas *et al.*, 2015). These two sources have resulted in low levels of contaminants (ng/L – μg/L) detected in water sources around the world (Caldwell, 2015; Cizmas *et al.*, 2015). Most PPCPs are water soluble and have low volatility so these contaminants are transported easily throughout the environment (Breton and Boxall, 2003). These contaminants have been found throughout the water environment, including groundwater, surface water, rivers, coastal waters, and treated drinking water (Randhir and Rolf, 2013; Taylor, 2015). Most PPCPs enter water sources through wastewater treatment plants (WWTP) (Zhang *et al.*, 2014, Caldwell, 2015) and become part of the water cycle; being reintroduced to the drinking water supply and subsequently back to WWTPs via sewage systems (Randhir and Rolf, 2013).

The goal of wastewater treatment is to limit water pollution and promote higher water quality that is suitable for reuse (Koopaei and Abdollahi, 2017). Secondary wastewater treatment processes currently in use do not effectively or efficiently remove PPCPs because they are designed to remove total suspended solids (TSS), pathogens, turbidity, odor, biological oxygen demand (BOD), and nutrients (e.g. nitrogen and phosphorus compounds) (Randhir and Rolf, 2013; Jasper *et al.*, 2013). As a result various

PPCP compounds are released into the environment when WWTPs discharge treated wastewater. A number of tertiary treatment processes are effective in removing PPCPs and can make the wastewaters suitable for reuse. However, many tertiary processes such as carbon filters, reverse osmosis, and ozonation have significant treatment costs and therefore, are not widely used (Zhang *et al.*, 2014). One tertiary treatment process, constructed wetlands, is both cost-effective to implement, maintain, and operate (Davis, 1994; Jasper *et al.*, 2013). In smaller communities where the costs of advanced tertiary processes are not available, constructed wetlands have the potential to improve the quality of wastewater before it is discharged into the environment. Historically, constructed wetlands have been effective in removal of TSS, BOD, nitrogen compounds, phosphorus compounds, heavy metals, and pathogenic microbes (Zhang *et al.*, 2014). However, the efficacy for constructed wetlands as a treatment process to remove PPCPs has not been fully evaluated and many of the mechanisms of removal are poorly understood.

Constructed wetlands commonly include a water column, substrate, plants, invertebrates, and microorganisms. Each wetland component plays a vital role in removal of pollutants such as CECs, organic matter, pathogens, and nutrients like nitrogen and phosphorus. Constructed wetlands vary in design and function for CEC removal. Flow designs range from surface flow (SF) to subsurface flow (SSF). Different plant species and substrate are used in various designs. SSF wetlands consist of a permeable substrate of rock and soil in which water flows horizontally or vertically below the surface of the ground. Water flow underneath the ground surface has advantages like tolerance to temperature changes, less animal and odor problems, and an increased removal of

pollutants compared to SF wetlands. Disadvantages include clogging due to presence of solid particles, lower volume capacity, and higher construction, repair, and maintenance costs. Resembling natural wetlands, SF constructed wetlands move water from one area to another through passive surface-flow. Esthetically pleasing, rooted aquatic plants aid the SF wetland in nutrient removal and reduce flow rates, while also providing a suitable environment for microorganism growth. Microbial communities exist throughout the water column, plant roots, and sediment. These communities assimilate and degrade chemical compounds, fix nitrogen, and decompose organic matter. Aerobic bacteria reside in the water column's oxygen rich upper portion while anaerobic bacteria reside in areas of limited or nonexistent oxygen concentrations like biomass covered or lower portions of the water column. Anaerobic bacteria also reside in the portions of sediment and plant roots or rhizomes where oxygen is limited. SF wetlands are both cost effective and esthetically desirable; however, a disadvantage is the large tract of land necessary to build and maintain a SF constructed wetland. Additionally, a hybrid version of SF and SSF wetlands also exists in which there are multiple stages of treatment divided into surface flow cells with subsurface flow stages in between surface flow cells (Davis, 1994).

2. Literature Review

2.1. Removal Processes

Evaluation of constructed wetlands for tertiary treatment of PPCPs in wastewaters has focused mostly on measuring pharmaceuticals in influent and effluent streams. This holistic approach is effective for measuring overall wetland removal efficiencies for

various contaminants. However, mechanisms of PPCP removal are complex and poorly understood because physical, chemical, and biological processes can occur simultaneously and are complex. This holistic approach cannot effectively identify individual mechanisms of removal, which are necessary to understand to optimize removal efficiencies. Photolytic degradation, sorption, plant uptake, phytodegradation, and microbial degradation are thought to be the most important mechanisms of removal for many PPCPs and are target areas for future research (Zhi and Ji, 2012; Zhang *et al.*, 2014). There is a need to understand these mechanisms of removal even at basic levels to optimize constructed wetland designs.

2.1.1. Photolytic Degradation

Photolytic degradation, or photolysis, is suggested to be the most important and predominant removal mechanism for certain PPCPs (Lin *et al.*, 2019, Lin and Reinhard, 2005). Some PPCPs have biologically toxic or inhibitory effects that prevent removal through biological adsorption, metabolism, or transformation. For these PPCPs, photolysis could be the most probable removal mechanism.

Two types of photolysis are possible within a constructed wetland, direct and indirect photolysis. Direct photolysis occurs when a compound absorbs light, causing an unstable, excited electron state, and leads to the cleavage of a chemical bond, decomposing the compound into fragments that are potentially more or less toxic. Susceptibility to photolysis depends on the absorbance spectrum of the PPCP and chemical bonds within the PPCP. Compounds with conjugated ring structures are highly susceptible to photolysis (Bear *et al.*, 2017). Some PPCPs, like diclofenac, are more

photo-reactive than other PPCPs, with photolysis driving its short half-life of less than 2 hours (Tixier *et al.*, 2003). Other PPCPs, like propranolol have been shown to be less photo-reactive with a half-life of multiple days and weeks (Andreozzi *et al.*, 2003). The effectiveness of photolysis will vary depending on the light intensity, duration, and attenuation. Photolysis is more effective during summer and fall seasons which have more intense and increased duration of solar radiation compared to winter season (Zhang *et al.*, 2018). Photolysis is less effective in areas of the water column that are shaded by biomass and the effect of photolysis attenuates as water depth increases (Zhang *et al.*, 2014).

Another factor affecting photolysis is the aquatic matrix, which influences the second type of photolysis, indirect photolysis. Indirect photolysis of PPCPs occurs when other chemicals in the water act as photosensitizers and aid in the degradation of PPCPs (Wang and Lin, 2014). Photosensitizers are formed when certain molecules or ions become excited by solar radiation and form radical species. Moieties present in surface waters that can be photosensitizers include dissolved organic materials (DOM), nitrates and nitrites, dissolved oxygen (DO), and bicarbonate and carbonate (Wang and Lin 2014). When photosensitizers are formed they immediately react with PPCPs of interest causing degradation (Wang and Lin, 2014). It is interesting to note that DO molecules can both inhibit, through quenching properties, and promote, by forming singlet oxygen compounds, indirect photolysis of PPCP compounds (Li *et al.*, 2017).

2.1.2. Sorption

Sorption is potentially a significant mechanism of removal for PPCPs by changing the fate of PPCPs in an aqueous environment. Compared to other mechanisms of removal, sorption does not usually degrade compounds but instead binds them to solid particles, creating a sink of PPCPs (Kümmerer, 2009). Sorption is the binding of compounds to a sorbent, typically found in the sediment of water bodies. When PPCP sorption occurs, these compounds become recalcitrant to microbial degradation and high levels of accumulation can occur within the constructed wetlands (Garcia *et al.*, 2010). A sorbent can be soil and other materials that contain organic carbon, mineral surfaces, and/or biofilms (Kadlec and Wallace, 2009) and can even include plant cell walls, suspended solids, and bacterial cell walls (Verlicchi *et al.*, 2012). Natural organic materials (NOM) can significantly affect sorption of PPCPs and are present at higher concentrations relative to PPCP concentrations (Choi *et al.*, 2007). Humic acids (HA), a major component of NOM, can act as a sorbent for PPCPs, promoting removal of PPCPs from wastewater. However, HA can compete for binding sites on other sorbents, negatively affecting sorption of PPCPs in wastewater (Kyzas *et al.*, 2017; Liu *et al.*, 2014). Sorption has been shown to depend on multiple factors of the compound such as its hydrophobic characteristics (i.e. log K_{OW}), stereo chemistry and chemical nature, water solubility, and acid/base properties (i.e. pKa). Important characteristics of the sorbent that will influence sorption are its fraction of organic carbon (fOC), oxidation-reduction potential (ORP), pH, temperature, and composition of cations and anions (Kümmerer, 2009). Additionally, aqueous environment conditions such as pH, temperature, and ORP can influence sorption.

Predicting which compounds are likely to sorb can be based on the hydrophobicity of the compound. Since compound characteristics can influence the tendency to partition in water or other materials, hydrophobicity should be a suitable predictor of sorption. Hydrophobicity can be measured with the partition coefficient log K_{OW}, which is the ratio of a compound's concentration between octanol and water. Higher levels of hydrophobicity (i.e. higher log K_{OW}) result in more sorption of a PPCP to sorbents. Low levels of hydrophobicity (i.e. lower log K_{OW}) result in less sorption of PPCP compounds. Some studies show sorption of PPCPs is relatable to the hydrophobicity of the compound (Lindberg *et al.*, 2006; Verlicchi *et al.*, 2012;). However, using log K_{OW} to predict the fate of PPCP compounds in constructed wetlands also has been shown to be a poor predictor of sorption (Martinez-Hernandez *et al.*, 2014). Additionally, the organic carbon partition coefficient, log K_{OC}, which is the ratio of a compound's concentration between octanol and carbon, can be used to predict sorption (Zhang *et al*, 2014). A compound with a higher log K_{OC} value will partition to soil and organic materials. A compound with a lower log K_{OC} value will be highly mobile in soil containing organic materials. Soils with greater amounts of carbon will sorb more compounds than soils with lesser amounts. The main difference between log KOW and log KOC is log KOW is able to predict movement in water and log KOC is able to predict movement in soil and organic materials.

Sorption can also occur when positively charged groups on the sorbent and negatively charged groups on the PPCP compound interact, or vice versa, known as electrostatic interactions (Kadlec and Wallace, 2009). PPCPs can contain neutral groups and positively or negatively charged groups. The overall charge of a PPCP may be

neutral, positive, or negative but it can still contain these charged groups, allowing sorption to occur. It is important to note that pH will also influence the charge of compounds based on the pKa of the compound. Free hydrogen can compete with PPCPs for binding sites, charged groups on the sorbent. Kümmerer (2009) found that pH influences the partitioning of ionizable compounds between water and organic material making sorption dependent on pH. However, Lindberg *et al.* (2006) found that sorption of two antibiotics, norfloxacin and ciprofloxacin, to sewage sludge was not dependent on pH as much as on hydrophobic and electrostatic interactions between the compounds and the sludge. Martinez-Hernandez *et al.* (2014) conducted experiments with neutral compounds and positively and negatively charged compounds and measured sorption rates onto sediment. The authors found that sorption of neutral compounds, acetaminophen and carbamazepine, was negligible Anions, negatively charged minerals, in the composition of the sediment can influence sorption of positively charged compounds.. Caffeine and atenolol, positively charged compounds, experienced high sorption rates in the negatively charged sediment, despite caffeine being highly water soluble and hydrophilic. Naproxen, a negatively charged compound, did not sorb to the negatively charged sediment due to repulsing electrostatic interactions. However, sorption to NOM occurred due to the high hydrophobicity characteristics of naproxen, and the NOM sorbed to cationic mineral surfaces (positively charged particles) in the sediment (Martinez-Hernandez *et al.*, 2014).

Sorption of PPCPs to sorbents in constructed wetlands is complex and not fully understood and desorption of PPCPs can occur, further complicating predictions of PPCP fate. Martinez-Hernandez *et al.* (2014) called attention to the lack of knowledge on what

may cause compounds to desorb in a wetland. The authors found that partial desorption occurred for all studied PPCPs that underwent measureable sorption: atenolol, caffeine, naproxen, and sulfamethoxazole. PPCP desorption may be influenced by competition for binding sites and the effects on sorption equilibrium caused by changes in pH, ORP, and temperature.

2.1.3. Plant Uptake and Phytodegradation - Phytoremediation

Vegetation plays a very important role in a constructed wetland by affecting overall PPCP removal (Hijosa-Valsero *et al.*, 2010; Matamoros *et al.*, 2007), increasing DO levels in the sediment via oxygen translocation, and increasing microbial community growth that may influence PPCP degradation in the roots and rhizospheres of plants (Ke and Tam, 2012). Vegetation also has a more direct role in removal of some PPCPs via uptake, accumulation, and translocation, leading to phytodegradation. Phytodegradation is the degradation of PPCPs due to metabolic transformation of PPCPs within a plant. Plant uptake is a non-destructive retention of PPCPs while phytodegradation is a decomposition of PPCPs into simpler compounds that can be volatilized or stored in the plants.

Information regarding the mechanisms of plant uptake and phytodegradation is limited and most of the studies on these processes have not been done in the field, but rather in hydroponic solutions (Zhang *et al.*, 2014). Plant uptake of PPCPs is thought to be predominately driven by the chemical characteristics of the PPCPs, especially hydrophobicity, because the cell membranes of plants do not contain specific transporters for PPCPs (Lv *et al.*, 2016; Stottmeister *et al.*, 2003). PPCP compounds with a log K_{OW}

in the range of 0.27 – 3.9 are ideal for uptake into plants via roots or rhizospheres (Lv *et al.*, 2016). Another study suggests a range of 0.5 – 3.0 log K_{OW} (Pilon-Smits, 2005). Compounds in these ranges are thought to be lipophilic enough to pass through the cell membrane of plants and hydrophilic enough to enter the cellular matrix. Lv *et al.* (2016) suggests that the enantiomeric fraction, the proportion of enantiomers of a PPCP in a plant versus in the water column of the constructed wetland, shows the ability of a plant to uptake a PPCP. Enantiomers are chiral molecules, mirror images of each other that are non-superimposable. Measuring the concentrations of an enantiomeric compound in a plant that is growing in a hydroponic solution with known concentrations of the enantiomers can determine a plant's ability to uptake certain compounds; this is also called enantiomeric selectivity. The uptake of PPCPs is also based on the composition and lipid content of cell membranes in the plant, indicating that certain plants will be favorable for phytoremediation of certain PPCPs (Lv *et al.*, 2016).

After a PPCP compound has been taken up by a plant, PPCPs may undergo two types of metabolism: 1) transformation (oxidations, reductions, hydrolysis) and 2) conjugation (addition of glutathione, sugars, amino acids) (Stottmeister *et al.*, 2003). These types of phytodegradation decompose parent molecules into metabolites which may or may not be toxic. Compartmentation (storage of PPCPs into plant tissues) and translocation (movement of PPCPs from one part of a plant to another) are also important mechanisms for phytoremediation (Stottmeister *et al.*, 2003; Collins *et al.*, 2006). Studies of PPCP metabolites in plants are limited and the long-term fate of PPCPs after phytoremediation is unclear (Zhang *et al.*, 2014). Knowing the fate of PPCPs after

phytoremediation will inform proper disposal of wetland biomass to prevent the reentry of PPCPs into the environment.

2.1.4. Microbial Degradation

Microbial degradation is influenced by many of the same factors that influence sorption and phytoremediation: pH, ORP, temperature, and DO (Meng *et al.*, 2014). Microbial degradation, the decomposition by microorganisms of organic substances into simpler chemical substances (Santos *et al.*, 2019), is commonly known to aid in nitrogen compound removal from wastewater in constructed wetlands as well as degradation of organic matter and other contaminants (Wu *et al.*, 2012; Cui *et al.*, 2013). In general, microbial degradation has also been shown to be effective for PPCP removal in constructed wetlands and for non-recalcitrant compounds such as ibuprofen and iohexol, it has been shown to be the main removal process, more so than photolytic degradation, sorption, and phytoremediation (Ke and Tam, 2012; Hijosa-Valsero *et al.*, 2016; Zhang *et al.*, 2017).

Microorganisms that exist in constructed wetlands include yeasts, molds, protozoa, algae, and, predominantly, bacteria, and can degrade PPCPs in aerobic and anaerobic conditions (Wu *et al.*, 2012). In general, aerobic degradation occurs when microorganisms oxidize contaminants using oxygen as an electron acceptor and carbon dioxide is released as a byproduct (Meng *et al.*, 2014; Faulwetter *et al.*, 2009). Anaerobic degradation occurs when microorganisms mineralize methane and carbon dioxide during methanogenesis and fermentation (Meng *et al.*, 2014; Faulwetter *et al.*, 2009). During microbial degradation, three functions can occur: 1) mineralization, 2) cleavage of certain

functional groups which forms a more hydrophobic compound, or 3) formation of a more hydrophilic compound after cleavage of functional groups.

Predicting the availability of PPCPs to microbial degradation is difficult because chemical structures of PPCPs, such as the long carbon chains, carbon rings, and functional groups, vary widely even within a particular class of drugs (Kümmerer, 2009). Biologically active PPCPs, like antibiotics, are thought to inhibit microbial degradation by inhibiting microorganism growth. However, Santos *et al.* (2019) found that while bacterial community structures were affected after exposure to two veterinary antibiotics, enrofloxacin and ceftiofur, in a constructed wetland mesocosm, microbial degradation of the compounds still occurred at greater than 85% removal rates. One explanation for the unaffected removal rates could be that the effects of enrofloxacin and ceftiofur only targeted certain microbial communities, leaving other microorganisms to degrade the compounds.

Microbial growth and the effectiveness of microbial degradation are affected by multiple factors within a constructed wetland. PPCP removal correlated positively with DO and negatively with CO_2 gas, indicating the importance of aerobic processes and the presence of DO (Matamoros *et al.*, 2007). ORP, a parameter dependent on DO, can influence the mineralization of PPCPs via microbial degradation (Faulwetter *et al.*, 2009). In addition to these factors, temperature has a strong influence on microbial growth and metabolic rates, influencing microbial degradation of PPCPs (Meng *et al.*, 2014). Higher temperatures result in higher microbial growth and higher microbial degradation rates; lower temperatures inhibit these processes (Zhang *et al.*, 2017; Meng *et al.*, 2014; Faulwetter *et al.*, 2009).

2.1.5. Abiotic Factors Affecting Removal Processes

The four most widely fluctuating and important abiotic factors in a constructed wetland are DO, ORP, pH, and alkalinity (Kadlec and Wallace, 2009). These factors, as well as seasonality in relation to photolysis, water quality parameters (i.e. DOM, nitrogen compounds, and carbon compounds), sediment matrix and composition, plant selection, and temperature in regards to microbial activity, affect the efficiency of the constructed wetland as a whole. In addition, the hydraulic retention time (HRT), a measure of time wastewater spends in the wetland from influent to effluent, as a design factor of the constructed wetland can affect the removal processes and overall efficiency of a constructed wetland (Zhang *et al.*, 2014). With a HRT that is too low, removal processes may not be possible due to a lack of time. Increased HRT will lead to longer dwell times and higher removal rates of PPCPs, but will decrease the volume of wastewater that is treated by the constructed wetland (Zhang *et al.*, 2014). The ideal HRT can optimize both the PPCP removal rates and the overall volume of treated wastewater for the constructed wetland. Furthermore, many of the factors mentioned above potentially interact and influence another. Temperature displays a great potential to interact with multiple other factors; thereby, giving temperature a profound influence in the wetland.

3. Study Site

The Sewanee Wetlands is a pilot, surface-flow constructed wetland in Sewanee, TN operated jointly by the Sewanee Utilities District (SUD) and The University of the South. The SUD operates a wastewater treatment plant (WWTP) that receives municipal wastewater from about 687 active sewage accounts in Sewanee (personal

communication, Emily Jackson, SUD). The municipal wastewater is initially filtered through a large metal screen where large solids are removed. The resultant wastewater is then pumped into two adjacent parallel lagoons (A and B; Figure 1.1). After 30 days, water from these two parallel lagoons is transferred to a third lagoon (C) where it remains for an additional 15 days. Finally, the wastewater from the Lagoon C is transferred to a disinfection system, chlorinated, and then sprayed into fields planted with trees, shrubs, and other forest vegetation. Lagoons A, B, and C are approximately 60,000-m^2 total (~20,000-m^2 each) and the wetland, three basins of varying size, is 1800-m^2 – 1900-m^2 total. Each wetland basin is approximately 0.5-m deep (personal communication, Deborah McGrath, University of the South).

Partially treated wastewater from Lagoon C is diverted to a 24,600-liter supply tank for storage and regulating wetland flow and hydraulic retention time. Water is gravity-fed from the supply tank to Basin 1 at approximately 100 L/min, flowing first over a small section of rocks (1-m^2) to promote oxygenation as the water enters Basin 1 (Figure 1.1). The first section of Basin 1 is approximately 1 m deep to prevent plant growth and 115-m^2 in area. This provides a photolysis zone where PPCPs can be degraded by solar radiation. The rest of Basin 1 is approximately 950-m^2 and contains soft-stem bulrush (*Schoenoplectus tabernaemontani,* Sedges), planted to aid in removal of excess nutrients and other wastewater contaminants. Wastewater then flows by gravity into Basin 2 (~380-m^2), which is designed to be a photolysis zone with nine small, planted mounds rising above the water surface. Designed to prevent erosion, mitigate short-circuiting (a preferred flow-path through the water column, resulting in reduced removal efficiencies), and enhance esthetics, the nine mounds contain boneset

(*Eupatorium perfoliatum,* Daisy), blue flag iris (*Iris versicolor,* Iris), rose mallow (*Hibiscus moscheutos,* Mallows), and swamp milkweed (*Asclepias incarnata,* Dogbanes). From Basin 2, wastewater flows by gravity into Basin 3 (~400-m^2 total). The first part of Basin 3 (~200-m^2) contains pickerelweed (*Pontederia cordata,* Pontederiaceae) for removal of excess nutrients. The second part of Basin 3 is another photolysis zone. From Basin 3, the wastewater flows back into Lagoon C (Figure 1.1). Currently, all photolysis zones are vegetated with duckweed (*Lemna lemnoideae,* Araceae). The duckweed is an unplanned third wetland plant which may impede intended PPCP photolysis.

3.1. Sewanee Wetlands Preliminary Water Quality Analyses (2016)

The Sewanee Wetlands construction was completed in June 2016 and weekly sampling periods occurred in the first four weeks. Water quality was evaluated for nitrite, nitrate, reactive phosphate, *E. coli,* and pH by Hopson *et al.* (2016) (Table 1.1). Water samples were taken from the effluent of five sites; Lagoon C, Inlet Pipe, Basin 1, Basin 2, and Basin 3 (Figure 1.1). Overall, total nitrogen, total phosphorus, and *E. coli* colony-forming units (CFU) decreased, indicating the potential capability to reduce nutrients and pathogens. However, *E. coli* CFU and nitrate both increased in the last cell, Basin 3, of the wetland. Also, the wastewater became more basic, higher pH levels, throughout the wetland. Finally, the levels of these water quality parameters were highly variable between sampling periods.

4. Conclusions and Study Objectives

Constructed wetlands are being evaluated to determine the efficacy as a cost effective, low maintenance tertiary wastewater treatment process. While constructed wetlands are known to effectively reduce TSS, BOD, nitrogen compounds, phosphorus compounds, heavy metals, and pathogenic microbes, removal of PPCPs and other emerging contaminants is still being studied. Studies are showing potential for constructed wetlands as an effective tertiary treatment, yet many studies only measure contaminant concentrations of wetland influent and effluent, leaving constructed wetlands to be viewed as a "black box". The removal mechanisms for PPCPs are poorly understood and therefore cannot be optimize to increase PPCP removal. Photolytic degradation, sorption, plant uptake, phytodegradation, and microbial degradation are target areas for future research.

This project seeks to evaluate and determine the efficacy of the Sewanee Wetlands, in its current configuration, as a tertiary treatment for partially treated wastewater from a WWTP. The first study will determine overall and basin-specific removal rates during eight sampling periods over one year by measuring reductions in PPCP concentrations throughout the wetland. Other objectives include investigating potential correlation between wetland characteristics and removal rates (% reduction of PPCPs). We hypothesize the wetland will have increased removal efficiencies with higher temperatures and increased DO. A second study will seek to determine the sorption potential of the sediment in the Sewanee Wetlands by conducting a mesocosm scale experiment to measure PPCP sorption over a 12-week exposure period. We hypothesize PPCPs with higher log K_{OW} values will sorb more compared to PPCPs with

lower log K_{OW} values. Conclusions from this research will add to the growing knowledge of constructed wetlands as tertiary treatments and illuminate characteristics of sorption for the studied PPCPs.

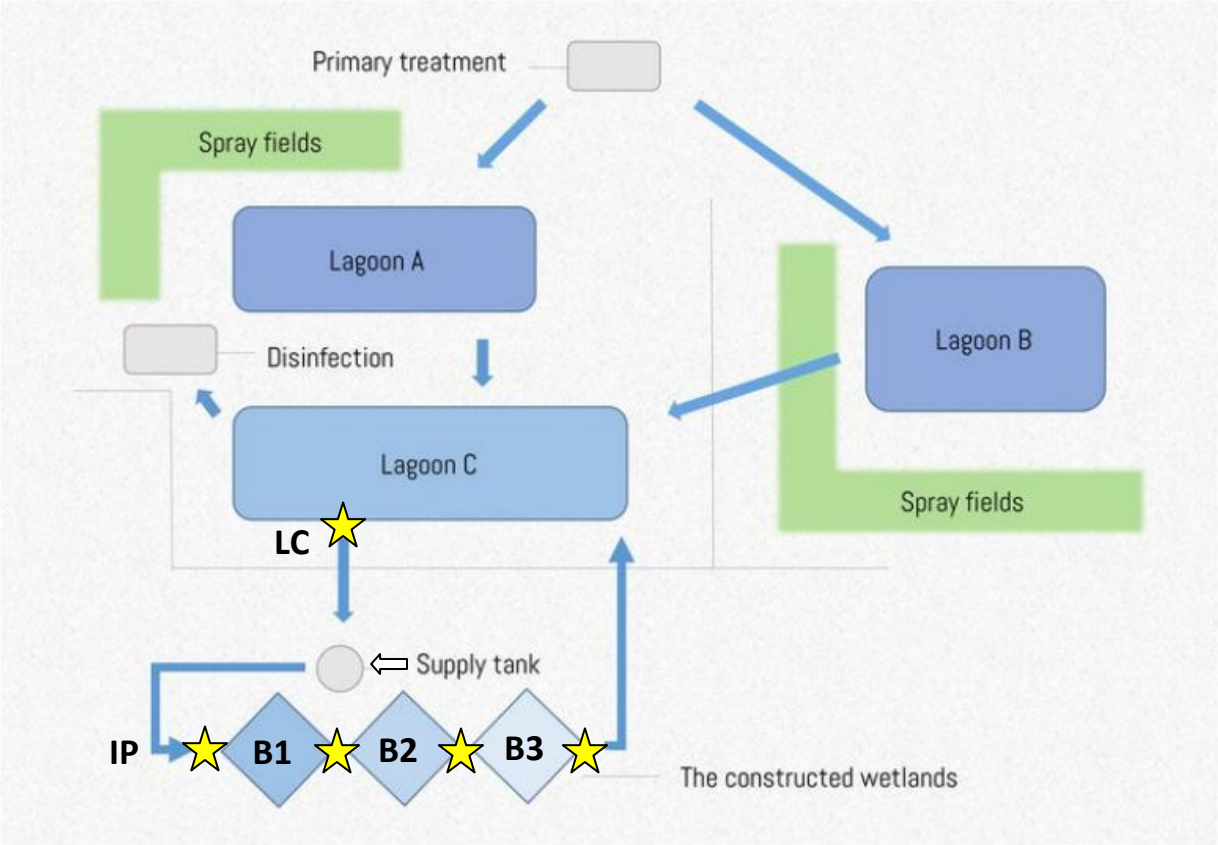

Figure 1.1. Layout design of the Sewanee Utility District wastewater treatment plant and the Sewanee Wetlands in Sewanee, TN.[1]

[1] Sampling sites are indicated by a yellow star. (LC = Lagoon C, IP = Inlet Pipe, B1 = Basin 1, B2 = Basin 2, B3 = Basin 3: modified from sewaneewetlands.org/ wetland-design/).

Table 1.1. Means ± SE for nutrient, *E. coli*, and pH levels for effluent samples at five sites in the Sewanee Wetlands taken over four weekly sampling periods (mid-June to mid-July 2016) (Hopson *et al.*, 2016).

Sample	Lagoon C	Inflow	Basin 1	Basin 2	Basin 3
Nitrite (mg (N)/L)	0.06 ± 0.05	0.05 ± 0.05	Bd[a]	Bd[a]	Bd[a]
Nitrate (mg (N)/L)	0.24 ± 0.13	0.13 ± 0.1	0.12 ± 0.04	0.11 ± 0.09	0.15 ± 0.12
Reactive phosphate (mg (P)/L)	1.8 ± 0.37	1.5 ± 0.51	1.1 ± 0.42	0.92 ± 0.31	0.79 ± 0.58
Total Nitrogen (mg (N)/L)	16 ± 5	11 ± 1.3	6.1 ± 0.8	5.6 ± 0.5	5.4 ± 0.5
Total Phosphorus (mg (P)/L)	2.8 ± 0.8	2.1 ± 0.5	1.1 ± 0.1	1.3 ± 0.1	0.9 ± 0.1
E. coli (CFU/100mL)	305 ± 115	162 ± 107	138 ± 116	146 ± 113	161 ± 104
pH	8.3 ± 0.2	8.1 ± 0.1	8.4 ± 0.6	8.7 ± 0.4	8.5 ± 0.2

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CHAPTER 2

EFFICACY OF A PILOT-SCALE, SURFACE-FLOW CONSTRUCTED WETLAND FOR REMOVING PHARMACEUTICALS AND PERSONAL CARE PRODUCTS FROM MUNICIPAL WASTEWATER[2]

[2] Wright, A.W., Black, M.C., Henderson, W.M., Torreano, S.J., McGrath, D.A. To be submitted to *Water Research*.

Abstract

Pharmaceuticals and personal care products (PPCP) are part of a larger group of contaminants of emerging concern that may negatively affect human health and aquatic life. Traditional secondary wastewater treatment is not designed to remove PPCPs, so they remain in some treated wastewater. Constructed wetlands, a tertiary wastewater treatment process, may be an economical, low energy requirement, and efficient process for removing PPCPs from treated wastewater. We examined the efficacy of a pilot, surface-flow wetland to determine the overall removal (%) and basin-specific removal rates of 14 PPCPs from treated wastewater. Triplicate 1-liter water samples were collected from five sites throughout the wetland during each of eight sampling periods from October 2017 – September 2018. PPCPs were extracted from samples with solid-phase extraction and analyzed using LCMS/MS analysis with single reaction monitoring. Of the 14 PPCPs studied, the wetland significantly removed atenolol ($p = 0.0002$), caffeine ($p = 0.0253$), and DEET ($p = 0.0367$). Caffeine, valsartan, diphenhydramine, DEET, and atenolol were the most abundant PPCPs in all five sample sites. The wetland significantly removed 73.32 ± 15.42% (mean ± SD, n=8) of Total PPCPs ($p = 0.0154$). The Sewanee Wetlands shows potential as a tertiary treatment to remove PPCPs and in general, constructed wetland processes are still being researched to optimize removal mechanisms.

1. Introduction

Pharmaceuticals and personal care products (PPCP) are part of a larger group of contaminants of emerging concern that are under investigation because of the potential to

negatively affect human health and the environment (Koopaei and Abdollahi, 2017). PPCPs include prescription drugs, illegal drugs, antibiotics, hormones, over-the-counter medications, and veterinary medicines, which remain bioactive in multiple species (Cizmas *et al.*, 2015) and are of particular concern because, when found in water sources, they are a potential threat to human health and aquatic life (USEPA, 2018).

The two main sources of PPCPs in the environment are: 1) human and animal excretion of PPCPs in metabolized and non-metabolized forms and 2) disposal of unused and expired PPCPs into wastewater (Caldwell, 2015; Cizmas *et al.*, 2015). These two sources have resulted in low levels of contaminants (ng/L – μg/L) detected throughout the water environment, including groundwater, surface water, rivers, coastal waters, and treated drinking water (Caldwell, 2016; Cizmas *et al.*, 2015; Randhir and Rolf, 2013; Taylor, 2015). Most PPCPs are water soluble and have low volatility so these contaminants are transported easily throughout the environment (Breton and Boxall, 2003).

The goal of wastewater treatment is to limit water pollution and promote higher water quality that is suitable for reuse (Koopaei and Abdollahi, 2017). Secondary wastewater treatment processes currently in use do not effectively or efficiently remove PPCPs because they are designed to remove suspended solids, pathogens, turbidity, odor, biological oxygen demand (BOD), and nutrients (e.g. nitrogen and phosphorus compounds) (Randhir and Rolf, 2013; Jasper *et al.*, 2013). As a result various PPCP compounds are released into the environment in treated wastewater discharges. A number of tertiary treatment processes, including carbon filters, reverse osmosis, and ozonation, are effective in removing PPCPs and can make wastewaters suitable for reuse. However,

these tertiary processes have significant treatment costs and are not widely used (Zhang *et al.*, 2014). One tertiary treatment process, constructed wetlands, is cost-effective to implement, maintain, and operate (Davis, 1994; Jasper *et al.*, 2013). In smaller communities where funding for advanced tertiary processes is not available, constructed wetlands have the potential to improve the quality of wastewater before it is discharged into the environment. Historically, constructed wetlands have been effective in removal of total suspended solids (TSS), BOD, nitrogen compounds, phosphorus compounds, heavy metals, and pathogenic microbes (Zhang *et al.*, 2014). However, the efficacy for constructed wetlands as a treatment process to remove PPCPs has not been fully evaluated and many of the mechanisms of removal are poorly understood.

Constructed wetlands commonly include a water column, substrate, plants, invertebrates, and microorganisms. Each wetland component plays a vital role in removal of pollutants such as CECs, organic matter, pathogens, and nutrients like nitrogen and phosphorus. Resembling natural wetlands, surface flow (SF) constructed wetlands move water from one area to another through passive surface flow. Substrate, such as highly organic soils, provides adsorption sites for contaminants. Esthetically pleasing, rooted aquatic plants aid the SF wetland in nutrient removal and reduce flow rates, while also providing a suitable environment for microorganism growth. Microbial communities exist throughout the water column, plant roots, and sediment of the wetland. These communities assimilate and degrade chemical compounds, fix nitrogen, and decompose organic matter. Aerobic bacteria reside in the water column's oxygen rich upper layer, while anaerobic bacteria reside in areas of limited or nonexistent oxygen concentrations, including the hypolimnion and areas under extensive biomass cover, such as algae and

duckweed. Anaerobic bacteria also reside in the portions of sediment and plant roots or rhizomes where oxygen is limited. SF wetlands are both cost effective and esthetically desirable; however, a disadvantage is the large tract of land necessary to build and maintain a SF constructed wetland.

1.1. Removal Processes

The mechanisms of removal for PPCPs in a constructed wetland include photolytic degradation, sorption, plant uptake and phytodegradation, and microbial degradation. These are thought to be the most important mechanisms for areas of future research (Zhi and Ji, 2012; Zhang *et al.*, 2014). Many factors affect removal mechanisms in constructed wetlands: DO, oxidation-reduction potential, pH, alkalinity, temperature, seasonality and weather conditions, water quality parameters (NOM, nitrogen compounds, carbon compounds), sediment composition, plant presence, and hydraulic retention time (Davis, 1994; Kadlec and Wallace, 2009; Zhang *et al.*, 2014). Understanding the removal mechanisms at even basic levels is important to optimize constructed wetland designs.

Some PPCPs are resistant to removal by other mechanisms but have molecular structures that are susceptible to direct or indirect photolysis. PPCP compounds with conjugated ring structures are highly susceptible to photolysis (Bear *et al.*, 2017). The effects of photolysis are more effective when solar radiation is more intense and has increased duration (Zhang *et al.*, 2018), such as summer seasons, and are less effective in shaded areas and as water depth increases (Zhang *et al.*, 2014).

Properties of PPCPs such as hydrophobicity, log K_{OW}, and the organic carbon partition coefficient, log Koc, can be used to predict sorption (Lindberg *et al.,* 2006; Verlicchi *et al.*, 2012; Zhang *et al*, 2014). Higher levels of log Kow and log Koc result in more sorption of PPCPs and lower levels of low Kow and low Koc result in increased mobility in the water column and sediment. Sorption can also be influenced by electrostatic interactions between negatively or positively charged PPCPs and negative or positive charges in the sediment (Kadlec and Wallace, 2009).

PPCP hydrophobicity is also considered a predominant driver in plant uptake and phytodegradation because plant cells do not contain specific transporters for PPCPs (Lv *et al.,* 2016; Stottmeister *et al.,* 2003). PPCPs with a log Kow range of 0.27 – 3.9 are ideal for plant uptake via passive diffusion (Lv *et al.*, 2016); these PPCPs are thought to be lipophilic enough to pass through the cell membrane of plant cells and hydrophilic enough to enter the cellular matrix. After plant uptake, PPCPs can be stored or translocated and then, two types of metabolism may occur: 1) transformation via oxidations, reductions, and hydrolysis and 2) conjugation via addition of glutathione, sugars, and amino acids (Stottmeister *et al.*, 2003).

Constructed wetland microorganisms can degrade PPCPs under aerobic and anaerobic conditions resulting in mineralization and biotransformation of PPCPs (Wu *et al.*, 2012). However, chemical structures of PPCPs, such as the long carbon chains, carbon rings, and functional groups, vary widely even within a particular class of drugs making predicting the availability of PPCPs to microbial degradation difficult (Kümmerer, 2009). Wetland factors such as increased DO and temperature can positively

influence microbial degradation (Zhang *et al.*, 2017; Meng *et al.,* 2014; Faulwetter *et al.*, 2009)

1.2. Study Objectives

This study seeks to determine overall and basin-specific removal rates during eight sampling periods over one year by measuring reductions in PPCP concentrations throughout the wetland. Other objectives include investigating potential correlation between wetland characteristics and removal rates (% reduction of PPCPs). We hypothesize the wetland will have increased removal efficiencies with higher temperatures and increased DO.

2. Methods and Materials

2.1. Study Site

The Sewanee Wetlands is a pilot, surface-flow constructed wetland in Sewanee, TN operated jointly by the Sewanee Utilities District (SUD) and The University of the South. The SUD operates a wastewater treatment plant (WWTP) that receives municipal wastewater from about 687 active sewage accounts in Sewanee (personal communication, Emily Jackson, SUD). The municipal wastewater is initially filtered through a large metal screen where large solids are removed. The resultant wastewater is then pumped into two adjacent parallel lagoons (A and B; Figure 1.1). After 30 days, water from these two parallel lagoons is transferred to a third lagoon (C), where it remains for an additional 15 days. Finally, the wastewater from Lagoon C is transferred to a disinfection system, where it is chlorinated and then sprayed onto fields planted with

trees, shrubs, and other forest vegetation. Lagoons A, B, and C have a total surface area of approximately 60,000 m^2 (~20,000 m^2 each) and the wetland comprised of three basins of varying size, is 1800 – 1900 m^2 (personal communication, Deborah McGrath, University of the South).

Partially treated wastewater from Lagoon C in the WWTP is diverted to a 24,600-liter supply tank for storage and regulating wetland flow and hydraulic retention time (HRT). Water from the supply tank flows by gravity via an Inlet Pipe at approximately 100 L/min over a small section of rocks (1-m^2) to promote oxygenation as the water enters Basin 1, the first of three wetland basins (Figure 1.1). The first section of Basin 1 is approximately 1-m deep to prevent plant growth and 115-m^2 in surface area, and was designed to provide a photolysis zone where PPCPs can be degraded by solar radiation. The rest of Basin 1 is approximately 950-m^2 and contains soft-stem bulrush (*Schoenoplectus tabernaemontani,* Sedges) to aid in removal of excess nutrients. Wastewater then flows by gravity into Basin 2 (~380-m^2), which is designed to be a photolysis zone with nine small, planted mounds rising above the water surface. Designed to prevent erosion, mitigate short-circuiting (a preferred flow-path through the water column, resulting in reduced removal efficiencies), and enhance esthetics, the nine mounds contain boneset (*Eupatorium perfoliatum,* Daisy), blue flag iris (*Iris versicolor,* Iris), rose mallow (*Hibiscus moscheutos,* Mallows), and swamp milkweed (*Asclepias incarnata,* Dogbanes). From Basin 2, wastewater flows by gravity into Basin 3 (~400 m^2 total). The first part of Basin 3 (~200 m^2) contains pickerelweed (*Pontederia cordata,* Pontederiaceae) for removal of excess nutrients. The second part of Basin 3 was designed to be another unplanted photolysis zone. From Basin 3, the wastewater flows back into

Lagoon C (Figure 1.1), as the wetland is not permitted for release of treated wastewater. Currently, all photolysis zones in Basins 1, 2 and 3 are vegetated with duckweed (*Lemna lemnoideae*, Araceae), providing an unplanned third wetland plant that may impede intended PPCP photolysis.

2.2. Wastewater Sampling

Wastewater samples from the wetland and Lagoon C were sampled every 6 weeks (± 1 week) from October 2017 to September 2018 for a total of eight sample periods. At each sampling period, triplicate 1000-mL samples of wastewater were collected in 1-L amber glass jars from the outflow of five sites: Lagoon C, Inlet Pipe, Basin 1, Basin 2, and Basin 3 (Figure 1.1). Triplicate field blanks (1000-mL Milli-Q water) were collected for each sampling period and served as controls for the analysis. Samples were passed through a 500-μm pore stainless steel sieve to remove plant materials and suspended particles. The sieve was rinsed with deionized (DI) water after each sample. In the field, caffeine-D3 and diphenhydramine-D5 (deuterated pharmaceutical compounds) were added to each sample and field blank as an internal standard (IS) for the LCMS/MS analyses (final IS concentration = 500 ng/L). Samples were stored in the collection jars on ice during transportation to the University of Georgia, Aquatic Toxicology Lab where samples were stored at 4 °C. Sample processing occurred within 7 days of field sampling. At each sampling session, pH, temperature (°C), dissolved oxygen (DO, mg/L), and conductivity (μS/cm) were determined at each sample location. Temperature, DO, and conductivity were measured with a YSI® PRO 2030 (YSI, Yellow Springs, OH); pH was measured with an Oakton® pHTestr (Oakton, Vernon Hills, IL).

2.4. Laboratory Sample Processing

Water samples were brought to room temperature prior to sample cleanup. Samples were vacuum filtered sequentially with 6 micron and 1.2 micron glass fiber filters to remove suspended particles. Target analytes were extracted by passing the pre-filtered1000-mL sample drop-wise at a rate of 10-mL/min through an Oasis HLB cartridge (6cc; 200 mg; Waters Corp., Milford, MA), which was preconditioned with 3 mL of HPLC-grade methanol followed by 3 mL of HPLC-grade water. Each HLB cartridge was eluted with 6-mL of HPLC-grade methanol. A slow stream of nitrogen gas was used to blow the methanol to near dryness, and sample residue was reconstituted in 1-mL of 10% acetonitrile. Samples were stored at 4°C until LCMS/MS analysis.

2.5. LCMS/MS Analysis

Reconstituted water samples were analyzed with an Accela HPLC coupled with a TSQ Quantum Ultra mass spectrometer (Thermo Scientific, Bellefonte, PA). At a flow rate of 200 μL/min, a gradient elution method was performed with a Kinetex 3 um C18 HPLC column (150 x 2.1 mm, Phenomenex, Torrance, CA) to achieve chromatographic separation. Samples were injected at a volume of 20 μL into initial mobile phase conditions composed of 95% water with 0.1% formic acid (mobile phase A) and 5% acetonitrile with 0.1% formic acid (mobile phase B). Initial mobile phase conditions were held for two minutes, ramped to 95% mobile phase B over 26 minutes, and held for four minutes. Initial mobile phase conditions were re-equilibrated for 15 minutes before the next sample injection, for an approximate 45 minute total run time. The SRM transitions (m/z) values and collision energy (V) for each analyte were: 267.2 to 145 for atenolol (23

V), 152.1 to 110 for acetaminophen (16 V), 195 to 138 for caffeine (18 V), 234 to 84 for methylphenidate (27 V), 260 to 183 for propranolol (17 V), 256 to 167 for diphenhydramine (10 V), 237 to 194 for carbamazepine (19 V), 310 to 148 for fluoxetine (8 V), 306 to 159 for sertraline 16 V), 192 to 119 for DEET (16 V), 299 to 109 for norethindrone (26 V), 436 to 235 for valsartan (30 V), 313 to 245.3 for norgestrel (16 V), and 345 to 123 for medroxyprogesterone (22 V).

2.6. Data Analysis

Mean concentrations of each PPCP were calculated from triplicate sample data and were used for all statistical analyses. The means of each sample were used to calculate PPCP removal rates (%) for each wetland cell or sample site. Total PPCP removal rates were calculated by summing the concentrations of each PPCP for each sample site and then calculating percent removal between sites.

All statistical analyses were performed using R (version 3.5.2). Data sets were tested for equal variances (Levene's Test). Linear mixed-effects (LME) models were made to test for significance in PPCP concentrations between sample sites with a random effects term (month) and included combinations of the following variables to identify potential water quality influences on PPCP concentrations: pH, DO, conductivity, and temperature. Using the second-order Akaike's information criterion AIC function, an AIC table was made to assess LME models for best-fit. The best-fit LME model residuals were tested for normality (QQnorm and QQplot) and a significance level was determined ($p < 0.05$). When required, a multiple comparisons test, Tukeys HSD, was also performed to determine which sample sites were significantly different.

3. Results and Discussion

3.1. Water Quality Parameters

Mean water quality parameters by sample site for cold versus warm sampling months showed a significant difference for temperature (W = 375, p-value = .0000002) and DO (t = -5.0942, df = 20.748, p-value = .00005) (Figure 2.1). December, February, and March were the three coldest sampling periods with a mean temperature of 8.51 ± 1.48 °C. October, May, July, August, and September were the five warmest sampling periods with a mean temperature of 22.65 ± 0.74 °C. Mean DO ranged from 2.76 ± 1.70 mg/L (Basin 1) to 9.35 ± 4.05 mg/L (Lagoon C) during cold sampling periods and 1.41 ± 0.60 mg/L (Inlet Pipe) to 3.19 ± 1.91 mg/L (Lagoon C) during warm sampling periods. Increased DO during colder months compared to warmer months are likely due to the increased solubility of oxygen in water at colder temperatures, decreased aerobic microbial activity, therefore, decreased consumption of DO, and the absence of duckweed on the surface of the water. During warmer months duckweed formed a thick mat covering the surfaces of the wetland water column, but throughout the colder months, duckweed senesced along with all other plants in the wetland, exposing the wetland's surface to increased oxygen exchange. Higher DO levels in Lagoon C can be explained because Lagoon C was never completely covered by duckweed during cold and warm sampling periods and varying wind directions changed which portions of it were covered throughout each day. Among sampling periods, mean pH ranged from 6.59 ± 0.08 (Basin 1) to 7.64 ± 0.52 (Lagoon C) for cold sampling periods and 6.59 ± 0.21 (Inlet Pipe) to 7.25 ± 0.91 (Lagoon C) for warm sampling periods, indicating that the wetland is slightly acidic to neutral throughout the year (Figure 2.1). Mean conductivity ranged from

192.55 uS/cm (Basin 3) to 266.85 uS/cm (Basin 1) during cold sampling periods and 188.82 ± 75.8 uS/cm (Basin 2) to 247.42 ± 71.37 uS/cm (Inlet Pipe) during warm sampling periods. Standard deviations for Basin 1 and Basin 3 conductivity values during cold sampling periods could not be calculated due to instrument error in the field during the February sampling period. Conductivity varied greatly during all sampling periods and varied moderately among sample sites (Figure 2.1).

3.3. Occurrence of PPCPs

Generally, caffeine, valsartan, diphenhydramine, DEET, and atenolol were the most abundant PPCPs in all five sample sites (Table 2.1), with caffeine having mean concentrations ranging from 3 to 1000-fold greater than any other PPCP. Although influent caffeine concentrations were highest among all PPCPs studied in this wetland (mean = 1640.39 ± 476.40 ng/L), caffeine concentrations were lower than seen in other studies. Vymazal *et al.* (2017) reported caffeine concentrations from 2,900 to 83,000 ng/L in wastewater effluent feeding four separate horizontal subsurface-flow wetlands. Hijosa-Valsero *et al.* (2016) reported mean caffeine concentrations of 19,200 ± 1,430 ng/L from raw wastewater. However, these studies used primary wastewater or raw wastewater to feed the wetlands and the Sewanee Wetlands receives secondary wastewater. The lower concentrations in the Sewanee Wetlands potentially indicates that the WWTP is able to remove significant amounts of caffeine before wastewater enters the wetland, however, we do not know the concentrations of PPCPs in the WWTP prior to the effluent of Lagoon C. Alternatively, methylphenidate, propranolol, carbamazepine, medroxyprogesterone were only detected at very low concentrations in wetland influent

(mean concentrations < 2 ng/L) and acetaminophen, fluoxetine, sertraline, norethindrone and norgestrel were found at low concentrations in wetland influent (mean concentrations 2 – 20 ng/L). For these nine PPCPs with mean concentrations < 20 ng/L, the wetland did not have consistent removal efficiencies and in many cases the PPCP concentrations increased throughout the wetland leading to an overall negative removal rate.

3.4. Individual PPCP Removal Rates

The constructed wetland removed varying amounts of PPCPs with mean removal rates ranging from -64.52 ± 91.06% (acetaminophen) to 88.71 ± 12.51% (caffeine) (Table 2.2). A negative removal rate indicates an increase in PPCP concentration as the wastewater passed through the wetland and a 0.00% removal rate indicates equal PPCP concentrations throughout the wetland (Table 2.2). When a PPCP's concentration was undetectable above the limit of detection (LOD), the value of the LOD was given in place of the PPCP concentration as a conservative value (Table 2.3). Removal of 0.00% of an analyte is likely an artifact caused by PPCP concentrations measured at or below above the LOD (Croghan *et al.*, 2003).

Removal rates (%) for each PPCP varied between sampling periods with some PPCPs removed at greater rates than others (Table 2.2). The wetland most effectively removed atenolol, caffeine, diphenhydramine, DEET, and valsartan. Concentrations of methylphenidate, propranolol, carbamazepine, and medroxyprogesterone concentrations were more or less unchanged after passing through the constructed wetland. The PPCPs with minimal to negative removal rates also displayed the lowest initial wetland concentrations or were not detected above the LOD.

The five most abundant PPCPs in the wetland also showed some of the highest removal rates in varying sampling periods. This finding is in contrast to Matamoros *et al.* (2016), who found a negative correlation between PPCP concentration and removal rate (%) showing that higher influent concentrations led to lower removal rates in a horizontal-flow constructed wetland. Llorens *et al.* (2009) found increased removal rates with lower concentrations of PPCPs in a tertiary surface-flow constructed wetland. Of all the PPCPs detected in the current study, caffeine was removed from the Sewanee Wetlands with the highest efficiency (mean = 88.71 ± 12.51%), similar to removal rates found in a lab-scale constructed wetland (98%) (Li *et al.*, 2017) and a surface-flow constructed wetland (90%) (Sgroi *et al.*, 2018). Caffeine was removed at rates greater than 85% during October, December, February, March, August, and September; removal rates were lower in May (67.16%) and July (71.07%). The wetland significantly removed caffeine ($p = 0.0253$) among all sampling periods (n=8).

Over all sampling periods atenolol was removed with a mean of -29.41 ± 281.54%; however, removal rates were greater than 50% for all sampling periods except in February (-725.73%), which skewed the mean removal to an overall negative rate with high error. Atenolol was significantly removed ($p = 0.0002$). Upon excluding the highly negative removal rate in February, the wetland removed 70.06 ± 11.11% during the rest of the study. Bear *et al.* (2017) found removal rates greater than 70% for atenolol in a surface-flow constructed wetland and attributed 70 – 90% of the removal to microbial degradation. The negative removal rate of atenolol in February is likely due to the lack of microbial degradation coinciding with the lowest recorded temperatures in the wetland (Figure 2.1).

Diphenhydramine was removed at moderate removal rates with a mean of 46.16 ± 73.02%. Similar to atenolol, diphenhydramine was removed at rates greater than 60% for all sampling periods except one, August (-133.44%). Gorito *et al.* (2018) found approximately 70% removal of diphenhydramine in a subsurface-flow microcosm wetland and Pi *et al.* (2017) found plant uptake to be an effective removal mechanism for diphenhydramine, especially under basic pH conditions. The pH of the Sewanee Wetlands, however, was slightly acidic. Diphenhydramine has been found to be recalcitrant to microbial degradation (Wu *et al.*, 2010), indicating other removal mechanisms, such as phytoremediation and sorption, are integral processes for its removal (Matamoros and Bayona, 2006).

Among all sampling periods, valsartan concentrations experienced highly variable removal, with rates ranging from -79.16% (October) to 82.23% (February) (Table 2.2). Five sampling periods had removal rates greater than 50%, while May had a 16.45% removal rate. Valsartan was the second most abundant PPCP studied with a mean concentration of 502.32 ± 290.17 ng/L in LC effluent (Table 2.1). There is a lack of data on constructed wetland removal of valsartan, a common antihypertensive drug, but Auvinen *et al.* (2017) found that increased dissolved oxygen leads to significantly higher removal rates of valsartan, 68% to 99%. Valsartan was removed consistently in cold sampling periods with increased DO but July and September also showed high removal rates when DO levels were relatively lower compared to cold sampling periods (Table 2.2).

DEET was significantly removed ($p = 0.0367$) by the wetland during all sampling periods (mean = 20.80 ± 45.33%). Removal rates were moderate and similar in October,

December, February, March, and September; ranging from 34.93% to 42.67%. The greatest removal of DEET occurred in May (61.40%) and the least in August (-81.76%). Sgroi *et al.* (2018) reported up to 63% removal rates for DEET which is similar to our findings, however, negative removal rates occurred in July and August. Similar to valsartan, DEET has been found to have higher removal rates with higher levels of DO (Sgroi *et al.*, 2018). However, DEET removal rates in cold sampling periods were not the highest observed removal rates (Table 2.2).

3.5. Total PPCP Removal Rates

The constructed wetland successfully removed 73.32 ± 15.42% (mean ± SD, n=8) of Total PPCPs from the treated wastewater. Total PPCP represents the cumulative concentrations of all PPCPs studied and is a measure to evaluate efficacy of treatment systems and occurrence of PPCPs in water sources (Padhye *et al.*, 2014). During cold sampling periods (n=3), the wetland removed 84.07 ± 10.35% of Total PPCPs and 66.88 ± 12.56% during warm sampling periods (n=5). Mean Total PPCP concentrations (n=8) ranged from a high of 2479.55 ± 601.94 ng/L in lagoon C to 583.83 ± 177.69 ng/L in Basin 3 (Table 2.1). The largest portion of Total PPCP concentrations were attributed to caffeine concentrations. Overall, Total PPCP removal rates were lowest in August (47.22%) and highest in February (94.02%), followed by December (88.39%; Figure 2.2). During the December and February sampling periods, soft-stem bulrush and pickerelweed were dormant and apparently unable to sequester PPCPs. Duckweed was also dormant and mostly absent, potentially resulting in higher DO levels and increased photo-degradation of PPCPs in photolysis zones. As discussed above, increased DO can

be caused by increased solubility of oxygen in water at colder temperatures, decreased aerobic microbial activity, and the absence of duckweed on the surface of the water. Increased levels of DO benefit aerobic microorganisms in the removal of ibuprofen, gemfibrozil, and DEET (Conkle *et al.*, 2012). Additionally, ketoprofen and diclofenac are removed more efficiently by photolysis (Zhang *et al.*, 2018). While increased DO levels during colder sampling periods provide opportunities for increased aerobic microbial activity, the lower temperatures may actually decrease microbial activity and cause substrate biofilms to degrade (Reyes-Contreras *et al.*, 2012). Among all eight sampling periods, the wetland significantly reduced the concentration of Total PPCP in the wetland wastewater ($p = 0.0154$).

Further analysis using LME, did not show significant differences between Total PPCP concentrations in Basin 1, Basin 2, and Basin 3; therefore, basins did not have significantly different removal rates. However, mean Total PPCP concentrations in Basin 1 versus Lagoon C approached significance ($p = 0.069$), potentially indicating a greater removal rate in Basin 1 compared to Basin 2 and Basin 3. This makes sense because Basin 1 is the first basin and receives higher concentrations of PPCPs, on average, than Basin 2 and 3. Water quality parameters were not found to have significant effects on Total PPCP removal. However, effects of increased DO on removal of Total PPCP approached significance ($p = 0.0805$).

3.6. Site-Specific Removal Rates and Effects of Water Quality Parameters

Ten PPCP data sets met the assumptions for LME analyses in R: atenolol, acetaminophen, caffeine, methylphenidate, propranolol, diphenhydramine, carbamazepine, DEET, valsartan, and norgestrel. However, the analyses revealed that

mean concentrations of Total PPCPs in Basin 1, Basin 2, and Basin 3 were not significantly different and therefore, removal rates were not significantly different among the basins. Figures 2.3.1, 2.3.2, and 2.3.3 show the removal rates (%) by site for atenolol, caffeine, and DEET for all eight sampling periods.

Atenolol ($p = 0.0002$), caffeine ($p = 0.0253$), and DEET ($p = 0.0367$) were found to be significantly removed by the wetland overall (Lagoon C compared to Basin 3) and further analyses showed DO significantly influenced removal of atenolol ($p = 0.0441$). DO effects on removal of caffeine approached significance ($p = 0.0934$). Removal of DEET was not significantly influenced by DO ($p = 0.4483$). Contradictory to our findings, Sgroi *et al.* (2018) and Conkle *et al.* (2012) found increased removal of DEET with increased DO. No other water quality parameters showed significant effects on PPCP removal rates.

3.7. Future Studies

Petrie et al. (2015) highlighted the lack of TSS analyses in wetlands and brought attention to the potential of PPCP sorption to TSS. When TSS is not analyzed for PPCP concentrations, pertinent data could be missed relating to PPCP removal in wetlands and efficiencies for removal, as PPCPs may pass through a wetland unchanged and unrecorded. Research and analyses of TSS could provide beneficial information about PPCP removal efficiencies.

Multiple research approaches are needed to gather a full understanding of factors impacting wetland efficiency. Seasonal and temperature influences, effects of retention time, and plant growth rates are areas of future investigation. In addition, the effects

duckweed has on DO and photolysis inhibition and whether removing duckweed with a wastewater-tolerant, herbivorous fish species may be beneficial to constructed wetlands are key areas on which to focus future research into the efficacy of constructed wetlands. Also, using non-targeted LCMS analyses would be beneficial for detecting a wider range of analytes which would include metabolites and parent compounds. This study only examined 14 parent compounds and many more compounds may be present as well as metabolites of these compounds.

The SUD has the ability to control retention time of water through the wetland. A slower flow rate may allow many PPCP removal processes to achieve a higher removal rates. Llorens *et al.* (2009) found longer retention times resulted in moderate to highly efficient removal rates of PPCPs except carbamazepine and clofibric acid, two recalcitrant PPCPs. However, even these recalcitrant PPCPs were removed at greater than 30% efficiency. Determining the ideal balance between retention time and higher volumes of treated wastewater will increase the wetland's output efficiency. The Sewanee Wetlands is currently a pilot wetland; increased capacity of a full-scale wetland will allow for greater volumes to be treated and longer retention times to increase removal efficiencies. As future inquires and studies into the efficacy of constructed wetlands continue, desired design features necessary for maximum PPCP removal may become evident.

4. Acknowledgements

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collaborative research on the wetland. We also thank Devon Boullion and Donovan Godbee (UGA) for contributions to sample preparation and David Brew (UGA) for training and assistance in sample analysis.

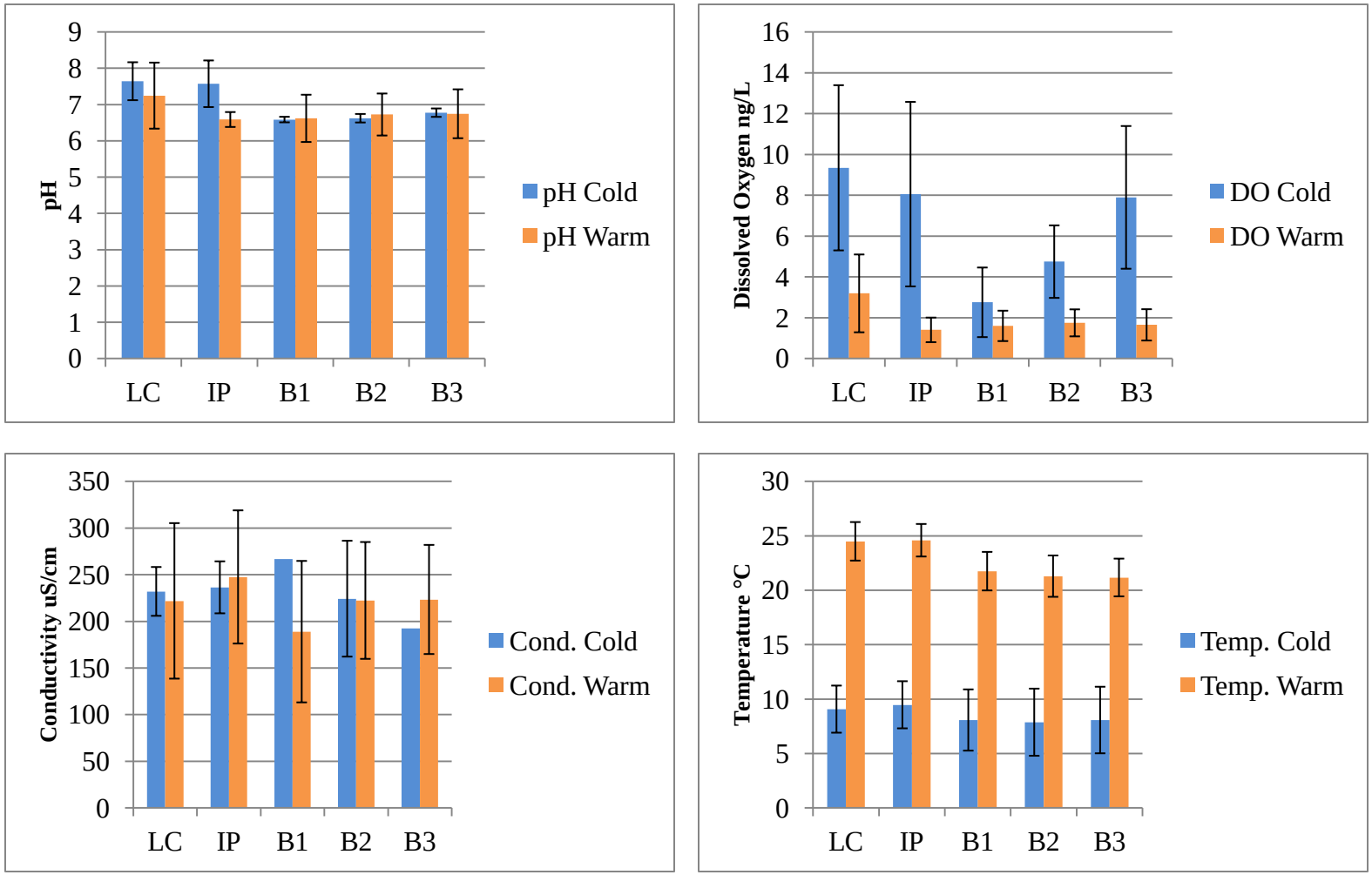

Figure 2.1. Average water quality data (± SD) by sample site for cold (n=3) versus warm (n=5) sampling periods. pH (Top Left), Dissolved Oxygen (DO; ng/L; Top Right), Conductivity (Cond.; uS/cm; Bottom Left), Temperature (Temp.; °C; Bottom Right). Sewanee Wetlands, Sewanee, TN. Lagoon C (LC), Inlet Pipe (IP), Basin 1 (B1), Basin 2 (B2), and Basin 3 (B3).

Table 2.1. Mean PPCP Concentrations ± SD (ng/L) for Lagoon C (LC), Inlet Pipe (IP), Basin 1 (B1), Basin 2 (B2), and Basin 3 (B3) for eight sampling periods (October 2017 - September 2018).

	Mean PPCP Concentrations ± SD (ng/L)				
	LC	**IP**	**B1**	**B2**	**B3**
Atenolol	20.49 ± 4.74	22.81 ± 4.69	10.45 ± 2.44	13.01 ± 5.24	10.44 ± 3.57
Acetaminophen	3.38 ± 0.83	3.50 ± 0.97	4.17 ± 0.94	4.99 ± 1.22	4.42 ± 0.92
Caffeine	1640.39 ± 476.40	1346.11 ± 294.59	360.72 ± 180.97	193.05 ± 93.94	140.73 ± 59.21
Methylphenidate	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
Propranolol	0.62 ± 0.19	0.77 ± 0.26	0.44 ± 0.08	0.46 ± 0.04	0.45 ± 0.04
Diphenhydramine	158.72 ± 57.84	144.11 ± 45.53	96.94 ± 42.72	84.11 ± 43.27	62.80 ± 22.39
Carbamazepine	0.91 ± 0.22	0.95 ± 0.23	1.06 ± 0.22	0.94 ± 0.24	0.97 ± 0.26
Fluoxetine	6.91 ± 2.31	7.17 ± 2.35	6.42 ± 2.09	5.60 ± 1.72	7.27 ± 3.98
Sertraline	5.90 ± 2.09	6.07 ± 2.74	3.47 ± 1.13	5.85 ± 2.38	3.61 ± 1.18
DEET	122.47 ± 12.57	109.73 ± 16.41	100.57 ± 10.32	90.26 ± 11.26	87.05 ± 10.43
Norethindrone	6.00 ± 2.47	6.62 ± 2.94	6.19 ± 2.65	5.95 ± 2.48	6.06 ± 2.45
Valsartan	502.32 ± 290.17	393.96 ± 183.65	241.79 ± 94.26	192.90 ± 73.14	248.09 ± 117.65
Norgestrel	9.86 ± 4.41	9.59 ± 4.19	14.29 ± 6.99	9.64 ± 4.17	9.79 ± 4.33
Medroxyprogesterone	1.56 ± 0.66	1.59 ± 0.74	1.08 ± 0.48	1.81 ± 0.86	1.95 ± 1.03
Total PPCP	2479.55 ± 601.94	2052.93 ± 441.92	847.80 ± 256.30	608.92 ± 166.78	583.83 ± 177.69

Table 2.2. Removal rates (% ± SD) of individual PPCPs and Total PPCP by the Sewanee Wetlands at each sampling period (October 2017 - September 2018).

Month	Atenolol	Acetaminophen	Caffeine	Methylphenidate	Propranolol	Diphenhydramine	Carbamazepine	Fluoxetine	Sertraline	DEET	Norethindrone	Valsartan	Norgestrel	Medroxyprogesterone	Total PPCP
Oct	69.63	0.00	97.09	0.00	0.00	82.55	0.00	2.11	15.80	40.54	0.00	-79.16	11.35	35.10	85.94
Dec	51.45	-1.60	98.09	0.00	0.00	80.25	0.00	3.46	29.93	36.48	0.00	67.23	-8.78	-164.34	88.39
Feb	-725.73	0.00	98.04	0.00	0.00	72.99	-12.99	N/A	0.00	34.93	0.00	82.23	0.00	-9.26	94.02
Mar	64.75	0.00	88.50	-31.78	54.18	77.98	0.83	100.00	43.96	36.79	0.00	59.17	-154.70	39.98	69.80
May	87.13	-199.66	67.16	0.00	15.98	64.01	-9.98	100.00	56.17	61.40	0.00	16.45	46.08	0.00	62.80
July	68.23	-215.68	71.07	43.67	-3.64	61.94	34.04	68.87	48.24	-4.66	12.89	68.91	0.00	0.00	66.79
Aug	78.45	-53.65	95.01	0.00	-32.52	-133.44	-19.55	81.83	17.81	-81.76	-138.47	-62.55	-105.79	0.00	47.22
Sept	70.78	-45.55	94.69	-30.27	-43.36	63.00	-17.98	-242.43	51.33	42.67	-6.16	50.38	2.05	90.74	71.63
Average	-29.41	-64.52	88.71	-2.30	-1.17	46.16	-3.20	16.26	32.90	20.80	-16.47	25.33	-26.22	-0.97	73.32
SD	281.54	91.06	12.51	23.27	29.59	73.02	17.12	121.37	20.18	45.33	49.58	62.55	67.57	73.83	15.42

Table 2.3. Limit of detection (LOD) values for each LCMS/MS analysis of pharmaceutical and personal care product concentrations from wastewater in the Sewanee Wetlands, Sewanee, TN (October 2017 – September 2018).

PPCP	LOD values (ng/L)			
	Oct., Dec., Feb.	Mar. and May	July and Aug.	Sept.
Atenolol	0.501	0.284	0.045	0.045
Acetaminophen	5.233	0.200	1.834	1.834
Caffeine	6.807	0.429	2.551	2.551
Methylphenidate	0.078	0.023	0.022	0.022
Propranolol	0.369	0.004	0.157	0.156
Diphenhydramine	0.486	0.058	0.334	0.334
Carbamazepine	0.053	0.031	0.051	0.051
Fluoxetine	7.032	0.000	0.369	0.369
Sertraline	0.560	0.301	0.433	0.433
DEET	0.158	0.526	0.241	0.241
Norethindrone	14.999	0.000	0.080	0.080
Valsartan	1.349	0.016	0.258	0.258
Norgestrel	24.866	0.012	0.221	0.221
Medroxyprogesterone	1.823	0.026	0.200	0.200

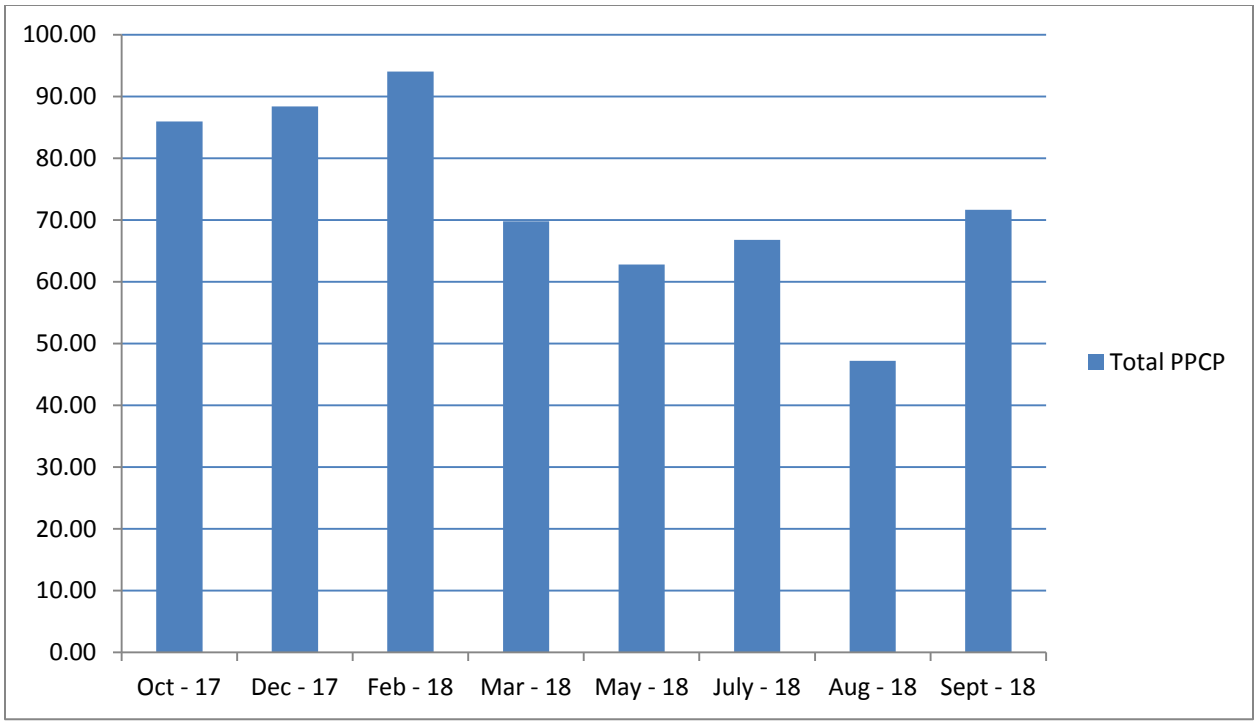

Figure 2.2. Total PPCP removal rates (%) for eight sampling periods (October 2017 - September 2018) by the Sewanee Wetlands.

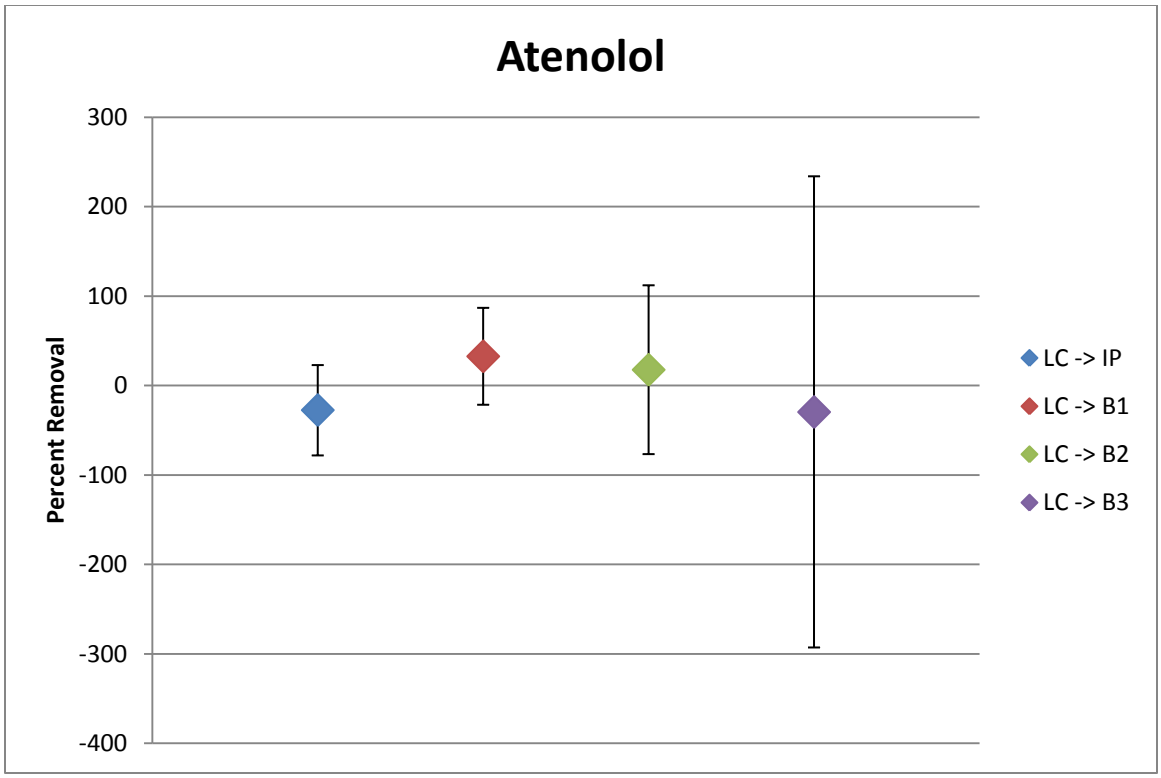

Figure 2.3.1. Mean atenolol removal rates (±SD) by sample site in the Sewanee Wetlands, Sewanee, TN (October 2017 - September 2018, n = 8).[3]

[3] Lagoon C (LC), Inlet Pipe (IP), Basin 1 (B1), Basin 2 (B2), Basin 3 (B3).

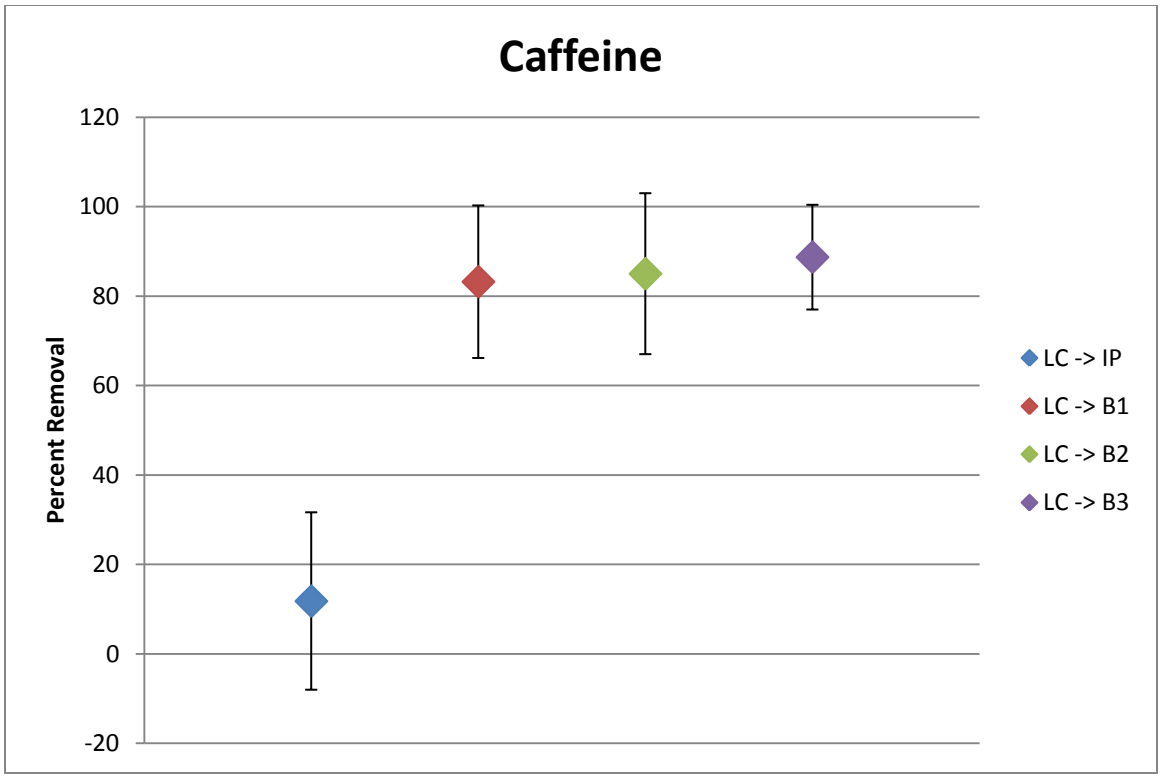

Figure 2.3.2. Mean caffeine removal rates (±SD) by sample site in the Sewanee Wetlands, Sewanee, TN (October 2017 - September 2018, n = 8).[4]

[4] Lagoon C (LC), Inlet Pipe (IP), Basin 1 (B1), Basin 2 (B2), Basin 3 (B3).

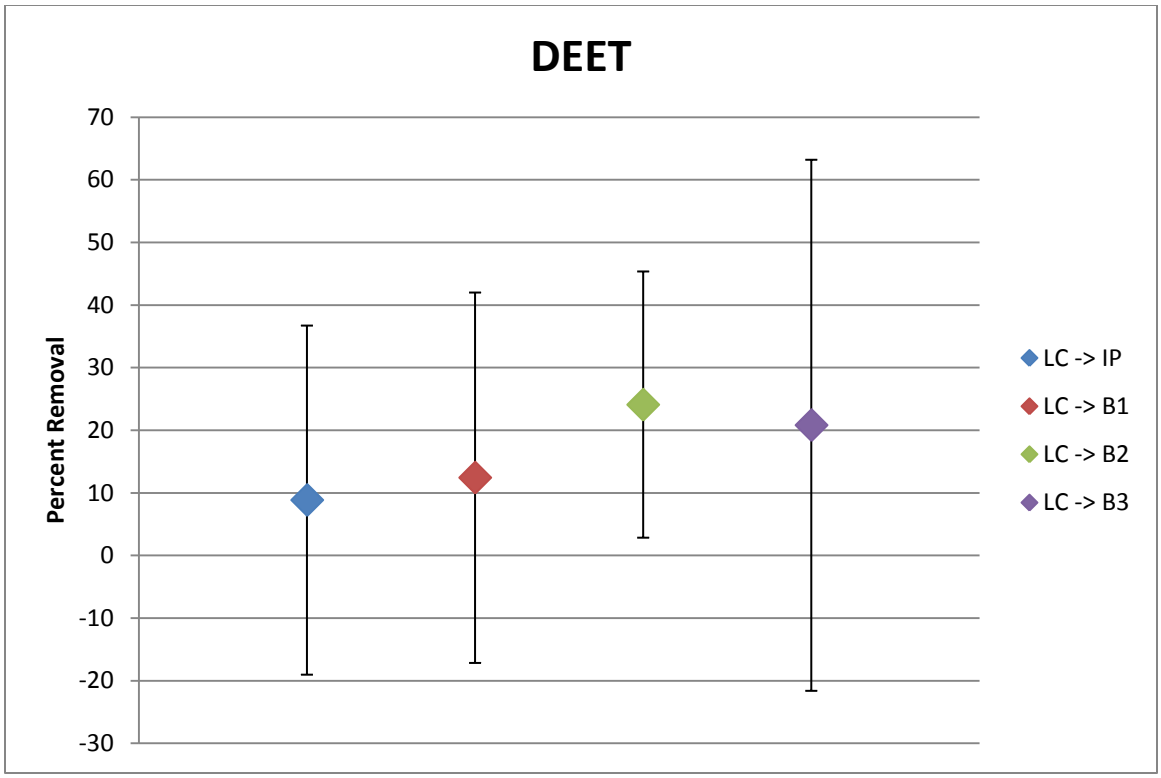

Figure 2.3.3. Mean DEET removal rates (±SD) by sample site in the Sewanee Wetlands, Sewanee, TN (October 2017 - September 2018, n = 8).[5]

[5] Lagoon C (LC), Inlet Pipe (IP), Basin 1 (B1), Basin 2 (B2), Basin 3 (B3).

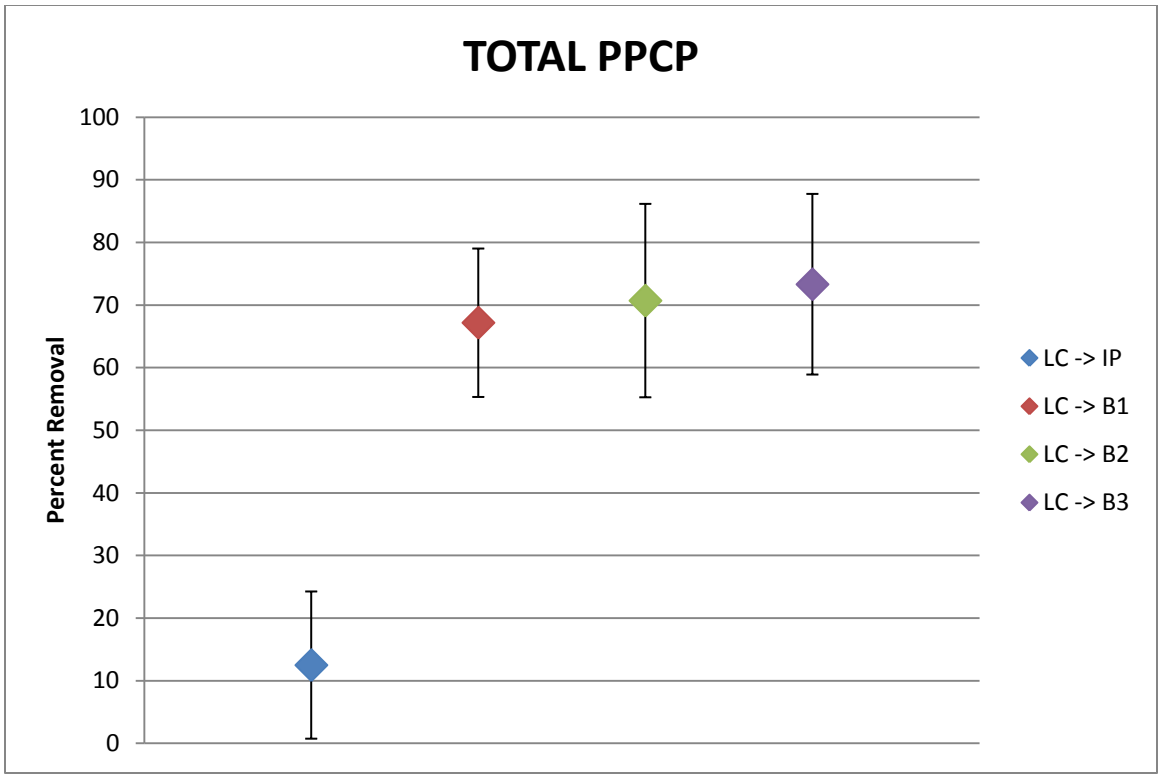

Figure 2.3.4. Mean Total PPCP removal rates (±SD) by sample site in the Sewanee Wetlands, Sewanee, TN (October 2017 - September 2018, n = 8).[6]

[6] Lagoon C (LC), Inlet Pipe (IP), Basin 1 (B1), Basin 2 (B2), Basin 3 (B3).

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CHAPTER 3

SORPTION OF SELECT PHARMACEUTICALS AND PERSONAL CARE PRODUCTS FROM WASTEWATER ONTO NATURAL SEDIMENT[7]

[7] Wright, A.W., Black, M.C., Henderson, W.M., McGrath, D.A., Torreano, S.J., Brew, D.W., and Leach, F. To be submitted to *Water Research*.

Abstract

Pharmaceuticals and personal care products (PPCP) are part of a rapidly growing group of contaminants of emerging concern found throughout water sources in the world. Constructed wetlands have shown potential to be a cost effective tertiary treatment to remove PPCPs from secondary wastewater treatment effluent, however, many of the mechanisms of removal are poorly understood. A mesocosm study was conducted with sediment from the same source used to construct the Sewanee Wetlands and the sediment was exposed to wastewater from the Supply Tank for 12 weeks. The sorption potential of sediment in the Sewanee Wetlands was evaluated to determine which of 14 PPCPs studied underwent sorption and log Koc values were estimated. Seven compounds showed increased concentration in sediment compared to water concentrations after 84 days of exposure to wastewater, indicating sorption: acetaminophen, methylphenidate, propranolol, carbamazepine, norethindrone, norgestrel, and medroxyprogesterone. Norethindrone had the highest sorption (log Koc = 4.22 ± 0.21) after 56 days of exposure and desorption occurred resulting in less sorption after 84 days of exposure (log Koc = 3.56 ± 0.12). While sorption occurred for 7 PPCPs, we interpret these results with caution due to the very low concentrations of the compounds in both sediment and water. Also, potential issues with the extraction method may have reduced the measured sorption.

1. Introduction

Pharmaceuticals and personal care products (PPCPs) are part of a rapidly growing group of contaminants of emerging concern found throughout water sources in the world (Caldwell, 2016; Cizmas *et al.*, 2015; Randhir and Rolf, 2013; Taylor, 2015). PPCPs

include prescription drugs, illegal drugs, antibiotics, hormones, over-the-counter medications, and veterinary medicines, which remain bioactive in multiple species (Cizmas *et al.*, 2015), and are of particular concern because of the potential to negatively affect human health and the environment (USEPA, 2018). Wastewater treatment processes do not effectively or efficiently remove PPCPs from wastewater before discharging treated wastewater into the environment, resulting in low levels of PPCPs (ng/L – μg/L) found in water sources around the world (Caldwell, 2015; Cizmas *et al.*, 2015). Constructed wetlands have been proposed as a tertiary treatment that is cost effective to implement, maintain, and operate (Davis, 1994; Jasper *et al.*, 2013). Designing a constructed wetland with the highest removal efficiencies will require a deeper understanding of the removal mechanisms. Sorption, in particular, is potentially a significant removal mechanism by changing the fate of PPCPs in an aqueous environment.

The Sewanee Wetlands is a pilot, surface-flow constructed wetland in Sewanee, TN operated jointly by the Sewanee Utilities District (SUD) and The University of the South. The SUD operates a wastewater treatment plant (WWTP) that treats municipal wastewater with three lagoons; A, B, and C. A portion of partially treated water is diverted to a 24,600-liter supply tank, which releases wastewater to the wetland at a rate of approximately 100 L/min. The wetland is designed with three basins that have photolysis zones, plants, microbial activity, and sorption processes to aid in PPCP removal.

1.1. Sorption - Removal Mechanism

Once wastewater enters a constructed wetland, PPCPs can transfer from contaminated water to sediment, total suspended solids (TSS), and other organic materials. The action of PPCPs binding to a sorbent, typically found in sediment, is called sorption. Sorbents in freshwater environments include soil and other particulate organic carbon materials, mineral surfaces, biofilms, plant cell walls, suspended solids, and bacterial cell walls (Kadlec and Wallace, 2009; Verlicchi *et al.*, 2012). Also, natural organic materials (NOM) can significantly affect sorption of PPCPs and are present at higher concentrations relative to PPCP concentrations (Choi *et al.*, 2007). Humic acids (HA), a major component of NOM, can act as a sorbent for PPCPs, promoting removal of PPCPs from wastewater. However, HA can compete for binding sites on other sorbents and negatively affect sorption of PPCPs in wastewater (Kyzas *et al.*, 2017; Liu *et al.*, 2014).

Unlike most other mechanisms of removal, sorption does not usually degrade compounds and instead creates a sink, preventing compounds from further transportation (Kümmerer, 2009). Sorption is influenced by characteristics of the compound (i.e. log Kow, log Koc, stereo chemistry, water solubility, and acid/base properties) and the sorbent (i.e. oxidation-reduction potential, pH, temperature, and composition of cations and anions) (Kümmerer, 2009). Of these characteristics, hydrophobicity, measured by log Kow, and the organic carbon partition coefficient, log Koc, are key predictors of which compounds are likely to sorb. Morissette *et al.* (2015) found that compounds with a log Kow below 3.0 had negligible sorption and above 3.0 showed considerable sorption. Higher log Kow and log Koc values result in more concentration of a compound to be

partitioned into sediments compared to the water column. Low log Kow and log Koc values result in less sorption of a PPCP to sorbents. However, only using these characteristics to predict the fate of PPCPs in constructed wetlands is not always reliable (Martinez-Hernandez *et al.*, 2014).

Sorption can also occur due to electrostatic interactions between positively charged groups on the sorbent and negatively charged groups on the PPCP compound, or vice versa (Kadlec and Wallace, 2009). The pH levels in the aquatic matrix surrounding PPCP compounds can influence sorption via competition for binding sites; free hydrogen can interact with charged groups on the sorbent and on the PPCP, altering its potential for sorption. Sorption due to electrostatic interactions also includes anion and cation interactions between positively and negatively charged functional groups on PPCPs and sorbents (Kümmerer, 2009). Additionally, desorption of PPCPs can occur when certain conditions in the wetland change (e.g., pH, oxidation-reduction potential, temperature, and composition of cations and anions), leading to uncertainty of PPCP fate (Martínez-Hernández *et al.*, 2014). Predicting sorption of PPCPs based on pH and electrostatic interactions is highly complex and sorbent and compound dependent (Liu *et al.*, 2014).

1.2. Study Objectives

This study seeks to determine the sorption potential of the sediment in the Sewanee Wetlands by conducting a mesocosm scale experiment to measure PPCP sorption over a 12-week exposure period. We hypothesize that PPCPs with log Kow values greater than 3.0 will sorb more to the mesocosm sediments compared to PPCPs with log Kow values less than 3.0. Total organic carbon in the sediment will greatly

influence sorption. Also, pH and pKa will influence compound-specific sorption of PPCPs.

2. Methods and Materials

2.1. Mesocosm Study Design

A mesocosm experiment was performed on-site of the Sewanee Wetlands in Sewanee, TN from July 3, 2018 through September 25, 2018 with exposures conducted in three replicate 265-liter mesocosms tanks (Rubbermaid, Atlanta, GA). Prior to use, the tanks were thoroughly cleaned and acid washed to remove contaminants. Each tank was plumbed with a flow-through design receiving water from the Supply Tank at a rate of 4.0 L/min (± 5%). A stand pipe was used in each tank to maintain a constant volume of approximately 66-liters in each tank. A canopy tent and bird netting were used to mitigate potential effects of photolysis, dilution by rainfall, radiant heating by the sun, and to prevent pests and wildlife from accessing the tanks. Seventy-two half-pint glass jars with an 8.25-cm opening were filled with 100 grams (± 5%) of homogenized sediment sourced from the same location used to construct the wetland. Each of the 72 sediment samples was given a unique label and 24 samples were randomly placed into each tank to begin the sediment exposure to wastewater from the Supply Tank in the Sewanee Wetlands. Additional sediment was collected for sample blank analyses as a control and for analyses of soil characteristics by the Agricultural Environmental Services Laboratories, College of Agriculture and Environmental Sciences, University of Georgia in Athens, GA.

2.2. Mesocosm Sampling

Using a predetermined, randomized order, 4 sediment samples were taken from each mesocosm tank during 6 sampling periods: 1.5 hours, 3 days, 10 days, 28 days, 56 days, and 84 days of wastewater exposure from the supply tank, providing increasing exposure over a period of 12 weeks. When samples were collected, excess water was carefully drained out of each sample container while preventing loss of sediment. Samples were stored on ice for transportation and frozen at -20 °C within 3 hours of collection. Samples were held at -20 °C until sample processing at the University of Georgia, Aquatic Toxicology Lab.

2.3. Sample Processing

Sediment samples were thawed over a time period of 1 hour and then placed in a 25 °C water bath for 30 minutes to bring to room temperature. Each sample was homogenized via vortexing and manual mixing with a spatula. Thirty grams (wet weight) of sample was weighed out and added to a 50-mL plastic test tube. After 2 minutes of centrifugation at 2500 RPM to settle sample material in the bottom of the test tube, 24-mL of HPLC-grade methanol, as an extraction solvent, and 5-µL of internal standard, containing caffeine-D3 and diphenhydramine-D5 (deuterated pharmaceutical compounds; final dilution = 500 µg/kg), were added to each test tube. Samples were vortexed to suspend the sediment in the extraction solvent and then placed in an ice bath sonicator for 30 minutes. After sonication, samples were centrifuged for 10 minutes at 2500 RPM and then the supernatant was transferred to a glass test tube. Sample supernatant was blown to near dryness under a slow stream of nitrogen and then reconstituted in 20 mL of HPLC-

grade water and vortexed for 10 seconds. Target analytes were extracted by passing the 20 mL reconstituted sample drop-wise at a rate of 10-mL/min through an Oasis HLB cartridge (6cc; 200 mg; Waters Corp., Milford, MA), which was preconditioned with 3 mL of HPLC-grade methanol followed by 3 mL of HPLC-grade water. Each HLB cartridge was eluted with 6-mL of HPLC-grade methanol. A slow stream of nitrogen gas was used to blow the methanol to near dryness, and sample residue was reconstituted in 1 mL of 10% acetonitrile. Samples were stored at 4°C until LCMS/MS analysis.

2.4. LCMS/MS Analysis

Soil extracts were analyzed with an Accela HPLC coupled with a TSQ Quantum Ultra mass spectrometer (Thermo Scientific, Bellefonte, PA). At a flow rate of 200 µL/min, a gradient elution method was performed with a Kinetex 3 um C18 HPLC column (150 x 2.1 mm, Phenomenex, Torrance, CA) to achieve chromatographic separation. Samples were injected at a volume of 20 µL into initial mobile phase conditions composed of 95% water with 0.1% formic acid (mobile phase A) and 5% acetonitrile with 0.1% formic acid (mobile phase B). Initial mobile phase conditions were held for two minutes, ramped to 95% mobile phase B over 26 minutes, and held for four minutes. Initial mobile phase conditions were re-equilibrated for 15 minutes before the next sample injection, for an approximate 45 minute total run time. The SRM transitions (m/z) values and collision energy (V) for each analyte were: 267.2 to 145 for atenolol (23 V), 152.1 to 110 for acetaminophen (16 V), 195 to 138 for caffeine (18 V), 234 to 84 for methylphenidate (27 V), 260 to 183 for propranolol (17 V), 256 to 167 for diphenhydramine (10 V), 237 to 194 for carbamazepine (19 V), 310 to 148 for fluoxetine

(8 V), 306 to 159 for sertraline 16 V), 192 to 119 for DEET (16 V), 299 to 109 for norethindrone (26 V), 436 to 235 for valsartan (30 V), 313 to 245.3 for norgestrel (16 V), and 345 to 123 for medroxyprogesterone (22 V).

2.5. Data Analysis

Mean concentrations of each PPCP were calculated from the 4 replicate sediment sample data for each sampling period from each tank. Using these means, estimates of sorption were determined using the following formulas:

$$Kd(estimated) = PPCP(sediment) / PPCP(water) \quad (1)$$

where Kd(estimated) is the solid/liquid partition coefficient, PPCP(sediment) is the measured concentration of PPCP in the sediment (µg/kg), and PPCP(water) is the measured concentration of PPCP in water (µg/L) from the Inlet Pipe that feeds the constructed wetland and,

$$Koc(estimated) = (Kd(estimated)) / \% TOC \quad (2)$$

where % TOC is the percent total organic carbon from the analyses of soil characteristics. Koc(estimated) was calculated for PPCPs in samples collected at 0.675, 56, and 84 days of exposure. Actual Koc values could not be calculated because wastewater PPCP concentrations were not measured directly in the mesocosms.

3. Results and Discussion

3.1. Soil Characteristics

The type of soil used to construct the Sewanee Wetlands and for use in the sediment mesocosms was slightly acidic and contained a large portion of clay (Table 3.1). Increased clay levels in sediment are capable of sorption of PPCPs at or greater than activated carbon (de Andrade *et al.*, 2018); though, the degree of sorption to clay depends on the pore structure and chemical nature of the clay (Babel and Kurniawan, 2003). Increased surface area on sediment particles results in increased sorption sites for PPCPs. In the slightly acidic sediment, free hydrogens (protons) would exist at the boundary of the sediment and water. PPCPs in this environment with a high pKa (e.g., caffeine, carbamazepine, norethindrone, norgestrel, and medroxyprogesterone) would be characterized as a strong Bronsted-Lowry base and will readily accept protons and become positive in charge (Table 3.2). Protonated PPCPs would be positively charged hydrophobic compounds and hydrophobic interactions would likely increase between PPCPs and sorbent.

3.2. Estimations of PPCP Sorption

PPCP concentrations in the blank samples analyses indicate the sediment used to construct the Sewanee Wetlands and for this study has very low levels of the contaminants investigated in this study (Table 3.3). Of the fourteen PPCPs studied, ten compounds increased in concentration in sediments after 1.5 hours of exposure (blank compared to first exposure interval), and four PPCPs had lower concentrations compared to the blank samples (Table 3.3).

Norethindrone showed an increase in sediment concentrations from 0.6751.5 hours to 84 days of exposure, ranging from 0.007 μg/kg ± 0.004 SD (1.5 hours of exposure) to 5.367 μg/kg ± 0.951 SD (28 days of exposure), and indicating sorption (Figure 3.1). Norethindrone sorption (log Koc) was greatest at 56 days of exposure and least at 1.5 hours of exposure (Table 3.4). Norethindrone showed the highest levels of estimated sorption among all PPCPs studied. Similar to the sorption of norethindrone in this study, Morissette *et al.* (2012) found log Koc values ranging from 1.1 to 2.0 L/kg for different wastewater sludge samples. The slightly acidic pH found in the sediment (mean = 6.03 ± 0.01; n = 3) and the water samples (mean = 6.53 ± 0.14; n = 3) could influence ionization of norethindrone making it positively charged and more hydrophilic, leading to greater sorption effects by potential electrostatic interactions with the sediment or partitioning out of water based on its hydrophobicity.

Many other compounds showed increases in concentration in sediment versus water from 1.5 hours to 84 days of exposure (Figures 3.2 – 3.7): acetaminophen, methylphenidate, propranolol, carbamazepine, norgestrel, and medroxyprogesterone. However, the PPCP concentrations in sediment were very low (less than 1 μg/kg to a few hundred or less ng/kg) and interpreting these values should be taken with caution. Even though concentrations in sediment were low, sorption was observed for six compounds at varying levels at 1.5 hours and 56 and 84 days of exposure: acetaminophen, methylphenidate, propranolol, carbamazepine, norgestrel, and medroxyprogesterone (Table 3.4). These six compounds and norethindrone all have one functional group in common: one or more carbonyl groups (Appendices S.1 – S.14). The carbon double bonded to oxygen in the carbonyl group is positively charged and clay, the main sorbent

in the sediment, is negatively charged. The sorption of these seven PPCPs is likely due to electrostatic interactions and covalent bonding.

Propranolol sorption did not vary much from 1.5 hours (log Koc = 2.21 ± 0.05 L/kg) to 84 days of exposure (log Koc = 2.36 ± 0.03 L/kg), showing rapid sorption and reaching equilibrium between sorption and desorption quickly, as sorption levels stopped increasing. Wang *et al.* (2017) reported propranolol log Koc values ranging from 3.39 to 4.15 L/kg in various types of sorbent with biochar and humic acid, showing much higher sorption than this study. Estimated log Koc values of acetaminophen and carbamazepine in this study compared to log Koc values found in the literature and indicate sorption occurred at slightly higher amounts than other studies. The highest estimation of log Koc = 1.05 ± 0.06 L/kg for acetaminophen in this study was similar to data from Martinez-Hernandez *et al.* (2014), who found a log Koc of 0.8 L/kg. Carbamazepine had an estimated log Koc = 1.32 ± 0.14 L/kg in this study, and Morissette *et al.* (2012) found a log Koc of 1.0 L/kg. Log Koc values from comparable studies for methylphenidate, norgestrel, and medroxyprogesterone could not be found in the literature. Norgestrel and medroxyprogesterone have log Kow values greater than 3.0, a good predictor of sorptive potential (Morissette *et al.*, 2013). Log Koc values of these two PPCPs in the current study ranged from 1.70 to 4.22 L/kg (norgestrel) and 0.98 to 1.84 L/kg (medroxyprogesterone; Table 3.4), indicating moderate to strong sorption.

3.3. Study Limitations

Although some results showed sorption, there were some issues with the methodology of this study that likely reduced the degree of sorption measured. During

the solid-phase extraction of PPCPs from sediment, significant dilution occurred when the entire 100 gram sample was homogenized, but only 30 grams were used for extraction. Sediment in a natural setting would allow permeation of water through the substrate, increasing exposures to PPCPs further into the substrate. In the mesocosm study, sediment was contained in impermeable glass jars that potentially only allowed exposure in the upper-most portion of sediment. Additionally, sediment in a natural setting experiences disturbances from benthic organisms and rainfall that would facilitate movement of water through sediment; these disturbances were not present in the mesocosms because the mesocosm tanks were protected from rainfall and other weather events by a tent and benthic organisms were not present in the sediment samples. Another potential error during extraction could be methanol not properly extracting all PPCPs from the sediment. A mixture of methanol and acetone (3:1) or methanol, acetonitrile, and water (1:1:2) has been proven to successfully extract many of the PPCPs in this study (Morissette *et al.*, 2013; Zhu and Chen, 2014).

4. Conclusion

This study showed potential sorption for seven PPCPs studied but, for most, the concentrations in the sediment were very low compared to the PPCP concentrations in water samples. Norethindrone was present at significantly higher concentrations in the sediment compared to water concentrations and the estimated log Koc of norethindrone is comparable to values in the literature; sorption of norethindrone occurred. Propranolol sorption also resulted in much greater concentrations in sediment compared to water concentrations. Improvements to the extraction method in the study will hopefully yield

more productive results and interpretations about PPCP removal from wastewater via sorption.

5. Acknowledgments

We thank Laurie Fowler of the University of Georgia (UGA), the University of the South-Sewanee and the Sewanee Utilities District for the opportunity to conduct collaborative research on the wetland.

Table 3.1. Soil characteristics of sample blank analyses (n = 3) from locally sourced sediment used for Sewanee Wetlands construction and sediment mesocosms in Sewanee, TN.[8]

		%				
Sample ID	**pH**	**TOC**	**Sand**	**Silt**	**Clay**	**Soil Type**
Replicate 1	6.02	0.33	13.96	47.96	38.08	Silty Clay Loam
Replicate 2	6.03	0.34	13.96	47.96	38.08	Silty Clay Loam
Replicate 3	6.05	0.34	13.96	47.96	38.08	Silty Clay Loam
Average:	6.03	0.33	13.96	47.96	38.08	
SD:	0.01	0.00	0.00	0.00	0.00	

[8] Total organic carbon (TOC).

Table 3.2. Target compounds and characteristics.

Compound		Water Solubility (mg/L; 25 °C)	pKa	Log Kow	MW
Atenolol	ATN	13300.00	9.60	0.16	266.34
Acetaminophen	ACT	14000.00	9.38	0.46	151.17
Caffeine	CAF	21600.00	14.00	-0.07	194.19
Methylphenidate	MPH	1255.00	8.77	0.20	233.31
Propranolol	PRP	61.70	9.42	3.48	259.35
Diphenhydramine	DPH	3060.00	8.98	3.27	255.36
Carbamazepine	CBZ	18.00	13.90	2.45	236.27
Fluoxetine	FLU	50.00	9.80	4.05	309.33
Sertraline	SRT	3.50	9.85	5.10	306.23
DEET	DEET	912.00	-0.95	2.02	191.27
Norethindrone	NOR	7.04	17.59	2.97	298.43
Valsartan	VLS	1.41	3.60	5.80	435.53
Norgestrel	NGL	2.05	17.91	3.48	312.45
Medroxyprogesterone	MED	22.20	17.82	3.50	386.53

Table 3.3. Mean PPCP concentrations ± SD (µg/kg) in sediment (n = 3) following microcosm exposures to wastewater from the Sewanee Wetlands Supply Tank (exposures = blank, 1.5 hours and 3, 7, 28, 56, 84 days).

	Time of Exposure													
	Blank		1.5 Hours		3 Days		7 Days		28 Days		56 Days		84 Days	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
ATN	0.0005	0.0002	0.0025	0.0013	0.0023	0.0002	0.0019	0.0006	0.0039	0.0007	0.0054	0.0006	0.0048	0.0003
ACT	0.0012	0.0005	0.0015	0.0000	0.0015	0.0000	0.0015	0.0000	0.0030	0.0017	0.0069	0.0016	0.0093	0.0012
CAF	0.0059	0.0015	0.4760	0.0298	1.1438	0.1084	0.7824	0.1293	0.6898	0.0863	0.3144	0.0416	0.1159	0.0062
MPH	0.0002	0.0000	0.0007	0.0003	0.0004	0.0000	0.0004	0.0000	0.0003	0.0001	0.0002	0.0000	0.0002	0.0001
PRP	0.0174	0.0030	0.0593	0.0067	0.0314	0.0012	0.0216	0.0034	0.0373	0.0046	0.0336	0.0089	0.0253	0.0018
DPH	0.0014	0.0014	0.0033	0.0000	0.0377	0.0486	0.0033	0.0000	0.0040	0.0010	0.0062	0.0010	0.0099	0.0043
CBZ	0.0005	0.0000	0.0011	0.0002	0.0029	0.0002	0.0021	0.0004	0.0066	0.0006	0.0087	0.0010	0.0074	0.0021
FLU	0.0013	0.0000	0.0135	0.0000	0.0223	0.0012	0.0155	0.0028	0.0342	0.0059	0.0150	0.0085	0.0084	0.0027
SRT	0.0086	0.0080	0.0170	0.0000	0.0170	0.0000	0.0170	0.0000	0.0010	0.0034	0.0204	0.0061	0.0133	0.0003
DEET	0.2750	0.0637	0.2077	0.0057	0.0606	0.0095	0.0307	0.0036	0.0261	0.0076	0.0811	0.0173	0.0960	0.0151
NOR	0.0115	0.0023	0.0072	0.0038	0.2454	0.0374	0.4080	0.1060	5.3675	0.9515	2.4936	1.2159	2.010	0.5196
VLS	0.0060	0.0022	0.0033	0.0006	0.0239	0.0034	0.0162	0.0063	0.0731	0.0069	0.0614	0.0086	0.0461	0.0027
NGL	0.0187	0.0086	0.0025	0.0000	0.0040	0.0018	0.0030	0.0005	0.0070	0.0016	0.0224	0.0077	0.0067	0.0014
MED	0.0017	0.0007	0.0014	0.0000	0.0014	0.0000	0.0014	0.0000	0.0043	0.0036	0.0059	0.0041	0.0027	0.0009

Table 3.4. Mean log Koc values (L/kg; n = 3) of 7 PPCPs sorbed to sediment in the mesocosm exposures

	Time of Exposure					
	1.5 Hours		56 Days		84 Days	
	Log Koc	± SD	Log Koc	± SD	Log Koc	± SD
ACT	0.41	0.00	0.60	0.11	1.05	0.06
MPH	1.54	0.19	1.53	0.04	1.62	0.15
PRP	2.21	0.05	2.12	0.11	2.36	0.03
CBZ	0.42	0.06	1.25	0.05	1.32	0.14
NOR	1.70	0.29	4.22	0.21	3.56	0.12
NGL	1.69	0.00	2.18	0.14	1.68	0.09
MED	1.32	0.01	1.84	0.32	0.98	0.16

Table 3.5. Limit of detection (LOD) values for each LCMS/MS analysis of pharmaceutical and personal care product concentrations from sediment in mesocosms at the Sewanee Wetlands, Sewanee, TN.[9]

	LOD values (µg/kg)	
	1.5 hours and 3, 6 days	**28, 56, 84 days**
ATN	0.00008	0.00035
ACT	0.00155	0.00060
CAF	0.00278	0.00148
MPH	0.00045	0.00015
PRP	0.00177	0.00209
DPH	0.00330	0.00027
CBZ	0.00026	0.00047
FLU	0.01354	0.00129
SRT	0.01698	0.00236
DEET	0.00200	0.00170
NOR	0.00192	0.00830
VLS	0.00233	0.00183
NGL	0.00247	0.00493
MED	0.00136	0.00116

[9] When a PPCP's concentration was undetectable above the limit of detection (LOD), the value of the LOD was given in place of the PPCP concentration as a conservative value.

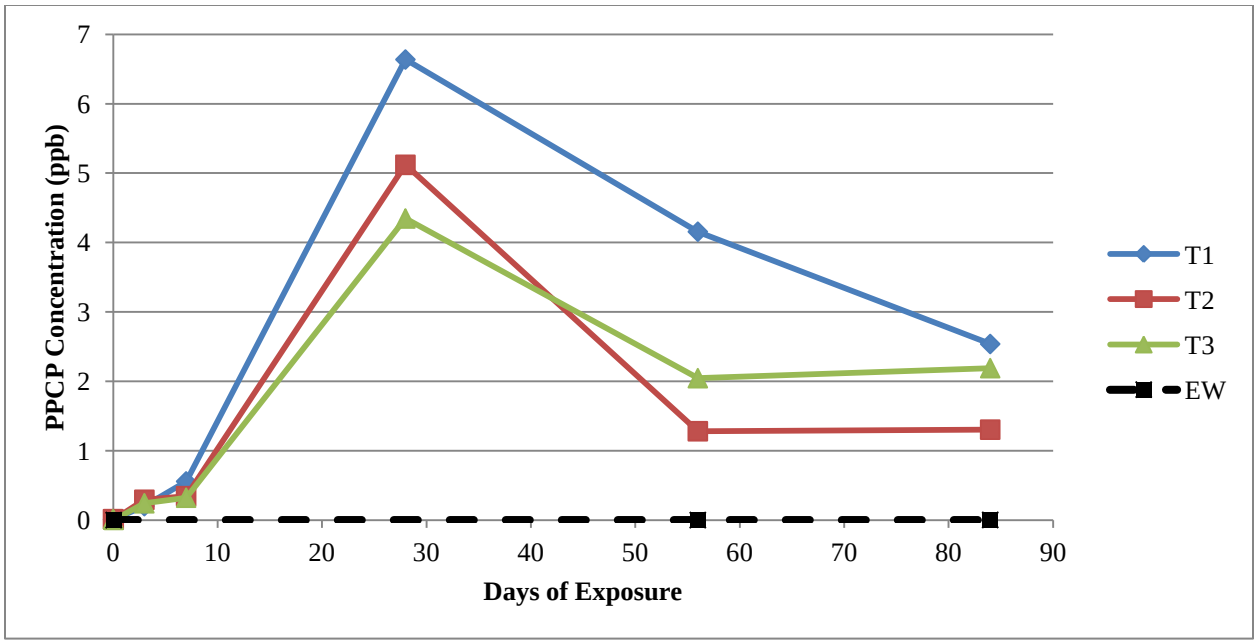

Figure 3.1. Norethindrone concentration in sediment over days of exposure (blank, 1.5 hours, and 3, 7, 28, 56, and 84 days) receiving wastewater from the Supply Tank in the Sewanee Wetlands, Sewanee, TN.[10]

[10] T1 = tank 1, T2 = tank 2, T3 = tank 3, EW = Exposure Water Concentration.

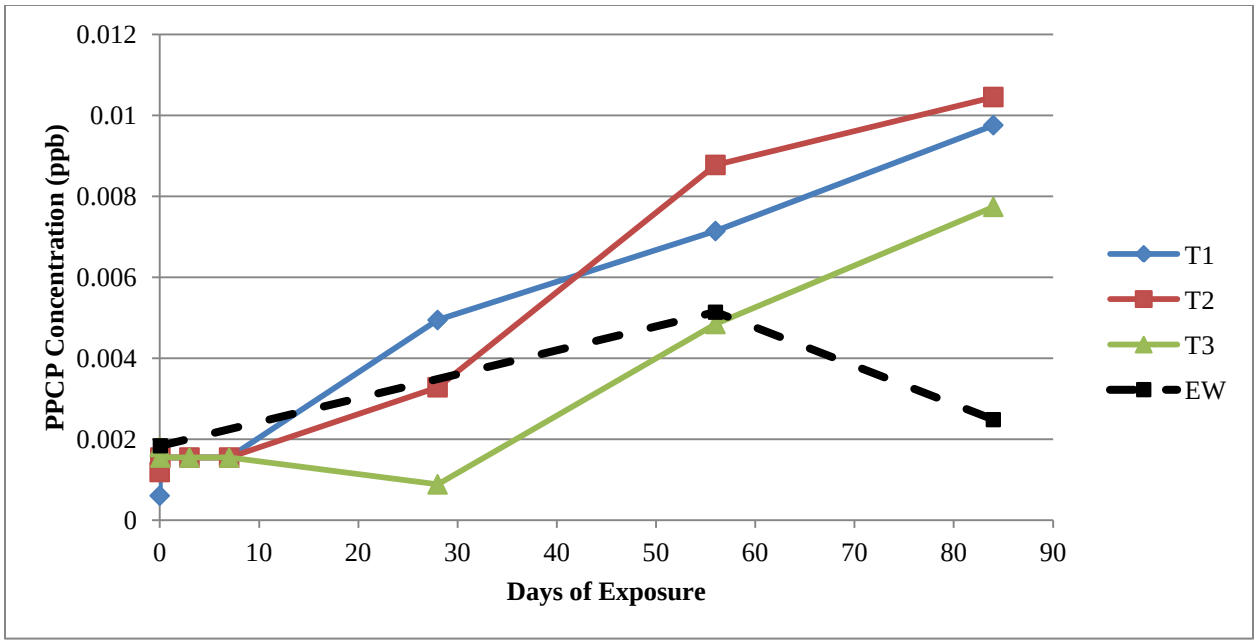

Figure 3.2. Acetaminophen concentration in sediment over days of exposure (blank, 1.5 hours, and 3, 7, 28, 56, and 84 days) receiving wastewater from the Supply Tank in the Sewanee Wetlands, Sewanee, TN.[11]

[11] T1 = tank 1, T2 = tank 2, T3 = tank 3, EW = Exposure Water Concentration.

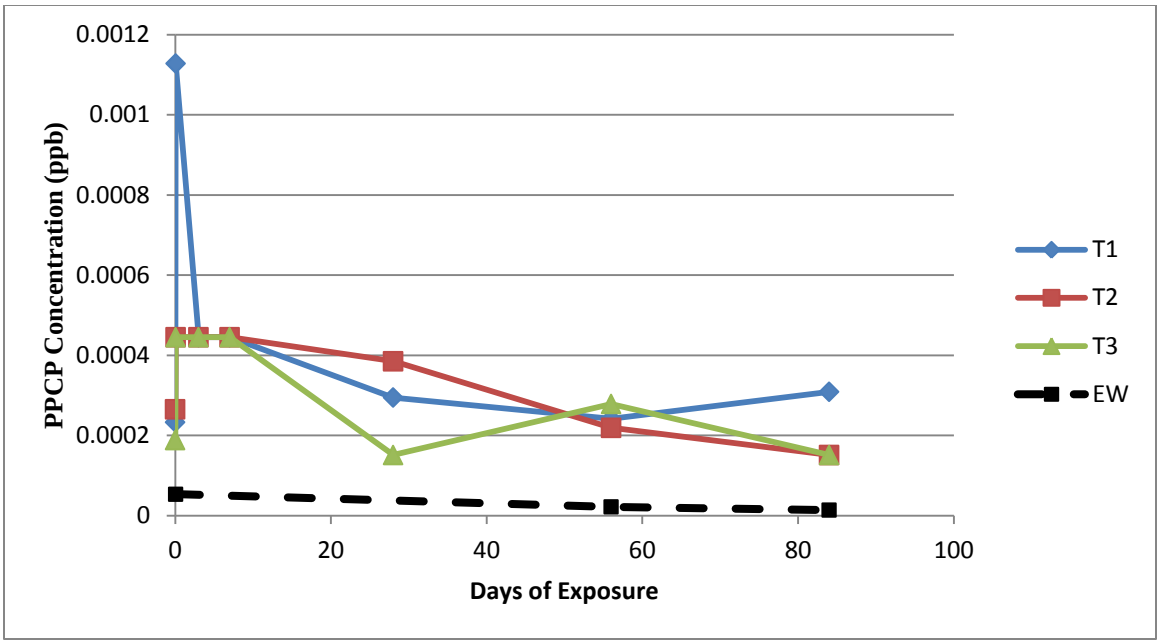

Figure 3.3. Methylphenidate concentration in sediment over days of exposure (blank, 1.5 hours, and 3, 7, 28, 56, and 84 days) receiving wastewater from the Supply Tank in the Sewanee Wetlands, Sewanee, TN.[12]

[12] T1 = tank 1, T2 = tank 2, T3 = tank 3, EW = Exposure Water Concentration.

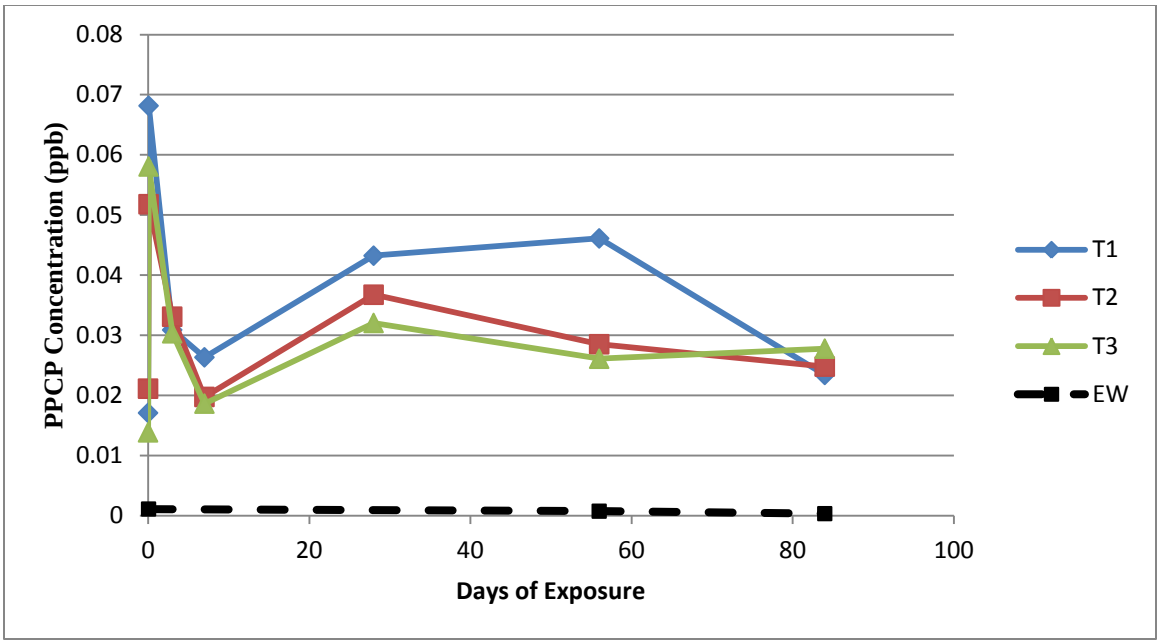

Figure 3.4. Propranolol concentration in sediment over days of exposure (blank, 1.5 hours, and 3, 7, 28, 56, and 84 days) receiving wastewater from the Supply Tank in the Sewanee Wetlands, Sewanee, TN.[13]

[13] T1 = tank 1, T2 = tank 2, T3 = tank 3, EW = Exposure Water Concentration.

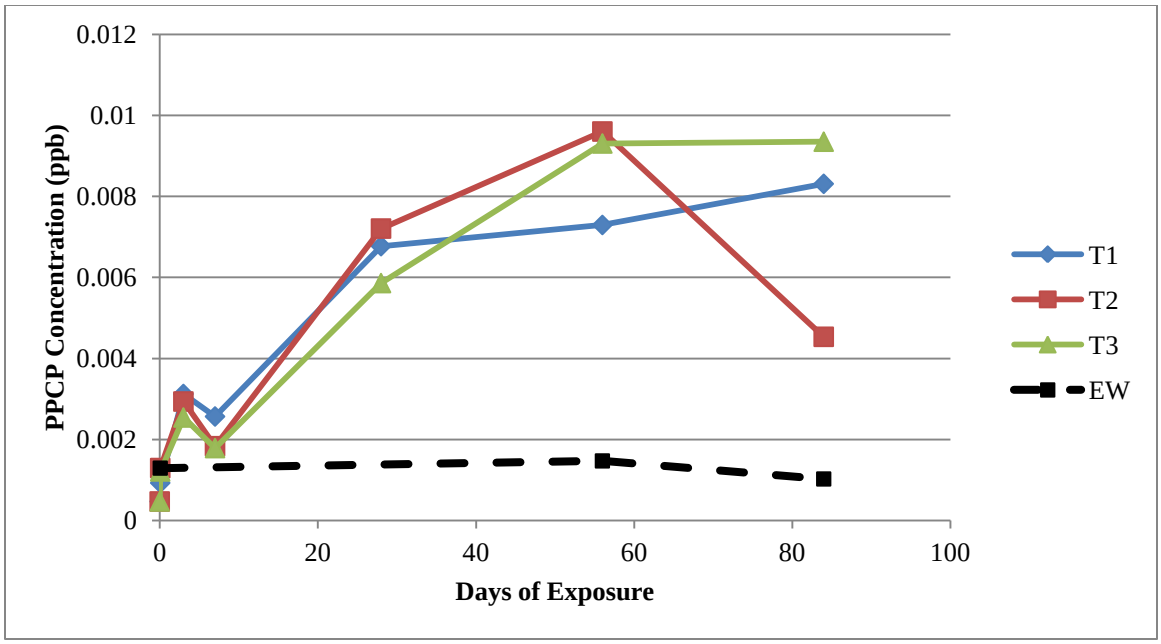

Figure 3.5. Carbamazepine concentration in sediment over days of exposure (blank, 1.5 hours, and 3, 7, 28, 56, and 84 days) receiving wastewater from the Supply Tank in the Sewanee Wetlands, Sewanee, TN.[14]

[14] T1 = tank 1, T2 = tank 2, T3 = tank 3, EW = Exposure Water Concentration.

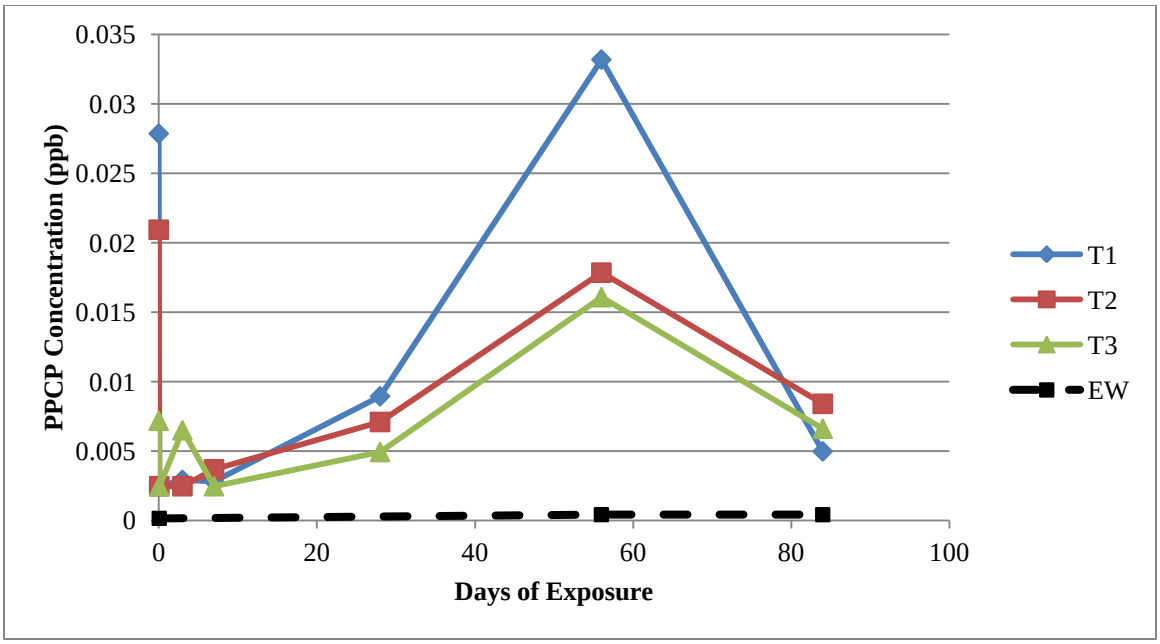

Figure 3.6. Norgestrel concentration in sediment over days of exposure (blank, 1.5 hours, and 3, 7, 28, 56, and 84 days) receiving wastewater from the Supply Tank in the Sewanee Wetlands, Sewanee, TN.[15]

[15] T1 = tank 1, T2 = tank 2, T3 = tank 3, EW = Exposure Water Concentration.

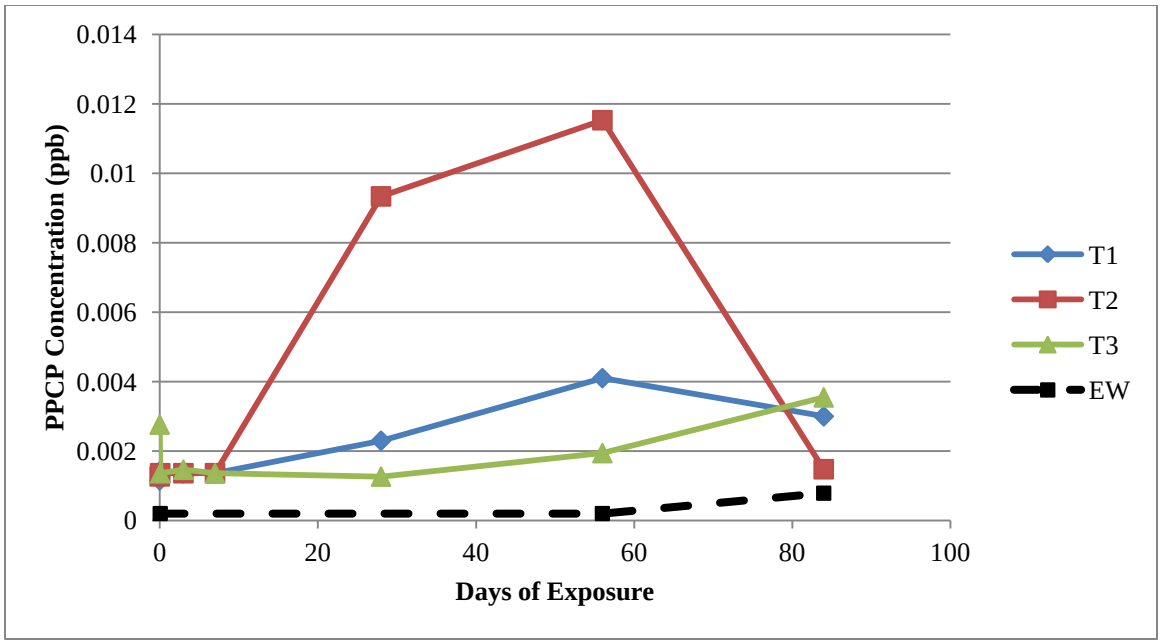

Figure 3.7. Medroxyprogesterone concentration in sediment over days of exposure (blank, 1.5 hours, and 3, 7, 28, 56, and 84 days) receiving wastewater from the Supply Tank in the Sewanee Wetlands, Sewanee, TN.[16]

[16] T1 = tank 1, T2 = tank 2, T3 = tank 3, EW = Exposure Water Concentration.

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CHAPTER 4

CONCLUDING REMARKS

Millions of pharmaceuticals and personal care products (PPCP) are used around the world every day and can be found at trace levels throughout the water environment, including groundwater, surface water, rivers, coastal waters, and treated drinking water (Randhir and Rolf, 2013; Taylor, 2015). The main source of PPCPs in the environment is through wastewater discharge because traditional wastewater treatment plants (WWTP) do not effectively remove these contaminants (Cizmas *et al*., 2015). Improving wastewater management technologies to provide clean water and protect human health and aquatic life from the harmful effects of wastewater contaminants will be an ongoing undertaking for future scientists and researchers. However, advanced technologies for wastewater reclamation are not accessible everywhere thus, cost effective, efficacious methods for removing contaminants from wastewater are needed. Constructed wetlands are cost effective to implement, maintain, and operate and are being researched to determine their efficacy as a treatment process to polish wastewater and remove contaminants. The studies presented in this thesis sought to evaluate the Sewanee Wetlands, a pilot, surface-flow constructed wetland, in the removal of PPCPs from partially treated wastewater from a WWTP.

The first study measured overall removal rates (%) of 14 PPCPs during eight sampling periods over one year by measuring PPCP concentrations throughout the wetland. Our results indicated that the constructed wetland successfully removed 73.32 ±

15.42% (mean ± SD, n=8) of Total PPCPs from the treated wastewater. The wetland significantly removed 3 of 14 PPCPs: atenolol ($p = 0.0002$), caffeine ($p = 0.0253$), and DEET ($p = 0.0367$). Caffeine, valsartan, diphenhydramine, DEET, and atenolol were the most abundant PPCPs in all five sample sites and alternatively, methylphenidate, propranolol, carbamazepine, medroxyprogesterone were only detected at very low concentrations in wetland influent (mean concentrations < 2 ng/L) and acetaminophen, fluoxetine, sertraline, norethindrone and norgestrel were found at low concentrations in wetland influent (mean concentrations 2 – 20 ng/L). These PPCPs with very low concentrations were not removed consistently among sampling periods and often, the concentrations increased slightly throughout the wetland. Llorens *et al.* (2009) found increased removal rates with lower concentrations of PPCPs in a tertiary surface-flow constructed wetland. The five most abundant PPCPs in the wetland showed some of the highest removal rates in varying sampling periods. This finding is in contrast to Matamoros *et al.* (2016), who found a negative correlation between PPCP concentration and removal rate (%) showing that higher influent concentrations led to lower removal rates in a horizontal-flow constructed wetland. Water quality parameters were measured during each sampling period for each sample site. Dissolved oxygen (DO) had significant effects on the removal of atenolol ($p = 0.0441$) and DO effects on removal of Total PPCP approached significance ($p = 0.0805$) with higher levels of removal with increased levels of DO. Overall, linear mixed model analyses did not show any other significant effects of pH, dissolved oxygen, temperature, or conductivity on the removal of PPCPs.

In the second study, we sought to evaluate the sorption effects of the sediment used to construct the Sewanee Wetlands. Occurrence of PPCP sorption was determined in

a mesocosm study that exposed sediment in glass jars to wastewater from the Supply Tank of the Sewanee Wetlands. The flow-through mesocosm design operated for 12 weeks and sediment was sampled at seven intervals: 0 days of exposure (control blank), 1.5 hours, and 3, 7, 28, 56, and 84 days of exposure. Seven of the fourteen PPCPs studied showed potential sorption: acetaminophen, methylphenidate, propranolol, carbamazepine, norethindrone, norgestrel, and medroxyprogesterone. Norethindrone showed the most potential sorption after 56 days of exposure (estimated log Koc = 4.22 ± 0.21) and desorption occurred after 84 days of exposure (log Koc = 3.56 ± 0.12. Of the other six PPCPs, sorption was estimated from log Koc = 0.98 L/kg to 2.36 ± 0.03 L/kg after 84 days of exposure. The results from this study must be taken with caution as potential issues in the extraction method may have reduced the degree of sorption measured.

Results from the sediment study showed that acetaminophen, methylphenidate, propranolol, carbamazepine, norethindrone, norgestrel, and medroxyprogesterone had moderate to great sorption capacity. These compounds were also present at some of the lowest concentrations in the wetland across all sampling periods from the first study. It may be possible that the lower concentration PPCPs in the wastewater entering the wetland were removed by the lagoons in the WWTP. The three lagoons have an approximate surface area of 60,000-m^2 combined. Assuming the sediment surface area is equivalent to the water surface area, there would be sufficient opportunities for sorption to occur during normal wastewater treatment. We suggest further research to test this hypothesis by measuring the concentrations of PPCPs in the raw wastewater entering Lagoons A and B as well as sampling the effluent from Lagoons A and B that feeds Lagoon C. Also, measuring the occurrence of PPCP in lagoon sediment will allow log

Koc values to be calculated. The results from this hypothesized study would further the knowledge of sorption in wastewater treatment systems.

Future research with the Sewanee Wetlands and other constructed wetlands should consider total suspended solids (TSS) in the measurements for PPCP removal. As highlighted by Petrie *et al.* (2015), TSS is a potential sorbent for PPCPs and when TSS is not analyzed for PPCP concentrations, those contaminants enter the wetland suspended in the water column and pass through, undetected. Measuring all potential sources of PPCPs in influent and effluent should be performed to properly evaluate removal efficiencies. Also, additional research is needed to deepen the understanding of removal mechanisms to be able to optimize constructed wetlands for PPCP removal. The processes affecting PPCP removal are complex and many mechanisms of removal occur simultaneously. Many of the processes transform PPCP compounds into metabolites so non-targeted LCMS analyses would be beneficial to detect the variety of parent and metabolite compounds present in the wastewater. In general, understanding the processes that effect PPCP removal will result in more efficient wastewater treatment and higher levels of contaminant removal in constructed wetlands.

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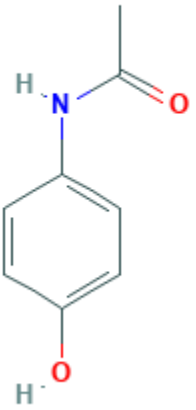
Appendix



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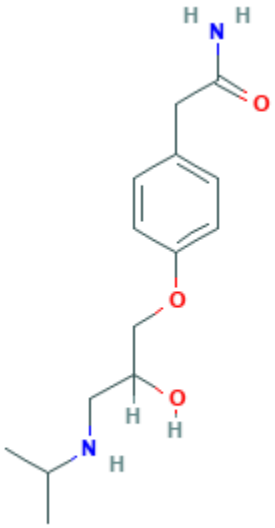

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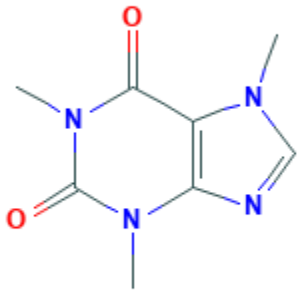

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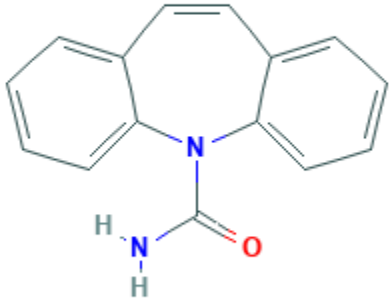

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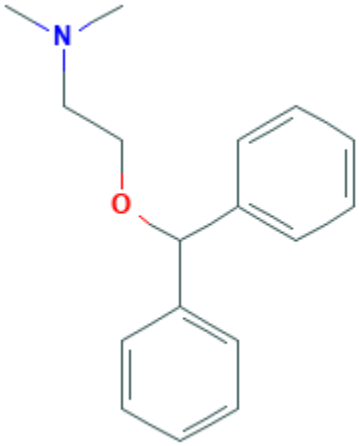

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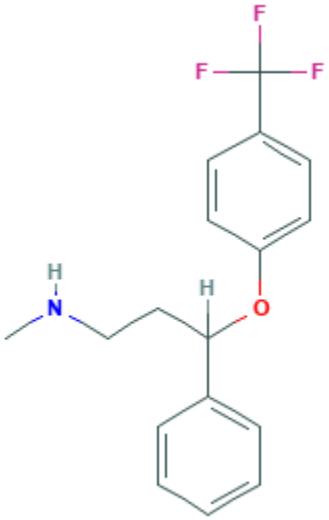

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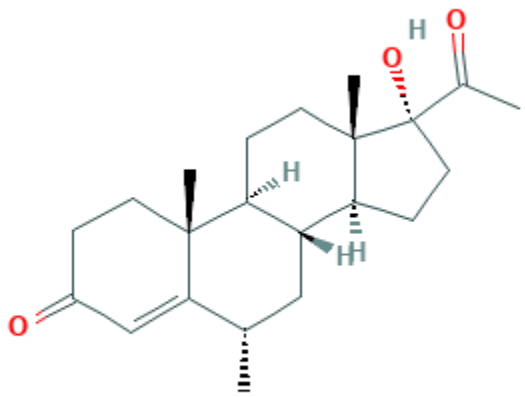

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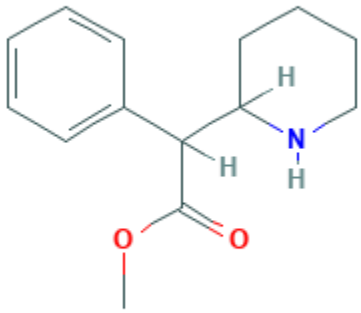

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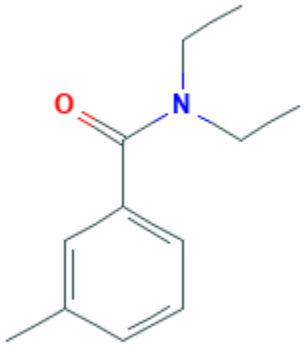

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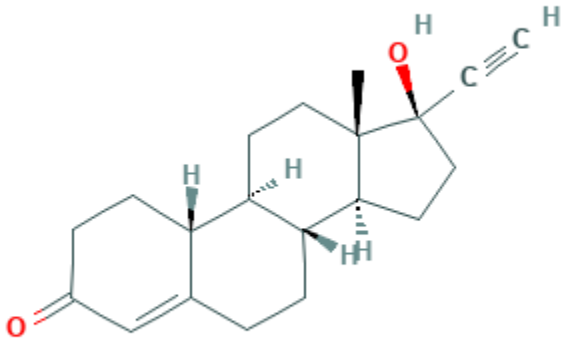

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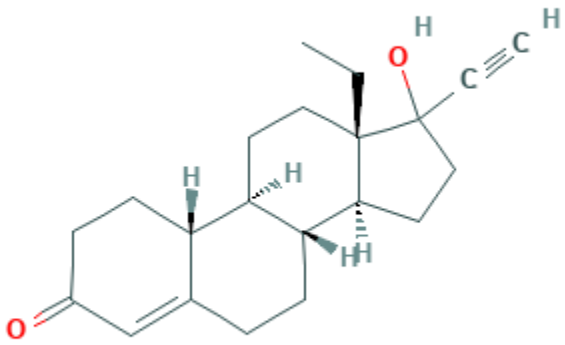

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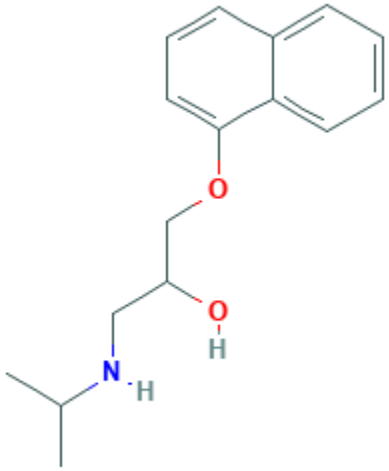

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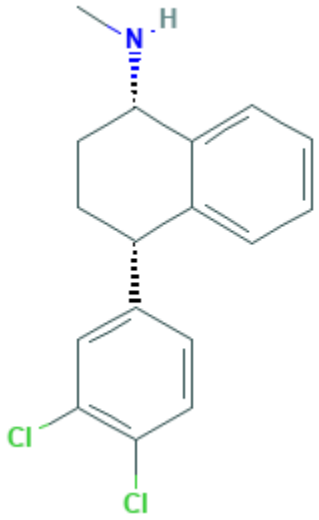

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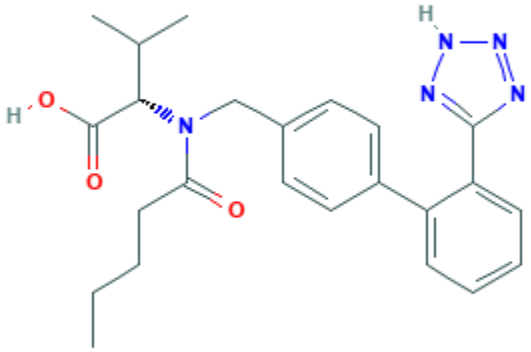

S.14. Valsartan chemical structure (Pubchem).