Summer 2012

Comparative removal of pharmaceuticals and antimicrobials in conventional and constructed wetland wastewater treatment in cold climate

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COMPARATIVE REMOVAL OF PHARMACEUTICALS AND ANTIMICROBIALS IN CONVENTIONAL AND CONSTRUCTED WETLAND WASTEWATER TREATMENT IN COLD CLIMATE

by

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A thesis submitted in partial fulfillment
of the requirements for the Master of
Science degree in Civil and Environmental Engineering
in the Graduate College of
The University of Iowa

July 2012

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Graduate College
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CERTIFICATE OF APPROVAL

MASTER'S THESIS

This is to certify that the Master's thesis of

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has been approved by the Examining Committee for the
thesis requirement for the Master of Science degree in
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ACKNOWLEDGEMENTS

I would like to thank my advisor Craig Just for his guidance, care, and support throughout my time at the University of Iowa. I would like to thank Brett Woods for all of his previous work on this research. I am grateful to have an exceptional thesis committee in Craig L. Just, Gene F. Parkin, and David M. Cwiertny and would like to thank them for the time they spent working with me to improve my thesis.

I would also like to express my immense gratitude to fellow graduate student Eric Redmond for all of his help and work on the wetland. I would like to thank Collin Just for all of his help in the laboratory and dealing with me pestering him all the time for ordering things. Also, I would like to express my gratitude to the EES department for all of the support and the great friendships I have developed here. Most of all, I would like to thank my family for all of their emotional support, love, and encouragement.

ABSTRACT

In this study we compare the ability of nitrifying activated sludge (NAS) and nitrifying trickling filter (NTF) wastewater treatment to remove the following contaminants: acetaminophen, caffeine, 1,7-dimethylxanthine, cotinine, ibuprofen, sulfamethoxazole, triclosan, and trimethoprim. Removal of acetaminophen, cotinine and caffeine was greater than 99% and removal 1,7-dimethylxanthine, ibuprofen, and triclosan was greater than 90% using NAS and NTF treatment. Sulfamethoxazole and trimethoprim were inadequately removed in both NAS and NTF treatments. The horizontal, subsurface flow treatment wetland showed removals of 45-89% for sulfamethoxazole and greater than 96% for trimethoprim. There was no statistical difference ($P>0.05$) between aeration, temperature and vegetation in the treatment wetland for the removal of sulfamethoxazole and trimethoprim.

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CHAPTER 1
INTRODUCTION AND OBJECTIVES

The last 15 years has brought growing concern among the general public, policy makers, and environmental scientists over the unknown consequences of pharmaceuticals and antimicrobials in the environment. They have been found in streams and rivers throughout the United States (Kolpin et al. 2002). It is widely believed that the primary source of these compounds in streams is discharge from wastewater treatment plants (WWTPs). Wastewater from industrial facilities which manufacture these compounds as well as hospital and domestic wastewaters are considered to be major contributors of these compounds to WWTPs (M.D. Hernando 2006). Since the major pathway of these compounds to the environment is through WWTPs, the release of these compounds to the environment ultimately depends on the ability of WWTPs to remove these compounds from wastewater. However, not all WWTPs use the same treatment technologies or have the same influent wastewater characteristics; therefore, the ability of WWTPs to remove these compounds may depend on the type of treatment being used and/or the characteristics of the wastewater.

Two common wastewater treatment technologies used in the U.S. are activated sludge and trickling filter treatment. When operated properly, both technologies are capable removing regulated constituents such as biochemical oxygen demand (BOD), suspended solids (TSS), and ammonia-nitrogen. However, the ability of activated sludge and trickling filters to remove certain pharmaceuticals and antimicrobials is not well understood. In fact, very little is known about removal of these compounds during wastewater treatment and associated treatment of sludge generated during treatment.

The pharmaceuticals and antimicrobials chosen for this study were acetaminophen, caffeine, 1,7-dimethylxanthine, cotinine, ibuprofen, sulfamethoxazole, triclosan, and trimethoprim (Table 1.1 and Figure 1.1). These compounds were among the 30 most frequently detected compounds in the Kolpin et al. (2002) study where reported concentrations ranged from approximately 0.01 to 10 μg/L. Acetaminophen, caffeine, and ibuprofen are nonprescription drugs. 1,7-dimethylxanthine is a metabolite of caffeine and cotinine is a metabolite of nicotine. Triclosan is an antibacterial agent used in many personal care products (Canosa et al. 2005). Sulfamethoxazole and trimethoprim are antibiotics prescribed to treat infectious diseases in both humans and animals (Perez et al. 2005). Typically sulfamethoxazole and trimethoprim are administered together to overcome the increase in bacterial resistance to sulfamethoxazole (Drillia et al. 2005).

Most literature reports describe the removal of these compounds over the entire treatment plant (i.e., plant effluent compared to raw wastewater concentrations) although some report on specific removals by activated sludge and trickling filtration. Caffeine is readily removed during wastewater treatment with Perez et al. (2005) reporting complete removal of 37 μg/L and Thomas and Foster (2005) reporting greater than 99% removal of approximately 43 μg/L. Reported removals of ibuprofen by activated sludge WWTPs were 40% to greater than 90%, yielding effluent concentrations of 0.01 to 0.2 μg/L (Castiglioni et al. 2006; Han et al. 2006; Paxeus 2004; Tauxe-Wuersch et al. 2005; Thomas and Foster 2005; Yu et al. 2006). Reported removals of acetaminophen were 9% to greater than 99% (Han et al. 2006; Yu et al. 2006). Batch experiments conducted by

Yu et al. (2006) showed complete removal of 50 µg/L of ibuprofen and acetaminophen within 14 days.

There is significantly more data on the fate of triclosan during wastewater treatment. Reported influent concentrations range from 0.8 to 17 µg/L, with effluent concentrations of 0.03 to 0.25 µg/L (McAvoy et al. 2002; Singer et al. 2002; Waltman et al. 2006; Yu et al. 2006). Removals at full-scale WWTPs ranged from 69 to 99%, with most removal occurring during biological treatment (Federle et al. 2002; McAvoy et al. 2002; Singer et al. 2002; Waltman et al. 2006; Yu et al. 2006). Separate batch experiments with activated sludge showed complete removal of 1 to 50 µg/L in 50 days (Yu et al. 2006). Federle et al. (2002) showed that 81 to 92% of added triclosan was mineralized to CO_2 or incorporated into biomass after 50 days. McAvoy et al. (2002) found activated sludge treatment to be more effective with a removal efficiency over 95% compared to a removal efficiency of 58% to 86% during trickling filter treatment.

Limited data indicates that trimethoprim is more completely removed than sulfamethoxazole in conventional wastewater treatment. Reported removals of trimethoprim were 77% to greater than 90%, with effluent concentrations of 0.05 to 0.1 µg/L (Gobel et al. 2005; Paxeus 2004; Perez et al. 2005). Removals of sulfamethoxazole are reported to be 17 to 71%, with effluent concentrations of 0.3 to 0.9 µg/L (Castiglioni et al. 2006; Gobel et al. 2005; Perez et al. 2005). The relatively low level of removal for some pharmaceuticals and antimicrobials, particularly for sulfamethoxazole, has led researchers to consider alternative treatment approaches to better safeguard the environmental and protect human health.

Constructed Wetlands for the Removal of Sulfamethoxazole and Trimethoprim

Three main types of engineered wetlands are typically used to treat wastewater including: free water surface (FWS), vertical flow (VF) and horizontal subsurface-flow (HSSF) (Kadlec and Wallace 2009). FWS wetlands are many times undesirable in populated areas where the growth of mosquitos and other flying insects would be a nuisance and increase the risk of negative human health outcomes. VF wetlands can typically remove higher levels of BOD_5, but they are also more expensive to operate due to increased pumping costs. Our research utilized HSSF treatment wetlands which can be vegetated and further insulated for use in cold climates with an organic rich mulch layer above the gravel treatment layer (Kadlec and Wallace 2009; Werker et al. 2002).

Studies on the removal of pharmaceuticals and antimicrobials showed that HSSF wetlands can remove many compounds of interest, even in cold climates. A treatment study in Mandeville, Louisiana utilized a series of aerations lagoons followed by a FWS wetland and then by an ultraviolet disinfection system showed reduction of almost all compounds of interest, including sulfamethoxazole, by greater than 90 percent. (Conkle et al. 2008).

Another study measured the mass discharge rate, into the River Besòs in Spain, for 12 pharmaceutical and personal care products from a typical. A one-hectare FWS wetland at the site showed removal efficiencies greater than 90 percent for 8 of the contaminants. The greater removal seen in the summer was likely caused by increased photo- and microbial degradation due to sun exposure and higher water temperatures. (Matamoros et al. 2008)

A similar study compared the use of two "Filtralite-P" units, two biological sand filters, five HSSF and four VF wetlands. Removal efficiencies for total suspended solids and BOD_5 were similar in all systems and pharmaceuticals were greater than 80 percent removed. The vegetated VF wetland showed the best overall performance (Matamoros et al. 2009).

It is suspected that deep rooted plants in HSSF treatment wetlands effect treatment by creating additional surface area for biofilm, by facilitating oxygen transfer, and by transformations mediated by plants exudates, fungi, and symbiotic bacteria in the rhizosphere (Kadlec and Wallace 2009). Hijosa-Valsero et al. compared removals of pharmaceuticals in wetlands with two types of vegetation, in an unplanted wetland, and in conventional wastewater treatment(Hijosa-Valsero et al. 2011) and found there was a greater removal in the vegetated wetlands (87±41%) than in the unplanted bed (73±35%) although the statistical significance of this finding remains in doubt (Hijosa-Valsero et al. 2011)

The use of cyclic aeration in constructed wastewater treatment wetlands is a newer concept that significantly improves removal of TSS, BOD_5 and nitrogen (Kadlec and Wallace 2009). The impact of cyclic aeration on the removal of sulfamethoxazole and trimethoprim has not been fully studied. In a comparison of 4 wastewater treatment plants with varying designs and operations, typical aeration levels showed little effect on the removal of sulfamethoxazole (Batt et al. 2007). But, sulfamethoxazole and trimethoprim were significantly removed in a pure oxygen primary treatment aeration system and during extended aeration secondary treatment. The pure oxygen system, which reportedly can handle a greater organic loading rate, had an HRT of only 1 hour

but significantly removed sulfamethoxazole and trimethoprim. The extended aeration secondary treatment operations had HRTs of 28-31 hours. One system with two aeration stages had solids retention times (SRT) of 6 days and 49 days with the authors suggesting that long SRTs favor the development of nitrifying bacteria that degrade trimethoprim (Batt et al. 2007).

This review indicates that limited data are available regarding the occurrence and fate of pharmaceutical and antimicrobial chemicals during wastewater collection and treatment. The objectives of this study were to 1) track acetaminophen, caffeine, 1,7-dimethylxanthine, cotinine, ibuprofen, sulfamethoxazole, triclosan, and trimethoprim through various stages of activated-sludge and trickling-filter WWTPs; 2) to measure these compounds in hospital, domestic, and industrial wastewaters; 3) to make these measurements during different seasons of the year, with the goal of better understanding the behavior of these compounds during wastewater collection and treatment and 4) determine the ability of horizontal, subsurface-flow treatment wetlands to remove sulfamethoxazole and trimethoprim from wastewater prior to discharge.

Table 1.1 Physical-chemical properties of the target compounds for this study.

Compound	CAS #	log K_{ow}	Solubility (mg/L)	pK_a
Acetaminophen	103-90-2	0.46	14,000 @ 25˚C	9.38
Caffeine	58-08-2	-0.07	21,600 @ 25˚C	10.4
Cotinine	486-56-6	0.07	999,000 @ 25˚C	n.a.
1,7-Dimethylxanthine	n.a.	n.a.	n.a.	7.5
Ibuprofen	15687-27-1	3.97	21 @ 25˚C	4.91
Sulfamethoxazole	723-46-6	0.89	610 @ 37˚C	5.7
Triclosan	3380-34-5	4.76	10 @ 20˚C	7.9
Trimethoprim	738-70-5	0.91	400 @ 25˚C	7.12

Source: Woods, Brett M. "Fate of Endocrine Disruptors, Antibiotics, and Pharmaceuticals in Wastewater Treatment Plants." Thesis. University of Iowa, 2006. Print.

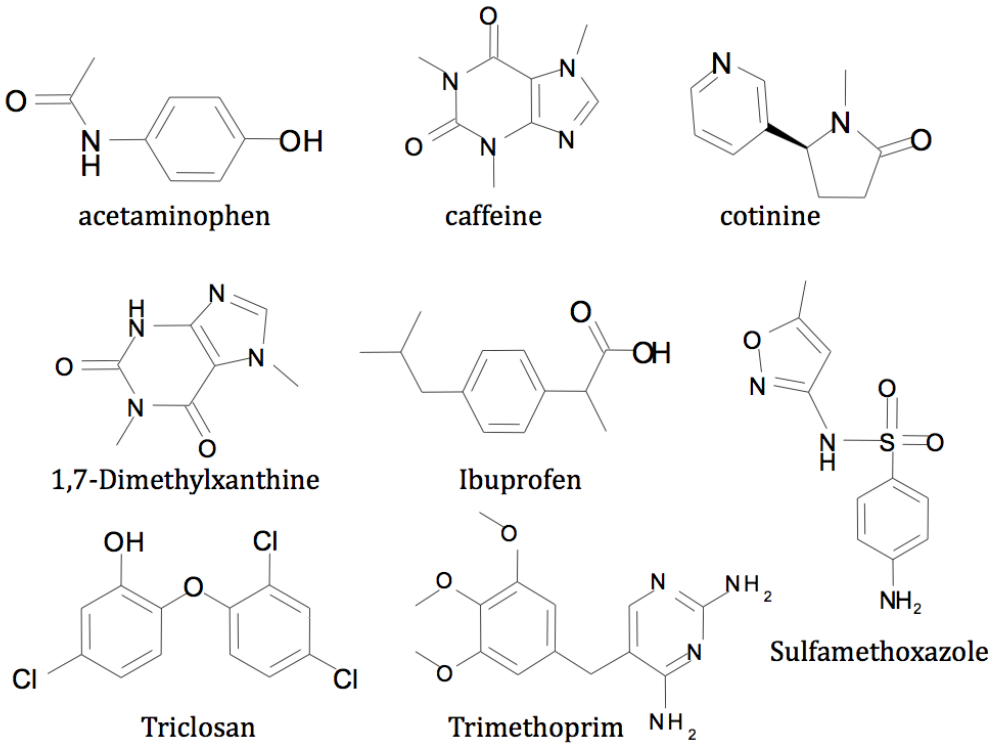

Figure 1.1 Names and molecular structures of the target compounds for this study.

CHAPTER 2
MATERIALS AND METHODS

Conventional Wastewater Treatment Studies

Two facilities, the Iowa City South WWTP and North WWTP, were examined for this research. The South WWTP utilizes nitrifying activated sludge (NAS) to treat 6-7 million gallons per day and the North WWTP utilizes nitrifying trickling filters (NTF) to treat 3-4 million gallons per day. The WWTPs treat similar wastewater and achieve similar effluent quality based on typical wastewater characteristics (Table 2.1).

The unit processes at the NAS WWTP include bar screens, grit removal, primary clarification, plug flow activated sludge with recycle, secondary clarification, and chlorination/dechlorination (Figure 2.1). Raw influent passes half-inch bar screens then through vortexing grit removal. Primary clarification occurs in four circular sedimentation tanks with a hydraulic detention time of approximately 5.5 hours. Activated sludge treatment occurs in four plug flow aeration basins with a hydraulic retention time of approximately 1.4 hours and a solids retention time of 6 to 20 days. The activated sludge effluent is distributed to four secondary clarifiers with a detention time of approximately 6 hours. The treated wastewater is chlorinated with hypochlorite and then dechlorinated with sulfur dioxide before discharge to the Iowa River.

The unit processes at NTF WWTP include bar screens, grit removal, primary sedimentation, trickling filtration with recycle, secondary sedimentation, and chlorination/dechlorination (Figure 2.1). Raw influent passes three-quarter inch bar screens and aerated grit removal just before primary clarification. Primary clarification occurs in one circular sedimentation tank with a hydraulic detention time of

approximately 4 hours. Four trickling filters, with a hydraulic detention time of approximately 8 hours, treat the primary effluent. The trickling filter effluent is distributed to two secondary clarifiers with a detention time of approximately 2 hours. The treated wastewater is chlorinated with hypochlorite, and then dechlorinated with sulfur dioxide before discharge to the Iowa River.

Sample Collection: Triplicate samples were taken from the wastewater collection network, in the spring season, at manholes near hospital outfalls, in a domestic area, and near an industrial manufacturer of shampoo, mouthwash and other personal care products. Triplicate samples were taken at the WWTPs from the 1) influent, 2) primary clarifier effluent, 3a) activated sludge effluent, 3b) trickling filter effluent, 4) secondary effluent and, 5) final effluent (Figure 2.1). The NAS WWTP was sampled four times, once during the fall season, once during the summer and twice during the winter. The NTF WWTP was sampled once during the winter season.

Samples were collected with PVC bailers then transferred (for manholes) or collected directly (at the WWTPs) in 950 mL amber, glass bottles. Latex gloves were worn during, and changed after, each collection and samples were stored at 4°C for no longer than 7 days prior to analysis preparation. The hospital wastewaters discharged to a branch of the collection network connected to the NTF WWTP. The wastewaters from the domestic area and the industrial facility were treated by the NAS WWTP.

Sample Preparation and Quantification: Solid phase extraction (SPE) was used to concentrate target compounds from wastewater. Samples were gravity settled in clean, glass beakers and then two ml of 0.5 M ammonium carbonate was added to 100 ml of the supernate in a separate, clean beaker. Oasis HLB SPE cartridges (Waters, Inc.) were

conditioned sequentially with 10 ml ethyl acetate, 10 ml methanol, and 10 ml ammonium carbonate (10 mM). The supernate was vacuumed through a SPE cartridge followed by 5 mL of beaker rinsate (10 mM ammonium bicarbonate). The cartridge was rinsed with 5 mL deionized water, under vacuum, before gravity elution with 15 mL ethyl acetate. The extract was evaporated to 0.5 mL with pure, dry nitrogen then transferred to a vial containing 0.1 mL acetonitrile and 0.4 ml deionized water.

The extracts were analyzed by a Waters Alliance Model 2695 HPLC system coupled to a Micromass Quattro mass spectrometer utilizing 100 uL injections. Analytical separation was done with a Zorbax SB C8, 5 μm, 150 x 3.0 mm reverse phase column and a dual mobile phase of 0.15% (v/v) acetic acid in water and 0.15% (v/v) acetic acid in 1:1 (v/v) methanol/acetonitrile at 0.6 ml/minute. Target compounds were quantified in positive or negative ion mode using tandem, MS/MS, mass spectrometry (Table 2.1).

Treatment Wetland Studies

Pilot-scale, HSSF wetlands at the Iowa City South Wastewater Treatment Plant (Iowa City, Iowa) were operated for this study. The treatment wetlands consisted of duplicate cells with 4 different treatments: (1) aerated and planted; (2) aerated and unplanted; (3) unaerated and planted; and (4) unaerated and unplanted. The aerated cells were designed as described by Wallace (2001). The planted cells were fitted with native Iowa, dark-green bulrush (*Scirpus atrovirens*) at approximately ten plants per square meter (about 55 plants per cell).

Each wetland cell was 2.44 m by 2.23 m, by 0.61 m deep (8 ft by 7.3 ft, by 2 ft. deep), and lined with a 45 mm impermeable ethylene propylene diene monomer (EPDM)

liner (Figure 2.2). Pea gravel occupying the bottom 0.3 m (1 ft) of each cell defined the saturated treatment zone at a volume of approximately 1.64 m^3 (58 ft^3). Approximately 0.25 m (10 in) of insulating mulch was placed on top of the treatment zone leaving 0.05 m (2 in) of freeboard. Six sampling ports, perforated throughout the treatment depth, were placed every 0.6 m (2 ft) along two lines parallel to wastewater flow. A treatment cell plan view (Figure 2.3) clearly shows the distribution of the sampling ports, the influent distribution header, the effluent collection piping and the perforated aeration tubes and aeration trunk line. The aeration lines were 1.9 cm (¾-inch) diameter flexible tubing, perforated along the entire length (2.3 m), attached to a main distribution line of 1.9 cm (¾-inch) PVC. The six lines in each of the aerated cells were about 0.55 m (1.8 ft) apart. Aeration was supplied by a Pondmaster AP-100 air compressor in six hour cycles at a rate of approximately 0.708-0.850 scmh (25-30 scfh).

Wastewater from the weir of a primary clarifier was delivered by a timer-controlled pump (Model 2JGA5, Dayton) through a 3.81 cm inside diameter, heat-tape wrapped and buried flexible pipe to a 208 L head tank in a 2.44 m by 2.44 m control shed. The head tank was drained by a 3.18 cm diameter PVC pipe to a Model 2800 pressure tank (Simer). The pressure tank discharged through a 3.18 cm diameter PVC pipe with pressure gauge to an 8 port manifold after passing a TM150-N model digital flow meter (GPI). Each port on the manifold was equipped with a Model 125-EFP-CB solenoid controlled by an ESP-LX Modular controller (RainBird®). The first cell received a 1 minute dose (about 17.5 L) followed by a 2.5 minute pause before the controller progressed to open the solenoid for the next wetland cell dose. The sequence

continued until all eight cells were dosed. The sequence was repeated four times for a total daily dose of approximately 265 L (70 gallons) per cell.

The wastewater dose was fed across the entire width of the cell by a level distribution manifold placed directly above the pea gravel bed (Figure 2.3). The treated effluent was collected by a manifold placed at the base of the wetland cell opposite the influent header. After collection in the manifold, the effluent from each cell was piped to the control shed and through a telescoping level controller made from 5.1 cm diameter PVC and with a rubber, slip fitting. Upon exiting the level controller, the effluent passed through custom-made tipping buckets (0.5 L per tip) equipped with a magnetic reed switch connected to a CR1000 datalogger (Campbell Scientific). The internal temperature of each wetland cell and ambient air temperatures were measured using CR200 dataloggers and Model 107 thermistor-style sensors (Campbell Scientific).

A bromide tracer test was conducted to assess the hydraulic characteristics of each wetland cell (Redmond et al. 2012). Potassium bromide (182 g Br^-) was added to each cell and samples were collected at the effluent daily for 12 days. Bromide concentrations were determined by ion selective electrode (Thermo Scientific; Waltham, MA).

Sampling, Preparation and Analysis of Sulfamethoxazole and Trimethoprim for Quantification: Influent and effluent samples were collected on three separate occasions representing internal wetland temperatures of 6, 9 and 24 degrees Celsius. An additional, cross-sectional sampling was performed at 2, 4, and 6 feet from the influent side of the wetland, along the water flow path. Samples (100 mL) for sulfamethoxazole and trimethoprim were collected in methanol rinsed glass bottles and then filtered with 0.45 glass microfiber filters. The samples collected at 24 C were prepared via solid phase

extraction to a final volume of 10 mL using Oasis 6 mL, 500 mg HLB SPE cartridges (Waters) conditioned with 6 mL of ACN followed by 6 mL of DI water. Prior to extraction, Na_2EDTA was added and the pH was lowered to 2.5-3 with hydrochloric acid. The analytes were eluted with 2 separate, 4 mL aliquots of ACN followed by 2 mL of ACN. The eluent was collected in 16 x 25 mm test tubes with a polypropylene line screw cap (Fisher Brand) and were then concentrated to a volume of 0.2 mL with a gentle stream of nitrogen in a 50°C water bath. Deionized water was added to reach a final extract volume of 1 mL. A sulfamethoxazole-$^{13}C_6$ (Cambridge Isotopes, lot SCJI-015) internal standard solution was prepared at 10 ug/mL and added to samples at a final concentration of 5 ng/mL. Samples collected at 6 C were prepared by the State Hygienic Laboratory at the University of Iowa using a similar solid-phase extraction method and the same internal standard.

Samples collected at 9 C were analyzed via a direct injection method, without solid phase extraction sample preparation. A caffeine-$^{13}C_3$ (Cerilliant, lot FN091611-02) internal standard solution was prepared at 10 ug/mL and added to samples at a final concentration of 5 ng/mL. All samples were analyzed by an ABI Sciex 4000 QT tandem mass spectrometer with a turbospray ESI source operated in positive ion, selected monitored reaction mode. Chromatographic separation was performed with an Agilent 1200 HPLC equipped with a Zorbax SB C8 column (3.5 um, 3.0 by 10 mm).

All samples were analyzed in batch with a 5-point or more calibration curve representing concentrations that bracketed the analyte concentrations in the samples. Two quality control analyses on known standards were performed and a minimum of two blanks were analyzed per batch. At least one matrix spike was analyzed per batch at a

nominal level of 10 ug/L and all recoveries were 85% or greater. Limits of quantitation were calculated as 10 times the standard deviation of a series of blank analyses for any given calibration and/or batch.

The data were statistically analyzed using SigmaPlot® (version 11.0) coupled with Microsoft Excel. A one-way repeated measures analysis of variance (OW-RM ANOVA) was used to determine significant differences ($P>0.05$) between treatments for sulfamethoxazole. A Friedman repeated measures analysis of variance on ranks (RM ANOV-R) was used to determine significant differences between treatments for trimethoprim. The OW-RM ANOVA measures the quality of means for a set of data collected under varying conditions, in this case temperature, aeration and vegetation. The RM ANOVA-R test also measures the quality of means, but was used instead of OW-RM ANOVA on the trimethoprim data because it was not normally distributed.

Table 2.1 Typical wastewater characteristics for this study.

Wastewater Characteristic	Activated Sludge WWTP		Trickling Filter WWTP	
	Influent	Final Effluent	Influent	Final Effluent
Temperature (°F)	54	48	58	53
pH	6.7	6.8	7.5	7.2
NH_3-N (mg/L)	23.2	0.5	23.4	0.3
$CBOD_5$ (mg/L)	253	4.5	119	7.3
Alkalinity (mg/L $CaCO_3$)	260	90	183	75
TSS (mg/L)	172	10	128	6.4

Source: Woods, Brett M. "Fate of Endocrine Disruptors, Antibiotics, and Pharmaceuticals in Wastewater Treatment Plants." Thesis. University of Iowa, 2006. Print.

Note: Wastewater characteristics are averages recorded by the City of Iowa City wastewater facility on the day of sampling.

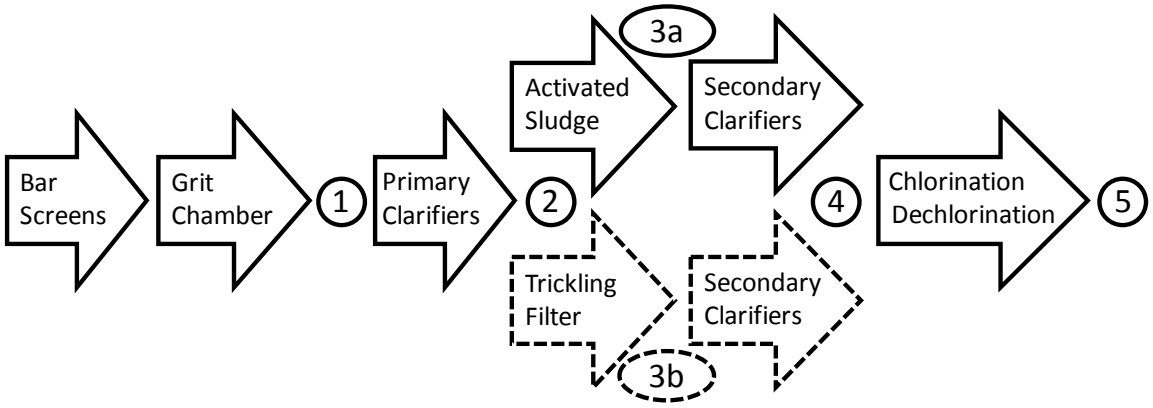

Figure 2.1 Unit operations and sampling locations for the NAS WWTP (1, 2, 3a, 4, and 5) and for the NTF WWTP (1, 2, 3b, 4, and 5).

Table 2.2 Mass spectrometry parameters used to identify and quantify the target compounds for this study

Compound	Ion Mode	Precursor Ion (m/z)	Product Ion (m/z)
Acetaminophen	+	151.9	109.9
Caffeine	+	195	137.9
Cotinine	+	176.9	79.9
1,7-Dimethylxanthine	+	181	123.9
Ibuprofen	-	205.1	161
Sulfamethoxazole	+	253.9	155.9
Triclosan*	-	288.9	n.a.
Trimethoprim	+	291	123.9

Source: Woods, Brett M. "Fate of Endocrine Disruptors, Antibiotics, and Pharmaceuticals in Wastewater Treatment Plants." Thesis. University of Iowa, 2006. Print.

*Analyzed in single ion monitoring mode

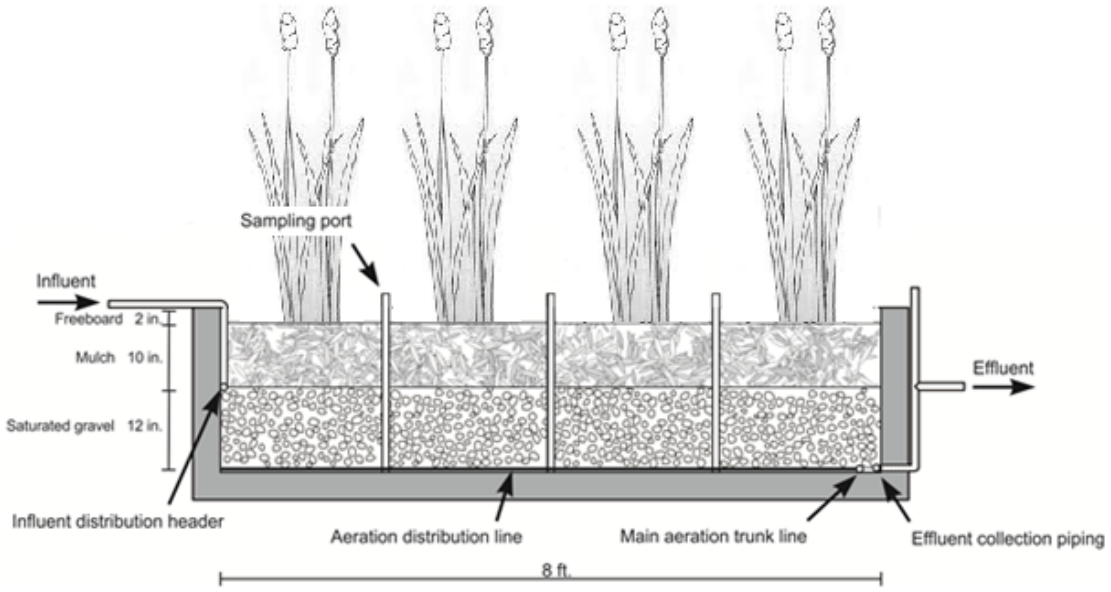

Figure 2.2 Cross-sectional view of a plant, horizontal flow, sub-surface, wastewater treatment wetland cell. From Redmond et al. (submitted).

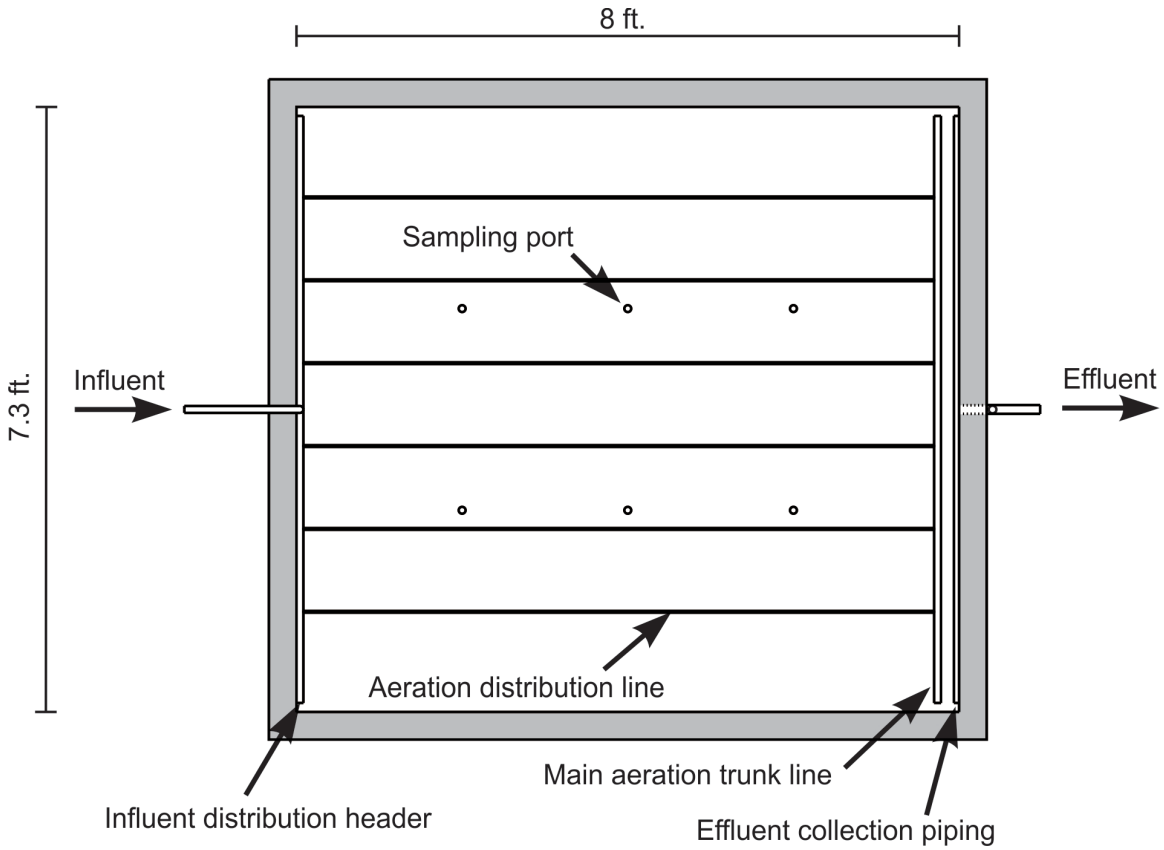

Figure 2.3 A plan view of an aerated horizontal flow, sub-surface, wastewater treatment wetland cell. From Redmond et al. 2012 (submitted).

CHAPTER 3
RESULTS AND DISCUSSION

Wastewater Characterization

Acetaminophen, caffeine, cotinine, ibuprofen, sulfamethoxazole, triclosan, and trimethoprim were found in the hospital and the domestic wastewater collection networks, but the industrial wastewater contained only acetaminophen and caffeine (Figure 3.1). The acetaminophen and caffeine in the industrial wastewater was likely human-waste derived since manufacturing processes at the facility do not utilize these compounds. The hospital wastewaters contained the greatest concentrations of measured compounds including acetaminophen and caffeine concentrations above 100,000 ng/L. Since sulfamethoxazole and trimethoprim are routinely prescribed in tandem they were, not surprisingly, found at similar concentrations in all samples. The domestic wastewater contained relatively high amounts of each measured compound with concentrations above 1,000 µg/L for acetaminophen, caffeine and ibuprofen (Woods 2006).

Removal During Conventional Wastewater Treatment

Acetaminophen, caffeine, 1,7-dimethylxanthine, cotinine, ibuprofen, sulfamethoxazole, triclosan, and trimethoprim were detected in the NAS WWTP influent wastewater in the month of December (Figure 3.2). Acetaminophen was measured at 140,000 ng/L, caffeine was 39,000 ng/L and ibuprofen was 12,000 ng/L. The influent sulfamethoxazole concentration was 2,000 ng/L while triclosan and trimethoprim concentrations were each found to be 700 ng/L (Woods 2006).

The aqueous concentrations of acetaminophen, caffeine, 1,7-dimethylxanthine, cotinine, ibuprofen, and triclosan in the final effluent were significantly less than the

plant influent concentrations (Figure 3.2). Sulfamethoxazole was removed to a lesser extent and trimethoprim was not removed at all. The final effluent concentrations for acetaminophen, cotinine and caffeine were below 20 ng/L, representing a greater than 99% removal. The final effluent concentrations of 1,7-dimethylxanthine, ibuprofen and triclosan were below 100 ng/L, representing a greater than 90% removal. The NAS WWTP removed 83% of the influent sulfamethoxazole with a measured final effluent concentration of 340 ng/L. Trimethoprim was not removed as evidenced by a consistent aqueous concentration near 700ng/L measured at each unit operation.

Acetaminophen, caffeine, 1,7-dimethylxanthine, cotinine, ibuprofen, triclosan, and sulfamethoxazole were mostly removed by the NAS unit operation. Slight removal was observed during primary clarification for acetaminophen, cotinine, ibuprofen, and sulfamethoxazole.

Acetaminophen, caffeine, 1,7-dimethylxanthine, cotinine, ibuprofen, sulfamethoxazole, and trimethoprim were found in the NTF WWTP influent wastewater in the month of February (Figure 3.3). The acetaminophen concentration was 123,000 ng/L, caffeine was 97,000 ng/L, cotinine was 900 ng/L and 1,7-dimethylxanthine was 16,000 ng/L. The influent concentration of ibuprofen was 5,800 ng/L, sulfamethoxazole was 2,000 ng/L and trimethoprim was 1,000 ng/L. Triclosan was not detected in the influent wastewater, but it was found in the primary effluent, trickling filter effluent and final effluent. The daily average NTF WWTP influent wastewater pH of 7.5, which is very near the pKa of triclosan (7.9), likely left only a fraction of the total triclosan in the quantifiable, protonated form. Samples collected later in the treatment process were at lower average pH (7.2) making triclosan detection more likely.

The aqueous concentrations of acetaminophen, caffeine, 1,7-dimethylxanthine, cotinine, ibuprofen, sulfamethoxazole and trimethoprim in the final effluent were significantly less than concentrations in the plant influent (Table 3.1). Acetaminophen was reduced 99%; caffeine decreased 92%; cotinine was reduced 56%; 1,7-dimethylxanthine decreased 84%; ibuprofen decreased 76%; sulfamethoxazole was reduced 45% and trimethoprim was reduced 30%.

The average influent/effluent concentration from four NAS WWTP sample events was compared to data for one NTF WWTP sampling event to determine the comparative, target compound removal efficiencies (Table 3.2). The NAS WWTP showed greater than 97% removal for all target compounds except sulfamethoxazole and trimethoprim, which were minimally removed or not removed at all. The NTF WWTP removed 99% of the acetaminophen, but unlike the NAS WWTP, the concentration of sulfamethoxazole and trimethoprim were reduced significantly (45% and 30%, respectively).

Removal of Sulfamethoxazole and Trimethoprim by Treatment Wetlands

The removal of sulfamethoxazole ranged from 45 to 89 percent across all temperatures studied (Table 3.3 and Figure 3.4, Figure 3.5, and Figure 3.6). There was no apparent correlation between removal and temperature as confirmed by the OW-RM ANOVA analysis ($P>0.05$). Trimethoprim was removed at greater than 96 percent regardless of temperature or any combination of wetland vegetation or wetland aeration. Therefore, all treatments were statistically similar for trimethoprim (RM ANOVA-R, $P>0.05$).

Vegetation played no significant role in the removal of sulfamethoxazole at the loading rates used in this study. Aeration was also an insignificant factor in sulfamethoxazole removal despite the measured increase in concentration in the aerated | unplanted treatment at 24 degrees C. The creation of sulfamethoxazole during wastewater treatment has been reported to result from the transformation of the human sulfamethoxazole metabolite, N^4- acetylsulfamethoxazole (Conkle et al. 2008; Gobel et al. 2007). But, the transformation of N^4- acetylsulfamethoxazole to sulfamethoxazole would be expected mostly under reducing conditions and not so much in an aerated wetland treatment cell as shown by our data.

Results of the cross-sectional sampling study (Figure 3.7) showed that sulfamethoxazole concentrations were somewhat variable at 2, 4 and 6 feet with most of the removal occurring between 2 and 4 feet. This result could be viewed as inconclusive since the sulfamethoxazole concentration actually increased in the first two feet of travel distance. The trimethoprim concentration remained mostly stable in the first two feet, but was almost completely removed before 4 feet.

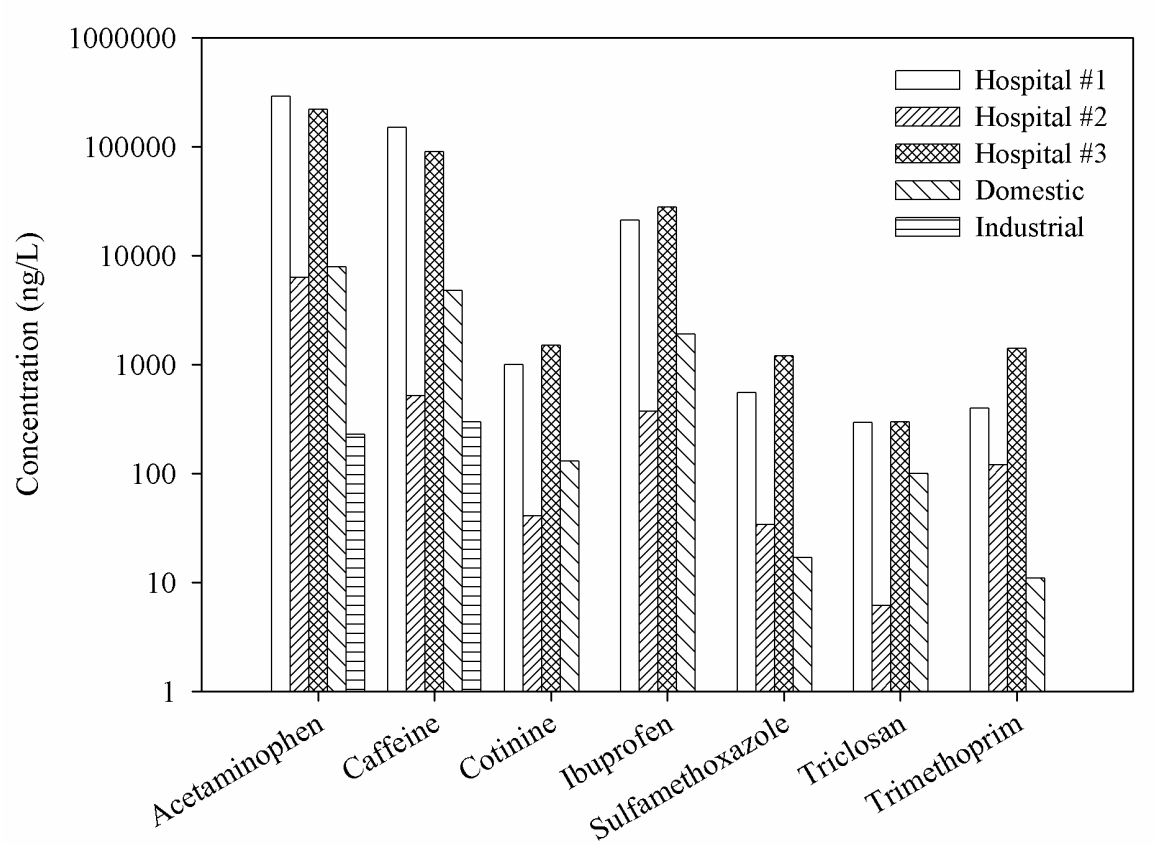

Figure 3.1 Concentration of acetaminophen, caffeine, cotinine, ibuprofen, sulfamethoxazole, triclosan and trimethoprim in hospital, domestic and industrial wastewaters (Woods 2006).

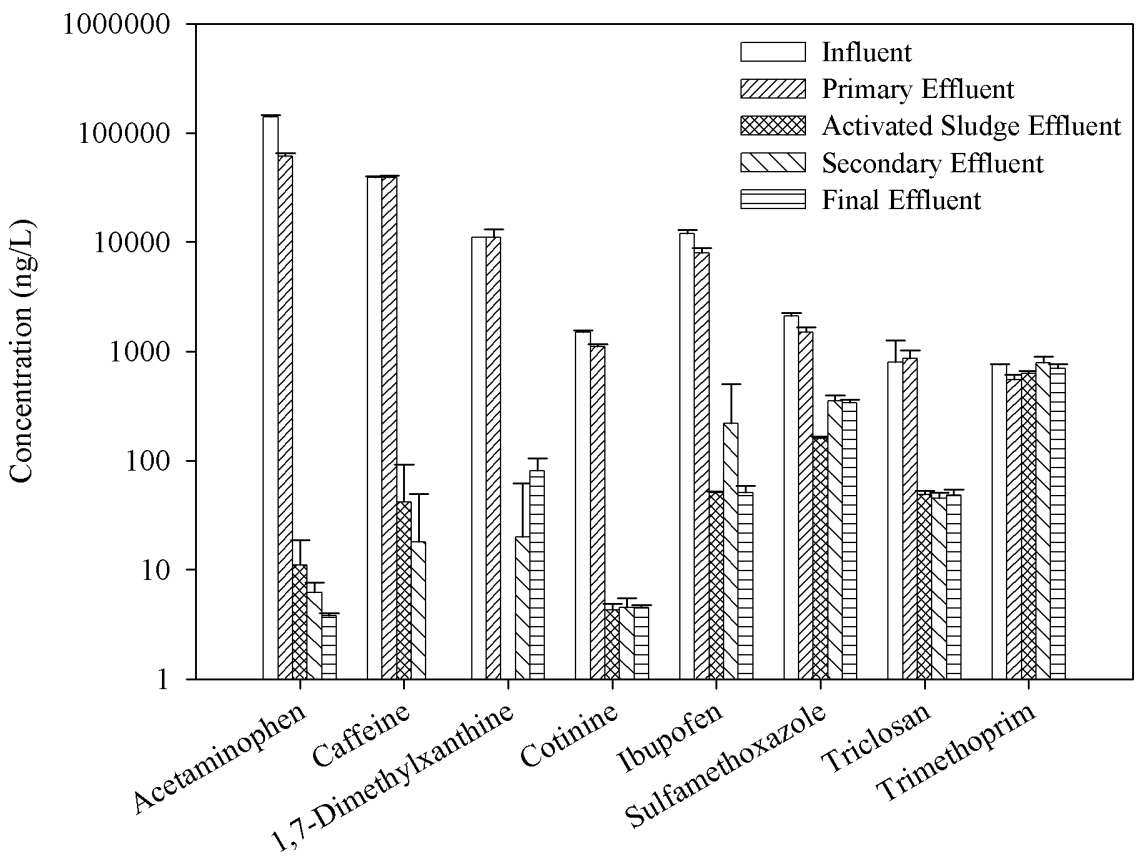

Figure 3.2 Concentration of acetaminophen, caffeine, 1,7-dimethylxanthine, cotinine, ibuprofen, sulfamethoxazole, triclosan and trimethoprim during various stages of a NAS WWTP. Samples were collected in the month of December. Error bars represent one standard deviation of triplicate analyses (Woods 2006).

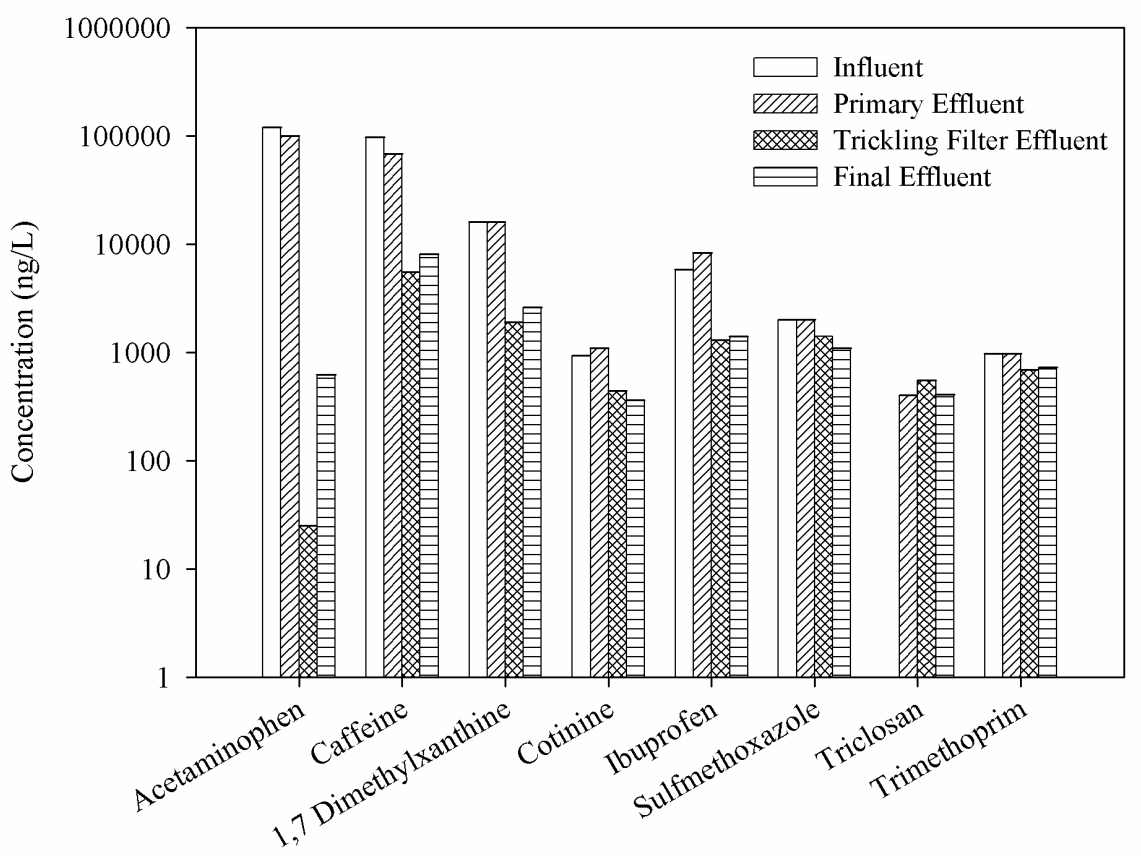

Figure 3.3 Concentration of acetaminophen, caffeine, 1,7-dimethylxanthine, cotinine, ibuprofen, sulfamethoxazole, triclosan and trimethoprim during various stages of a NTF WWTP. Samples were collected in the month of February. Error bars represent one standard deviation of triplicate analyses (Woods 2006).

Table 3.1 Target compound influent concentrations and percent removals for the NAS WWTP (average of four sample events) compared to the NTF WWTP (single event).

	NAS WWTP Concentrations (ug/L)			NTF WWTP Concentrations (ug/L)		
Target Compound	Influent	Effluent	% Removal	Influent	Effluent	% Removal
Acetaminophen	100	0.023	>99	123	0.6	>99
Caffeine	56	0.082	>99	97	8.1	92
Cotinine	2.9	0.023	>99	0.9	0.4	56
1,7-Dimethylzanthine	11	0.083	>99	16	2.6	84
Ibuprofen	12	0.047	>99	5.8	1.4	76
Sulfamethoxazole	2.7	3.5	0	2.0	1.1	45
Triclosan	0.95	0.033	97	<0.05	0.4	inconclusive
Trimethoprim	1.8	3.3	0	1.0	0.7	30

Source: Woods, Brett M. "Fate of Endocrine Disruptors, Antibiotics, and Pharmaceuticals in Wastewater Treatment Plants." Thesis. University of Iowa, 2006. Print.

Table 3.2 Seasonal comparison of target compound influent concentrations and percent removals for the NAS WWTP.

	NAS WWTP Influent Concentrations (ug/L) and Percent Removals							
	Fall		Winter A		Winter B		Summer	
Target Compound	Influent	%	Influent	%	Influent	%	Influent	%
Acetaminophen	75	>99	137±5.8	>99	147±11	>99	570	>99
Caffeine	68	>99	38±0.0	>99	85±9	>99	350	>99
Cotinine	2.0	>99	1.5±0.0	>99	5.8±0.2	>99	2.3	>99
Ibuprofen	18	>99	13±0.6	>99	14±0.6	>99	11	>99
Sulfamethoxazole	0.19	0	2.1±0.2	84	5.4±0.1	71	1.3	58
Triclosan	7.3	>99	0.9±0.4	95	<0.02	n.a.	<0.02	n.a.
Trimethoprim	<0.002	n.a.	0.7±0.0	5.0	5.3±0.1	59	0.43	9

Source: Woods, Brett M. "Fate of Endocrine Disruptors, Antibiotics, and Pharmaceuticals in Wastewater Treatment Plants." Thesis. University of Iowa, 2006. Print.

Table 3.3 Percent removals of sulfamethoxazole and trimethoprim in treatment wetland cells at various temperatures.

		WETLAND Influent Concentrations (ng/L) and Percent Removals					
	HRT	6 C		9 C		24 C	
Target Compound	(days)	Planted	Unplanted	Planted	Unplanted	Planted	Unplanted
Unaerated							
Sulfamethoxazole	3.4	64 (470)	64 (470)	50 (650)	45 (710)	77 (78)	88 (40)
Trimethoprim		100 (<2)	100 (2)	100 (<10)	100 (<10)	100 (1.5)	100 (1.3)
Aerated							
Sulfamethoxazole	3.6	72 (360)	69 (400)	54 (600)	48 (670)	89 (39)	0 (390)
Trimethoprim		100 (4.5)	99 (15)	100 (<10)	100 (<10)	100 (1.5)	96 (14)

Source: Woods, Brett M. "Fate of Endocrine Disruptors, Antibiotics, and Pharmaceuticals in Wastewater Treatment Plants." Thesis. University of Iowa, 2006. Print.

Influent sulfamethoxazole was 1300, 1300, and 340 ng/L for the 6, 9 and 24 C sample sets respectively.
Influent trimethoprim was 1200, 550, and 320 ng/L for the 6, 9 and 24 C sample sets respectively.

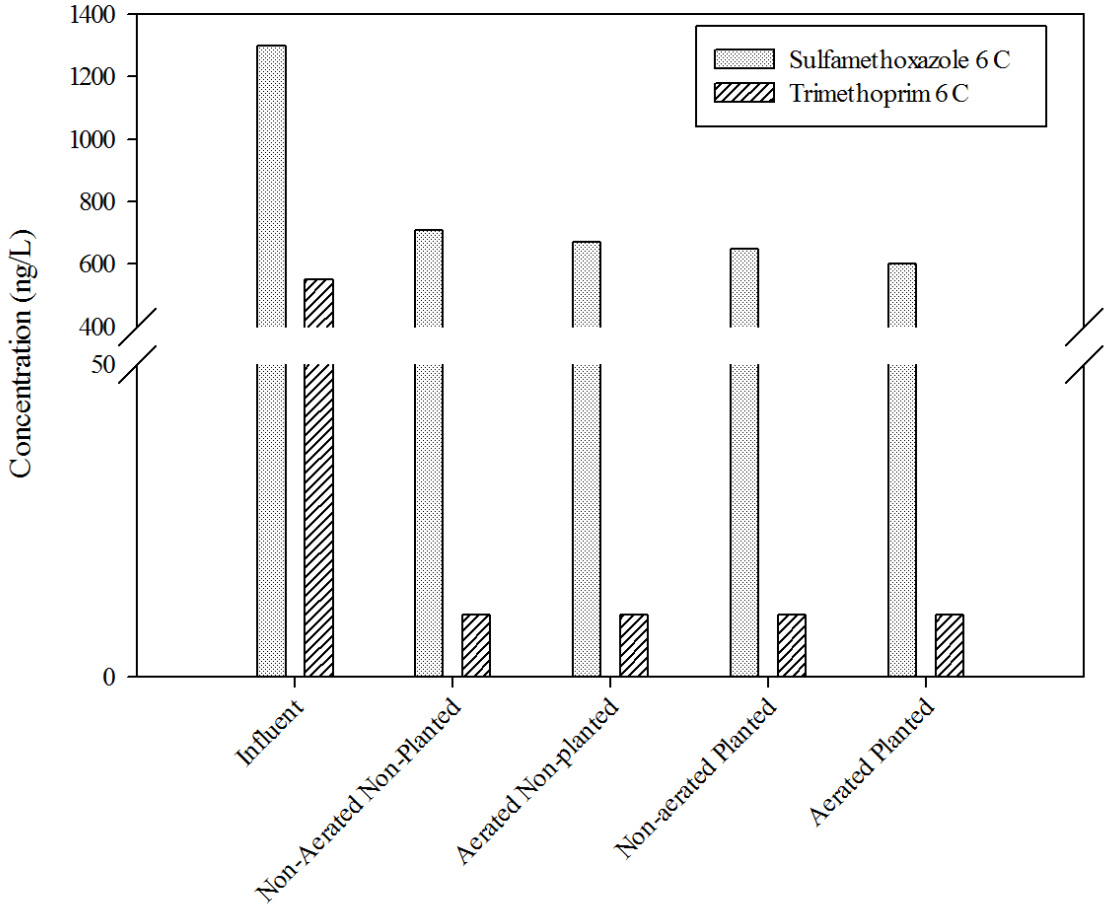

Figure 3.4 Influent and effluent concentrations (ng/L) of sulfamethoxazole and trimethoprim for various wetland treatments at 6C.

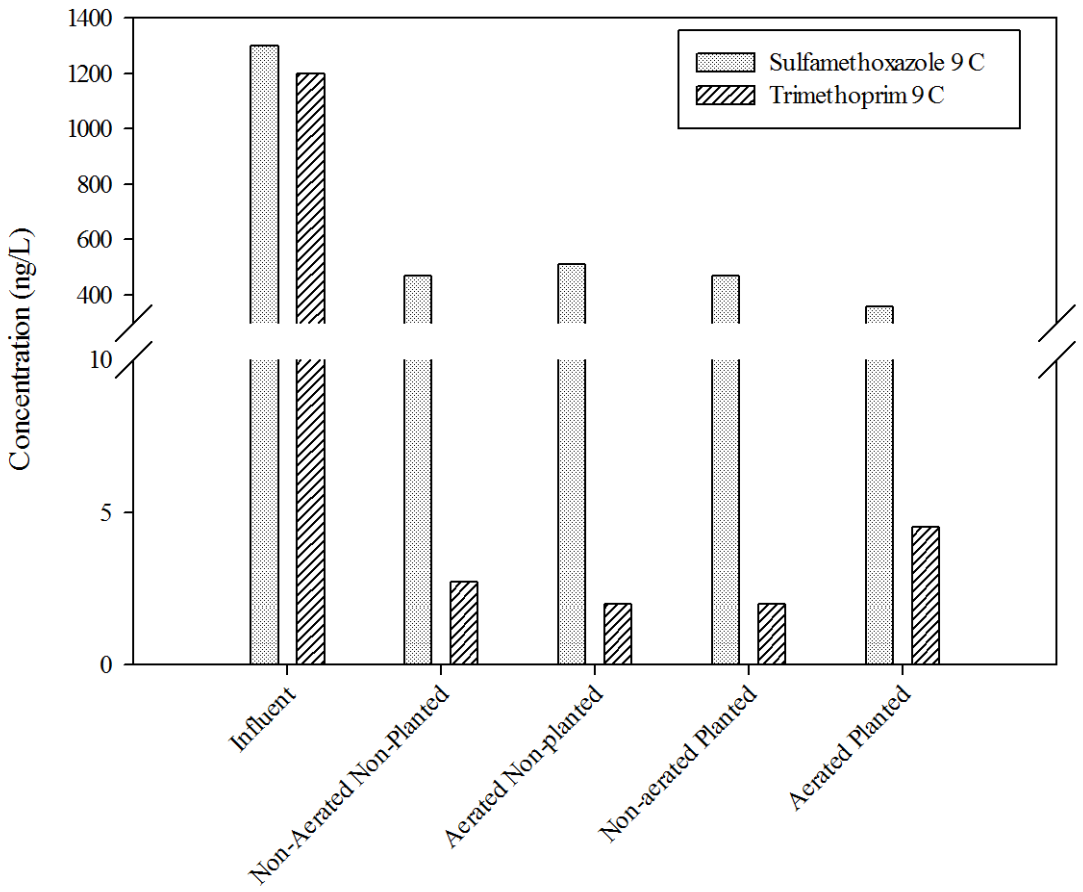

Figure 3.5 Influent and effluent concentrations (ng/L) of sulfamethoxazole and trimethoprim for various wetland treatments at 9C.

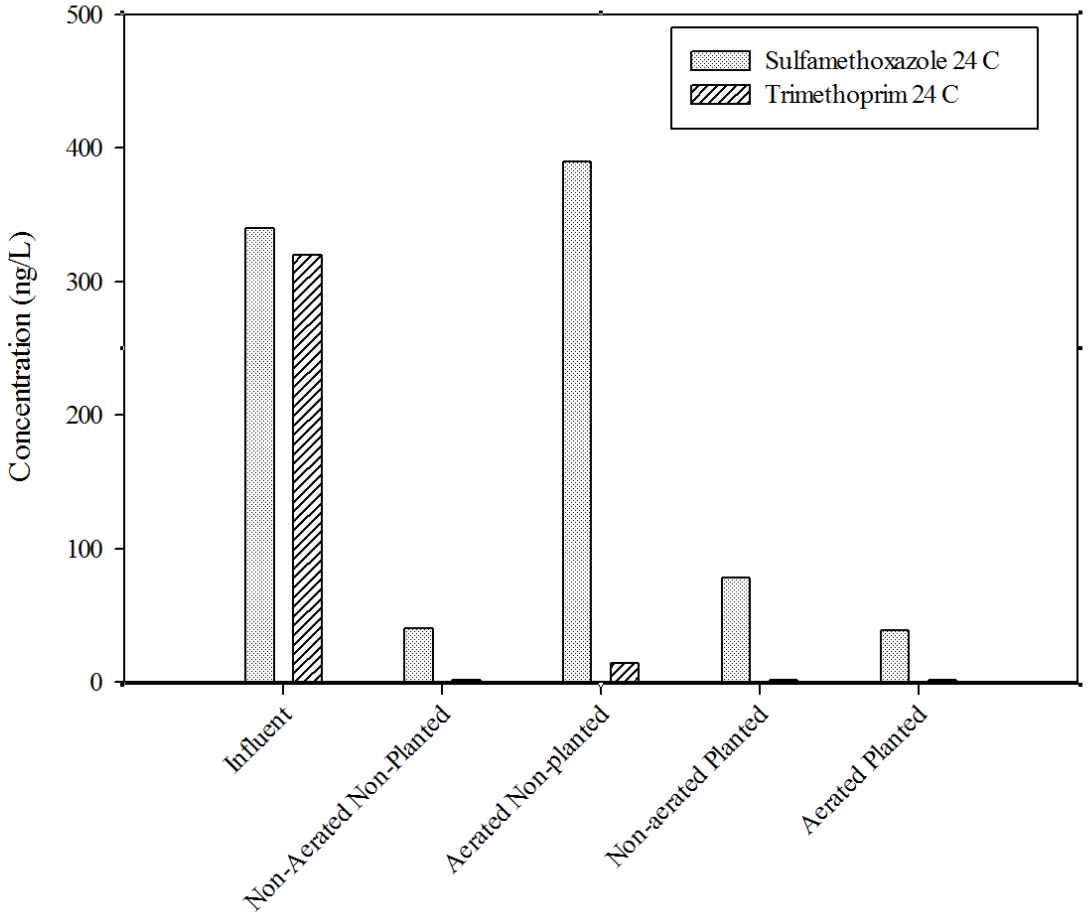

Figure 3.6 Influent and effluent concentrations (ng/L) of sulfamethoxazole and trimethoprim for various wetland treatments at 24C.

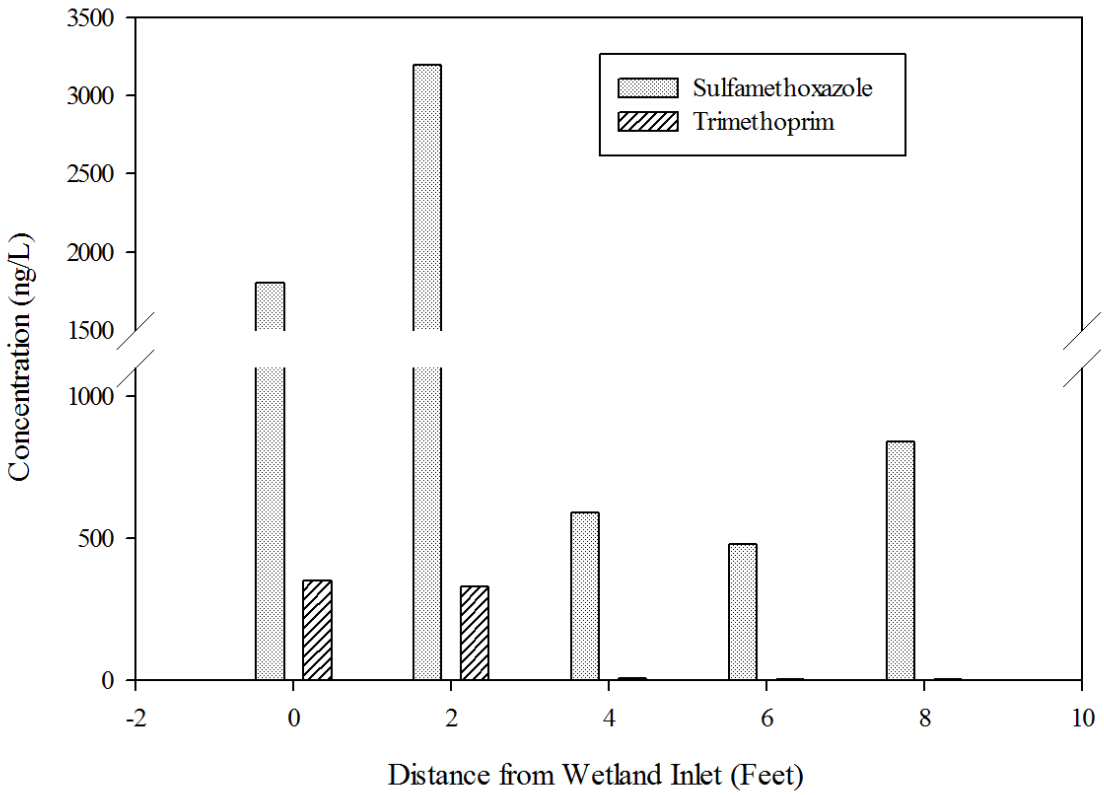

Figure 3.7. Cross-sectional concentrations of sulfamethoxazole and trimethoprim compared to their corresponding concentration in an unaerated | unplanted wetland treatment cell at 9C. Trimethoprim shows nearly 100 percent removal within the first 4 feet. Sulfamethoxazole is variable throughout the wetland but decreases overall.

CHAPTER 4
CONCLUSIONS

The results of this study demonstrate a comparison of a NAS WWTP, a NTF WWTP, and a horizontal, subsurface-flow wastewater treatment wetland and their ability to remove pharmaceuticals and antimicrobials prior to discharge to surface water. The pharmaceuticals and antimicrobials of interest; acetaminophen, caffeine, 1,7-dimethylxanthine, cotinine, ibuprofen, sulfamethoxazole, triclosan, and trimethoprim; were detected in all stages of the NAS WWTP, the NTF WWTP, and in the hospital waste stream, with the exception of tricolsan in the influent of the NTF WWTP. However, only acetaminophen and caffeine were detected in the industrial waste stream, which was likely the result of human excretion. The NAS WWTP removed acetaminophen, cotinine, and caffeine with a removal efficiency of greater than 99% and 1,7-dimethylxanthine, ibuprofen, and triclosan resulted in greater than 90% removal. In the NAS WWTP sulfamethoxazole was hardly removed while trimethoprim had no removal. The NTF WWTP removed acetaminophen by 99%, caffeine by 92%, cotinine by 56%, 1,7-dimethylxanthine by 84%, ibuprofen by 76% and variant from the NAS WWTP sulfamethoxazole and trimethoprim were remove by 45% and 30% respectively. Sulfamethoxazole and trimethoprim were the compounds tested for removal in the horizontal, subsurface-flow wastewater treatment wetland with aeration, planting and temperature as the treatments applied to the waste stream. The results showed no statistically significant relationship between the removal of each compound and the treatments applied. Sulfamethoxazole removal ranged from 45-89 percent and trimethoprim was removed greater than 96 percent. The aerated subsurface flow

wetlands offer a significantly higher removal of sulfamethoxazole and trimethoprim than the NAS WWTP and the NTF WWTP. The data from the wetland indicates the removal is due to a greater HRT in the wetland than in a typical WWTP.

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