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EFFECTS OF HYDROLOGY, VEGETATION, AND AERATION ON EMERGING CONTAMINANT REMOVAL FROM SECONDARILY TREATED WASTEWATER EFFLUENT IN TREATMENT WETLAND MESOCOSMS

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EFFECTS OF HYDROLOGY, VEGETATION, AND AERATION ON EMERGING CONTAMINANT REMOVAL FROM SECONDARILY TREATED WASTEWATER EFFLUENT IN TREATMENT WETLAND MESOCOSMS

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BY THE COMMITTEE CONSISTING OF

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Abstract

Indirect potable reuse is an expanding practice which returns portions of treated wastewater to municipal supplies. However, traditional wastewater treatment is not designed to remove the wide variety of "emerging contaminants" (EC), including personal care products, pharmaceuticals, industrial and agricultural compounds, and their transformation byproducts, found in modern wastewater. Treatment wetlands are an ecologically engineered approach designed for water quality improvement via natural biogeochemical, physiochemical, microbiological, and ecological processes, including sorption, volatilization, photodegradation, biodegradation, and phytoremediation. Mesocosm-scale wetlands provide the experimental flexibility required to identify appropriate combinations of wetland design parameters (e.g., hydrologic design, vegetation scheme, hydraulic retention time (HRT)) that optimize EC removal. A mesocosm treatment wetland compound comprised of 52 individual 220-L batch reactors was constructed at the Norman Water Reclamation Facility. This research focused on half of the mesocosms which received secondarily treated municipal wastewater. The experimental design included five open water control (OWC), ten free water surface (FWS) and ten subsurface flow (SSF) mesocosms. Half of each of the FWS and SSF mesocosms were planted with soft rush (*Juncus effusus*), while the remaining half remained unplanted. After an initial experiment determined an appropriate HRT of seven days, an aeration experiment was conducted in which active aeration was provided to 14 of the mesocosms by solar powered fish-pond aerators. Evaluation of removal efficiencies for sulfamethoxazole (SMZ), carbamazepine (CMP), and bisphenol-A (BPA) was conducted by measuring influent and effluent aqueous concentrations via enzyme linked immunosorbent assay (ELISA) testing. SMZ removal was greater in FWS and OWC, averaging 83% and 82% respectively, compared to 16% in SSF and was not significantly affected by vegetation ($p = 0.303$)

or aeration ($p = 0.228$). CMP removal was impacted by hydrologic design, with removal rates in FWS, SSF, and OWC averaging 73%, 66%, and 56%, respectively, with a significant difference ($p = 0.001$) between OWC (56%) and FWS (73%). CMP removal was not affected by vegetation or aeration. The BPA concentration in 84% of the effluent samples was below the minimum detection limit (MDL) of 50 ng/L, limiting BPA removal efficiency calculations to a maximum removal efficiency of 64%. BPA removal efficiency averaged 50% or greater for all treatment combinations. BPA removal efficiency was not affected by hydrology, vegetation, or aeration. Greater removal efficiencies for all target ECs as compared to previous studies may be due to a combination of factors including the high quality of the influent, extended hydraulic retention time, maturity of the systems, or other environmental influences. The results of this study provided evidence for the effectiveness of TWs for the removal of ECs from secondarily treated wastewater.

1.0 Introduction

Equitable access to treatable water is a basic human requirement that is increasingly scarce in today's environment (United Nations, 2021). One option for securing a Public Water Supply (PWS) is Indirect Potable Reuse (IPR). IPR returns a portion of secondarily treated wastewater to the PWS through an environmental buffer such as a river, wetland, or aquifer. Public acceptance of IPR is dependent upon the understanding of, and trust in, the effectiveness of both the secondary wastewater treatment (WWT) processes and the environmental buffer. Conventional WWT is primarily designed to address three wastewater characteristics: Biochemical Oxygen Demand (BOD), suspended solids, and hydrogen ion concentration (pH) (Davis, 2010). Conventional WWT is not designed to remove a wide variety of recalcitrant compounds found in modern wastewater (Zhang et al., 2014). Municipal wastewaters are known to contain thousands of personal care products, pharmaceuticals, industrial/agricultural compounds, and their transformation products (Oberg and Leopold, 2019). Multiple terms are used to collectively refer to these compounds including: micropollutant (MP), emerging contaminant (EC), contaminant of emerging concern (CEC), organic micropollutant, emerging pollutant, and emerging organic contaminant (Overton et al., 2023). Most of these compounds are found in trace amounts (i.e., microgram to nanogram per liter; Murray et al., 2010), yet still are cause for concern as ECs can persist in the environment (Oros et al., 2003) and produce unintended and undesirable consequences (Keerthanan et al., 2021). Communities considering IPR must find economically viable options for removal of ECs from wastewater prior to entry into the PWS.

Treatment wetlands (TWs) are engineered ecosystems designed specifically to improve water quality through a series of natural processes (Mitsch and Jørgensen, 2004). TWs are an

established technology for the treatment of municipal wastewater, mine drainage, and even highly concentrated agricultural waste (Vymazal, 2011). Research has shown that TWs can remove a variety of ECs commonly found in municipal wastewater through a combination of natural processes including photolysis, sorption, aerobic and anaerobic microbial degradation, oxidation-reduction cycles, and phytoremediation (Salah et al., 2023). As indicated by the repeated use of "emergent" when describing these compounds, quantified awareness of their presence and their environmental impacts is in its infancy (Munné et al., 2023) Likewise, research in nature-based solutions addressing ECs is an emerging field. More information concerning EC removal rates, loading capabilities, hydrologic designs, vegetation schemes, seasonal performance, and regional considerations are needed for scientists, engineers, stakeholders, and the public to consider TWs in their water supply cycle (Wilkinson et al., 2017).

2.0 Literature Review

2.1 Emerging Contaminants

ECs are not new to the environment. Anthropogenic compounds have made their way into air, soil, and water since before the industrial revolution (Rhind, 2009). There are currently over 350,000 synthesized compounds available on the international market with more being created on a constant basis (Wang et al., 2020). Many ECs, such as expanded polytetrafluoroethylene (Gore-Tex ™), are designed to resist biodegradation, while others, such as amoxicillin, are surprisingly recalcitrant due to unknown environmental interactions (Chowdhury et al., 2022). In addition to the numerous ECs introduced to the environment, the degradation of these compounds can result in the creation of more toxic byproducts, of which we know less about than the parent compound (Ruggeri et al., 2013). Furthermore, many ECs are readily degraded, but are continually present due to constant release into the environment (Ellis,

2006). Detection of these compounds was previously impossible due to the dilute concentrations at which they are found in the natural environment. Advancements in Liquid Chromatography coupled with Mass Spectrometry (LC/MS) as well as Gas Chromatography/ Mass Spectrometry (GS/MS) have increased analytical capabilities and lowered detection limits enabling these compounds to be measured at concentrations in the microgram to nanogram per liter range (Boyd et al., 2003). With increasing awareness of ECs comes increasing concern over the ecological impacts and human health risks associated with their presence, evidenced by the fact that peer-reviewed journal articles focused on ECs have increased from around 10 per year in the 1980s to 1000 per year in 2017 (Oberg and Leopold, 2019).

2.1.1 Prevalence in Public Water Supplies

The Oklahoma Department of Environmental Quality (ODEQ) defines ECs as a group of synthetic or naturally occurring chemicals that are not currently regulated under the Clean Water Act (CWA) or Safe Drinking Water Act (SDWA) and are not currently monitored in the environment (ODEQ, 2022). ECs include Pharmaceuticals and Personal Care Products (PPCPs), veterinary medicines, Endocrine-Disrupting Chemicals (EDCs), industrial compounds, household cleaners, fertilizers, and pesticides. Studies indicate that ECs are continuously introduced into the environment via discharge of treated sewage into receiving water bodies (Boyd et al., 2003; Ellis, 2005; Rhind, 2009). Those same receiving water bodies often serve as PWS for downstream communities, a scenario termed de facto reuse. In addition to ECs from WWT discharge, many PWS also receive non-point source discharge containing fertilizers, pesticides, and other ECs found in stormwater runoff. As ECs are unmonitored and unregulated, quantification and characterization of their presence in PWS is often non-existent. Due to dilute environmental concentrations, the wide array of possible analytes, the expense of testing, and a

lack of regulatory guidelines, EC monitoring is not currently standard practice in the United States (US). However, recent announcements from the United States Environmental Protection Agency (USEPA) regarding proposed Maximum Contaminant Levels (MCLs) of 4 ng/L (4 parts per trillion) for Perfluorinated Alkylated Substances (PFAS) in drinking water indicate regulatory guidelines for ECs with known health risks are on the way (USEPA, 2023). Currently, MCLs are based not only on known ecological and human health risks, but also on the feasibility of treatment and limits of detection. Although the ecological and human health risks of several individual ECs such as atrazine and PFAS are well documented, the additive and long-term effects of the various combinations of ECs at environmental concentrations found in PWSs are unknown (Wilkinson et al., 2017). Although removal of ECs is now possible via advanced treatment processes such as membrane separation and ozonation, those systems are prohibitively expensive for many communities (Zagklis et al., 2022; Zhang et al., 2014). Due to advancements in LC/MS and GC/MS, detection of ECs at environmental concentrations is now possible, but capable laboratories are not readily available in all regions, routine analysis is sometimes prohibitively expensive, and results can take weeks or months, leaving operators lacking the information needed to adjust operations appropriately. Considering these hurdles, MCLs for most ECs will not be determined in the near future.

2.1.2 Ecological Impacts

Although ECs are ubiquitous in surface waters across the US, their ecological impacts are largely unknown (USEPA, 2008). Laboratory studies have identified the effects of a few individual ECs on select species, but the varying combinations and concentrations of ECs in the environment make it difficult to conduct a true assessment of their ecological impacts (USFWS, 2019). However, Aquatic Life Criteria (ALC) are being developed for ECs with an observable

impact. Field observations of intersex in wild populations of aquatic vertebrates downstream from WWTPs has prompted the USEPA to begin determining ALC for EDCs, a subgroup of ECs including synthetic estrogens and androgens, as well as other compounds capable of modulating hormonal functions in aquatic organisms (USEPA, 2008). ALC for ECs for which the effects are not as obvious are difficult to establish under current USEPA procedures, a discussion beyond the scope of this thesis but detailed in the USEPA white paper: Aquatic Life Criteria for Contaminants of Emerging Concern (USEPA, 2008). In 2019, the U.S Fish & Wildlife Service examined the results of 123 laboratory studies of 38 of the most frequently detected and potentially dangerous ECs in the Great Lakes Basin to produce effect-specific and mean Screening Values (SVs) for freshwater fish (USFWS, 2019). SVs are produced as pairs to provide an upper limit, SV_{HIGH}, the water concentration above which adverse health effects are expected, and a lower limit, SV_{LOW}, below which no adverse effects are expected. The studies examined the Lowest Observable Adverse Effect Concentration (LOAEC) and No Observable Adverse Effect Concentration (NOAEC) levels regarding behavior, blood and circulation, life stage development, endocrine function, genotoxicity, gross pathology, growth, histopathology, immunological function, mortality, neurological development, metabolic function, and reproductivity. The effect-specific SVs were adjusted with Uncertainty Factors (UFs) and then used to calculate the mean SVs for 14 ECs which are provided in Table 1 (USFWS, 2019).

Table 1: Screening Values for 14 ECs determined by the USFWS (USFWS, 2019).

Compound	Use	SV_{HIGH} (µg/L)	SV_{LOW} (µg/L)	Confidence
4-Androstene-3, 1 7-dione	Hormone	0.852	0.000204	Very Low
Bisphenol A	Plasticizer	118	0.0318	High
Carbamazepine	PPCP (anticonvulsant)	139	0.00865	High
Citalopram	PPCP (antidepressant)	0.222	0.000102	Very Low
N,N-diethyl-meta-toluamide (DEET)	Insect repellant	22	0.236	Very Low
Diphenhydramine	PPCP (antihistamine)	1.26	0.00846	Very Low
Estrone	Hormone	0.00665	0.0000144	Low
Hexahydrohexamethylcyclopentabenzopyran (HHCB)	PPCP (fragrance)	21.3	0.0649	Low
Ibuprofen	PPCP (anti-inflammatory)	10.5	0.0153	Moderate
Lidocaine	PPCP (antiarrhythmic)	890	2.4	Low
β-Sitosterol	Phytohormone	18.4	0.0604	Low
Tris(2-butoxyethyl)phosphate (TBEP)	Flame retardant	267	0.448	Low
Triclosan	PPCP (antibacterial)	12.8	0.00254	Low
Venlafaxine	PPCP (antidepressant)	0.155	0.000638	Very Low

It should be noted that the reported confidence levels for many SVs are “Low-Very Low” due to combinations of UFs. The UFs associated with each SV are based on errors due to extrapolation from laboratory to field conditions. The UFs and a brief explanation of each are provided in Table 2.

Table 2: Uncertainty factors impacting SV calculations (USFWS, 2019).

Uncertainty Factor	Explanation
Chemical Complexity	Adjusts single EC LOAEC/NOAEC from laboratory studies to an exposure scenario involving the same EC in a mixture
Inter-Species Sensitivity	Adjusts LOAEC/NOAEC from laboratory studies that tested for effects in a single species to an estimated effect concentration in a receptor species or group of species that are more sensitive to the subject EC
Intra-Species Sensitivity	Adjusts LOAEC/NOAEC from laboratory studies that tested for effects in one class of individuals within a fish species to an estimated effect concentration in a more sensitive class of individuals in the same species
Exposure Duration	Adjusts LOAEC/NOAEC from laboratory studies that tested for effects after acute or sub-chronic exposure to a presumed equivalent effect concentration after chronic exposure
Effect Concentration	Adjusts an unbounded LOAEC to a corresponding NOAEC for the same EC, endpoint, species, life stage, exposure route and duration, including adjustment for nominal exposure concentrations reported in laboratory studies
Database Adequacy	Adjusts the mean SV for uncertainty associated with limited quantity and breadth of reliable and available ecotoxicity information

Although the study examined 123 laboratory experiments with 38 ECs, the quantity and breadth of reliable data limited the final report to 14 ECs. This effort by the USFWS is one of the more comprehensive to date, yet still only addresses 14 ECs and their effects on freshwater fish in the Great Lakes Basin. The ranges between SV_{HIGH} and SV_{LOW}, often several orders of magnitude in difference, are representative of the current lack of knowledge regarding the ecological impacts of ECs.

2.1.3 Implications for Indirect Potable Reuse

Intentional potable reuse, both IPR and Direct Potable Reuse (DPR), is a growing practice in the United States. Although federal guidelines and regulations for IPR/DPR do not exist outside of the Clean Water Act (CWA) and Safe Drinking Water Act (SDWA), several states have adopted policies to establish both IPR and DPR treatment requirements. Of the 17 states with IPR/DPR policies, California (CA) and Oklahoma (OK) are the only states that

address ECs in their treatment requirements. Practicing IPR since 1962, CA has the oldest and most stringent IPR regulations in the US. CA allows indirect potable reuse through Groundwater Replenishment (GWR) via surface application, GWR via direct injection, and Surface Water Augmentation (SWA). All three applications require advanced treatment which must include reverse osmosis and advanced oxidation to meet minimum log reduction requirements for enteric viruses, *Giardia lamblia*, *Cryptosporidium,* and 1,4-dioxane (SWRCB, 2018). It should be noted that CA originally developed these policies with an eye towards the greatest known human health risks associated with wastewater: bacteria, viruses, and protozoa. The regulations have evolved to reflect increased knowledge of pollutants and trace chemicals found in modern WWT. Currently, treatment must also satisfy all MCLs specified in CA's Drinking Water Regulations, Notification Limits (NLs) specified in CA's Drinking Water Notification Levels , and Monitoring Trigger Levels (MTLs) specified in CA's Recycled Water Policy (SWRCB, 2018). These MCLs, NLs, and MTLs apply to a wide range (over 40 contaminants) of organic and inorganic chemicals, radionuclides, disinfection byproducts, lead, copper, health-based ECs, performance indicator ECs, and bioanalytical screening tools for ECs. Table 3 (SWRCB, 2018) provides the health-based and performance indicator ECs and reporting limits currently applied in CA.

Table 3: Health and performance-based EC limits for IPR in California (SWRBC, 2018)

Compound	Indicator Type	Limit (µg/L)
Groundwater Recharge – Surface Application		
1,4-dioxane	Health	0.1
N - Nitrosodimethylamine (NDMA)	Health	0.002
N - Nitrosomorpholine (NMOR)	Health	0.002
Perfluorooctanesulfonate (PFOS)	Health	0.0065
Perfluorooctanoic acid (PFOA)	Health	0.007
Gemfibrozil	Performance	0.01
Iohexal	Performance	0.05
Sucralose	Performance	0.1
Sulfamethoxazole	Performance	0.01
Reservoir Augmentation and Groundwater Recharge – Direct Injection		
1,4-dioxane	Health	0.1
N - Nitrosodimethylamine (NDMA)	Health	0.002
N - Nitrosomorpholine (NMOR)	Health	0.002
Perfluorooctanesulfonate (PFOS)	Health	0.0065
Perfluorooctanoic acid (PFOA)	Health	0.007
Sucralose	Performance	0.1
Sulfamethoxazole	Performance	0.01

With these treatment requirements, CA is confident that all known health risks associated with IPR have been minimized. The OK policy on IPR was established in May 2023 by ODEQ Title 252, Chapter 628. The treatment requirements for conventional parameters are essentially identical to those of CA, with a focus on pathogens, however the requirements for ECs are loosely defined and will be evaluated by ODEQ on a case-by-case basis. The policy states "Constituents of Emerging Concern…are to be evaluated in IPR Source Water treatment. Examples of CECs are chemicals in the following categories...Selected constituents may be surrogates for a broader list of constituents for use in evaluating overall levels in treated IPR Source Water or for reservoir evaluation." There are currently no IPR applications in OK,

therefore no specific list of monitored ECs has been determined by ODEQ. The lack of regulation from other states is reflective of the lack of guidance from the USEPA. The USEPA does acknowledge that ECs are an important consideration for IPR and should be addressed on a case-by-case basis in the "2017 Potable Reuse Compendium" (USEPA, 2017), which is a supplement to the "2012 Guidelines for Water Reuse" (USEPA, 2012). Both documents iterate the lack of scientific consensus regarding human health risks of these compounds at such dilute concentrations. Although federal guidelines provide recommendations for EC considerations, the only legal requirements are those provided in the CWA and SDWA (USEPA, 2012).

2.2 Treatment Wetlands

2.2.1 Historical Background

Natural wetlands, due to their landscape position and historic underappreciation, have received and treated wastewater for millennia. Wetlands foster a wide range of naturally occurring processes including sorption, photolysis, phytoremediation, anaerobic and aerobic microbial degradation, and oxidation-reduction cycles that remove both organic and inorganic matter from the water column, a function that has earned them the nickname "nature's kidneys" (Mitsch & Jorgensen, 2004). Exploration of this function began in earnest in the 1950s with Käthe Seidel of The Max-Planck Institute in Germany (Vymazal, 2011). Seidel's experiments found that gravel beds planted with emergent macrophytes were effective at removing bacteria and organic and inorganic materials from wastewater. Seidel's work led to the development of the *Max-Planck-Institute process* which has been refined into the various forms of Sub-Surface Flow (SSF) TWs operating today. Unlike the SSF designs of their European counterparts, initial TW designs in the US utilized a free water surface (FWS) that mimic natural wetlands. Continued research into the natural processes occurring in both FWS and SSF TWs led to TWs

being designed to treat wastewaters originating from domestic and industrial sources, agricultural runoff, acid mine drainage, landfill leachate, and urban stormwater runoff (Kadlec and Wallace, 2009). TWs have evolved to include multiple hydrologic designs which will be discussed in the following section.

2.2.2 Hydrologic Designs

TWs can be divided into two general hydrologic designs, FWS and SSF. FWS TWs (Figure 1) are characterized by water flowing at some depth above a substrate, typically hydric soil. SSF TWS (Figure 2) are characterized by water flowing through a highly permeable substrate such as gravel or sand. Although Figures 1 & 2 depict vegetation, TWs are not always vegetated.

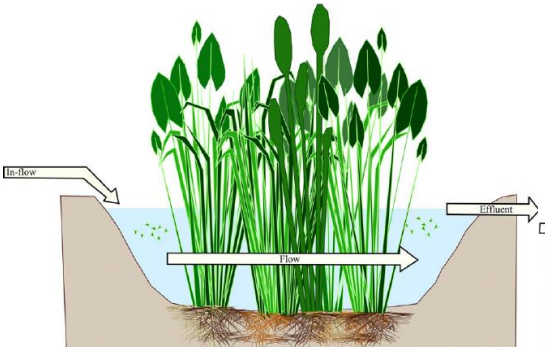


Figure 1: FWS TW flow schematic (White,2013)

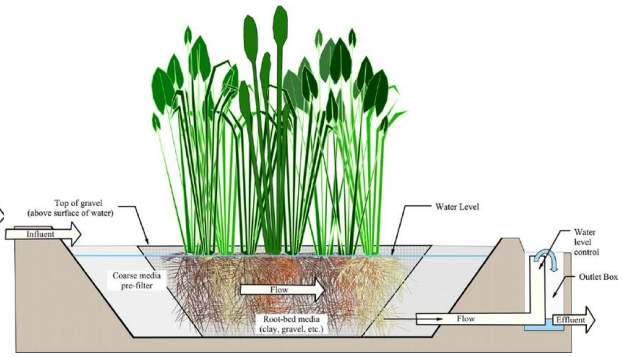


Figure 2: SSF TW flow schematic (White, 2013)

A more comprehensive discussion of removal mechanisms is provided in the following sections, but in general, FWS TWs promote aerobic microbial degradation, oxidation, nitrification, and photolysis while SSF TWs promote anaerobic microbial degradation, reduction, denitrification, and sorption.

2.2.3 Removal Mechanisms

The major removal mechanisms for ECs in TWs are sorption, phytoremediation, photodegradation, and microbial degradation (Kadlec and Wallace, 2009). Sorption can refer to either or both process(es) of adsorption of ECs onto the surface of substrate particles and biofilms, or absorption of ECs into the volume of substrate particles or microorganisms. Sorption is governed by the physicochemical characteristics of the sorbate including hydrophobicity/ hydrophilicity, water solubility (S_W), chemical structure, partitioning coefficients, and ionizability as well as sorbent properties including particle size, porosity, specific surface area, micropore structure, surface charge, organic carbon content, clay content, and cation exchange capacity (Mihelcic and Zimmerman, 1992). The relationship between the concentration of sorbent in the liquid phase and the concentration of the adsorbed phase is frequently described with Freundlich, Langmuir, and linear isotherm models which can be used to compare sorption capacities (Overton et al., 2023). Isotherm models are sorbate-sorbent specific, created empirically, and are not available for many ECs. Therefore, sorption is often predicted based on an EC's hydrophobicity, represented by the octanol-water partitioning coefficient (K_{OW}) or octanol-water distribution coefficient (D_{OW}) , and ionizability, represented by the pKa(s) of the compound. As K_{OW} and D_{OW} increase, hydrophobicity and sorption potential increase. ECs with high K_{OW} values, Log K_{OW} > 3.0, including diclofenac, triclosan, naproxen, and ketoprofen are considered highly susceptible to sorption. ECs with Log K_{OW} < 2.5, including caffeine, atenolol, and ciprofloxacin are considered unlikely to be adsorbed (Chowdhury et al., 2022). The pKa of an EC determines the state of ionization for a given system pH, which influences sorption through electrostatic attraction/repulsion. ECs with low pKa will exist in cationic form at circumneutral pH and will sorb strongly to negatively charged substrates such as clay. However,

if system pH is greater than pKa, ECs will exist as neutral or anionic species resulting in charge repulsion and minimized sorption. In general, compounds with high pKa values are also highly soluble. Highly soluble compounds such as caffeine (pKa = 10.4, S_W = 21600 mg/L) are hydrophilic and therefore resist sorption (Salah et al., 2023). However, experiments have shown some ECs such as sulfamethoxazole (Log K_{OW} = 0.89, S_w = 610 mg/L) with low K_{OW} and moderate S_W to be removed via sorption, indicating that predicting sorption based on physicochemical properties is prone to error due to simultaneous and possibly competing mechanisms in TWs (Chowdhury et al., 2022). To further complicate the matter, changes in system pH or influent composition can result in desorption of ECs, increasing concentrations in the water column. More research is needed to identify ideal sorbents, sorption capacities, competition over sorption sites in complex waters, and potential for desorption.

Photolysis, or photodegradation, is a removal mechanism for many pharmaceuticals in aquatic environments (Wilkinson et al., 2017). Photolysis can occur directly or indirectly, depending on EC structure and system properties. Direct photolysis refers to a change in the molecular structure due to solar radiation. The presence of aromatic rings, conjugated pi systems, chromophores, and other functional groups facilitate the direct adsorption of solar radiation in the UV-C, UV-B, and UV-A ranges resulting in bond cleavage (Challis et al., 2014). Indirect photolysis occurs via reactions with photo-induced species including singlet oxygen ($^1O_2$), hydroxyl radicals ($OH^\bullet$), and the triplet excited states of chromophoric dissolved organic matter ($^3CDOM^*$) which are formed via photosensitizers such as nitrate, nitrite, iron complexes, and dissolved organic matter (Lastre-Acosta et al., 2019). Direct photolysis can be estimated by relating quantum yield, which quantifies the ability of a particle to release absorbed electromagnetic radiation, to photodegradation constants and resulting half-life. Indirect

photolysis is more difficult to predict due to the role of photo-induced species and system properties. Lastre-Acosta et al. (2019) examined the effects of direct and indirect photolysis on the antibiotic enoxacin and found both mechanisms to be responsible for removal with the dominant mechanism dependent upon initial concentrations, system pH, and the presence of dissolved organic matter. A subsequent study focused on 2-chlorobiphenyl determined that reactions with hydroxyl radicals ($OH^{\bullet}$) was the primary removal mechanism and direct photolysis was insignificant (Lastre-Acosta et al.,2022). Zhou et al. (2015) conducted similar experiments on a group of antivirals and determined that despite similar structures, zidovudine is prone to direct photolysis while lamivudine and acyclovir are prone to indirect photolysis. The presence of nitrate was found to increase photolysis of all three antivirals, while the presence of bicarbonate increased photolysis of acyclovir, and DOM increased photolysis of acyclovir and lamivudine. Few studies have separated the effects of indirect and direct photolysis, with most of the research focused on photolysis in concert with other removal mechanisms (Ilyas and Hullebusch, 2020). More research is needed to examine this removal mechanism as photolysis cannot be predicted, is highly dependent upon system properties, and has been proven effective in the removal of ECs that resist biotic degradation.

Phytoremediation is a general term that encompasses multiple ways in which plants remove pollutants from soil and water. Phytoremediation includes phytoextraction, translocation, rhizodegradation, phytodegradation, phytovolatilization, and phytostabilization. Several studies indicate the key factor determining susceptibility to phytoremediation is hydrophilicity/ hydrophobicity as indicated by the Kow and/or Dow (McCorqoudale-Bauer et al., 2023; Chowdhury et al., 2022). This perspective assumes that the main pathway is via plant water uptake. However, this may be an oversimplified approach due to substantial differences in the

macrophytes employed in various studies. Phytoremediation begins in the rhizosphere, the region of substrate under direct influence from the plant's roots system and associated microbiological community. The rhizosphere of each species is unique and varies depending on environmental conditions and stresses. A recent critical review concluded that due to the high soluble organic carbon found in rhizospheres, hydrophobic compounds can be immobilized and /or mineralized in the biofilms of the region (McCorquodale-Bauer et al., 2023). Additionally, due to differences in plant cytoplasmic membranes and lipid structures, using K_{ow} values to estimate partitioning in plants is inaccurate (Miller et al., 2016). Although it is accepted that the presence of vegetation generally improves EC removal, the pathway(s) appear to be specific to each combination of EC and vegetation. For example, it has been observed that in the rhizosphere of *Juncus effusus* (soft rush), bacteria form iron plaque which develop into iron hydroxides capable of strongly sorbing and immobilizing ECs such as azithromycin and clarithromycin (Tai et al., 2017). Studies with *Lactuca sativa* (lettuce) concluded that clarithromycin was transported primarily into the lettuce leaves and degraded, while sulfadiazine was contained primarily in the roots and remained undegraded (McCorquodale-Bauer et al., 2023). While both studies indicate phytoremediation effectively removed clarithromycin, the specific pathways were very different. This comparison is just one example, of which there are dozens, to be found in the swath of research currently being conducted in this field (e.g., Avila et al., 2021; Ilyas et al., 2019; Brunhoferova et al., 2021). One of the challenges in assessing the role of plants is the wide variety of species employed in various climates, variations in experimental setups, and a wide range of source waters and influent EC concentrations. Due to these variables, there are currently no guidelines or predictive models for EC removal via phytoremediation.

Microbial degradation is the most widely studied removal mechanism of ECs in TWs (Overton et al., 2023). Microbes inhabit multiple regions of TWs including the water column, inside plant tissue, the rhizosphere of macrophytes, and in biofilms. Although the specific microbial degradation pathway of ECs in TWs is not certain, it is hypothesized that some ECs are completely mineralized, and others are transformed to more hydrophobic (or more hydrophilic) compounds to be acted upon by other removal mechanisms (Nguyen et al., 2019). Susceptibility to biodegradation is predicted based on chemical structure. Specific functional groups make predictions difficult, but in general, simple molecules are more readily degradable while larger molecules containing branched alkanes, aromatic rings, and amides are more recalcitrant (Rout et al., 2021). For example, molecules belonging to the same class of therapeutic pharmaceuticals with similar physicochemical properties, such as diclofenac and ketoprofen, can exhibit significantly different removal rates due to minute differences in structure (Matamoras et al., 2006). Aerobic microbial degradation has been cited as the removal mechanism of multiple ECs such as 4-nonylphenol, bisphenol-A, atenolol, nicotine, caffeine, and gemfibrozil (Avila et al., 2010; Matamoras et al., 2012; Carvalho, 2020). Anaerobic degradation has been cited as the removal mechanism for more recalcitrant ECs such as diclofenac, tonalide, ofloxacin trimethoprim, and sulfamethoxazole (Park et al., 2009; Chen et al., 2016; Liang et al., 2018). However, microbial degradation in most research is not directly measured but determined indirectly through changes in concentrations in the aqueous phase (Zhang et al., 2014; Chowdhury et al., 2022) . Therefore, it is often difficult to discern between removal due to microbial degradation and removal due to other mechanisms.

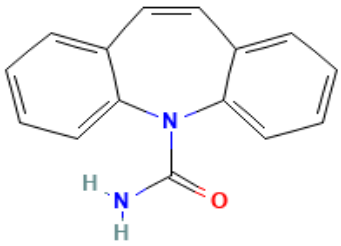
2.2.4 Mesocosm and Pilot Scale Studies

Researchers around the world have conducted numerous experiments to determine removal mechanisms and predict removal rates of ECs in TWs (Avila et al., 2019; Chen et al., 2019; Park et al., 2009; Carranza-Diaz et al. 2014). Much of these data have been compiled into critical reviews to identify correlations between EC removal and TW design, hydraulic retention time, substrate, vegetation, and other design variables (Chowdhury et al., 2021; Wilkinson et al., 2019; McCorquodale-Bauer et al., 2023; Overton et al., 2023). Despite the number of critical reviews, little consensus has been reached. This lack of consensus is not surprising, as the number of variables between experiments makes comparison almost impossible. For example, in a review published by ASCE in 2022, the authors examine over 120 peer-reviewed articles to guide TW design specifically for EC removal. However, when comparing removal mechanisms and rates, they make no distinction between TW size (bench scale, mesocosm, pilot) or influent water quality (raw, artificial, secondarily treated) when drawing their conclusions (Chowdhury et al., 2022). As a result, some of the expected removal rates are reported as a wide range such as 10-80%. Even when comparing similar systems with comparable influents, consensus is elusive. A 2018 comparison of three TWs receiving secondarily treated municipal wastewater with low organic and nutrient content concluded that EC removal in all three wetlands was much lower than expected based on literature review (He et al., 2018). The investigators correlate a low organic loading rate with a low EC removal rate, reinforcing previous studies that link high BOD, TSS, and NH_4-N removal with high EC removal (Matamoras et al., 2008) . Conversely, a 2023 study examining TWs receiving secondarily treated municipal wastewater with low organic and nutrient content reported high removal rates for a variety of ECs and concluded that the high DO, low COD, and low NH_4-N were critical for the high EC removal rates in TWs (Wang et al.,

2023). Although these experiments vary in their setup, they often share common target analytes including carbamazepine (CMP) , sulfamethoxazole (SMZ), and bisphenol-A (BPA).

CMP, a widely prescribed anticonvulsant used to treat seizures, diabetic neuropathy, and bipolar disorder (Mann, 2023) is one of the most detected and studied ECs (Overton et al., 2023) and is a proposed anthropogenic marker to assess water quality (Hai et al., 2018). CMP, at environmentally relevant concentrations, has been shown to alter larvae development and behavior of aquatic organisms (Qiang et al., 2016; Yan et al., 2020) and significantly alter gene expression in laboratory rats (Santariova et al., 2023). The physio-chemical properties of CMP (Table 4) have been used to predict the fate and transport of the molecule in previous studies.

Table 4 : Physio-chemical properties of CMP

Structure [a]	**Formula**	**Log K_{OW}** [b]	**SW** [c]	**pKa** [d]
	$C_{15}H_{12}N_2O$	2.45	17.7	13.9

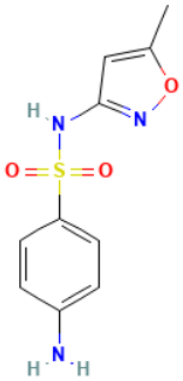
[a] (NCBI, 2024)
[b] Octanol-water partition coefficient (log K_{OW}).
[c] Water solubility (25 ˚C) (mg L^{-1}).
[d] Ionization constant (pKa).

Zhang et al. (2014) concluded that due to its recalcitrance and low water solubility (17.7 mg/L), microbial degradation and photolytic degradation have minimal impact on CMP, making sorption and plant uptake the main removal mechanisms in SSF TWs. Experiments with light expanded clay aggregates and vermiculite by Dordio et al. (2009) conclude that sorption is the main removal mechanism for CMP in SSF TWs. Subsequent work by Dordio et al. (2010) concluded that due to a log Kow of 2.45, CMP is subject to plant uptake and translocation.

Carvalho et al. (2021) concluded that CMP removal in FWS TWs was trivial due to its resistance to photolytic degradation.

SMZ, much like CMP, is a widely prescribed pharmaceutical that is commonly studied (Overton et al., 2023). SMZ is an antibiotic used to treat infections of the urinary tract, respiratory system, and gastrointestinal tract. SMZ is considered a persistent pollutant in aquatic environments as it is poorly removed in conventional WWT and continuously introduced to the environment through municipal wastewater (Hernández-Pérez et al., 2020). The presence of SMZ in the aquatic environment is a concern due to the impacts on microbial communities and antibiotic resistant genes (Kergoat et al., 2021). The physio-chemical properties of SMZ (Table 5) have been used to predict the fate and transport of the molecule in previous studies with limited success.

Table 5: Physio-chemical properties of SMZ

Structure [a]	**Formula**	**Log K_{OW}** [b]	**SW** [c]	**pKa** [d]
	$C_{10}H_{11}N_3O_3S$	0.89	610	1.6, 5.2

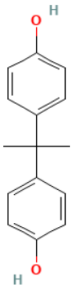
[a] (NCBI, 2024)
[b] Octanol-water partition coefficient (log K_{OW}).
[c] Water solubility (25 ˚C) (mg L^{-1}).
[d] Ionization constant (pKa).

Prior research indicates SMZ may be affected by aerobic and anaerobic microbial degradation, sorption, photolytic degradation, and phytoremediation (Chowdhury et al., 2020). However, despite the numerous mechanisms cited in SMZ removal, removal efficiencies vary

greatly between studies with little agreement on the fate of the compound. Liang et. al. (2018) examined TWs with SMZ removal efficiencies greater than 70% and concluded that sorption was primarily responsible for SMZ removal. Park et. al. (2009) examined the soils and macrophytes of TWs with SMZ removal rates of 15-85% and found no detectable quantities of SMZ, suggesting sorption and phytoremediation are not major removal mechanisms. Button et. al. (2018) examined SMZ in aerated TWs and concluded that sorption to soil and biofilms were responsible for SMZ removal with greater removal efficiencies in vegetated wetlands. This lack of consensus may be due to the physio-chemical properties of SMZ. With an octanol-water partitioning coefficient of 0.89, SMZ is not lipophilic and not prone to adsorption. However, the deprotonation of the amide group (pKa = 1.6) and sulfide moiety (pKa = 5.2) will result in a negatively charged molecule (anion) with greater solubility and greater potential to bind with cationic species. In addition to deprotonation, SMZ can deconjugate and conjugate in biological treatment systems (Sochacki et al., 2018), thereby increasing concentration. While there is no lack of research into SMZ removal, the only consensus is that the fate and transport of the compound cannot be predicted based on the physio-chemical properties of the molecule (Chowdhury et al., 2020).

BPA is another widely studied EC due to its designation as an EDC and its prevalence in surface waters (Canesi and Fabbri, 2015). BPA is a plasticizer used in polycarbonate plastics and epoxy resins. According to the USEPA, releases of BPA to the environment exceed one million pounds per year (USEPA, 2015). In April 2023, The European Food Safety Authority issued an updated scientific opinion that deemed BPA a consumer health risk (EFSA, 2023). The physio-chemical properties of BPA (Table 6) indicate BPA is removed by adsorption and aerobic microbial degradation (Avila et al., 2010).

Table 6 : Physio-chemical properties of BPA

Structure [a]	Formula	Log K_{OW} [b]	SW [c]	pKa [d]
	$C_{15}H_{16}O_2$	3.32	300	9.6

[a] (NCBI, 2024)
[b] Octanol-water partition coefficient (log K_{OW}).
[c] Water solubility (25 ˚C) (mg L^{-1}).
[d] Ionization constant (pKa).

Tondera et al. (2019) examined BPA removal in TWs receiving combined sewer overflow and found BPA removal to be seasonally dependent, with removal efficiencies of 90% in summer reduced to 50% during winter. This seasonal dependance suggests that microbial degradation is a major BPA removal mechanism. This seasonal dependance was not observed for CMP or SMZ, suggesting that abiotic removal mechanisms such as sorption and photolysis are responsible for their removal.

Although previous research indicates TWs remove CMP, SMZ, and BPA, more research is needed to establish removal efficiencies and loading rates. One thing is clear from the wealth of information available: TWs are complex systems that encourage physical, chemical, and biological processes that may occur simultaneously or sequentially. Furthermore, the effectiveness of those processes is dependent upon a wide range of variables including hydraulic design, choice of substrate, presence and type of vegetation, quality of influent, and target analytes.

Aeration is commonly used to increase substrate removal rates in biological treatment systems that utilize aerobic heterotrophs. Providing a sufficient (or excessive) amount of

terminal electron acceptor is critical to ensure that the rate limiting factor in the system is the substrate. Previous studies on the effects of aeration on EC removal in TWs receiving secondarily treated wastewater are scarce. However, studies on TWs receiving primary wastewater indicate that aeration improves EC removal along with organic and nutrient removal (Avila et al., 2021; Al Falahi et al., 2021). However, the experimental setup in both studies makes it hard to draw solid conclusions. Al Falahi et al.(2021) conducted a mesocosm-scale study in a greenhouse using domestic septic tank effluent spiked with raw (unmetabolized) ibuprofen to a concentration of 0.6 mg/l. This modification is representative of a widespread problem in this field of research: actual concentrations of ECs in wastewater are often near the detection limit, so researchers spike the water with a concentration several orders of magnitude above the detection limit. This procedure allows them to say, for example, this system removed 98% of ibuprofen by lowering the concentration from 600 µg/l to 12 µg/l. Although it may be accurate, it is not very applicable when the ibuprofen concentration in primary wastewater is commonly in the nanogram per liter range. Avila et al. (2021) studied three pilot scale wetlands receiving primary municipal wastewater without spiking, and several of the target analytes were below detection limits during sampling events. However, results indicate that aeration significantly improved removal rates of all target ECs, COD, and NH_4-N. Although these results are valid and promising, they may not be applicable to tertiary polishing, where the influent has low COD and nutrient content.

3.0 Hypotheses and Objectives

The objective of this research was to evaluate the effectiveness of mesocosm-scale treatment wetlands for removal of SMZ, BPA, and CMP in secondarily treated municipal wastewater and determine which combination(s) of TW design parameters provides the greatest removal efficiencies to aid in future design. This study examines the effects of hydrologic design, vegetation, and aeration on removal rates of SMZ, BPA, and CMP in addition to traditional water quality parameters including BOD, TSS, nitrate-N, nitrite-N, dissolved reactive phosphate, sulfate, and a suite of metals.

It is hypothesized:

1. Aerated wetlands will have greater SMZ and BPA removal efficiencies than non-aerated wetlands.
2. CMP removal efficiency will not be affected by aeration.
3. Vegetated wetlands will have greater removal efficiencies for all target analytes.

4.0 Approach and Methods

TWs are ecologically engineered ecosystems that utilize a wide range of biogeochemical processes found in natural wetlands and are a potential solution for the removal of ECs found in municipal wastewater effluent (Bayati et al.,2021). TWs can be designed to improve water quality through a combination of natural mechanisms including hydrolysis, volatilization, sorption, microbial biodegradation, phytoremediation, and photodegradation (Mitsch & Jorgensen, 2004). Mesocosm scale wetlands are experimental ecosystems that mimic natural ecosystems and allow for controlled manipulation of various forcing functions (Lyu et al., 2018). In this study, several experimental units in a mesocosm treatment wetland compound of 52 individual mesocosm wetlands were manipulated through artificial aeration to explore removal mechanisms and removal efficiencies for a selected suite of ECs. For this study, removal efficiency is defined as percent reduction of aqueous concentration (Equation 1).

$$Removal\ effeciency = \frac{C_{IN} - C_{OUT}}{C_{IN}}\ x\ 100\% \qquad (1)$$

Where C_{IN} represents the influent concentration and C_{OUT} represents the effluent concentration.

4.1 Mesocosm Compound

A treatment wetland mesocosm compound consisting of 52 individual 220-L drainable stock tanks acting as batch reactor TWs was constructed on-site at the Norman Water Reclamation Facility (NWRF). Of the 52 tanks, 25 were dedicated to stormwater experiments, 25 were dedicated to wastewater experiments, and 2 served as plant nurseries. Figure 3 is an aerial photograph of the mesocosm compound at the NWRF.

Figure 3: Aerial photograph of mesocosm compound at NWRF. Image generated by CREW unoccupied aerial system (2022)

4.2 Hydrologic Design

TWs employ various hydrologic designs to encourage multiple biogeochemical processes. Two hydrologic designs were used in these experiments: Free Water Surface (FWS) and Subsurface Flow (SSF). FWS wetlands are defined by the USEPA as "wetland systems where the water surface is exposed to the atmosphere" (USEPA, 2000) and bear more resemblance to natural wetlands than SSF TWs. In Figure 4, a FWS mesocosm schematic, the water surface is maintained above the hydric soil layer, which is prevented from entering the perforated sampling port by the rock pack.

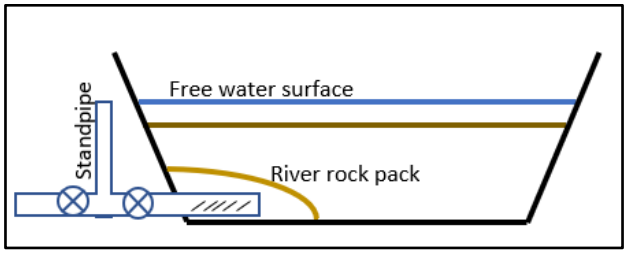


Figure 4: Schematic of FWS mesocosm wetland

SSF mesocosm wetlands, illustrated in Figure 5, are designed to maintain the water level below the top surface of the substrate, which is typically gravel, river rock, or sand.

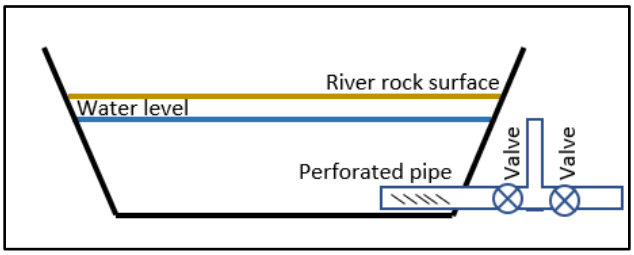


Figure 5: Schematic of SSF mesocosm wetland

In the wastewater portion of the mesocosm wetland compound, 10 mesocosms are FWS, 10 are SSF, and five are open water control tanks. Each mesocosm is a 220-liter drainable stock tank of high-density polyethylene (HDPE). Each tank is 0.33 meters deep with a surface area of 0.67 square meters. FWS mesocosms have a locally sourced hydric soil substrate classified as a clay loam. SSF mesocosms have a river rock substrate. Open water control tanks have no substrate.

4.3 Vegetation Design

The presence of vegetation in TWs facilitates several biogeochemical processes known to remove ECs from the water column (Chowdhury et al., 2022). To examine the effects of vegetation, half of the TWs (five FWS and five SSF) are planted with soft rush, *Juncus effusus.* In addition to planted vegetation, the seed bank of the hydric soil was allowed to express itself and no volunteer vegetation was removed. Half of the TWs (five FWS and five SSF) are unplanted and all volunteer vegetation was removed. Table 7 provides a summary of the hydrologic design and vegetation combinations in the mesocosm compound.

Table 7: Summary of mesocosm compound configuration by hydrologic design and vegetation.

Wastewater Treatment Wetland Mesocosm Design Plan		
	Planted	Unplanted
FWS	5	5
SSF	5	5
OWC	5	
Total	25	

4.4 Aeration Design

Aerobic and anaerobic biodegradation are known removal mechanisms for ECs (Avila et al.,2021). While FWS and vegetated wetlands promote aerobic conditions near the free surface and in the rhizome, most of the water column in both FWS and SSF wetlands is anoxic (USEPA, 2000). TWs are often aerated to promote an oxidizing environment that may not occur naturally. To examine the effects of aeration on EC removal, 3 OWC, 5 SSF, and 6 FWS mesocosms were aerated using commercially available, solar-powered fishpond aerators providing a maximum flow rate of 2 LPM at a maximum pressure of 66 kPa (Figure 6).

Figure 6 : Solar-powered aerator installation for one FWS, one OWC, and two SSF mesocosms.

FWS wetlands were aerated from the top of the hydric soil substrate (air diffuser is visible as a blue dot in Figure 6) as it is impractical to aerate hydric soil. This was intended to create an aerobic/oxidizing environment in the water column while creating an anoxic-anaerobic/reducing environment in the substrate; however, due to the relatively shallow depth of the mesocosms and the circulation effect of an aerator, it is possible the entire column became aerobic. SSF wetlands were aerated from the bottom river rock substrate so that the entire TW would be aerobic/oxidizing. OWC tanks were aerated from the bottom of the tank. Aeration only occurred during daylight hours as the aerators are solar powered.

4.5 Data Generation

4.5.1 Sample Collection

The mesocosm influent was secondary effluent from NWRF. Influent was pumped to the mesocosms from a manhole in the NWRF treatment train following final aeration and prior to UV treatment. Influent samples to be analyzed for TSS, BOD, cations (Ag, Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, S, Se, and Zn), and anions (nitrite, nitrate, chloride, sulfate, and dissolved reactive phosphate) were collected directly from the pump transfer hose and preserved in HDPE bottles following Standard Method 1060 (Standard Methods Committee, 2018). Samples to be analyzed for EC were collected and preserved in acid-washed amber glass vials following Standard Method 1060 (Standard Methods Committee, 2018). SSF mesocosm effluent samples were drawn from the bottom of the tank through the sampling port after a 2-liter flush volume. FWS effluent samples were collected from the top of the FWS mesocosms. All mesocosm effluent not collected for analysis was pumped back into the NWRF treatment train to undergo UV treatment prior to final discharge.

4.5.2 Field Analyses

In-situ measurements of several physical and chemical parameters of influent and effluent samples including water temperature, specific conductance, resistivity, total dissolved solids, salinity, pH, dissolved oxygen, turbidity, and oxidation-reduction potential were collected with a YSI 600XL multiparameter data sonde from Yellow Springs Instruments following all manufacturer's guidelines for calibration and quality assurance/quality control. For influent measurements, the probe was lowered into the manhole and logged data every two minutes for the entire filling operation. For effluent measurements, the probe was placed directly into the water column of FWS and OWC mesocosms and allowed to equilibrate before manually logging data. Measurements in the SSF mesocosms required filling a bucket in which the probe was placed. As a result of this conveyance through the sampling port, dissolved oxygen measurements from SSF mesocosms may have been affected.

4.5.3 Laboratory Analyses

4.5.3.1 Standard Methods

All influent and effluent samples were tested for a suite of anions including nitrite, nitrate, chloride, dissolved reactive phosphate, and sulfate via a SEAL AQ300™ Discrete Analyzer following AWWA Standard Methods: 4500-NO_2 B (nitrite), 4500-Cl-E (chloride), 4500-P (O-phosphate), 4500-NO_3 (nitrite + nitrate), and ASTM D516-11(sulfate). All influent and effluent samples were tested for a suite of cations including: Ag, Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, S, Se, and Zn via Inductively-Coupled Plasma – Optical Emission Spectrometry (ICP-OES) following EPA Method 6010C on a Varian Vista-Pro simultaneous axial ICP-OES after hot acid microwave digestion following EPA Method 3015 using a CEM MARS. The BOD_5 concentration in all influent and effluent samples was measured

following ASTM Method 5210B. The TSS concentration in all influent and effluent samples was measured following EPA Standard Method 160.2.

4.5.3.2 ELISA

Enzyme-Linked Immunosorbent Assay (ELISA) test kits from Gold Standard Diagnostics (GSD) were used to measure concentrations of the selected suite of ECs (SMZ, CMP, BPA) in all influent and effluent samples. Proprietary SOPs provided by GSD were followed for individual EC test kits. The test is a direct competitive ELISA that allows the detection of a target analyte. It is based on the recognition of the analyte by specific antibodies. The tests are designed to minimize cross-reactivity with other related compounds but may react with metabolites of the parent compound. For example, the CMP test has a 97% cross-reactivity to 10, 11-Dihydrocarbamazepine and 78% cross-reactivity to 10, 11-Epoxycarbamazepine.

Although all three tests are very similar, the following text details the CMP ELISA test principle. CMP, when present in a sample, and a carbamazepine-horseradish peroxidase (HRP) analogue compete for the binding sites of mouse anti-carbamazepine antibodies in solution. The CMP antibodies were then bound by a second antibody immobilized in the plate. After a washing step and addition of the substrate solution, a color signal was generated. The intensity of the color signal was inversely proportional to the CMP concentration in the sample. After a specified time, the color reaction was stopped, and the color was evaluated using a Bio-Rad iMark Microplate Reader and Bio-Rad Microplate Manager 6 software. Each test kit contains a microtiter plate coated with an antibody (goat anti mouse), six standards (0, 0.025, 0.050, 0.10, 0.25, 0.50, and 2.5 µg/L), antibody solution (mouse anti-carbamazepine), carbamazepine-HRP, diluent, wash buffer concentrate, color solution, and stop solution. The laboratory must be equipped with micro-pipettes (50-250 µL), multi-channel pipette (50-250 µL), reagent reservoirs

for multichannel pipettes, and a microtiter plate reader (450 nm wavelength). The seven-step procedure is outlined below.

1. Add 75 µL of the standard solutions, control, or samples into the wells of the microplate working in duplicates or triplicates.
2. Add 50 µL of the enzyme conjugate solution to the individual wells successively using a multi-channel pipette.
3. Add 50 µL of the antibody solution to the individual wells successively using a multi-channel pipette. Cover the wells with parafilm and mix the contents on an orbital shaker for 30 seconds. Incubate strips for 90 minutes at 2-8 ℃.
4. After incubation, remove the parafilm and vigorously shake the contents of the wells into a sink. Wash the strips four times using the wash buffer solution. Use at least 250 µL of wash buffer for each well and each washing step. The remaining buffer in the wells should be removed by patting the plate dry on a stack of lint-free chem-wipes.
5. Add 100 µL of substrate/color solution to the wells using a multi-channel pipette. Incubate the strips at room temperature for 30 minutes. Protect the strips from sunlight.
6. Add 50 µL of stop solution to the wells in the same sequence as for the substrate/color solution using a multi-channel pipette.
7. Read the absorbance at 450 nm using a microplate reader (photometer) within 15 minutes after stopping the reaction.

The evaluation of the ELISA was performed using a commercial ELISA evaluation program utilizing a four-parameter fit to generate a calibration curve from the provided standards. The concentrations of the samples were determined using the constructed standard curve. ELISA results should be verified by LC/MS if possible. However, in the absence of

independent verification, specific calibration parameters can be used to determine the accuracy of the ELISA assay. Krall et al. (2022) compared results from ELISA to mass spectrometry for six unregulated ECs and found good agreement (79%-100%) between methods for both SMZ and CMP if the following conditions were met:

1. The coefficient of variance (CV) must be less than or equal to 10% for each calibration standard pair, OR
2. A single calibration standard pair can have a CV less than or equal to 15% if the assay curve coefficient (R^2) is greater than or equal to 0.98.

These criteria were used as validation in this study, with all tests meeting the requirements except for one BPA test. The test that did not meet the criteria had a single CV greater than 15% for the 10 µg/L standard. However, all other standard CVs for the test (0, 0.05, 0.3, and 1.0 µg/L) were less than or equal to 10% and the R^2 for the test was 0.998. The results of this test were determined to be acceptable due to fact that influent BPA concentrations were between 0.1 and 0.4 µg/L, placing them in the lowest range of the test which should not be affected by the highest standard.

4.5.4 Meteorological Data

To collect local meteorological data at the mesocosm compound, a HOBO U30 Weather Station (HOBO U30-NRC) was constructed. The station monitored and stored data for the following parameters: air temperature (HOBO S-THC-M002), relative humidity (HOBO S-THC-M002), wind speed (HOBO S-WSB-M003), wind direction (HOBO S-WDA-M003), precipitation (DAVIS S-RGE-M002), solar radiation (HOBO S-LIB-M003), and photosynthetically active radiation (HOBO S-LIA-M003).

5.0 Results and Discussion

All results were analyzed for statistical significance. Normality distribution of each variable was evaluated using the Shapiro-Wilks and Anderson-Darling tests. Significant differences were determined using ANOVA analysis of variance and the Bonferroni post hoc pair-wise test for normally distributed variables or the Kruskal-Wallis and Dunn's procedure for non-normally distributed variables. A confidence interval of 95% ($\alpha = 0.05$) was set for all variables. All statistical analyses were carried out with Lumivero XLSTAT v.2023.3.1.1416 software.

5.1 Meteorological Data

This experiment was conducted during early September 2023 in Norman, Oklahoma (35˚10'35.3"N 97˚26'31.2"W). The hydraulic retention time (HRT) for this experiment was seven days, based on the results of previous experiments conducted in the mesocosm compound during the spring of 2023. The daytime high temperatures (˚C) varied from 39 to 23 and the overnight low temperatures varied from 16 to 21. The mesocosms received 17 mm of precipitation during the experiment. Potential evapotranspiration (ET) was estimated to be 25 mm using the corrected Thornthwaite method (Thornthwaite calculations and supplemental meteorological data provided in Appendix Figures A1-A4).

5.2 Influent Characteristics

5.2.1 Traditional Parameters

The influent for this study, characterized in Table 8, was drawn from the final stages of the NWRF treatment train, following secondary treatment and aeration. One distinction between this experiment and previous experiments examining the role of aeration in TWs is the quality of the influent. Table 9 provides traditional wastewater quality parameters from similar studies.

Table 8: Influent water quality parameters for the current study.

Parameter (units)	NWRF effluent / Mesocosm influent
Temperature (˚C)	26.6 ± 0.02
pH (SU)	7.06 ± 0.08
TDS (mg/L)	356 ± 122
TSS (mg/L)	1.8 ± 1.6
BOD_5 (mg/L)	2.7 ± 1.0
Turbidity (NTU)	1.02 ± 0.59
Conductivity (mS/cm)	0.56 ± 0.19
DO (mg/L)	5.0 ± 0.07
DO (%)	62 ± 0.91
$NO_2 + NO_3$ (mg N/L)	6.8 ± 0.11
DRP (mg P/L)	2.1 ± 0.1

Table 9: Influent water quality parameters for previous studies examining the role of aeration in EC removal efficiency in TWs.

Reference	Influent Source	DO (mg/L)	COD (mg/L)	NH_4-N (mg /L)	NO_3-N (mg /L)
Auvinen et al., 2016	hospital wastewater post - primary		168-667	24-42	0.2-2.3
	municipal wastewater post - primary		168-933	24-66	0.2-4.3
Sossalla et al., 2022	septic tank	0.6	320	74	
Avilla et al., 2021	septic tank	0.6	106	13	8.2
Kahl et al., 2017	municipal wastewater post - primary	1.0	315 [a]	77	

[a] Reported as $CBOD_5$

Sossalla et al. (2022) examined the effects of aeration on EC removal in full scale horizontal SSF TWs and concluded that removal rates of ibuprofen, diclofenac, benzotriazole, and naproxen were all improved with constant aeration while carbamazepine removal rates were degraded. However, the influent of the TWs was municipal wastewater from a dual septic tank system with $CBOD_5$ concentrations of 320 ± 99 mg/L. Avila et al. (2023) conducted similar experiments with pilot scale horizontal SSF TWs and observed improved removal efficiencies for all target ECs, including carbamazepine, in TWs receiving intermittent aeration. Much like Sossalla et al.(2022), TWs in the Avila et al. (2021) study were fed with settled wastewater from an office building with COD concentrations of 106 ± 23 mg/L. Auvinen et al. (2016) conducted mesocosm scale studies using aerated vertical SSF TWs receiving raw hospital sewage with COD as high as 900 mg/L and observed negative removal rates for sulfamethoxazole.

The influent quality is a significant factor in EC removal, as previous studies examining TWs as tertiary polishing suggest that EC removal efficiencies may exceed those observed in TWs providing primary or secondary treatment (Wang et al., 2023; Gornick et al., 2020; He et al., 2018). Wang et al. (2023) investigated several full-scale TWs providing tertiary treatment and suggested that DO concentrations of 6 mg/L or greater in the secondary effluent may result in higher EC removal efficiencies due to increased aerobic degradation and oxidative pathways. Additionally, lower NH_4 and COD concentrations in secondary effluent compared to primary effluent may contribute to higher EC removal efficiencies as organic matter and NH_4 exert an oxygen demand, decreasing DO concentrations which reduces pollutant degradation via oxidative pathways (Wang et al., 2023). Alternatively, when most of the readily biodegradable carbon sources have been utilized, microorganisms may use ECs as a carbon and electron source (Gornick et al., 2020).

The influent for this study (Table 8) has relatively low amounts of readily labile carbon and nutrients, as evidenced by the BOD_5, $NO_3^- + NO_2^-$, and DRP concentrations, and is comparable to the influent examined by Wang et al. (2023) and Gornick et al. (2020). The influent DO concentration of 5.0 ± 0.07 mg/L exceeds the BOD_5 concentration of 2.7 ± 1.0 mg/L, indicating a high level of secondary treatment from the NWRF. An influent turbidity of 1.02 ± 0.59 NTU and TSS of 1.8 ± 1.6 mg/L are also indicative of highly treated wastewater with little left to remove in the way of traditional parameters. The low turbidity and TSS also allow for sunlight to penetrate the water column with minimal diffraction or absorbance by background components such as natural organic matter, therefore promoting photolysis (Zhou et. al., 2015). Due to the high quality of the influent, removal efficiencies in this study were expected to be greater than those observed in studies utilizing influent with greater carbon and nutrient concentrations.

5.2.2 Emerging Contaminants

The mean influent concentrations of CMP, SMZ, and BPA were 1580 ng/L, 400 ng/L, and 140 ng/L, respectively (Figure 7).

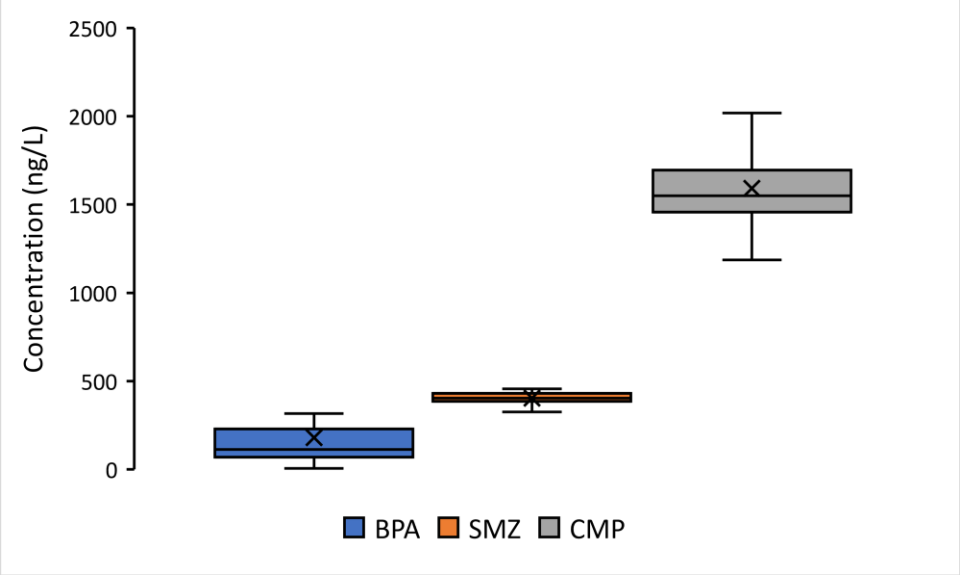


Figure 7 : Box and whiskers plot of influent concentrations of BPA, CMP, and SMZ. (n = 25). The top whisker represents the maximum value, the top of the box represents the third quartile, the × represents the mean, the midline represents the median , the bottom of the box represents the first quartile, and the bottom whisker represents the minimum value.

The mean influent CMP concentration of 1580 ng/L exceeds levels reported in previous studies on NWRF effluent by Thornton (2017), Crowley and Mattingly (2009), and Overton (2023), but is consistent with other studies examining secondary effluent (Table 10).

Table 10: CMP concentrations in secondary WWTP effluent from previous research.

Reference	CMP (ng/L)
Thornton (2017)	480
Crowley and Mattingly (2009)	400
Spongberg and Witter (2008)	2300
Overton (2023)	580-1200
Hai et al. (2018)	30 - 6300
Kostich et al. (2014)	97 - 240
Matamoros and Salvado (2012)	100 - 1200
Wang et al. (2023)	10-40
He et al. (2018)	230-320

These differences in measured concentrations could be due to seasonal and daily variability of CMP consumption (Auvinen et al., 2016, Matamoras and Salvado, 2012), or due to cross reactivity with CMP byproducts in the ELISA (Gold Standard, 2023). Influent CMP concentrations were within the detectable range of the ELISA (25-2500 ng/L) and therefore did not require dilution.

The mean influent SMZ concentration of 400 ng/L is lower than reported in previous studies on NWRF effluent by Thornton (2017) and Crowley and Mattingly (2009) but consistent with other studies examining secondary effluent (Table 11). Influent SMZ concentrations were within the detectable range of the ELISA (15-1000 ng/L) and therefore did not require dilution.

Table 11: SMZ concentrations in secondary WWTP effluent from previous research.

Reference	SMZ (ng/L)
Crowley and Mattingly (2009)	1300
Tewari et al. (2013)	3-89
Thornton (2017)	5200
Chandra et al. (2021)	40-370
Straub (2015)	224-826
Spongberg and Witter (2008)	79-472
He et al. (2018)	70-450

Historical data on BPA concentrations in NWRF effluent is limited to the 2022 NWRF pilot scale study, which detected BPA concentrations of 126 -1400 ng/L at various stages of the treatment train. Influent BPA concentrations were consistent with other studies examining secondary effluent (Table 12).

Table 12: BPA concentrations in secondary WWTP effluent from previous research.

Reference	BPA (ng/L)
Flint et al. (2012)	1000
Zielenska et al. (2016)	10-86000
Mohapatra et al. (2011)	400
Chiriac et al. (2020)	75
Petrie et al. (2019)	62-892

Except for one influent sample, all influent BPA concentrations were within the detectable range of the ELISA (50-10000 ng/L), but just above the lower detection limit of 50 ng/L.

5.3 Carbamazepine Removal

CMP removal efficiencies (mean ± 1 standard deviation) were higher in non-aerated mesocosms (69 ± 12%) as compared to aerated mesocosms (65 ± 7%) but not significantly different (p = 0.085) (Figure 8).

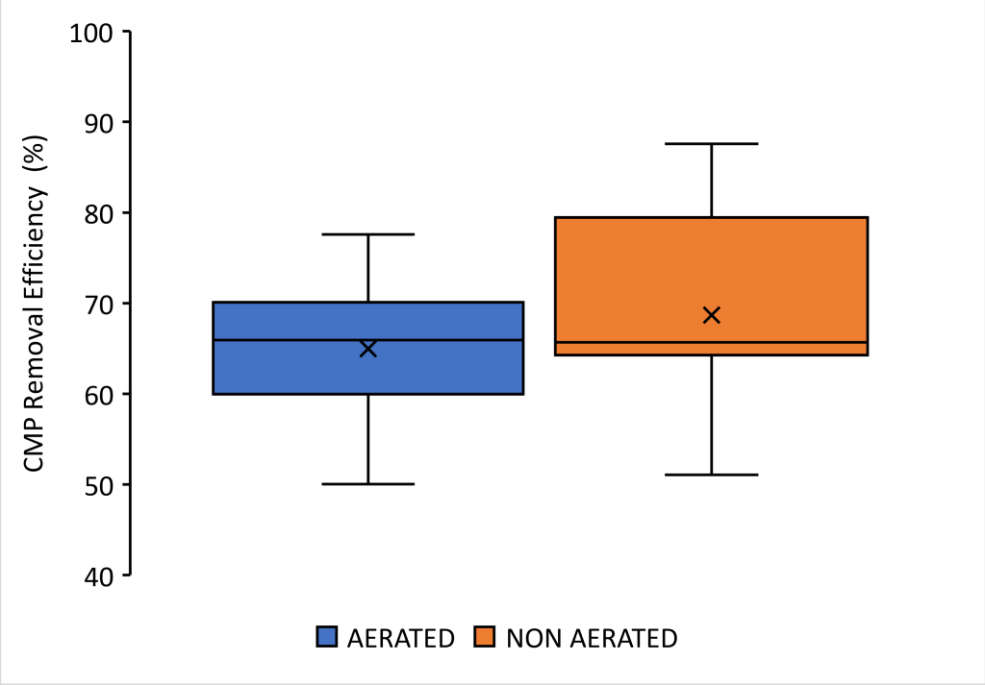


Figure 8 : Box and whiskers plot of CMP removal efficiencies (%) by aeration strategy (n = 14 Aerated, n = 11 Non-Aerated) The top whisker represents the maximum value, the top of the box represents the third quartile, the × represents the mean, the midline represents the median , the bottom of the box represents the first quartile, and the bottom whisker represents the minimum value.

The higher CMP removal efficiencies in the non-aerated mesocosms supports previous studies that suggest aerobic biodegradation is not a significant removal mechanism for CMP due to the extremely stable tricyclic structure (Table 4) of the molecule (Chowdhury et al., 2022, Zhang et al., 2014). The reduction in CMP removal efficiencies in aerated mesocosms also supports previous findings by Sossalla et al.(2022), which observed lower CMP removal efficiencies in aerated TWs. The CMP removal efficiencies for all mesocosms were higher than expected from literature (Table 13).

Table 13: CMP removal efficiencies from previous studies.

Reference	TW design	System scale	Influent water type	HRT (days)	Influent CMP concentration (ng/L)	CMP removal efficiency
He et al., 2018	FWS-V	Full	Municipal post-secondary	4	230	0-2 %
	FWS-V	Full	Municipal post-secondary	0.82	204	0-(-7) %
Wang et al., 2023	SSF-V	Full	Municipal post-secondary	0.2	28	0-14 %
Auvinen et al., 2017	SSF-V-A	Pilot	Hospital post-primary	2	5200	0-10 %
Conckle et al., 2008	FWS-V	Full	Municipal post-primary	1.5	80-100	-53 %
Matamoras et al., 2017	SSF-V	Full	Municipal post-secondary		1900	12-28 %
Matamoras and Salvado, 2012	FWS-V	Full	Municipal post-secondary	8.5	300-1500	5-51%
Verlicchi et al., 2013	SSF-V	Pilot	Municipal post-secondary	1	370±70	-4%

The most cited removal mechanisms for CMP are sorption and phytoremediation (Chowdhury et al., 2022; Zhang et al., 2014; He et al., 2018; Avila et al., 2021; Brunhoferova et al., 2021), due to the physio-chemical properties and structure of CMP (Table 1). With a pKa of 13.9, CMP is a neutral (non-polar) molecule at environmentally relevant pH values. The log K_{OW} value of 2.45 places CMP in a mildly hydrophobic/ mildly lipophilic category. A water solubility of 17.7 mg/L (at 25˚C) is practically insoluble. ECs with low water solubility and high log K_{OW} values (> 3) are mainly retained by adsorptive process and tend not to bind to organic matter (Dordio et al., 2010; Chowdhury et al., 2022; Zhang et al., 2014). In addition to sorption, studies have identified phytoremediation as a potential, but less effective removal mechanism for CMP in TWs (Avila et al., 2021; Brunhoferova et al., 2021). ECs with log K_{OW} values ranging from 0.5 to 3, such as CMP, are lipophilic enough to move through the lipid bilayer of membranes yet

soluble enough to travel into cell fluids (Zhang et al., 2014). Examination of full-scale TWs vegetated with *Phragmites australis* by He et al.(2018) concluded that CMP was not removed by vegetated TWs. However, the removal of CMP may be dependent on the species of vegetation as suggested by the results of Brunhoferova et al.(2021), who observed CMP removal efficiencies of 30% in TWs vegetated with *Lythrum salicaria* as compared to negative CMP removal efficiencies in TWs vegetated with *Phragmites australis.* In addition to sorption and phytoremediation, photodegradation may also play a role in CMP removal. Examinations by Chiron et al. (2006) and Novikov et al. (2023) determined that the absorption spectrum of CMP consists of a long wavelength adsorption band, suggesting that photodegradation in natural sunlight is possible due to multiple chromophores in the CMP molecule (Table 4).

Comparison of CMP removal efficiencies by hydrologic design (Figure 9) illustrates the higher removal efficiencies of FWS and SSF mesocosms, 73 ± 9% and 66 ± 5%, respectively, as compared to OWC (56 ± 7%). Bonferroni post hoc analysis (Table 14) indicates a significant difference between OWC and FWS ($p = .001$), but not between SSF and FWS or between SSF and OWC.

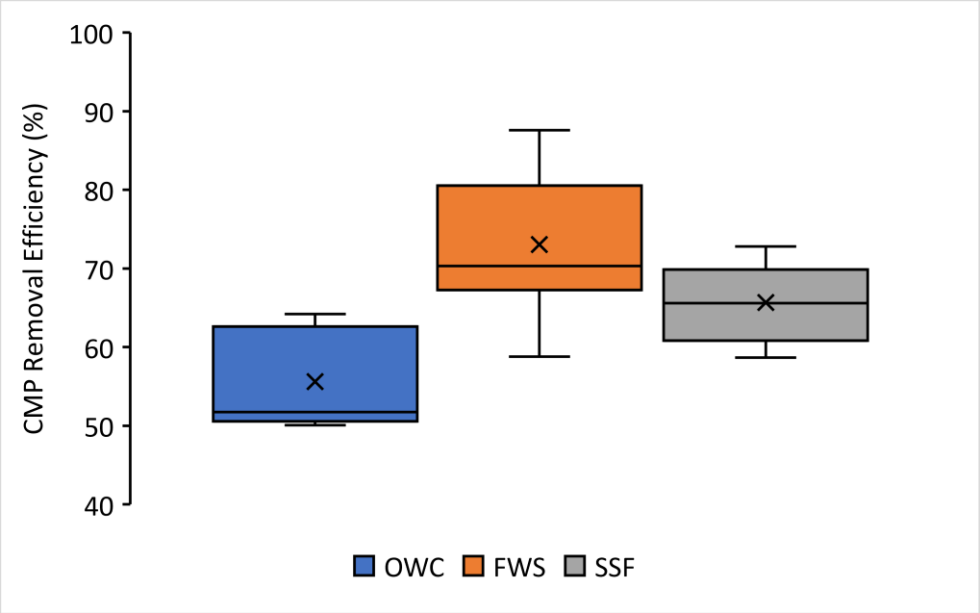


Figure 9 : Box and whiskers plot of CMP removal efficiencies (%) by hydrologic design (n = 5 OWC, n = 10 FWS, n = 10 SSF) The top whisker represents the maximum value, the top of the box represents the third quartile, the × represents the mean, the midline represents the median , the bottom of the box represents the first quartile, and the bottom whisker represents the minimum value.

Table 14: Bonferroni analysis of differences, CMP removal by hydrologic design; 95% confidence interval; modified significance level = 0.017

Hydrologic Design	FWS	SSF	OWC
FWS	1	0.018	0.001*
SSF	0.018	1	0.089
OWC	0.001*	0.089	1

* Indicates significance.

Further analysis considering hydrologic design in combination with aeration (Figure 10) indicates that aeration decreased CMP removal efficiencies in SSF and FWS mesocosms, but improved CMP removal efficiencies in OWC mesocosms.

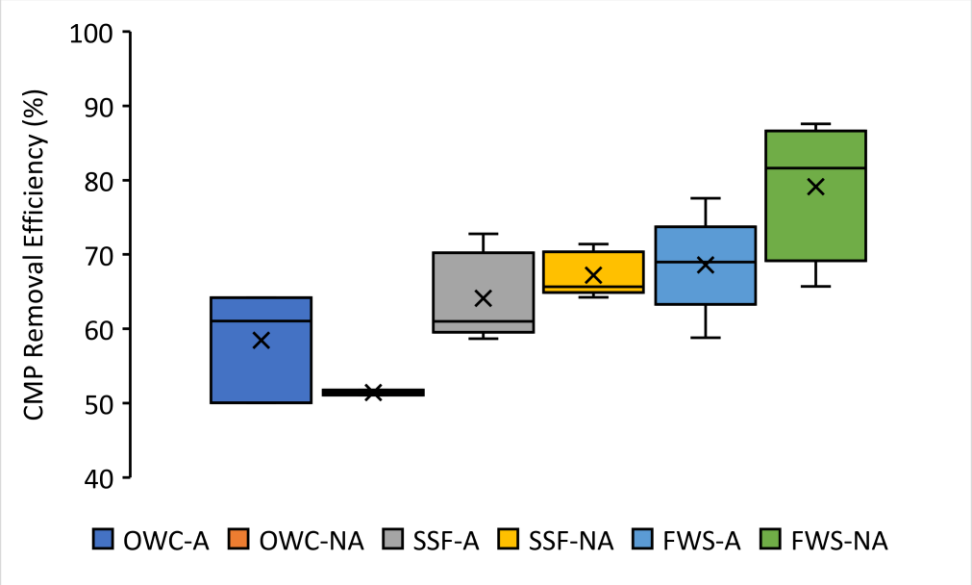


Figure 10 : Box and whiskers plot of CMP removal efficiencies (%) by hydrologic design and aeration strategy (OWC – A, n = 3 ;OWC – NA, n = 2 ; SSF-A, SSF-NA, n = 5;FWS-A, n = 6; FWS-NA, n = 4). The top whisker represents the maximum value, the top of the box represents the third quartile, the × represents the mean, the midline represents the median , the bottom of the box represents the first quartile, and the bottom whisker represents the minimum value.

The lower removal efficiencies in the OWC mesocosms as compared to FWS and SSF were expected based on previous experiments citing sorption as the dominant removal mechanism for CMP (Park et al., 2018; Sharif et al., 2014, Dordio et al., 2010). However, the removal efficiencies of the OWC mesocosms are much greater than expected as there is no substrate or vegetation, leaving photolysis and aerobic degradation as the major removal mechanisms. Iron and nitrate, known photosensitizers (Andreozzi et al., 2002), were present in the influent at concentrations of 0.077 mg Fe/L and 6.7 mg N/L, and may have contributed to an increased rate of photolysis. Additionally, the shallow depth of the OWC mesocosms and low influent turbidity (1.02 ± 0.59 NTU) allowed for sunlight to affect the entire water column. While the OWC mesocosms do not have a substrate, they do receive some deposit of windblown dust and organic matter from the adjacent road and neighboring compost facility. This deposition was not measured or quantified for this experiment but was observed on several days during the

duration of the experiment which experienced a maximum sustained wind speed of 7.8 mph and maximum wind gust of 18 mph. Although the wind-blown deposition was not directly measured, TSS concentrations in OWC mesocosms increased from 1.75 ± 1.6 mg/L in the influent to 11.1 ± 5.2 mg/L in the effluent, and TDS concentrations increased from 356 ± 122 to 487 ± 6 mg/L. Additionally, algae were growing in OWC and FWS mesocosms (Figure 11) which may have provided sufficient sorption sites or provided some phytoremediation to account for the high CMP removal rate.

Figure 11 : OWC mesocosm with green algae and multiparameter data sonde on final day of experimentation.

CMP removal efficiencies by vegetation scheme were 73 ± 9%, 66 ± 6%, and 56 ± 7% for unvegetated (UV), vegetated (V), and OWC mesocosms, respectively (Figure 12). Post hoc analysis indicates a significant difference between OWC and UV mesocosms ($p = 0.001$) (Table 15).

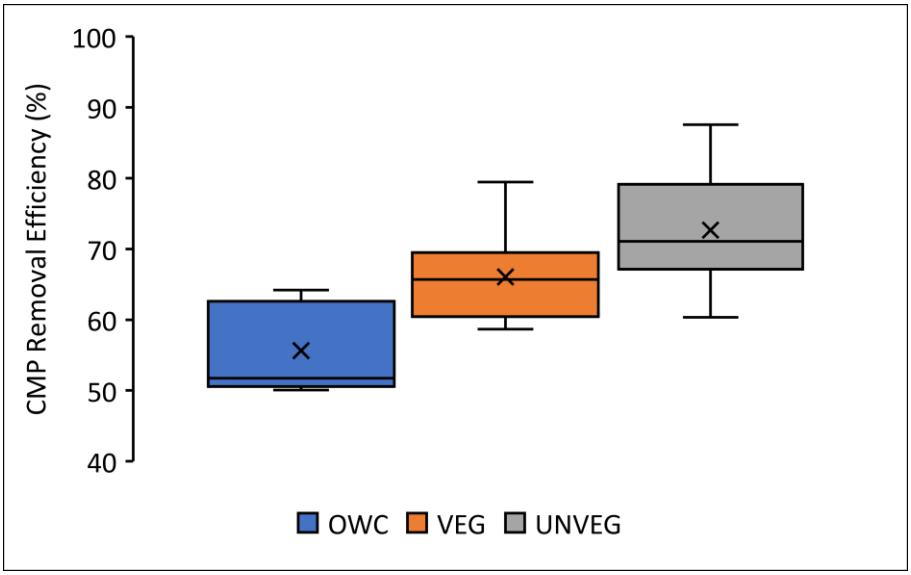


Figure 12 : Box and whiskers plot of CMP removal efficiencies (%) by vegetation scheme (n = 5 OWC, n = 10 VEG, n = 10 UNVEG) The top whisker represents the maximum value, the top of the box represents the third quartile, the × represents the mean, the midline represents the median , the bottom of the box represents the first quartile, and the bottom whisker represents the minimum value.

Table 15: Pairwise comparison of CMP removal by vegetation using Dunn's procedure, confidence interval = 95%

Vegetation Scheme	V	UV	OWC
V	1	0.101	0.053
UV	0.101	1	0.001*
OWC	0.053	0.001*	1

* Indicates Significance

The lower CMP removal efficiencies in the unvegetated mesocosms were unexpected based on literature. As the mesocosms were planted in early 2023, this difference in CMP removal efficiencies could be due to the young age of vegetation and lack of established rhizospheres. Comparison of CMP removal efficiencies by all combinations of hydrologic design, vegetation scheme and aeration strategy (Figure 13) confirms previous conclusions that aeration and vegetation were not significant factors in CMP removal.

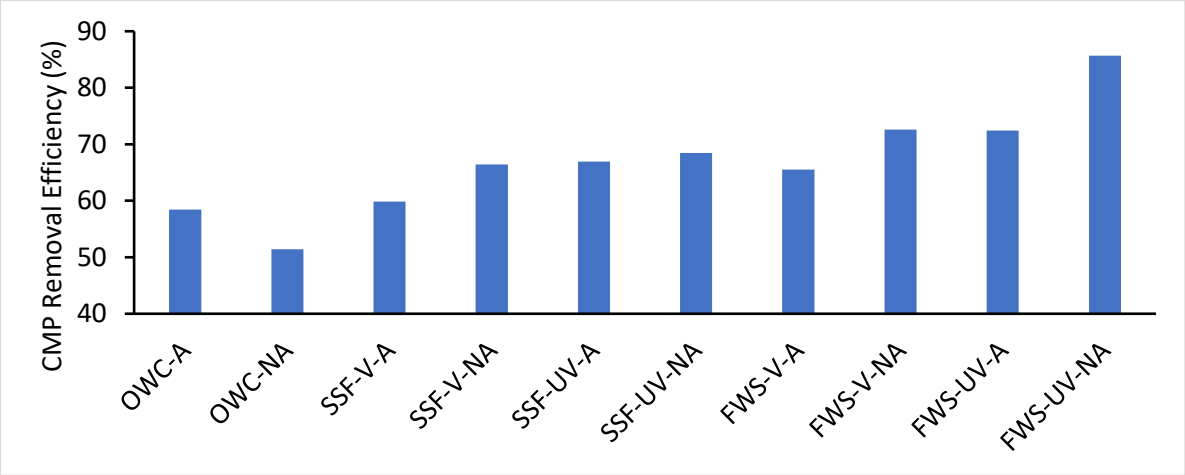


Figure 13 : CMP removal efficiencies by hydrologic design, vegetation scheme, and aeration strategy (n = 3: OWC-A, SSF -V-NA, SSF-UV-A, FWS-V-A, FWS-UV-A; n = 2: OWC-NA, SSF-V-A, SSF-UV-NA, FWS-V-NA, FWS-UV-NA)

The higher removal efficiencies observed in this study may be due to the relatively high quality of the influent (Table 8) and the combined effects of low COD (< 30 mg/L), high DO (> 5 mg/L) which promotes aerobic degradation and oxidative pathways (Wang et al., 2023). Although biodegradation is not cited as a removal mechanism for CMP, studies by Gornick et al.(2020), suggest that microorganisms may use recalcitrant ECs such as CMP as a carbon and electron source when most of the readily biodegradable carbon sources have been utilized.

Mean influent and effluent CMP concentrations are provided in Figure 14.

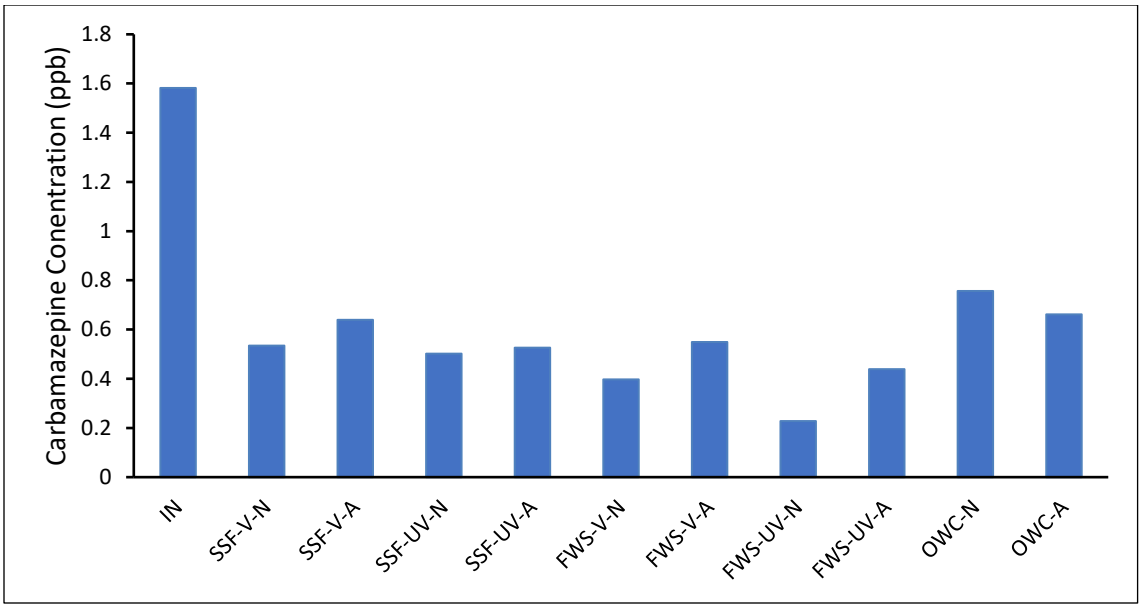


Figure 14 : CMP concentrations (ppb) for influent and effluent of all treatment combinations (n = 25: IN; n = 3: OWC-A, SSF -V-NA, SSF-UV-A, FWS-V-A, FWS-UV-A; n = 2: OWC-NA, SSF-V-A, SSF-UV-NA, FWS-V-NA, FWS-UV-NA).

The effluent CMP concentrations (523±148 ng/L) of all treatment combinations indicate that CMP concentrations are effectively reduced from the influent concentration (1580 ± 200 ng/L) by the mesocosm TWs.

5.5 Sulfamethoxazole Removal

SMZ removal efficiencies for aerated and non-aerated mesocosms, 55 ± 33% and 57 ± 44%, respectively, were highly variable (Figure 15) but not significantly different (p = 0.228).

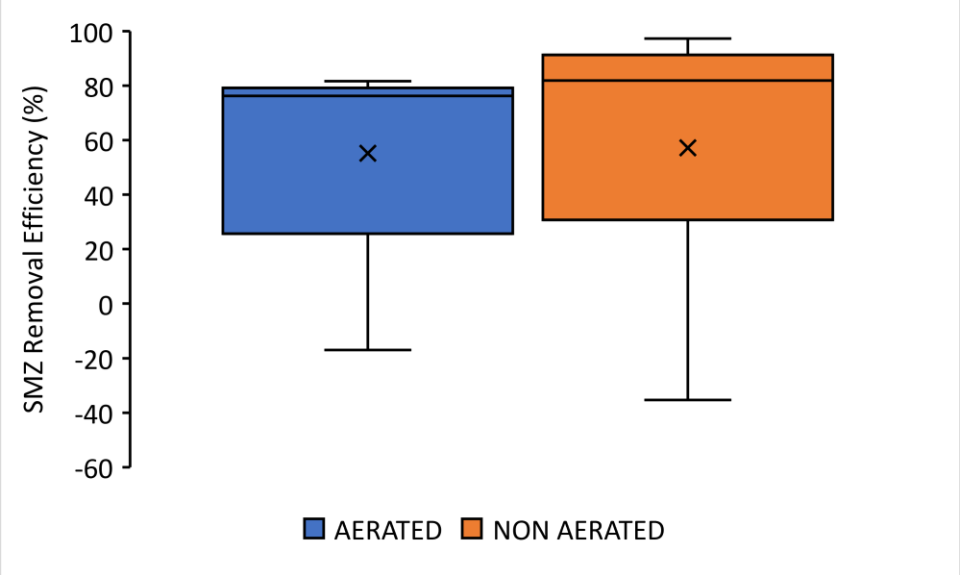


Figure 15 : Box and whiskers plot of SMZ removal efficiencies (%) by aeration strategy (n = 14 Aerated, n = 11 Non-Aerated) The top whisker represents the maximum value, the top of the box represents the third quartile, the × represents the mean, the midline represents the median , the bottom of the box represents the first quartile, and the bottom whisker represents the minimum value.

While Figure 15 illustrates mean positive removal for both aeration strategies, negative removal efficiencies were observed for both aerated and unaerated mesocosms. This initial comparison agrees with previous studies that have found SMZ removal to be highly variable and possibly negative in TWs (Table 16).

Table 16: SMZ removal efficiencies and influent concentrations from previous studies.

Reference	TW design	System scale	Influent water type	HRT (days)	Influent SMZ concentration (ng/L)	SMZ removal efficiency
Wang et al. 2023	SSF-V	Full	Municipal post-secondary	0.23	40±20	0-5%
Auvinen et al. 2017	SSF-V	Pilot	Hospital post-primary	2	60±80	0-10%
He et al. 2018	FWS-V	Full	Municipal post-secondary	4	74±4	25%
	FWS-V	Full	Municipal post-secondary	0.82	362±8	-23%
Conckle et al. 2008	FWS-V	Full	Municipal post-primary	1.5	920±460	92%
Park et al. 2008	FWS-V	Full	Municipal post-secondary	0.25	50-75	30-55%
Verlicchi et al. 2013	SSF-V	Pilot	Municipal post-secondary	1	214±35	16%

Sochacki et al. (2018) examined the role of aeration in mesocosm-scale SSF and FWS TWs and found that SMZ removal was higher in non-aerated SSF TWs (> 77%) as compared to aerated SSF TWs (26%) but found no difference between aerated (50%) and unaerated (50%) FWS TWs, suggesting that both aerobic and anaerobic degradation pathways are possible. The role of both aerobic and anaerobic degradation was previously established by Mahott et al.(2011) which identified SMZ transformation products under aerobic, anoxic, and anaerobic conditions, indicating that SMZ is subject to a wide variety of biotic and abiotic transformations. Conversely, studies have also shown that the metabolites and transformation products of SMZ can undergo deconjugation and conjugation in biological treatment systems, resulting in negative removal efficiencies (Sochacki et al., 2018; Alvarino et al., 2016; Wang et al., 2023).

SMZ removal efficiencies by hydrologic design were 82 ± 12%, 83 ± 5%, and 16 ± 28% for OWC, FWS, and SSF, respectively (Figure 16).

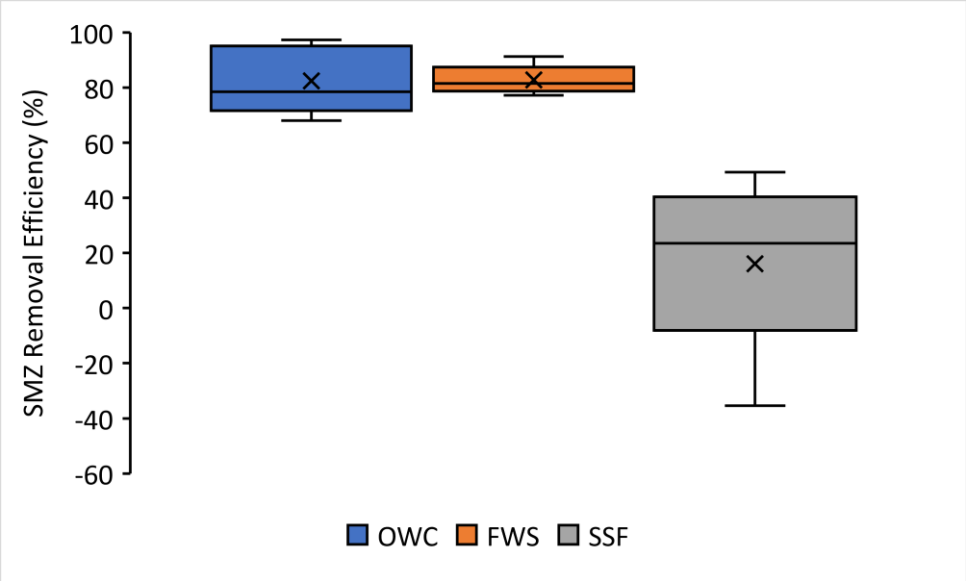


Figure 16 : Box and whiskers plot of SMZ removal efficiencies (%) by hydrologic design (n = 5 OWC, n = 10 FWS, n = 10 SSF) The top whisker represents the maximum value, the top of the box represents the third quartile, the × represents the mean, the midline represents the median , the bottom of the box represents the first quartile, and the bottom whisker represents the minimum value.

Post hoc analysis (Table 17) indicates significant differences between FWS and SSF ($p < 0.0001$) and between OWC and SSF ($p = 0.004$).

Table 17: Pairwise comparison of SMZ removal by hydrologic design using Dunn's procedure, confidence interval = 95%

Hydrologic Design	FWS	SSF	OWC
FWS	1	< 0.0001*	0.766
SSF	< 0.0001*	1	0.004*
OWC	0.766	0.004*	1

* Indicates significance.

The significantly lower removal efficiencies of the SSF mesocosms could be explained by the lack of photolysis, as the water column in SSF mesocosms do not receive any sunlight. Previous studies have established that SMZ is subject to photolytic degradation (Boreen et al., 2004; Andreozzi et al., 2002; Straub et al., 2016), with an estimated environmental half-life of 17-58 hours, based on direct photolysis. Andreozzi et al. (2002) found that a humic acid concentration of 5 mg/L or a nitrate concentration of 10 mg/L to reduce the half-life of SMZ by

68% and 77%, respectively. The significantly lower removal efficiencies of the SSF mesocosms could also be contributed to the hydrophilicity of SMZ. SMZ, with a log K_{OW} of 0.89, is not prone to adsorption (Park et al., 2009; Dan et al.,2013; Chowdhury et al., 2022). Park et al. (2009) examined the soils and macrophytes of TWs with SMZ removal rates of 15-85% and found no detectable quantities of SMZ, suggesting sorption and phytoremediation are not major SMZ removal mechanisms, which is supported by a comparison of SMZ removal efficiencies by vegetation scheme for the current study (Figure 17).

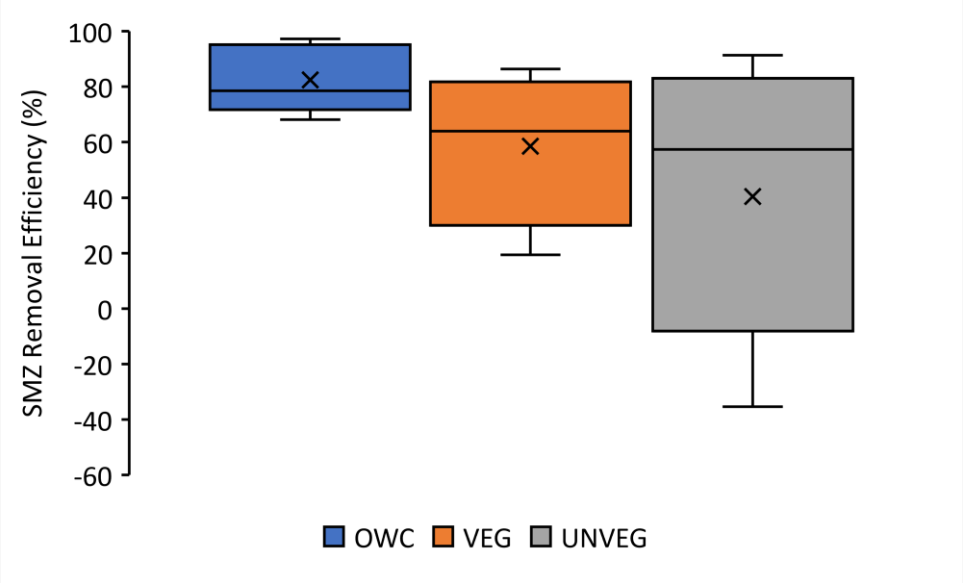


Figure 17 : Box and whiskers plot of SMZ removal efficiencies (%) by vegetation scheme (n = 5 OWC, n = 10 VEG, n = 10 UNVEG) The top whisker represents the maximum value, the top of the box represents the third quartile, the × represents the mean, the midline represents the median , the bottom of the box represents the first quartile, and the bottom whisker represents the minimum value.

SMZ removal efficiencies of 82 ± 12%, 59 ± 26%, and 40 ± 49% for OWC, V, and UV, respectively, were not significantly different and in agreement with experiments by Dan et al. (2013) which compared various substrates (gravel, vesuvianite, and zeolite) and species of vegetation (*Thalia, Arundo*) in pilot-scale TWs and determined that SMZ removal was not affected by the presence or species of vegetation, or the type of substrate used. Comparisons of

treatment combinations are provided in Figures 18-21, which confirm previously drawn conclusions of significance while accounting for other treatment variables.

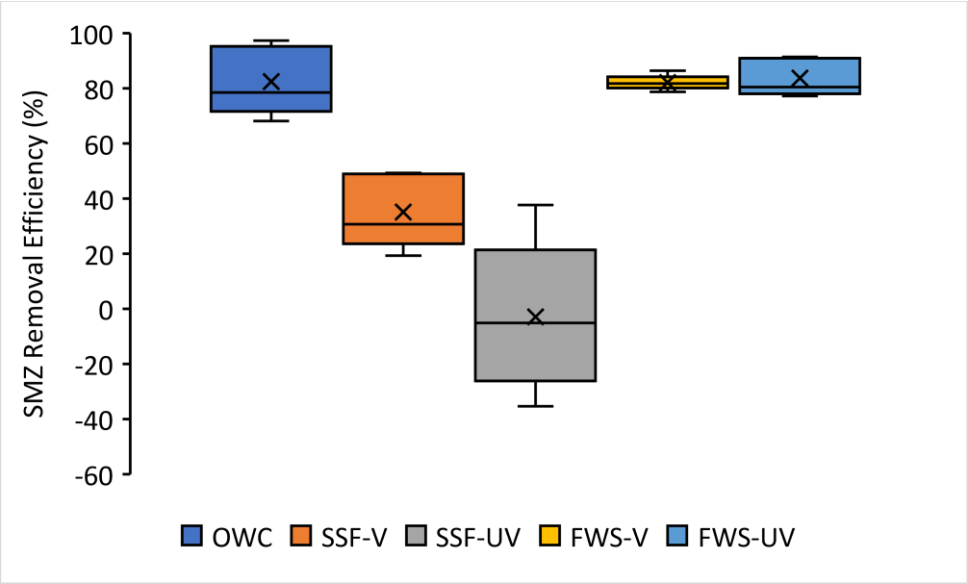


Figure 18 : Box and whiskers plot of SMZ removal efficiencies (%) by hydrologic design and vegetation scheme.(n = 5 for all treatment combinations). The top whisker represents the maximum value, the top of the box represents the third quartile, the × represents the mean, the midline represents the median , the bottom of the box represents the first quartile, and the bottom whisker represents the minimum value.

Although the lowest SMZ removal efficiencies in Figure 18 are from SSF-UV mesocosms, there is no statistical significance between SSF-V and SSF-UV removal efficiencies ($p = 0.321$). While not significantly different from each other, the differences between SSF-V and SSF-UV (Figure 18) are mimicked by the differences between SSF-A and SSF-NA (Figure 19).

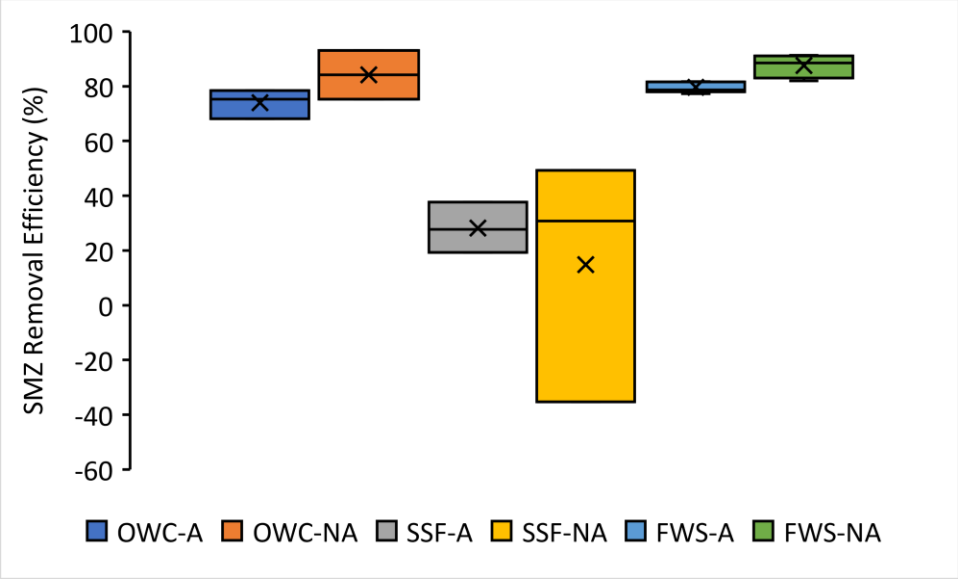


Figure 19 : Box and whiskers plot of SMZ removal efficiencies (%) by hydrologic design and aeration.(n = 5 for all treatment combinations). The top whisker represents the maximum value, the top of the box represents the third quartile, the × represents the mean, the midline represents the median , the bottom of the box represents the first quartile, and the bottom whisker represents the minimum value.

The similarity between SSF-A and SSF-V as well as between SSF-NA and SSF-UV suggest that while phytoremediation may not be a removal mechanism for SMZ, the presence of vegetation may induce an aerobic environment in the rhizosphere, facilitating aerobic degradation which is not promoted in the unvegetated or unaerated SSF mesocosms. Removal efficiencies by vegetation scheme and aeration strategy (Figure 20) reinforce previous conclusions that vegetation and aeration do not affect SMZ removal efficiencies.

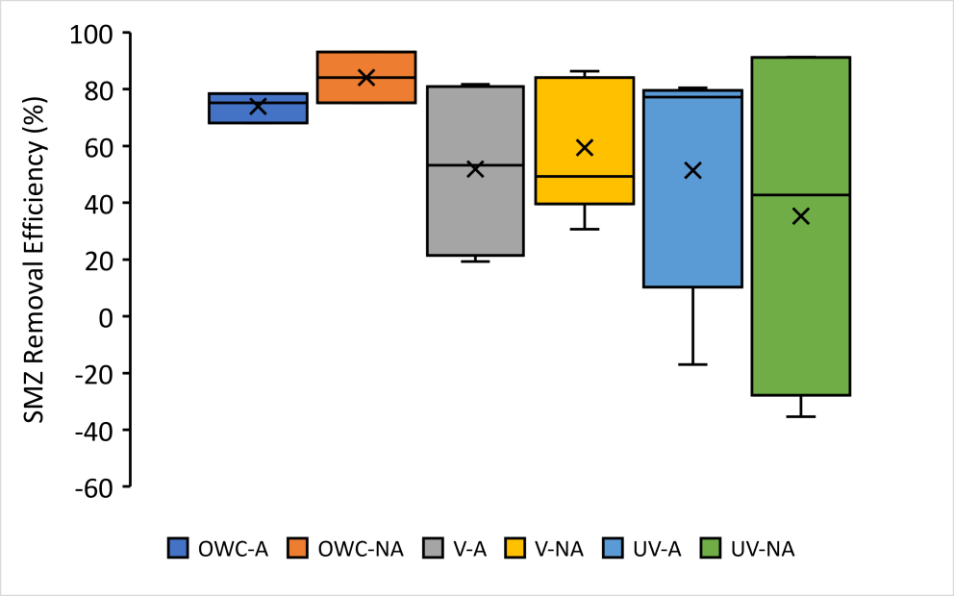


Figure 20 : Box and whiskers plot of SMZ removal efficiencies (%) by vegetation and aeration.(n = 5 for all treatment combinations). The top whisker represents the maximum value, the top of the box represents the third quartile, the × represents the mean, the midline represents the median , the bottom of the box represents the first quartile, and the bottom whisker represents the minimum value.

Figure 21 provides SMZ removal efficiencies of all treatment combinations. The box and whisker format may not be appropriate for the number of samples for each treatment combination (n = 2 or 3) but is the clearest visual representation of the differences between SMZ removal efficiencies.

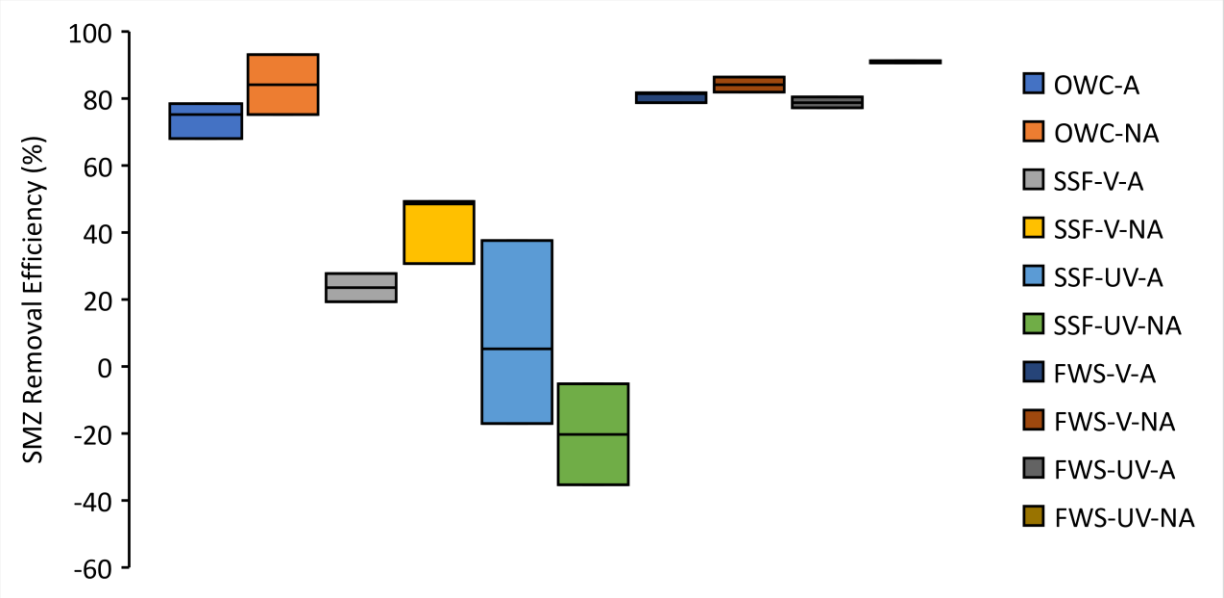


Figure 21 : SMZ removal efficiencies for all treatment combinations (n = 3: OWC-A, SSF -V-NA, SSF-UV-A, FWS-V-A, FWS-UV-A; n = 2: OWC-NA, SSF-V-A, SSF-UV-NA, FWS-V-NA, FWS-UV-NA). The top of the box represents the third quartile, the midline represents the median, and the bottom of the box represents the first quartile.

The effluent SMZ concentrations for all treatment combinations (Figure 22) reinforce previous conclusions that SMZ removal is not affected by aeration or vegetation and that SSF TWs do not effectively remove SMZ.

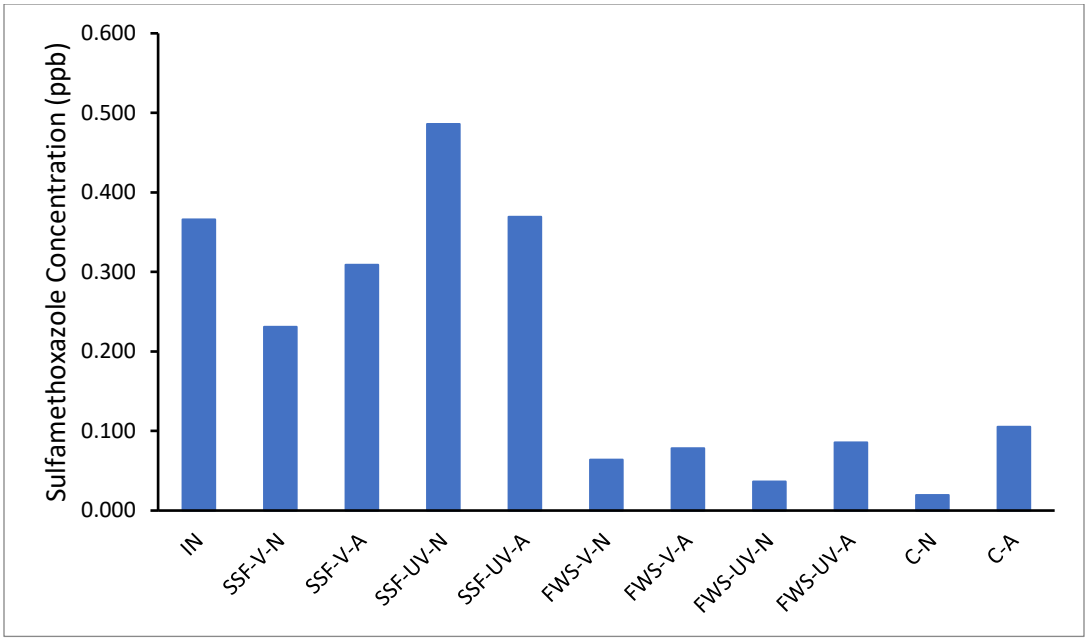


Figure 22 : SMZ concentrations (ppb) for influent and effluent of all treatment combinations.(n = 25: IN; n = 3: OWC-A, SSF -V-NA, SSF-UV-A, FWS-V-A, FWS-UV-A; n = 2: OWC-NA, SSF-V-A, SSF-UV-NA, FWS-V-NA, FWS-UV-NA) .

5.6 BPA Removal

BPA concentrations in 84% of effluent samples were below the minimum detection limit (MDL) (50 ng/L) of the ELISA tests. Effluent concentrations below the MDL were set equal to the MDL for rudimentary analysis. Outliers were included due to the limited number of measurable concentrations. Comparison of removal by hydrologic design (Figure 23) illustrates removal efficiencies of 61 ± 5%, 58 ± 14%, and 56 ± 17% for OWC, FWS, and SSF respectively.

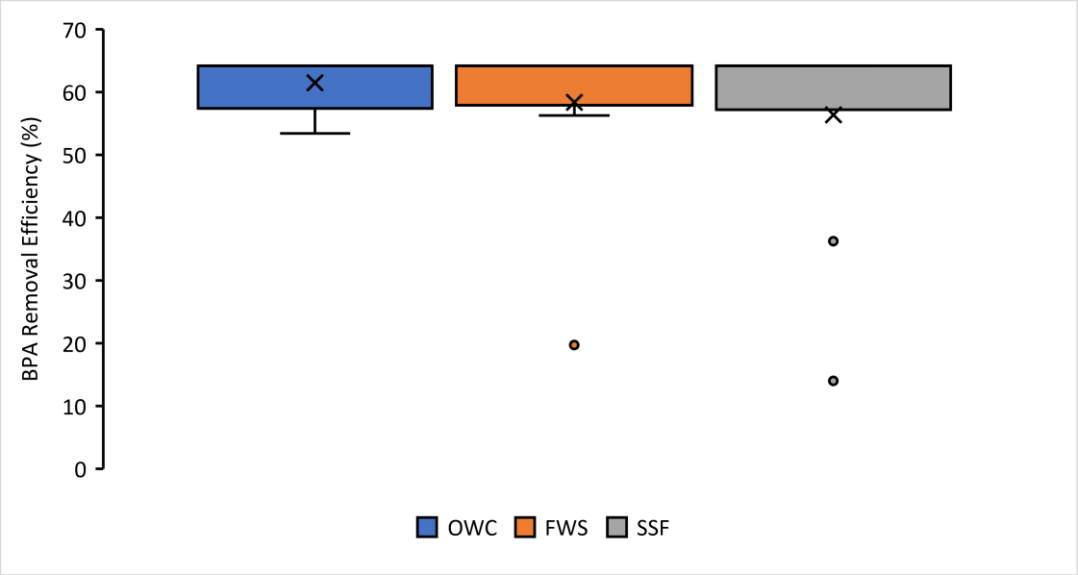


Figure 23 : Box and whiskers plot of BPA removal efficiencies (%) by hydrologic design (n = 5 OWC, n = 10 FWS, n = 10 SSF) The top whisker represents the maximum value, the top of the box represents the third quartile, the × represents the mean, the midline represents the median , the bottom of the box represents the first quartile, and the bottom whisker represents the minimum value.

Due to the high percentage of effluent concentrations below the MDL and the equal distribution of said concentrations amongst treatment types, further statistical analysis was not conducted. However, the mean BPA concentration (80 ng/L) in effluent samples above the MDL (n = 7), as compared to the influent concentration (140 ng/L) indicates that BPA concentrations were effectively reduced by all mesocosm treatment combinations. BPA removal efficiencies for this study agree with those found in literature (Table 18).

Table 18: BPA removal efficiencies and influent concentrations from previous studies.

Reference	TW design	System scale	Influent water type	HRT (days)	Influent BPA concentration (ng/L)	BPA removal efficiency
He et al. 2018	FWS-V	Full	Municipal post-secondary	4 d	1232 (±1107)	-29%
	FWS-V	Full	Municipal post-secondary	0.82 d	3682 (±1847)	26%
	FWS-V	Full	Municipal post-secondary	1.7 d	870 (±319)	-65%
Avila et al. 2010	SSF	Pilot	Municipal post-secondary	3.5 d	1000	83%
Tondera et al. 2019	SSF-V	Full	Combined sewer overflow	Varied	330-1164	74-98%

Previous studies have identified sorption and aerobic biodegradation as major removal mechanisms for BPA in TWs (Tondera et al., 2019; Chowdhury et al., 2022; Avila et al., 2010). Tondera et al. (2019) examined full scale TWs receiving combined sewer overflow over a period of ten years and determined that BPA is readily biodegradable with removal efficiencies greater than 90% in summer months and greater than 50% in winter months. These BPA removal efficiencies were consistent over the course of the study, as opposed to CMP and SMZ removal which were initially greater than 50% but declined as the system aged, leading to the hypothesis that both CMP and SMZ were initially removed by sorption with removal decreasing as sorption sites became occupied. The consistency of BPA removal indicates that the compound is being fully mineralized and not just sequestered to the substrate media. Avila et al.(2015) examined BPA removal in full-scale hybrid TWs and reported 99% removal in FWS TWs, which was

attributed to a combination of sorption, photolysis, and aerobic microbial degradation. Given the high quality of the influent for this study and the biodegradability of BPA, the reported removal efficiencies of 64% are lower than expected and may not be representative of actual removal efficiencies.

5.7 Effluent Water Quality

A summary of effluent water quality by hydrologic design, vegetation scheme, and aeration is provided in Table 19.

Table 19: Summary of effluent water quality by hydrologic design, vegetation scheme, and aeration strategy. Mean concentrations (± std. deviation).

Treatment	n	pH	TSS (mg/L)	TDS (mg/L)	DO (mg/L)	NO_2^-+NO_3^- (mg N/L)	DRP (mg P/L)
Influent		7.06	1.8	356	5.0	6.8	2.1
		(±0.08)	(±1.6)	(±122)	(±0.1)	(±0.11)	(±0.1)
Hydrology							
FWS	10	7.66	10.7	847	10.1	0.02	2.2
		(±0.06)	(±7.1)	(±159)	(±0.9)	(±0.01)	(±0.6)
SSF	10	7.56	1.72	732	9.6	0.22	0.6
		(±0.24)	(±1.6)	(±102)	(±0.8)	(±0.13)	(±0.2)
OWC	5	9.80	11.1	487	10.9	2.91	0.6
		(±0.15)	(±5.2)	(±6)	(±0.9)	(±0.22)	(±0.1)
Vegetation							
V	10	7.33	6.0	806	10.2	0.14	1.4
		(±0.10)	(±5.6)	(±63)	(±0.6)	(±0.14)	(±1.0)
UV	10	7.89	6.3	786	9.5	0.04	1.4
		(±0.39)	(±8.0)	(±191)	(±0.9)	(±0.03)	(±0.9)
Aeration							
A	14	8.16	7.7	693	9.9	0.12[a]	1.4
		(±0.98)	(±6.9)	(±123)	(±0.9)	(±0.14)	(±1.0)
NA	11	7.90	6.7	786	10.3	0.11[a]	1.0
		(±1.14)	(±6.3)	(±224)	(±1.0)	(±0.11)	(±0.7)

[a] Calculation excludes OWC mesocosms.

Quality assurance requirements for effluent BOD_5 testing were not met, therefore data are not available. However, with the relatively low influent BOD_5 concentration of 2.7 mg/L, an increase in BOD to a nonzero background concentration should be expected (Kadlec and Wallace, 2009). Statistical analysis of DO concentrations indicates no significant difference between aerated (9.9 ± 0.9 mg/L, n = 14) and unaerated mesocosms (10.3 ± 1.0 mg/L, n = 11) ($p = 0.324$). Measured DO concentrations during effluent sampling may not have reflected the impact of aeration as sampling occurred in the early morning, when the aerators had been off for 10-12 hours (since preceding nightfall). Additionally, SSF samples were drawn from the bottom of the mesocosms into a collection bucket for DO analysis, which may have increased the DO concentration in the samples. The effluent pH of the OWC mesocosms (9.8 ± 0.15) was found to be significantly higher ($p < 0.002$) than FWS (7.66 ± 0.06) and SSF (7.56 ± 0.24). Hydrologic design had an impact on TSS, with SSF mesocosms removing TSS and both OWC and FWS mesocosms producing biological TSS in the form of algae. Combined nitrate and nitrite (NO_2+ NO_3) effluent concentrations were 2.91 ± 0.22, 0.22 ± 0.13, and 0.02 ± 0.01 mg/L for OWC, SSF, and FWS mesocosms, respectively. NO_2+ NO_3 removal efficiencies for aerated (98.1%) and unaerated (98.4%) mesocosms were practically identical. Hydrologic design had a significant impact on NO_2+ NO_3 removal efficiencies of 57, 97, and 99% for OWC, SSF, and FWS mesocosms, respectively ($p < .001$). Dissolved reactive phosphorous (DRP) concentrations were significantly lower ($p < .001$) in SSF (0.6 ± 0.2 mg/L) and OWC (0.6 ± 0.1 mg/L) effluents than in FWS effluents (2.2 ± 0.6 mg/L). DRP removal efficiencies for both SSF and OWC mesocosms were 72%, as compared to -3% for FWS mesocosms. The DRP in the FWS mesocosms is most likely being released from the hydric soil, which is a clay loam that had a phosphorus concentration of 120 mg/kg when the mesocosms were constructed. A closer look at

DRP concentrations by treatment combinations (Figure 24) reveals the increase only occurred in the aerated FWS mesocosms.

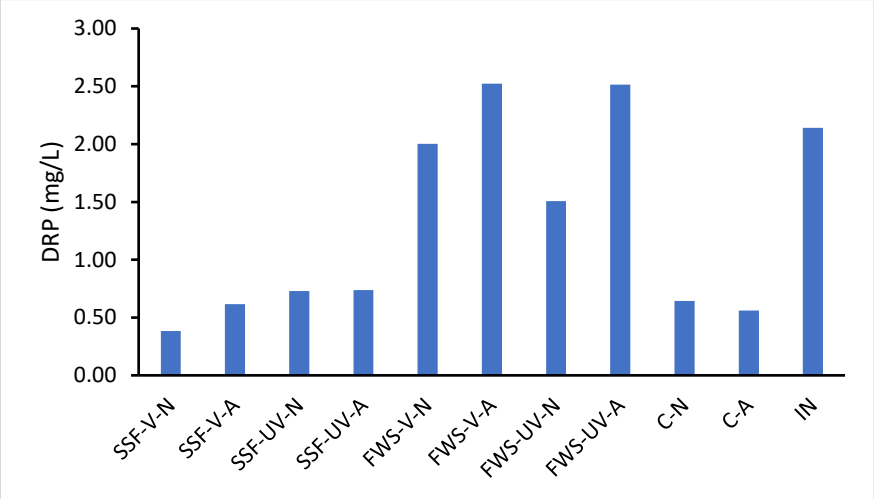


Figure 24 : DRP concentrations by treatment combinations.

Iron and aluminum concentrations (Figures 25 & 26) followed a similar trend as DRP in FWS mesocosms, supporting the hypothesis that the hydric soil is contributing to said concentrations.

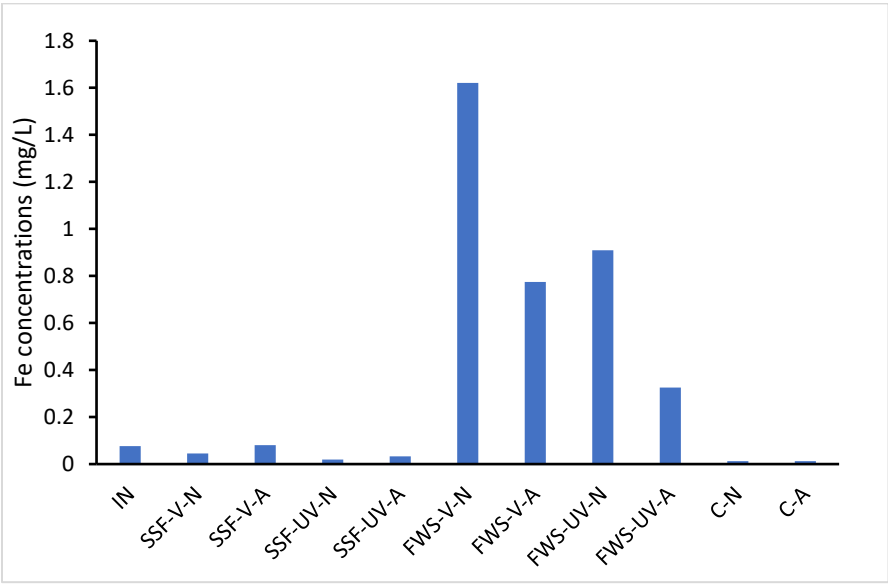


Figure 25 : Iron (Fe) concentrations by treatment combinations.

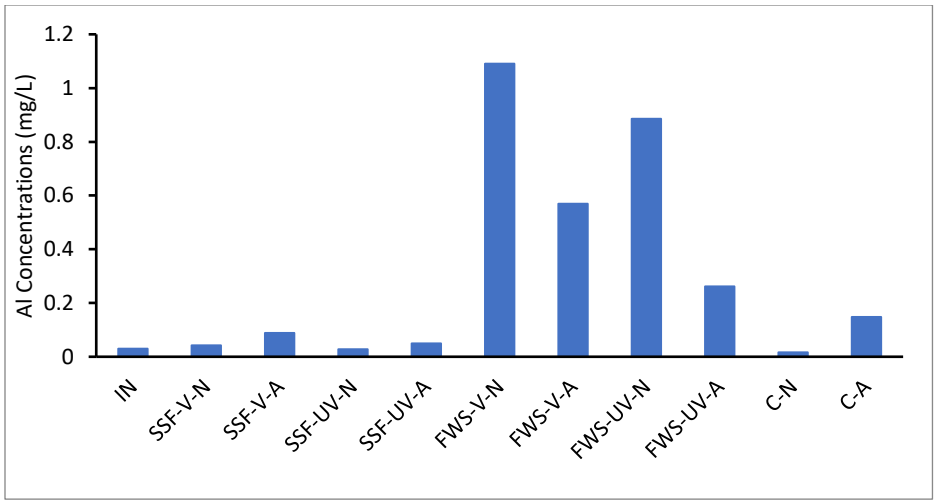


Figure 26 : Aluminum (Al) concentrations by treatment combinations.

Additional metals data is provided in Appendix Table A4.

6.0 Conclusions

CMP removal was significantly affected by hydrologic design, but not by vegetation scheme or aeration, supporting the hypothesis that aeration would not improve CMP removal efficiency. CMP removal efficiencies for all hydrologic designs, vegetation schemes, and aeration strategies (> 50%) were greater than expected from the literature. This result could be due to a combination of factors including the scale of the system, influent water quality, low hydraulic loading, seasonal effects, the maturity of the system, and other influences. Although results indicate CMP was effectively removed from the water column, further testing is necessary to determine the fate of CMP. If CMP is sorbed to the substrate without further degradation, CMP removal may decline as the system ages and sorption sites become occupied. Although CMP removal efficiencies were not affected by vegetation in this study, this may be due to the species of vegetation (*Juncus effusus*) and maturity of the plants in the mesocosms.

SMZ removal efficiencies were highly variable among treatment types, ranging from negative 20% to 80%. SMZ removal was affected by hydrologic design, but not by vegetation

scheme or aeration, with the highest removal efficiencies (> 70%) occurring in the OWC and FWS mesocosms. These results do not support the hypothesis that SMZ removal efficiencies would be greater in aerated mesocosms or the hypothesis that vegetated mesocosms would have higher removal efficiencies for all target ECs. SMZ removal efficiencies were below 50% for all SSF mesocosms, and negative (-20%) for the unvegetated, unaerated SSF mesocosms. This result agrees with previous findings that SMZ can deconjugate and conjugate under anoxic or anaerobic conditions, thereby increasing concentration in biological treatment systems. This result warrants further investigation into the fate of SMZ and its transformation products and conjugates. While removal of SMZ from the water column is the primary objective, the proliferation of antibiotic resistant genes is of concern if SMZ is sequestered in the substrate and not further degraded. Although photolysis was not directly measured for this study, the SMZ removal efficiencies in OWC mesocosms support previous studies that suggest photodegradation plays a role in SMZ mineralization.

BPA removal efficiencies were greater than 50% for all mesocosms. However, due to the high percentage of effluent concentrations below the detection limit of the ELISA, coupled with an even distribution of said concentrations amongst hydrologic designs, vegetation schemes, and aeration strategies, further conclusions about the removal mechanisms involved cannot be drawn. These results are not conclusive, and neither support nor reject the hypothesis that BPA removal efficiencies would be greater in aerated mesocosms. The results of this study agree with previous studies examining TWs as tertiary polishing which suggest that EC removal efficiencies may exceed those observed in TWs providing primary or secondary treatment (Wang et al., 2023, Gornick et al., 2020, He et al., 2018).

The results of this experiment are contingent on the accuracy of the ELISA test kits. Significant results should be verified with further experimentation and validated with LC-MS/MS or equivalent testing methods. However, the results of this experiment suggest that TWs can effectively remove ECs from secondarily treated wastewater. Furthermore, the removal efficiencies observed in FWS and OWC mesocosms suggest that a simple system consisting of a flow equalization basin (equivalent to an OWC) followed by a FWS TW may produce sufficient EC removal in secondarily treated wastewater.

7.0 References

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8.0 Appendix.

Table A1: Mean (± std. deviation) EC removal efficiencies (%) by treatment combination.

TREATMENT	n	CMP	SMZ	BPA
FWS	10	73.0 (±8.8)	82.9 (± 4.9)	58.4 (±13.9)
SSF	10	65.7 (±4.7)	16.1 (±28.5)	56.4 (±17.3)
V	10	66.1 (±6.2)	58.6 (±26.3)	56.4 (±17.3)
UV	10	72.6 (±8.3)	40.4 (±49.3)	58.4 (±13.9)
OWC	5	55.6 (±6.5)	82.4 (±12.3)	61.4 (4.7)
A	14	65.0 (±7.1)	55.2 (±33.7)	58.2 (±13.5)
NA	11	68.7 (±11.6)	57.2 (±44.6)	58.1 (±14.9)
FWS-V-A	3	65.5(±5.9)	80.6(±1.6)	64.2 (±0.0)
FWS-V-NA	2	72.6(±9.7)	84.2(±3.1)	64.2 (±0.0)
FWS-UV-A	3	72.4(±4.5)	78.8(±1.6)	64.2 (±0.0)
FWS-UV-NA	2	85.7(±2.7)	91.0(±0.5)	57.4(±1.5)
SSF-V-A	2	59.8(±1.6)	23.6(±5.9)	50.2(±19.8)
SSF-V-NA	3	66.4(±2.6)	42.9(±10.5)	47.5(±29.0)
SSF-UV-A	3	66.9(±6.2)	8.6(±27.5)	64.2(±0.0)
SSF-UV-NA	2	68.5(±4.2)	-20.2(±21.3)	64.2(±0.0)
OWC-A	3	58.4(±7.4)	73.9(±5.3)	60.6(±6.2)
OWC-NA	2	51.4(0.5)	95.2(±3.0)	62.7(±2.0)

Table A2: Traditional water quality parameters of influent and effluent by treatment combination.

TREATMENT	n	T (˚C)	pH	SC (mS/cm)	DO (mg/L)	TSS (mg/L)	TDS (mg/L)
Influent	25	26.6 (±0.1)	7.06 (±0.08)	0.56 (±0.19)	5.0 (±0.07)	1.8 (±1.6)	356 (±122)
FWS	10	20.2 (±0.4)	7.66 (±0.06)	1.32 (±0.23)	10.1 (±0.9)	10.7 (±7.1)	847 (±159)
SSF	10	19.9 (±0.2)	7.56 (±0.24)	1.13 (±0.16)	9.6 (±0.8)	1.72 (±1.6)	732 (±102)
V	10	20.3 (±0.3)	7.33 (±0.10)	1.24 (±0.10)	10.2 (±0.6)	6.0 (±5.6)	806 (±63)
UV	10	19.8 (±0.1)	7.89 (±0.39)	1.21 (±0.29)	9.5 (±0.9)	6.3 (±8.0)	786 (±191)
OWC	5	19.6 (±0.2)	9.80 (±0.15)	0.75 (±0.01)	10.9 (±0.9)	11.1 (±5.2)	487 (±6)
A	14	20.0 (±0.4)	8.16 (±0.98)	1.07 (±0.19)	9.9 (±0.9)	7.7 (±6.9)	693 (±123)
NA	11	20.0 (±0.3)	7.90 (±1.14)	1.21 (±0.34)	10.3 (±1.0)	6.7 (±6.3)	786 (±224)
FWS-V-A	3	20.5 (±0.2)	7.38 (±0.05)	1.16 (±0.07)	10.2 (±0.5)	8.8 (±6.6)	755 (±44)
FWS-V-NA	2	20.5 (±0.0)	7.25 (±0.05)	1.32 (±0.05)	10.7 (±0.1)	12.2 (±1.4)	857 (±35)
FWS-UV-A	3	19.9 (±0.1)	8.22 (±0.11)	1.21 (±0.07)	9.4 (±1.3)	6.7 (±4.7)	789 (±47)
FWS-UV-NA	2	20.0 (±0.2)	7.67 (±0.51)	1.73 (±0.06)	10.5 (±0.6)	11.8 (±12.2)	1122 (±36)
SSF-V-A	2	20.1 (±0.0)	7.49 (±0.07)	1.23 (±0.04)	10.4 (±1.1)	1.3 (±0.4)	798 (±25)
SSF-V-NA	3	20.1 (±0.2)	7.23 (±0.03)	1.27 (±0.14)	9.9 (±05)	2.2 (±1.9)	827 (±90)
SSF-UV-A	3	19.7 (±0.1)	7.77 (±0.03)	1.03 (±0.02)	9.3 (±0.6)	2.8 (±1.9)	668 (±15)
SSF-UV-NA	2	19.8 (±0.1)	7.82 (±0.05)	0.96 (±0.09)	8.9 (±0.2)	2.2 (±1.9)	757 (±247)
OWC-A	3	19.6 (±0.2)	9.74 (±0.17)	0.75 (±0.01)	10.3 (±0.6)	9.6 (±4.7)	490 (±6)
OWC-NA	2	19.7 (±0.3)	9.88 (±0.11)	0.74 (±0.01)	11.7 (±0.5)	8.0 (±2.0)	482 (±4)

Table A3: Anion concentrations of influent and effluent by treatment combination.

TREATMENT	n	$NO_2^- + NO_3^-$ (mg N/L)	DRP (mg P/L)	Cl^- (mg/L)	SO_4^{-2} (mg/L)
INFLUENT	5	6.76 (±0.13)	2.12 (±0.07)	75.3 (±1.1)	56.1 (±2.8)
FWS	10	0.03 (±0.03)	2.21 (±0.59)	148.6 (±24.3)	96.7 (±26.0)
SSF	10	5.06 (±9.19)	0.60 (±0.17)	140.9 (±28.2)	88.6 (±25.8)
OWC	5	4.17 (±2.83)	0.59 (±0.11)	82.7 (±1.1)	44.2 (±21.9)
V	10	0.14 (±0.14)	1.40 (±1.02)	152.6 (±19.9)	91.7 (±22.4)
UV	10	4.96 (±9.25)	1.42 (±0.89)	136.9 (±29.8)	93.6 (±22.9)
A	14	1.95 (±3.16)	1.45 (±1.01)	123.7 (±27.0)	81.8 (±26.8)
NA	11	4.05 (±8.79)	0.99 (±0.67)	143.3 (±40.4)	84.5 (±37.9)
FWS-V-A	3	0.01 (±0.01)	2.52 (±0.43)	130.2 (±11.2)	75.1 (±12.6)
FWS-V-NA	2	0.08 (±0.07)	2.00 (±0.24)	157.1 (±0.5)	96.3 (±22.9)
FWS-UV-A	3	0.03 (±0.01)	2.51 (±0.61)	134.6 (±5.2)	94.3 (±17.1)
FWS-UV-NA	2	0.03 (±0.02)	1.51 (±0.57)	188.5 (±9.4)	133.2 (±24.5)
SSF-V-A	2	0.33 (±0.12)	0.62 (±0.02)	164.9 (±11.0)	112.9 (±30.1)
SSF-V-NA	3	0.18 (±0.13)	0.38 (±0.03)	163.8 (±21.9)	91.1 (±22.5)
SSF-UV-A	3	0.89 (±1.42)	0.74 (±0.09)	120.1 (±3.0)	90.8 (±3.7)
SSF-UV-NA	2	NA[1]	0.73 (±0.04)	114.0 (±23.8)	57.1 (±28.6)
C-A	3	5.11 (±3.57)	0.56 (±0.10)	82.5 (±1.2)	46.1 (±20.7)
C-NA	2	2.77 (±0.10)	0.64 (±0.15)	83.0 (±1.2)	41.3 (±32.1)

1. Concentrations for SSF-UV-N determined to be outliers, mean = 19.14, n = 2.

Table A4: Metals concentrations of influent and effluent by treatment combination.

TREATMENT	n	Al (µg/L)	Ba (µg/L)	Ca (mg/L)	Cu (µg/L)	Fe (µg/L)	K (mg/L)	Mg (mg/L)	Na (mg/L)	Ni (µg/L)	S (mg/L)	Si (mg/L)	Zn (µg/L)
INFLUENT	5	30 (±2)	73 (±1)	30.6 (±0.1)	4 (±1)	77 (±1)	15.0 (±0.1)	12.9 (±0.2)	68.5 (±0.3)	9 (±0)	20.4 (±0.1)	3.5 (±0.0)	25 (±3)
FWS	10	653 (±497)	138 (±29)	79.3 (±17.0)	7 (±2)	843 (±620)	101.2 (±23.0)	20.2 (±4.8)	101.3 (±11.6)	31 (±10)	46.7 (±27.0)	8.1 (±2.9)	12 (±6)
SSF	10	51 (±26)	230 (±39)	66.9 (±8.7)	10 (±2)	44 (±24)	20.1 (±7.8)	18.9 (±3.3)	110.2 (±13.3)	28 (±4)	45.3 (±6.3)	7.0 (±0.8)	29 (±10)
OWC	5	20 (±5)	38 (±7)	31.7 (±0.6)	5 (±2)	12 (±4)	16.9 (±0.4)	13.5 (±1.3)	78.9 (±1.4)	8 (±1)	23.1 (±0.4)	3.0 (±0.4)	9 (±3)
V	10	403 (±510)	200 (±61)	76.0 (±9.9)	8 (±2)	565 (±715)	55.2 (±35.9)	21.2 (±2.1)	92.5 (±43.6)	33 (±7)	43.9 (±8.3)	8.7 (±2.2)	27 (±10)
UV	10	276 (±414)	174 (±55)	70.3 (±17.8)	9 (±3)	293 (±429)	61.5 (±53.2)	18.1 (±4.9)	104.1 (±9.8)	26 (±5)	47.7 (±24.9)	6.5 (±1.4)	16 (±11)
A	14	178 (±285)	147 (±72)	62.7 (±14.5)	8 (±2)	234 (±361)	47.4 (±36.1)	16.9 (±2.8)	100.4 (±15.9)	25 (±11)	35.5 (±11.1)	6.8 (±2.6)	17 (±13)
NA	11	379 (±537)	189 (±77)	77.6 (±22.3)	9 (±3)	581 (±719)	59.3 (±53.1)	20.9 (±4.9)	102.3 (±16.5)	28 (±9)	51.6 (±23.1)	7.1 (±2.5)	21 (±11)
FWS-V-A	2	569 (±679)	131 (±26)	74.2 (±12.7)	7 (±1)	774 (±697)	85.6 (±14.7)	18.8 (±2.3)	89.4 (±4.6)	33 (±14)	32.6 (±3.4)	9.4 (±3.8)	14 (±1)
FWS-V-NA	2	1091 (±174)	167 (±5)	88.2 (±1.1)	7 (±2)	1621 (±121)	98.2 (±7.7)	22.2 (±0.4)	107.5 (±0.3)	40 (±7)	42.1 (±0.5)	11.1 (±1.5)	19 (±1)
FWS-UV-A	3	262 (±150)	113 (±30)	62.2 (±7.0)	5 (±1)	326 (±193)	89.0 (±6.8)	15.5 (±2.4)	105.0 (±14.0)	25 (±9)	30.2 (±15.6)	6.7 (±2.7)	6 (±3)
FWS-UV-NA	2	886 (±687)	154 (±12)	101.4 (±1.3)	9 (±5)	909 (±685)	138.3 (±12.0)	26.7 (±2.7)	NA	27 (±2)	90.0 (±18.4)	5.9 (±0.6)	11 (±6)
SSF-V-A	2	89 (±7)	200 (±12)	65.0 (±1.6)	9 (±1)	81 (±5)	26.8 (±2.9)	20.8 (±1.7)	128.5 (±NA)	31 (±4)	47.0 (±3.7)	7.7 (±1.0)	34 (±5)
SSF-V-NA	3	42 (±23)	268 (±42)	76.3 (±6.4)	9 (±1)	46 (±19)	25.4 (±9.6)	22.3 (±2.0)	125.5 (±NA)	29 (±3)	50.7 (±7.5)	7.2 (±0.5)	35 (±2)
SSF-UV-A	3	50 (±20)	229 (±30)	61.3 (±1.7)	11 (±1)	33 (±8)	14.6 (±0.4)	16.6 (±0.9)	107.3 (±5.2)	29 (±2)	43.3 (±1.9)	7.0 (±0.4)	27 (±12)
SSF-UV-NA	2	28 (±1)	205 (±27)	56.4 (±4.1)	11 (±4)	19 (±3)	13.7 (±1.0)	15.4 (±2.0)	97.9 (±10.8)	22 (±3)	38.4 (±4.5)	6.0 (±0.3)	17 (±10)
OWC-A	3	23 (±5)	39 (±3)	31.7 (±0.6)	4 (±1)	12 (±1)	17.1 (±0.3)	12.9 (±1.3)	79.4 (±1.5)	7 (±1)	23.3 (±0.4)	2.7 (±0.2)	9 (±4)
OWC-NA	2	16 (±3)	36 (±14)	31.7 (±1.0)	6 (±2)	13 (±7)	16.5 (±0.1)	14.3 (±1.1)	78.2 (±1.2)	9 (±1)	22.9 (±0.4)	3.3 (±0.0)	9 (±2)

Figure A1: Daytime high and overnight low temperatures (˚C) during experimentation as measured by the HOBO weather station at NWRF.

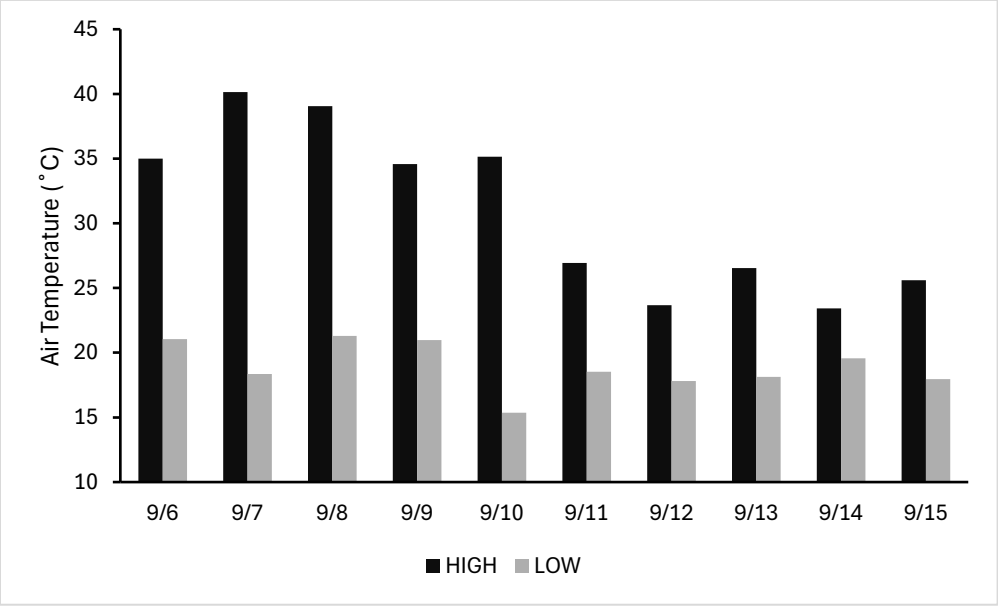

Figure A2: Peak and average solar radiation (W/m$^{2)}$ *during experimentation as measured by the HOBO weather station at NWRF.*

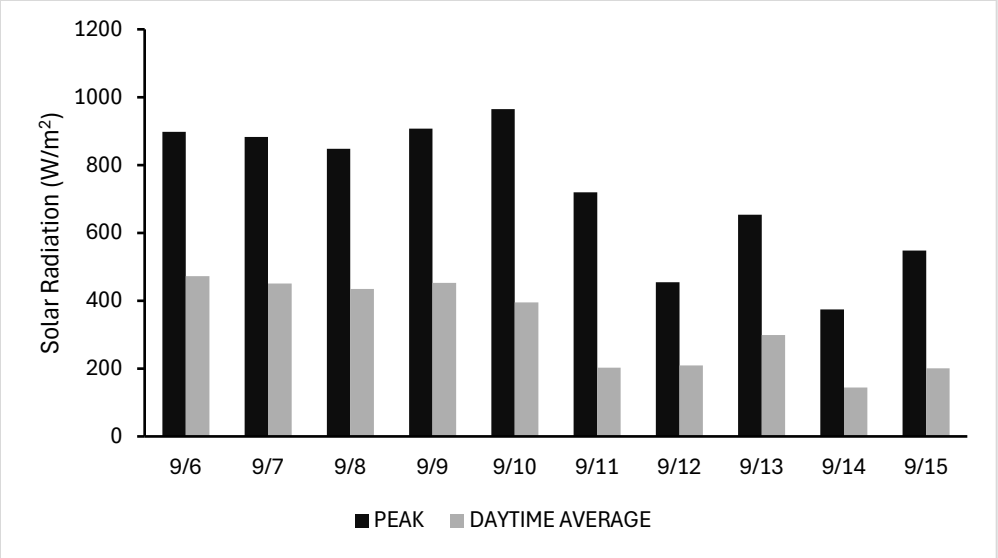

Figure A3 : Precipitation (mm) during experimentation as measured by the HOBO weather station at NWRF.

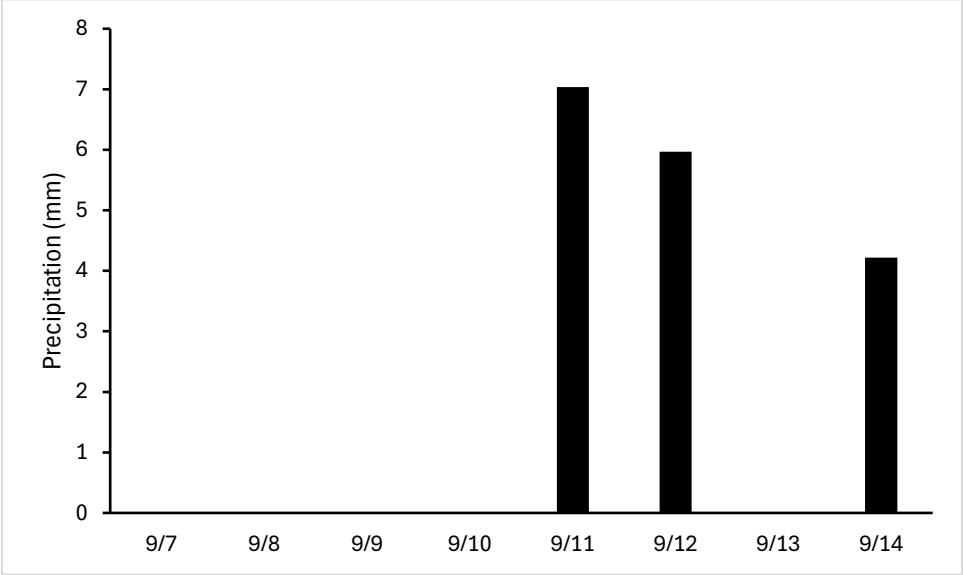

Table A5: Thornthwaite PET calculations for Norman, Oklahoma, 2023. $i = (\frac{T}{5})^{1.514}$*, where T = mean monthly temperature (˚C), the Annual Heat Index (I) is the sum of the Monthly Heat Indices:* $I = \sum_{i=1}^{12} i$*, A PET estimation is obtained for each month, based on the assumption that a month has 30 days and there are 12 hours of sunshine per day, applying the following equation:*

$$PET_{non\ corrected} = 16 * (\frac{10 * t}{I})^{\alpha}$$

Where $\alpha = 675*10^{-9} *I^3 - 771*10^{-7} * I^2 + 1792*10^{-5} + 0.49239$

Obtained values are then corrected according to the real length of the month and theoretical sunshine hours for the latitude of interest, with the formula:

$$PET = PET_{non\ corrected} * \frac{N}{12} * \frac{d}{30}$$

Where N = theoretical sunshine hours for each month and d = days for each month.

Month	**MMT (°C)**	**i**	**PET*nc* (mm)**	**n (h/d)**	**d**	**PET (mm)**
January	4.44	0.84	5.22	10.1	31	4.54
February	6.67	1.55	10.94	10.9	28	9.28
March	11.67	3.61	30.24	12	31	31.25
April	16.67	6.19	57.82	13.1	30	63.12
May	21.11	8.85	88.82	14	31	107.08
June	26.11	12.21	130.71	14.5	30	157.94
July	28.89	14.23	157.10	14.2	31	192.09
August	28.33	13.82	151.61	13.4	31	174.94
September	23.31	10.29	106.35	12.4	30	109.90
October	17.22	6.50	61.34	11.3	31	59.68
November	10.55	3.10	25.18	10.3	30	21.61
December	5.1	1.03	6.72	9.8	31	5.67
						I = 82.21 α = 1.82