

Onsite Remediation of Pharmaceutical and Personal Care Products in Domestic Wastewater  
using Alternative Systems Including Constructed Wetlands

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ABSTRACT

Pharmaceuticals, personal care products (PPCPs) and other trace organic contaminants (TOrcs) encompass a diverse group of chemicals that are not currently monitored or regulated in US drinking water or wastewater. Researchers have found low levels of TOrcs in aquatic and terrestrial environments all over the globe, and observed negative effects on impacted biota. The primary source of TOrcs in the environment is domestic wastewater discharges. Centralized wastewater treatment plants present greater risks on a global scale, but on a local scale, onsite treatment systems may have more potent impacts on resources that are invaluable to residents, including groundwater, surface waters, and soils.

The objective of this thesis is to identify and characterize promising treatment technologies for onsite TOrc remediation. Receptors who could be impacted by TOrc discharges are assessed, and applications that may require alternative treatment are identified. The best treatment technologies are recognized as those that protect sensitive environmental receptors, provide permanent removal pathways for as many TOrcs as possible, and are not prohibitively expensive to install or maintain. Findings from a pilot study show increased removal of conventional pollutants and TOrcs in an aerobic treatment unit (ATU), two types of biofilter, and a hybrid constructed wetland, all relative to septic tank effluent. The constructed wetland achieved the highest nutrient removals with TN concentrations below 10 mg/L throughout the study. A system with an ATU and peat biofilters achieved the highest removals of persistent pharmaceuticals carbamazepine and lamotrigine (>85% and >95%, respectively).

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GENERAL AUDIENCE ABSTRACT

Trace organic contaminants (TOrcs) are chemicals found in pharmaceuticals, laundry detergents, shampoo, flame retardants, food preservatives, and many other products used in a typical home, which are not currently monitored or regulated in US drinking water or wastewater. Researchers have found low levels of TOrcs in waters and soils all over the globe, and observed negative effects on the plants and animals that live in those environments. The primary source of TOrcs in the environment is treated wastewater from centralized wastewater treatment plants, which is usually released to rivers, lakes, and other surface waters. People in rural communities also have TOrcs in their wastewater, which is normally treated using a septic system. Water released in the septic field can add TOrcs to septic field soils, groundwater sources, or nearby surface water sources, and from there these chemicals have the potential to impact human health, soil fertility, livestock health, or fish and other living things in surface waters.

The objective of this thesis is to identify and characterize promising treatment technologies that would prevent or limit TOrc impacts to these important resources, which are called “receptors.” Receptors who could be impacted by TOrc discharges are assessed, and the situations in which these treatment technologies would be necessary are identified. The best treatment technologies are recognized as those that protect sensitive environmental receptors, remove as many TOrcs as possible, and are affordable to install or maintain. An experiment was designed to compare the performance of three different technologies that could remove TOrcs from septic tank effluent, including a peat filter and a constructed wetland. The constructed wetland removed the most nitrogen (total nitrogen <10 mg/L throughout the study), and a system with a peat filter removed the greatest amounts of persistent pharmaceuticals carbamazepine and lamotrigine (averaging >85% and >95%, respectively).

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## Acronyms

2-OH-CBZ	2-hydroxy-CBZ
3-OH-CBZ	3-hydroxy-CBZ
ACE	Acetaminophen
ADI	Adult daily intake
ANOVA	Analysis of variance
AOP	Advanced oxidation process
ARG	Antibiotic resistance gene
ATU	Aerobic treatment unit
BOD	Biochemical oxygen demand
CBZ	Carbamazepine
CBZ-E	CBZ-10,11-epoxide
Cl	Chloride
COD	Chemical oxygen demand
CS	Conventional septic
CW	Constructed wetland
CW4	Constructed wetland Tank 4
CW6	Constructed wetland Tank 6
DiOHCBZ	trans-10,11-dihydroxy-10,11-dihydro-CBZ
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DWEL	Drinking water equivalency level
EDC	Endocrine disrupting compound
ETU	Enhanced treatment unit
F	Fluoride
FOG	Fats, oils, and greases
FWS	Free water surface
GAC	Granular activated carbon
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
HRT	Hydraulic retention time

HSD	Honest significant difference
HSSF	Horizontal subsurface flow
IS	Internal standard
LAM	Lamotrigine
LECA	Light expanded clay aggregate
LEV	Levetiracetam
LMG-N2-G-TP430	Glucuronide metabolite of lamotrigine
LOAEL	Lowest observable adverse effect level
LOQ	Limit of quantification
LWA	Light weight aggregate
MET	Metformin
MHD	10,11-dihydro-10-hydroxy-carbamazepine
MTD	Minimum therapeutic dose
NAPQI	N-acetyl-p-benzoquinone imine
NDV	N-desmethyl-venlafaxine
NH <sub>4</sub>	Ammonia
NO <sub>2</sub>	Nitrite
NO <sub>3</sub>	Nitrate
NOAEL	No observable adverse effect level
N,O-DDM-VEN	N,O-didesmethyl-venlafaxine
ODV	O-desmethyl-venlafaxine
OXO-LAM	Synthetic impurity of lamotrigine
O <sub>3</sub>	Ozone
PO <sub>4</sub>	Phosphate
PPCP	Pharmaceutical and personal care product
SF	Surface flow
SO <sub>4</sub>	Sulfate
SSF	Subsurface flow
STE	Septic tank effluent
TCS	Triclosan

TIN	Total inorganic nitrogen
TN	Total nitrogen
TOP	Topiramate
TOrC	Trace organic contaminant
TP	Total phosphorus
TSS	Total suspended solids
UCB L057	Acid metabolite of levetiracetam
UV	Ultraviolet
V <sub>2</sub>	Metabolite of venlafaxine
VEN	Venlafaxine
VSS	Volatile suspended solids
VSSF	Vertical subsurface flow
WRF	White rot fungi
WWTP	Wastewater treatment plant

# 1 Introduction

Trace organic chemicals (TOrcs) encompass a diverse group of tens of thousands of chemicals that are not currently monitored or regulated in US drinking water or wastewater. Researchers have found low levels of TOrcs in aquatic and terrestrial environments all over the globe (Ternes, 1998; Kolpin *et al.*, 2002; Barnes *et al.*, 2008; Schlusener *et al.*, 2015), and observed negative effects on impacted biota (Writer *et al.*, 2010; Fong and Hoy, 2012).

The primary source of TOrcs in the environment is domestic wastewater discharges, including those of centralized and decentralized origin (Conn *et al.*, 2006; Li *et al.*, 2014). On a global scale, TOrcs discharged in effluents from centralized wastewater treatment plants (WWTPs) can have greater impacts for sensitive environmental receptors because they process such large volumes of wastewater and often discharge directly to surface water resources. However, on a local scale, onsite treatment systems have the potential to impact resources that are invaluable to residents.

TOrcs are extremely difficult, if not impossible, to remove from wastewater by biological treatment alone, and varying levels of many parent compounds and metabolites have been found in effluents from both centralized and decentralized wastewater treatment systems (Rudel *et al.*, 1998; Conn *et al.*, 2006; Joss *et al.*, 2006; Carrara *et al.*, 2008; Writer *et al.*, 2010; Gurke *et al.*, 2015; Phillips *et al.*, 2015). Septic systems, the most commonly used onsite treatment technology in the US, may be particularly ineffectual for TOrc remediation because they provide limited mechanisms for removal during primary treatment (US EPA, 2002). Secondary treatment in subsurface dispersal fields relies on site specific factors including soil conditions and groundwater dilution. While these strategies can provide effective remediation of conventional pollutants, they may be insufficient for TOrcs. Several studies have shown that TOrcs can be found in groundwater, soils, and ecosystems impacted by septic systems (Miller and Meek, 2006; Godfrey *et al.*, 2007; Carrara *et al.*, 2008; Phillips *et al.*, 2015; Subedi *et al.*, 2015). Because onsite treatment systems are often in rural locations, sited close to homes, they may be adjacent to valuable groundwater, surface water, or soil resources, which residents may use for drinking water, food production, and/or recreation. Impacts to aquatic ecosystems may be particularly devastating, even when chemicals are present at levels as low as a couple nanograms per liter (Lange *et al.*, 2001;

Fenske *et al.*, 2005; Kidd *et al.*, 2007; Palace *et al.*, 2009; Writer *et al.*, 2010; Fong and Hoy; 2012).

Advanced physicochemical treatments, including advanced oxidation processes (AOPs) and membranes, are currently regarded as the most effective means of remediating wastewater-borne TOrCs (Ternes *et al.*, 2002). For most TOrCs, these treatments are able to remove parent compounds as well as bioactive metabolites and metabolites that could be converted back to the parent compound. As such, they have received a lot of attention in the literature and are being employed at various municipal wastewater reuse facilities where TOrC removal is an important goal. However, they also have various disadvantages. First, there are TOrCs that can resist degradation by certain advanced oxidation methods, including metformin which is resistant to oxidation by UV/H<sub>2</sub>O<sub>2</sub>, and lamotrigine which is resistant to ozonation (De la Cruz *et al.*, 2012; Keen *et al.*, 2014). Other researchers have observed the formation of toxic byproducts by AOPs, particularly in the early stages of oxidation (Dantas *et al.*, 2011). Perhaps their most important limitation is that they are prohibitively expensive and difficult to operate, so that they currently have limited utility in onsite applications. Treatment with UV/H<sub>2</sub>O<sub>2</sub> could be considered, but it would not be possible to treat STE directly; an additional treatment step would be required to reduce the concentrations of radical scavengers.

Enhanced treatment units (ETUs) improve the performance of a septic system by adding a treatment step between the septic tank and the septic field. Often, that step involves aeration or contact with some kind of media, like sand or peat. Both aerobic treatment units (ATUs) and media filters are described in detail in the text by Jantrania and Gross (2006) and multiple researchers have considered remediation of PPCPs using these systems (Hinkle *et al.*, 2005; Conn and Siegrist, 2009; Stanford and Weinberg, 2010; Du *et al.*, 2014; Subedi *et al.*, 2015). Design configurations may vary widely between manufactured systems, but they do not include macrophytes and typically have residence times of a few days. Constructed wetlands (CW) have also been used as a secondary treatment step for decentralized treatment or as an alternative to conventional WWTPs (Conn *et al.*, 2006; Conkle *et al.*, 2008; Matamoros *et al.*, 2009; Hijosa-Valsero *et al.*, 2010a; Stanford and Weinberg, 2010). Wetland design can vary widely as well, from flow regime, loading mode (continuous, intermittent), and hydraulic retention time (HRT), to the types of plants and

media included, and the types of microorganisms that are encouraged to grow. Generally, wetlands are classified most broadly by their flow regime, either free water surface (FWS), surface flow (SF), horizontal subsurface flow (HSSF), or vertical subsurface flow (VSSF). Each type is known for promoting different removal mechanisms. The use of CWs for TOrC remediation has been reviewed by several researchers (Imfeld et al, 2009; Li *et al.*, 2014; Verlicchi and Zambello, 2014; Carvalho *et al.*, 2014).

Findings from the literature clearly indicate that both CWs and ETUs have a greater capacity for TOrC remediation than CS tanks alone (Conn et al, 2006; Matamoros *et al.*, 2009; Stanford and Weinberg, 2010; Du *et al.*, 2014). Furthermore, CWs and ETUs that include aeration, either forced or through the passive use of unsaturated filters, have been found to be more effective for the removal of TOrCs, particularly endocrine disrupting compounds (EDCs) (Stanford and Weinberg, 2010). However, both CWs and ETUs are more expensive to install and operate, and the advantages of using them when septic fields are operating properly has been questioned (Conn and Siegrist, 2009).

Every application of onsite treatment technology is unique with regard to the relative priority of TOrC remediation. For systems that have adequate septic fields, sufficient groundwater dilution, and are physically separated from other onsite systems, farmland, groundwater resources, and surface waters, and/or where residents do not emit large quantities of persistent TOrCs, there may be no need to improve TOrC remediation. However, researchers are continually finding situations where insufficient treatment leads to concerning concentrations of TOrCs in adjacent resources and environments (Swartz *et al.*, 2006; Writer *et al.*, 2010). In these situations, it may be extremely difficult for users to identify alternatives with greater TOrC remediation capacity or determine whether the investment is worth making.

This thesis is comprised of two articles that attempt to tackle different issues related to onsite remediation of pharmaceuticals and personal care products (PPCPs), and other TOrCs. The first is a literature review which considers potential impacts to the environment and human health from onsite discharges of TOrCs, as well as the relative performance and utility of different onsite treatments for TOrC remediation. Although there have been many reviews of the environmental fate of TOrCs and potential remediation strategies, the “onsite” focus of this review is unique. In

order to assess human and environmental health effects, findings from studies related to centralized WWTP discharges were translated to onsite applications. From there, potential impacts from onsite discharges of TOrCs could be used to develop qualitative metrics for assessing treatment alternatives.

The second article describes a pilot study comparing the PPCP remediation capacity of a hybrid CW and two different ETUs treating real septic tank effluent (STE) over a period of seven months. The site of the project was an 80-person community for intellectually-disabled adults in rural Virginia. The community was interested in alternative onsite treatment options for their wastewater because septic fields on the property are sited over groundwater resources and under agricultural land. Seven pharmaceuticals were chosen for monitoring throughout the study based on relatively high consumption rates in the community: acetaminophen, carbamazepine, lamotrigine, levetiracetam, metformin, topiramate, and venlafaxine. In addition, one personal care product, the antimicrobial triclosan, was also monitored.

A few studies in the literature have compared grab samples from ETUs and CWs processing real septic tank or primary treated effluents (Conn and Siegrist, 2009; Matamoros *et al.*, 2009; Stanford and Weinberg, 2010). One study compared the performance of an ATU that included a settling basin with that of a CW treating STE and the septic tank alone (Du *et al.*, 2014). However, this study is unique in the following respects:

- The ETUs and CW were processing the same STE
- The ETUs and CW were operated and samples collected over a relatively long duration (seven months)
- The consumption rates for most of the monitored PPCPs were known and consistent from day to day
- Mycoremediation of CW effluents was considered
- Some of the monitored PPCPs, including levetiracetam, lamotrigine, metformin, and topiramate, have received little prior attention in the decentralized treatment literature

## 2 Literature Review

### Abstract

This paper reviews issues related to trace organic contaminants (TOrcs) discharged from domestic, onsite wastewater treatment systems. Many environmental fate and impact studies have focused on issues related to centralized wastewater treatment plant (WWTP) discharges. However, onsite discharges may have more pertinent implications for localized environments and human communities. First, the routes that TOrcs may take between domestic use and downstream environments are mapped. Then, findings from impact assessments of direct discharge or agricultural applications of WWTP effluents are translated to subsurface discharges in septic fields. Both environmental and human health impacts are considered. Next, the TOrc remediation capacities of various alternative onsite treatment technologies – including constructed wetlands (CWs), aerated treatment units, and biofilters – are compared to those of conventional septic (CS) systems. Finally, conclusions are drawn about the comparative ability of the different technologies to protect sensitive receptors, as well as their suitability for onsite treatment. The applications of conventional septic systems that are most likely to cause environmental pollution or raise human health risks are identified. Research gaps are indicated, including the effect of pretreatment on septic field performance. A primary focus of the review is to define the problem of onsite TOrc discharges in order to support the logical development of solutions.

**Keywords:** critical review, trace organic contaminants (TOrcs), pharmaceuticals and personal care products (PPCPs), constructed wetlands, enhanced treatment (units), septic systems

### 2.1 Introduction

TOrcs encompass a diverse group of tens of thousands of chemicals that are not currently monitored or regulated in US drinking water or wastewater. Researchers have found low levels of TOrcs in aquatic and terrestrial environments all over the globe (Ternes, 1998; Kolpin *et al.*, 2002; Barnes *et al.*, 2008; Schlusener *et al.*, 2015), and observed negative effects on impacted biota (Writer *et al.*, 2010; Fong and Hoy; 2012).

The primary source of TOrCs in the environment is domestic wastewater discharges, including those of centralized and decentralized origin (Conn *et al.*, 2006; Li *et al.*, 2014). Centralized WWTPs typically collect wastewater from a large area and treat it to relatively high standards (at least secondary treatment) before discharging it directly into a surface water source. Decentralized or onsite wastewater treatment refers to the treatment of wastewater from one or a handful of homes, and is the focus of this paper. CS systems are the most commonly used onsite treatment system in the United States (US EPA, 2002). CS systems treat wastewater in an anaerobic tank to primary standards followed by subsurface soil application.

TOrCs are extremely difficult, if not impossible, to remove from wastewater by biological treatment alone, and varying levels of many parent compounds and metabolites have been found in effluents from both centralized and decentralized wastewater treatment systems (Rudel *et al.*, 1998; Conn *et al.*, 2006; Joss *et al.*, 2006; Carrara *et al.*, 2008; Writer *et al.*, 2010; Gurke *et al.*, 2015; Phillips *et al.*, 2015). Septic systems, the most commonly used onsite treatment technology in the US, may be particularly ineffectual for TOrC remediation because they provide limited mechanisms for removal during primary treatment (US EPA, 2002). Secondary treatment in subsurface dispersal fields relies on site specific factors including soil conditions and groundwater dilution. While these strategies can provide effective remediation of conventional pollutants, they may be insufficient for TOrCs. Several studies have shown that TOrCs can be found in groundwater, soils, and ecosystems impacted by septic systems (Miller and Meek, 2006; Godfrey *et al.*, 2007; Carrara *et al.*, 2008; Phillips *et al.*, 2015; Subedi *et al.*, 2015). Because onsite treatment systems are often in rural locations, sited close to homes, they may be adjacent to valuable groundwater, surface water, or soil resources. Impacts to aquatic ecosystems may be particularly devastating, even when chemicals are present at levels as low as a couple nanograms per liter (Lange *et al.*, 2001; Fenske *et al.*, 2005; Kidd *et al.*, 2007; Palace *et al.*, 2009; Writer *et al.*, 2010; Fong and Hoy; 2012).

It is important to recognize that each onsite treatment system is unique with regard to the relative priority of TOrC remediation. For systems that have adequate septic fields, sufficient groundwater dilution, and are physically separated from other onsite systems, farmland, groundwater resources, and surface waters, and/or where residents do not emit large quantities of persistent TOrCs, there

may be no need to improve TOrC remediation. However, researchers are continually finding situations where insufficient treatment leads to concerning concentrations of TOrCs in adjacent resources and environments (Swartz *et al.*, 2006; Writer *et al.*, 2010). In these situations, it may be extremely difficult for users to identify alternatives with greater TOrC remediation capacity or determine whether the investment is worth making. They must answer questions, like: What are the potential environmental and human health impacts associated with TOrCs released via onsite treatment systems? Are there alternative treatment systems that provide more effective TOrC remediation than CS, and are there features of these technologies that limit their use in onsite applications?

The goal of this review is to comb the literature for answers to these questions. The considered treatment options will provide secondary treatment to septic tank effluent (STE) and will discharge to a septic field. The review will consider removal mechanisms and knowledge gaps for each system and overview potential drawbacks, including solid waste production and other potential environmental impacts.

There are several reasons why it is difficult to generalize about TOrC remediation strategies:

1. There are a vast number of TOrCs with a wide array of chemical characteristics. A unique pattern of chemicals and concentrations will be present in every wastewater source.
2. In part because of the vast numbers of TOrCs, the chemical interactions between them, their metabolic paths, and the chemical characteristics/toxicity of their metabolites are not well understood.
3. The effectiveness of septic systems, specifically the septic fields, depends on site conditions, including soil composition, depth to groundwater, groundwater flow rates, locations of wells and agricultural areas, and proximity to surface water resources.

Therefore, an additional goal of this review will be to establish qualitative metrics for comparing the successful remediation of TOrCs by onsite wastewater treatment systems.

## 2.2 Trace Organic Contaminants (TOrcs) in Domestic, Onsite Wastewater

TOrcs found in pharmaceuticals and personal care products (PPCPs) as well as various other consumer products can be conveyed to onsite wastewater systems through many portals, including toilets, sinks, dishwashers, showers, and laundry machines. Some of these chemicals may be modified from their original form by the time they reach the treatment system. For example, pharmaceuticals can be partially or fully metabolized while passing through the human digestive system (Daughton and Ternes, 1999).

When TOrcs are discharged into a septic field, a particular compound's fate will depend on its chemical attributes and the characteristics of the septic field, including media type, redox conditions, gradient, and climate. In general, more polar compounds could be expected to leach into groundwaters or downstream surface waters (Daughton and Ternes, 1999) while compounds with moderate to high Log  $K_{ow}$  values that are not removed in the septic tank could be expected to remain sorbed in the soil ( $>2$ ), or to be taken up by plants (1 to  $<3.5$ ) (Dietz and Schnoor, 2001; Imfeld *et al.*, 2009). Compounds with moderate to high Log  $K_{ow}$  values ( $\geq 5$ ), including pharmaceuticals designed to cross the blood-brain barrier, are more likely to pose bioconcentration or bioaccumulation risks for terrestrial biota (Daughton and Ternes, 1999; Arnot and Gobas, 2006).

Significant work has been done on the environmental fate and effects of TOrcs, particularly pharmaceuticals, released from centralized treatment plants to surface waters and soils (Jjemba, 2002; Ferrari *et al.*, 2003; Fenske *et al.*, 2005; Ying *et al.*, 2007; Chen *et al.*, 2013; Carter *et al.*, 2014; Mrozik and Stefanska, 2014). Like biosolids, TOrcs released via septic systems are also applied to terrestrial ecosystems; however, the location of release and the types of chemicals released are different. Biosolids application would be expected to return significantly more compounds with high to moderate Log  $K_{ow}$  values ( $>2$ ). Reclaimed water irrigation might contain compounds that have lower Log  $K_{ow}$  values, similar to those in STE; however, reclaimed water has undergone primary, secondary, and potentially even tertiary treatment, as well as disinfection. Both biosolids application and reclaimed water irrigation release TOrcs closer to the surface of the soil where removal mechanisms such as aerobic degradation, plant uptake, organic sorption, and photodegradation may be more effective than in a subsurface drain field. Figure 2.1 below

maps the routes that TOrCs may take from the home to environmental receptors and indicates chemical characteristics that determine fate.

The presence of TOrCs in the environment has been described as “pseudo-persistent” – even chemicals that break down rapidly in the environment are always present because they are being emitted consistently (Daughton and Ternes, 1999). However, some TOrCs are persistent in their own right (Howard and Muir, 2011). We must conclude that, if emitted consistently at a higher rate than the rate at which they can be degraded in the natural environment, these TOrCs will accumulate, resulting in higher observed concentrations in the environment. An illustration of this is that organic contaminants which were banned decades ago can still be detected in the environment today (Aitken *et al.*, 2004).

The four primary exposure routes for TOrCs discharged into septic fields to human, plant, and animal receptors are:

1. Leaching into groundwater and subsequent discharge to surface waters with impacts on aquatic life
2. Partitioning to soil pore water and upward movement in soils with impacts on soil fertility and plant health
3. Leaching into groundwater and subsequent direct or indirect (via livestock) consumption by humans
4. Uptake by plants and subsequent direct or indirect consumption by humans

The implications of these four exposure routes will be described in more detail below.

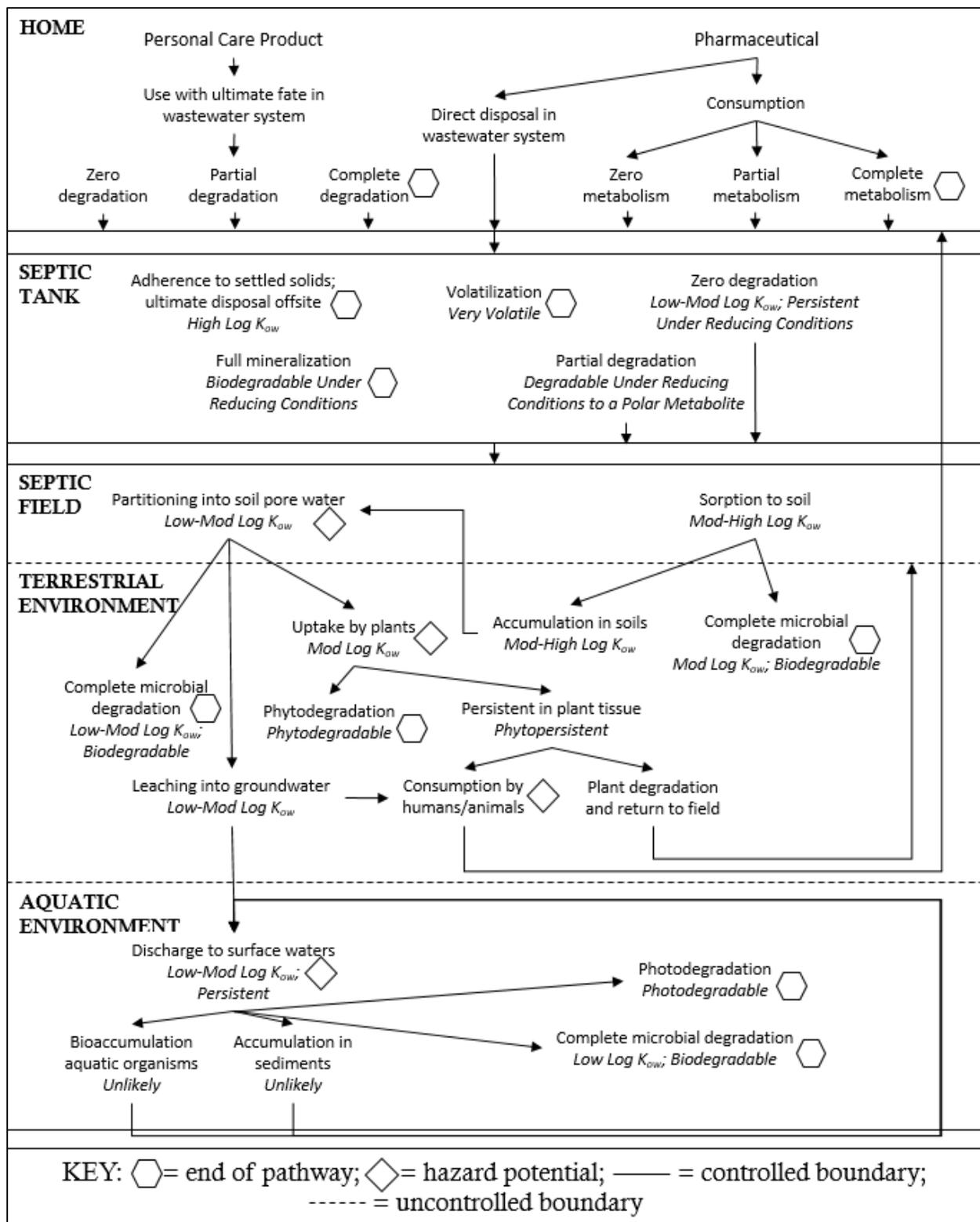


Figure 2.1: Fate map for trace organic contaminants in conventional septic systems

### 2.2.1 Impact on Aquatic and Terrestrial Environments

The risks of TOrC exposure to aquatic biota is often considered a greater concern than the risks to terrestrial biota, in part because WWTP discharges are often made directly to surface waters (Daughton and Ternes, 1999). As a result, many more investigations have examined toxic and chronic effects of single and combinations of TOrCs on aquatic organisms compared to organisms from other ecosystems. However, there has been some contention in the literature over the severity of aquatic toxicity risks from observed TOrC concentrations in waters receiving WWTP effluent discharges. A paper by Ferrari *et al.* in 2003 concluded that chronic aquatic toxicity risks from carbamazepine in WWTP effluents were high. The researchers predicted no adverse effect levels (NOAELs) based on acute toxicity tests with carbamazepine, clofibric acid, and diclofenac, and then compared those concentrations to predicted maximum WWTP discharge concentrations. The calculated hazard quotients (effluent concentrations divided by NOAELs) were less than unity for clofibric acid and diclofenac, and approximately 50 for carbamazepine.

A couple years later, a review by Fent *et al.* (2006) was published which came to the opposite conclusion. The researchers catalogued the known ecotoxicity of a large number of TOrCs and determined that chronic effects were unlikely at environmentally relevant concentrations. They compared chronic lowest observed adverse effect levels (LOAELs) and NOAELs on phytoplankton, benthos, zooplankton, and fish to highest observed treatment plant effluent concentrations. In general, the NOAELs were one to three orders of magnitude higher than the maximum observed concentrations. Propranolol and carbamazepine NOAELs were the same order of magnitude as the highest observed WWTP effluents, however effluents were found to be about one order of magnitude higher than observed surface water concentrations. Although hazard quotients were not calculated in the paper, they would have been less than 0.1 for the considered TOrCs.

Because the Fent *et al.* (2006) paper was based on chronic toxicity tests and observed WWTP effluents and surface water concentrations, its findings are more reliable than those of Ferrari *et al.* (2003). However, the review was still limited by the lack of available chronic toxicity data, and its lack of consideration for endocrine disrupting compounds (EDCs). There is growing evidence in the literature that there are serious aquatic toxicity risks from observed environmentally-relevant

concentrations of TOrCs, particularly for EDCs but also for other chemicals. Chronic exposure to synthetic steroid 17- $\alpha$  ethinylestradiol has been found to affect reproductive development and have population-level effects in various fish species including fathead minnows and zebrafish at concentrations on the order of 1 ng/L (Lange *et al.*, 2001; Fenske *et al.*, 2005; Kidd *et al.*, 2007; Palace *et al.*, 2009). Fong and Hoy (2012) found that exposure of freshwater snails to the antidepressants citalopram and venlafaxine caused acute effects on their ability to attach to substrate at concentration magnitudes as low as 0.1 to 10 ng/L. Cleuvers (2003) found that, when exposed to combinations of pharmaceuticals, half maximal effective concentrations were additive in nature for *Lemna minor* and *Daphnia magna*. Although he was working at environmentally-unrealistic concentrations of 10-100 mg/L, his findings have important implications for how toxicity risks are considered for TOrCs in the environment.

Although STEs are not applied directly to groundwater or surface water, TOrCs can be conveyed from the septic field through groundwater to surface water resources, particularly when concentrated septic systems are located near streams or lakes. Standley *et al.* (2008) found combined concentrations of EDCs of up to 10 ng/L in Cape Cod ponds impacted by septic systems and concentrations were correlated with septic system density. Writer *et al.* (2010) observed endocrine disruption in resident fish from several lakes in Minnesota impacted by septic systems and stormwater runoff, and not receiving any WWTP discharges. The concentrations of EDCs affecting the fish were at the nanogram per liter level. Thus, the preponderance of data shows that there is reason to expect toxic effects to aquatic organisms at environmentally relevant TOrC concentrations as a result of decentralized treatment discharges.

As for terrestrial environments, several researchers have considered impacts on plant health from the presence of antibiotics in the soil, and have had trouble differentiating between the health of the plants and supporting soil microorganisms. These studies considered exposure via biosolids application or wastewater irrigation, and may therefore overestimate the risks posed by septic fields. Jjemba (2002) tested the impact of increasing concentrations of anti-protozoa agents on the health of soybean (*Glycine max*) plants as well as associated soil protozoa populations. In general, higher concentrations correlated with reduced plant health and protozoa populations. However, very high exposure levels were required to see any impacts – roughly 1 to 10 mg/g soil. On the

other hand, Migliore *et al.* (2003) observed a continuum between crop plant toxicity and hormesis when exposed to varied levels of the antibiotic enrofloxacin. Impacts on plant growth were solely ascribed to uptake via the roots and subsequent DNA modification by the chemical.

Mixed information was found regarding terrestrial impacts by TOrCs besides antibiotic agents at environmentally-relevant levels. Winker *et al.* (2010) did not see any phytotoxic effects to ryegrass (*Lolium perenne*) from carbamazepine (0.06 mg/L) or ibuprofen (0.8 mg/L) exposure via urine fertilization. Eggen *et al.* (2011) found that metformin significantly inhibited plant growth for various carrot species (*D. carota* subspecies *sativus* cultivars Amager, Rothild and Nutri Red) but not for barley (*Hordeum vulgare*) or turnip rape (*Brassica campestris*) when present at about 10 mg/kg soil. The significance of growth limitations measured for meadow fescue (*Festuca pretense*) and wheat (*Triticum aestivum*) was not clearly indicated. Ying *et al.* (2007) did not see any inhibition of microbial activity by triclosan or triclocarban at soil concentrations intended to mimic biosolids application to soil. Because exposure concentrations from septic fields are likely to be so much lower than the concentrations used in these studies, phytotoxic or microbial effects are probably unlikely to result from TOrC releases in septic fields. However, Underwood *et al.* (2011) observed significant impacts to cell growth and nitrate reduction potential when microbes cultured from a pristine groundwater environment were exposed to the antimicrobial sulfamethoxazole. Environmentally-relevant concentrations of only 5 ng/L sulfamethoxazole were enough to reduce nitrate reduction potential by nearly 50%. Sulfamethoxazole is one of the most commonly detected TOrCs in United States groundwaters, and septic systems are believed to be a primary source (Barnes *et al.*, 2008; Segura *et al.*, 2009).

### **2.2.2 Current Knowledge of Human Health Impacts**

Many studies have shown that septic fields can contribute TOrCs to drinking water sources (Rudel *et al.*, 1998; Swartz *et al.*, 2006; Godfrey *et al.*, 2007; Schaidler *et al.*, 2011; Schaidler *et al.*, 2014; Subedi *et al.*, 2015). It has also been demonstrated that crop plants can take up pharmaceuticals and other TOrCs when biosolids are applied or when they are irrigated with wastewater (Eggen *et al.*, 2011; Karnjanapiboonwong *et al.*, 2011; Shenker *et al.*, 2011). Studies considering TOrC uptake by plants grown over septic fields were not found in the literature, but it is conceivable that wastewater-borne TOrCs could be drawn up towards plant roots via capillary action or by

pressures exerted by the roots themselves. It is also possible that TOrCs could accumulate in animals that consistently consume TOrC-contaminated plants. Therefore, humans can be exposed to TOrCs released via septic systems by consuming contaminated plants, livestock, and/or drinking water.

Most drugs are safe for human consumption at doses that are orders of magnitude higher than those observed in these environmental compartments, and therefore these exposure routes initially seem to be of little concern. Furthermore, there is little concrete evidence in the literature of chronic effects on humans from long term, low level exposure to a combination of TOrCs, although EDCs (including estradiol and nonylphenol) have raised alarm bells for their potential impacts on human fertility (Aitken *et al.*, 2004). The lack of data reflects how difficult it is to collect results on chronic effects with human subjects and to predict the interactions between drug combinations and the human body. However, toxic effects on human embryonic cells have been observed when they were exposed to very low levels of a combination of pharmaceuticals (Pomati *et al.*, 2006). The researchers exposed the cells to 13 pharmaceuticals at nanogram per liter levels and observed 10 to 30% decreases in cell proliferation. It is important to note that the exposure route of the cells in this experiment to the pharmaceuticals (i.e. submersion) may not be realistic, so it is difficult to extrapolate these findings.

Because concrete data is not available, hazard assessments have been used to estimate human health risks from long term, low level exposures to TOrCs in drinking water and plants (Snyder *et al.*, 2008; Prosser and Sibley, 2015). In general, an acceptable daily intake (ADI) value (typical units of mg/kg/day) is calculated by applying uncertainty and/or conversion factors to NOAELs, LOAELs, or minimum therapeutic doses (MTDs). NOAELs and LOAELs are determined based on animal or human toxicity studies. The ADI is defined as the rate at which an average adult can consume a particular chemical for an extended period of time *without seeing any adverse health effects*. By assuming a constant weight and water consumption rate (70 kg and 2 L per day for adults in the United States), the ADI can be converted to a drinking water equivalency level (DWELs, typical units of µg/L or ng/L). The DWELs can be compared directly to observed drinking water concentrations. A hazard quotient is obtained by dividing the highest observed concentration by the DWEL.

Using maximum groundwater concentrations found in the literature at sites impacted by human or animal wastewater discharges and the procedure in Snyder *et al.* (2008), hazard quotients were calculated for a handful of TOrCs. Results are shown in Table 2.1 below. Relatively few chemicals could be found both in the referenced studies and in Snyder *et al.* (2008). However, all hazard quotients were below 1, with the highest value calculated for phenytoin (cancer endpoint) at 0.4. These results confirm that the concentrations of TOrCs in groundwater are not a health risk when chemicals are considered individually.

**Table 2.1: Hazard quotients calculated for human exposure to observed concentrations of TOrCs in impacted groundwaters**

<sup>1</sup>Maximum observed groundwater concentration found in the literature. <sup>2</sup>Values from this source based on tap water concentrations. TOrCs = trace organic contaminants; DWEL = drinking water equivalent levels; EDCs = endocrine disrupting compounds.

TOrC	Observed Groundwater Concentration (µg/L) <sup>1</sup>	DWEL (µg/L)	Adult Hazard Quotient	Reference for Groundwater Concentration
<i>DWELs based on non-cancer endpoints</i>				
<i>non-EDCs</i>			4E-03	
Atenolol	0.0195	93	2E-04	Subedi <i>et al.</i> , 2015 <sup>2</sup>
Carbamazepine	0.226	350	6E-04	Phillips <i>et al.</i> , 2015
Diazepam	0.0011	35	3E-05	Phillips <i>et al.</i> , 2015
Fluoxetine	0.056	34	2E-03	Barnes <i>et al.</i> , 2008
Gemfibrozil	0.0012	1,100	1E-06	Schaider <i>et al.</i> , 2014
Meprobamate	0.0054	260	2E-05	Schaider <i>et al.</i> , 2014
Phenytoin	0.690	2,000	3E-04	Phillips <i>et al.</i> , 2015
Simvastatin	0.014	23	6E-04	Schaider <i>et al.</i> , 2011
Sulfamethoxazole	0.113	18,000	6E-06	Schaider <i>et al.</i> , 2014
Trimethoprim	0.0007	6,700	1E-07	Schaider <i>et al.</i> , 2014
<i>EDCs</i>			3E-01	
Bisphenol A	2.550	1,800	1E-03	Barnes <i>et al.</i> , 2008
17β-estradiol	0.045	1.8	3E-02	Swartz <i>et al.</i> , 2006
Estrone	0.120	0.46	3E-01	Swartz <i>et al.</i> , 2006
4-nonylphenol	84.000	1,800	5E-02	Swartz <i>et al.</i> , 2006
<i>DWELs based on cancer endpoints</i>				
Atenolol	0.0195	70	3E-04	Subedi <i>et al.</i> , 2015 <sup>2</sup>
Carbamazepine	0.226	12	2E-02	Phillips <i>et al.</i> , 2015
Gemfibrozil	0.0012	14	9E-05	Schaider <i>et al.</i> , 2014
Phenytoin	0.690	1.7	4E-01	Phillips <i>et al.</i> , 2015
Simvastatin	0.014	0.19	7E-02	Schaider <i>et al.</i> , 2011

The hazard quotient calculations fall into three different categories: risks from non-EDCs based on non-cancer endpoints, risks from EDCs based on non-cancer endpoints, and risks from non-EDCs based on cancer endpoints. The DWELs for some EDCs are remarkably low. Although no groundwater concentrations of 17- $\alpha$  ethinylestradiol could be found in the literature, its DWEL is only 0.0035  $\mu\text{g/L}$ . In the supporting information for the Stanford and Weinberg (2010) study, the septic tank effluent concentration of 17- $\alpha$  ethinylestradiol for a girl's boarding school dormitory is on the order of 0.1  $\mu\text{g/L}$ .

The DWELs based on non-cancer endpoints are calculated using a different method (Snyder *et al.*, 2008). Because the risk of cancer is never considered to be zero, the ADI is conventionally defined as the maximum dose at which the risk of cancer is less than one in one million. For carbamazepine, the ADI for cancer can be converted to a DWEL of 12  $\mu\text{g/L}$ . This value is two orders of magnitude lower than the ADI for non-cancer effects. However, it is important to keep in mind that the cancer risk of one in one million is insignificant compared to the overall lifetime risk of developing cancer for men and women in the United States (42% and 38%, respectively) (ACS, 2016). A risk of one in ten thousand or one in one thousand might be a more appropriate benchmark given these statistics. Therefore, it may be inappropriate to measure hazard quotients based on cancer endpoints on the same scale as those based on non-cancer endpoints.

Many researchers have postulated that risks would be additive for humans consuming small quantities of a large number of drugs (Pomati *et al.*, 2006; Prosser and Sibley, 2015). Risks would increase if those same people were already consuming one or more pharmaceuticals at standard dose levels. When used in combination, drugs can react with each other with adverse effects on human health. An example of this is the interaction of lamotrigine and carbamazepine, two anticonvulsants. Pharmacological studies have found that when patients who were already taking carbamazepine added lamotrigine to their regime, the two drugs reacted and the toxic byproduct carbamazepine-10,11-epoxide was formed. Patients experienced dizziness, nausea, and double-vision, and symptoms stopped when the carbamazepine dose was stopped (Warner *et al.*, 1992).

Because of observations like these, it is recommended that hazard assessments consider summed hazard quotients for all detected chemicals. In Table 2.1 above, hazard quotients were summed for each grouping of chemicals. Although hazard quotients were only calculated for four EDCs, the

sum was 0.3, comparable to the hazard quotient of 0.5 for cancer endpoints of non-EDCs and much larger than the 0.004 for non-cancer endpoints of non-EDCs. Based on this analysis, it is possible that human health risks from EDCs and based on cancer endpoints may be a concern for people, especially sensitive individuals, drinking groundwater that has been impacted by septic system discharges.

A similar assessment can be run for the risks of consuming TORCs via plants. A review by Prosser and Sibley (2015) estimated hazard quotients  $\leq 0.1$  for human exposure to most PPCPs through consumption of plants receiving biosolids or manure amendment, or irrigation with reclaimed wastewater. Exceptions included exposure to carbamazepine and exposure to many PPCPs for young children, or exposure to multiple PPCPs at once. The maximum/median hazard quotients for consumption of plants amended with biosolids, manure, and irrigated with wastewater were 0.48/0.01, 0.08/0.002, and 0.08/0.003, respectively. The calculations assumed a vegetable consumption rate that represents the 95<sup>th</sup> percentile for US consumers.

Another potential exposure route would be indirect consumption of contaminated plants via livestock, although this issue has received little attention in the literature, particularly for onsite treatment applications (Clarke and Cummins, 2015). Organic compounds with high Log  $K_{ow}$  values ( $\geq 5$ ) are most likely to present bioaccumulation risks (Arnot and Gobas, 2006), but these compounds are not likely to be taken up through plant roots because of their strong affinity for soil organic matter (Dietz and Schnoor, 2001). Triclosan, with its Log  $K_{ow}$  of 4.8, is a good compound to assess the potential risk for bioaccumulation in terrestrial organisms. Triclosan has been detected at concentrations of approximately 50  $\mu\text{g}/\text{kg}$  (dry weight) in soils receiving biosolids application for 3 decades (Xia *et al.*, 2010), and has been reported to have an uptake rate of approximately 40 in ryegrass (*Lolium perenne*), where uptake rate is defined as the ratio between the triclosan concentration in ryegrass and the corresponding concentration in soil (dry weight) (Carter *et al.*, 2014). Based on these observations, the expected concentration of triclosan in ryegrass grown over fields with long term application of biosolids would be 2,000  $\mu\text{g}/\text{kg}$ . A cow consuming ryegrass from that field at a rate of 20 lb/d or 10 kg/d would consume approximately 20 mg of triclosan per day. If 1% of that triclosan was retained in the edible portions of the cow, and the cow was slaughtered after 2 years yielding 250 kg of edible meat, that meat would contain

0.5 mg/kg of triclosan, and a human consuming 0.1 kg of beef per day (USDA ERS, 2016) would ingest 0.05 mg of triclosan per day from beef consumption. The ADI for triclosan has been estimated as 0.075 mg/kg-d or 5.6 mg/d (Snyder *et al.*, 2008) so the hazard quotient for consumption of triclosan through this route is approximately 0.01. Given the relatively low number of TORCs that are expected to accumulate in plants *and* bioaccumulate in livestock, the combined hazard quotient for all TORCs consumed by this route is not expected to exceed 1 for situations that involve biosolids application.

Because the considered practices involve application of PPCPs directly to the soil surface, they almost certainly overestimate the risks posed by septic fields. However, Prosser and Sibley noted that fields receiving irrigation or soil amendments year after year may present a higher risk since persistent PPCPs would build up in the soil (2015). In onsite treatment systems, septic fields may receive contaminated waste streams for decades. Currently, there is a lack of available data on the expected concentrations of TORCs in the root-zone layers of the soils above septic fields. Therefore, it is only possible to say that human health impacts would be unlikely, but conceivable, especially for sensitive individuals and people consuming TORCs through other routes at higher rates (e.g. pharmaceuticals).

Some researchers have expressed concern over the potential to generate antibiotic resistance genes (ARGs) and bacteria by exposure to wastewater-borne antibiotics (Daughton and Ternes, 1999; Chander *et al.*, 2005; Zhang *et al.*, 2009). Although onsite treatment systems usually do not receive the same antibiotics continuously, it is possible for ARGs to persist in the environment even after the selective pressure is gone (Salyers and Amabile-Cuevas, 1997). Therefore, it may be possible for onsite treatment systems to contribute ARGs and anti-infective resistance to the wider environment. This pathway has not been considered widely in the literature; hospital and intensive agricultural wastes are thought to be the primary sources of ARGs in the environment (Zhang *et al.*, 2009). The spread of ARGs is a global threat, so the relative contribution ARGs from decentralized, domestic treatment is thought to be negligible.

### **2.2.3 Mechanisms for Trace Organic Contaminant (TOrc) Removal from Wastewater**

The mechanisms by which TORCs can be removed from wastewater include:

1. Chemical degradation
2. Physical separation
3. Microbial degradation
4. Plant uptake
5. Photodegradation
6. Volatilization
7. Sorption

The effectiveness of each is dependent on the chemical characteristics of the particular TOrC or its metabolites, as well as environmental conditions, such as pH, temperature, and redox potential (Kümmerer, 2009). Before delving into the utility of each removal mechanism, it is important to establish what TOrC removal means in the context of onsite wastewater treatment, and to define different degrees of removal.

First, the system boundary must be defined. The goal of an onsite wastewater treatment system should be to remove pollutants within the system boundary so that they cannot have negative effects on the downstream environment. Thus, the system boundary should be defined as the wastewater phase between the septic tank inlet and the edge of the septic field, with limits on the septic field boundary set by the root zone above and the water table below, with no lateral limits. By this definition, a particular chemical is “removed” as long as it does not cross the system boundary.

Second, it is important to distinguish between degrees of removal mechanisms. Other researchers have used the term “primary elimination” to describe the removal of only the parent compound (Kümmerer, 2009). This is a good term to use when describing the approach of an experiment that measures initial and final compound concentration with minimal analysis of metabolites or carbon dioxide production. However, parent compounds that have undergone “primary elimination” during a particular treatment process may be converted back to their original form once they enter the environment, or they may desorb from media within the system boundary if environmental conditions (including temperature, pH, background concentrations) change. Therefore, when discussing the effectiveness of different treatment approaches, it may be more useful to distinguish

between permanent and temporary removal. This idea has been considered by other reviewers, including Imfeld *et al.* (2009), who referred to “destructive” and “non-destructive” processes.

Permanent removal is achieved when chemicals are removed and are unable to return to the system, regardless of changing environmental conditions. These permanent removal mechanisms include conversion to innocuous species via chemical reaction, microbial degradation, phytodegradation or photodegradation; physical separation; plant uptake if plants are trimmed and removed from the site; and sorption with relatively frequent media replacement. For the purposes of onsite wastewater treatment, volatilization and phytovolatilization could be considered mechanisms of complete removal, since negligible amounts of TOrCs can re-enter the system once they have been transferred to air. Imfeld *et al.* (2009) have reviewed advantages and air quality concerns associated with volatilization and phytovolatilization. Other removal mechanisms, like sorption without media replacement, plant uptake without plant trimming, or incomplete degradation, may provide temporary sinks for TOrCs, which can later become a source. For example, some metabolites of parent compounds can easily be converted back to their original form (Ternes, 1998; Conn *et al.*, 2006; Carvalho *et al.*, 2014). In some cases, incomplete degradation may lead to the formation of chemicals that are more toxic than the parent compound, in which case the “removal” mechanism is simultaneously a “production” mechanism (Marco-Urrea *et al.*, 2009).

The permanent removal strategies are certainly preferred, especially those that do not generate a contaminated solid waste stream (as would treatment systems that rely on sorption or plant uptake). This is why advanced physicochemical treatments are being implemented at centralized WWTPs. These processes include advanced oxidation, ozonation, and membranes, and are regarded as the most effective means of remediating wastewater-borne TOrCs (Ternes *et al.*, 2002). As such, they have received a lot of attention in the literature and are being employed at various municipal wastewater reuse facilities where TOrC removal is an important goal. However, they also have various disadvantages. Perhaps the most important of these is that they are prohibitively expensive and difficult to operate, so that they are currently of limited utility in onsite applications.

Advanced oxidation processes (AOPs) use a highly reactive radical, often a hydroxyl radical, to degrade the target TOrC. Ideally, AOPs transform TOrCs into biologically inactive or biodegradable products (Keen *et al.*, 2014). Different AOPs generate radicals in different ways,

and include the use of UV/H<sub>2</sub>O<sub>2</sub>, Fenton's reagent, photo-Fenton, and ozone. AOPs are a very effective removal strategy for TOrCs that otherwise resist biological degradation, like carbamazepine. However, there are some TOrCs that are resistant to degradation by both biological treatment and AOPs, such as the antidiabetic drug, metformin (De la Cruz *et al.*, 2012). De la Cruz *et al.* (2012) found that metformin was the only TOrC among 32 examined that could resist degradation when exposed to UV<sub>254</sub> and H<sub>2</sub>O<sub>2</sub> at 50 mg/L for 30 minutes (while TOC was 18.8 mg/L and total iron 1.48 mg/L) following activated sludge treatment. Only 11% of metformin was removed, as compared with  $\geq 99\%$  (or non-detect) for the other considered TOrCs. When 5 mg/L of iron II was added under the same conditions, 88% of metformin could be removed, but other TOrCs were removed at lower rates, like triclosan (44% compared with 100%). Similarly, Keen *et al.* (2014) found that lamotrigine was resistant to degradation by ozone, and that only 45% of the parent molecule could be transformed when exposed to UV light and 10 mg/L H<sub>2</sub>O<sub>2</sub>. Other researchers have observed the formation of toxic byproducts by AOPs, particularly in the early stages of oxidation. For example, Dantas *et al.* (2011) found that, although propranolol had been completely consumed after an 8-minute exposure to ozone (effective dose of 0.47 mmol/L), more than 60 minutes of exposure time (effective dose greater than 3.5 mmol/L) was required to reduce acute toxicity (measured by Microtox) below the level of the original solution.

Based on the reviewed research, it seems unlikely that advanced physicochemical treatment systems will be available or desirable for onsite wastewater treatment. Treatment with UV/H<sub>2</sub>O<sub>2</sub> could be considered, but it would not be possible to treat STE directly; an additional treatment step would be required to reduce the concentrations of radical scavengers. Therefore, it is only recommended that onsite users consider advanced physicochemical treatment options after other types of alternative treatment have been installed and found insufficient.

Advanced physicochemical treatments aside, the only viable permanent removal mechanisms for onsite wastewater treatment include microbial degradation, photodegradation, sorption, and mechanisms related to plant uptake. The latter two options require relatively frequent media replacement or plant trimming, and generate a contaminated solid waste stream to be managed in some way (solid phase biodegradation via composting or mycoremediation may be considered). Photodegradation and microbial degradation both require a long residence times for persistent

TOrCs, and in these cases temporary removal mechanisms, acting as retention strategies, could be very helpful. Although in most cases sorption makes TOrCs unavailable for microbial degradation, it has been suggested that reversible sorption processes can slow the movement of TOrCs through media once a sorption-desorption equilibrium has been reached (Imfeld *et al.*, 2009).

Dilution is another critically important remediation mechanism, although it does not represent removal. For aquatic organisms and humans, the concentration of a chemical within water dictates the rate of consumption of that chemical, and therefore the toxicity. When TOrCs cross the lower boundary of the septic field into groundwater, they are dissolved in a relatively large body of water compared to original volume of water discharged to the septic field. This dilution may be enough to eliminate toxicity concerns, depending on the flow rate of groundwater below the septic field and the degree of mixing in groundwater between the point of entrance and the receptor. Detailed knowledge of aquifer characteristics may allow for the approximation of the maximum possible concentration of TOrCs in the groundwater, given septic tank effluent concentrations and wastewater discharge rates. This may be a valuable tool for risk assessment at sites where septic fields have not reached steady-state in terms of sorption.

## **2.3 Onsite Wastewater Treatment**

### **2.3.1 Introduction**

Several inherent characteristics of onsite wastewater treatment limit the types of technologies that are appropriate for these applications. First, they almost always serve relatively small populations, from one to a handful of homes. Thus there are no economies of scale – treatment infrastructure, operation, and maintenance must be durable and low cost. Second, wastewater flow rates have high daily variability for smaller populations, so technologies must have hydraulic flexibility, and preferably limit influxes from precipitation, infiltration, and inflow. Third, onsite treatment systems are often sited relatively close to homes, so they must not be unsightly or emit offensive odors. Fourth, although onsite systems can typically occupy a larger area per person equivalent than a centralized system, available space for onsite systems may be limited in terms of size, slope, or soil quality.

Based on these criteria, septic systems, which are the most popular onsite wastewater technology in the US today, score high marks (US EPA, 2002). They require maintenance only every couple of years, and the basic infrastructure can last decades. Appropriately-sized septic tanks can handle hydraulic fluctuations. Finally, they can be buried and go largely unnoticed by residents unless they malfunction.

However, according to US Census data and EPA studies, more than half of US septic systems are over 30 years old, and at least 10% are malfunctioning (US EPA, 2002). Septic systems have strict requirements for the size and quality of land for the septic field, which, if not met, can lead to early failure of the system.

Because treatment systems are often in rural locations, sited close to homes, they may be adjacent to valuable groundwater, surface water, or soil resources, which residents may use for drinking water, food production, and/or recreation. Although generally considered freely available resources, these amenities have a real value to residents and their neighbors. Just as oxygen demand and nutrient pollution can impact these resources, so too can pharmaceuticals and other TOrCs. Therefore, installing and maintaining onsite wastewater treatment systems should be seen as investments with tangible returns.

The effectiveness of septic systems and other onsite treatment technologies are often measured against the performance of conventional WWTPs. Conventional WWTPs were originally designed to treat only the most biodegradable compounds, so they are not well-equipped to degrade persistent TOrCs (Zhang *et al.*, 2015). Still, the removal percentages achieved in conventional treatment plants are considered to be a good benchmark for onsite treatments prior to subsurface soil application. As noted previously, advanced treatment options are not currently realistic for onsite applications, so many add-on treatment units to CS systems therefore use an aerobic biological reactor to mimic the performance of conventional WWTPs (Garcia *et al.*, 2013; Du *et al.*, 2014).

## 2.3.2 Conventional Septic Systems

### 2.3.2.1 Overview

Because so many people in the U.S. use onsite wastewater treatment systems – 20% nationwide and as high as 85% in more localized areas – there has been a lot of research into the transport of wastewater-borne contaminants from septic fields into adjacent ground and surface waters (US Census Bureau, 2006). Many of these studies have focused on nitrate because of its health impacts and its utility as an indicator of other contaminants (Hinkle *et al.*, 2005; Verstraeten *et al.*, 2005). Research on transport of organic contaminants has historically been more limited, primarily because it is so difficult to measure contaminants at environmentally-relevant concentrations. Some studies in the literature, including Hinkle *et al.* (2005) and Conn *et al.* (2006), used analytical procedures with a minimum reporting limit of 0.5 µg/L or more for some compounds. It is difficult for researchers to quantify effluent concentrations when using such high reporting limits, and comparisons between treatment technologies that are made based on these studies have limited utility.

Despite the analytical limitations, multiple studies have shown that pharmaceuticals and other TORCs can be detected in soils, groundwaters, and surface waters impacted by septic systems (Rudel *et al.*, 1998; Hinkle *et al.*, 2005; Conn *et al.*, 2006; Swartz *et al.*, 2006; Godfrey *et al.*, 2007; Carrara *et al.*, 2008; Writer *et al.*, 2010; Phillips *et al.*, 2015). Most of the research in the literature has focused on TORC releases from septic fields in shallow, sandy aquifers (Rudel *et al.*, 1998; Swartz *et al.*, 2006; Godfrey *et al.*, 2007; Phillips *et al.*, 2015). In many ways, these fields could be considered to represent the worst case scenario because STE has a very low residence time in a field that is not conducive to sorption or biological activity, therefore biological degradation would be extremely limited (Standley *et al.*, 2008). On the other hand, the high porosity in sandy soils could be expected to have greater air flow and therefore more oxidized conditions, which could facilitate TORC degradation (Stanford and Weinberg, 2010).

### **2.3.2.2 Conventional Pollutants**

Septic tanks alone are designed as a “primary treatment” step and their purpose is to remove settleable and floatable solids. They typically remove around 60% of the biochemical oxygen demand (BOD), 80% of total suspended solids (TSS), and 80% of fats, oils, and greases (FOG). However, dissolved pollutants are not removed to a great extent, and nutrient removal rates are not expected to exceed 5% for total nitrogen (TN) or 10% for total phosphorus (TP) (Crites and Tchobanoglous, 1998). The organic nitrogen may be removed via settling or converted to ammonia.

Septic fields are required as a “secondary treatment” step to remove dissolved nutrients and oxygen demand. Ammonia generated in the septic tank can be nitrified in aerobic zones at the top of the septic field, and with potential for limited denitrification in the anoxic zones at lower elevations. Not all nitrate will be converted; a certain amount will make its way into the groundwater where it can impact well water quality and/or adjacent streams. Performance is very site specific, and even CS systems that are not obviously failing can release pathogens, nutrients, and other conventional pollutants into the groundwater (Geary, 2005). Septic system density may have the most potent impacts on the risk of well water contamination by nitrate, bacteria, and viruses (Yates, 1985). The performance of the septic field is also dependent on the septic tank. If tanks are not pumped out frequently enough, built-up solids in the tank can impede settling, resulting in higher suspended solids concentrations in the effluent. This can speed clogging and failure of the septic field (Siegrist, 1987).

### **2.3.2.3 TOrC Removal Mechanisms**

Septic tanks only provide two major mechanisms for TOrC removal: facultative/anaerobic microbial degradation and sorption to settled or floating solids. Many TOrCs cannot be degraded under anaerobic conditions and/or are polar in nature, preferring to stay in the aqueous phase as they move through the septic tank (Conn *et al.*, 2006). Conn *et al.* (2006) found that less than half of most TOrCs measured were removed in the septic tank, so that TOrC concentrations in septic tank influent and effluent could be expected to have roughly the same order of magnitude.

Various factors can impact the TOrC remediation capacity of septic fields. Perhaps the most important determinant of fate is the chemical attributes of a particular TOrC, including its hydrophobicity, ionic form at environmental pH, volatility, and biodegradability. TOrCs with high hydrophobicity, high volatility, and/or aerobic degradation pathways can be mitigated by the septic field. Conn and Siegrist (2009) found that caffeine was removed below the detection limit (representing 2-log removal) within 60 cm of the application point in sandy loam septic fields. This relatively rapid removal was attributed to the high biodegradability of caffeine, and has been observed by various other researchers (Swartz *et al.*, 2006; Godfrey *et al.*, 2007). Phillips *et al.* (2015) found that plant and animal derived biochemicals with high Log  $K_{ow}$  values (>6) were readily removed in the septic tank and field due to sorption, while more polar TOrC compounds had much lower removal rates in the septic field.

Conditions in septic field plumes, including groundwater flow rate, microbial population and redox conditions can also impact the removal rates of TOrCs (Barber, 1988; Swartz *et al.*, 2006; Carrara *et al.* 2008). Carrara *et al.* (2008) measured TOrC concentrations and redox conditions in three septic system plumes in Ontario, Canada and found the highest concentrations of most TOrCs in nitrate-, manganese (IV)-, and iron (III)-reducing zones of the plumes, and fewer in the aerobic, sulfate-reducing, and methanogenic zones. Distribution of individual TOrCs within the zones of the plume varied for each chemical, indicating that the preferred redox conditions for degradation depends on chemical characteristics. Similarly, Swartz *et al.* (2006) studied releases of TOrCs to groundwater from leach pits on Cape Cod and found that TOrCs were degraded more rapidly on the oxic edges of a septic field plume than they were in the center of the plume. Groundwater flow also impacts the dilution of TOrCs discharged via septic fields, and therefore the effective consumption rate for humans drinking impacted groundwater.

#### **2.3.2.4 Knowledge Gaps**

Across the literature, observed removal rates in the septic field have been quite variable. The reason for the inconsistency could be attributed in part to differences in analytical methods and reporting limits, and whether researchers looked for major metabolites. For example, Conn and Siegrist (2009) reported “high removal efficiencies” of TOrCs in a sandy loam septic field. However, the reporting limit for all target compounds was 0.5  $\mu\text{g/L}$  or more – at least 2 orders of

magnitude greater than concentrations which are known to have effects on downstream aquatic environments (Lange *et al.*, 2001; Fenske *et al.*, 2005; Kidd *et al.*, 2007; Palace *et al.*, 2009). Similarly, the findings in Godfrey *et al.* (2007) indicated that carbamazepine could be removed at rates from 16 to 87% over 2 m of a sandy soil septic field. However, the authors acknowledged that their analytical methods could only be considered a “screening level” and that results should be more accurately quantified using updated methods. Furthermore, the authors were only measuring concentrations of the parent carbamazepine compound, although various metabolites of carbamazepine have been reported at similar or higher levels than carbamazepine in the environment (Writer *et al.*, 2013).

Another cause for inconsistency is the variation in septic field characteristics between studies. Phillips *et al.* (2015) analyzed the onsite wastewater treatment system for an extended health-care facility. They found that total TOrC concentrations could decrease by as little as one order of magnitude compared with septic tank influent concentrations 50 m downgradient from the septic fields. For carbamazepine, concentrations in groundwater were actually higher than those in septic tank effluents for groundwater samples taken 30 and 50 m downgradient from the septic field. Part of the difference observed between this study and Godfrey *et al.* (2007) with regard to carbamazepine removal rates could be attributed to differences in aquifer depth. The septic field soil in the Phillips *et al.* (2015) study was also relatively porous – comprised of silty sand with a “shallow” aquifer (depth from ground surface not provided). However, the aquifer was confined by fractured bedrock at a depth of 3.6 to 7.6 m, unlike the unconfined aquifer in the Godfrey *et al.* study (2007). It is likely that lower dilution rates in a smaller effective aquifer volume would lead to lower observed removal rates.

In general, there is little conclusive information in the literature regarding septic field performance. The reviewed factors indicate that the remediation capacity of a septic fields is extremely site specific. However, the sites in which TOrCs have been detected in downstream groundwater or surface water resources fall into general categories, as indicated in Table 2.2 below. There were no reports in the literature found for sites that did not fall into one of these categories.

**Table 2.2: Characterization of septic field studies in the literature**

<b>Category</b>	<b>References</b>
Sandy/porous soils with shallow aquifers and wells	Rudel <i>et al.</i> , 1998; Seiler <i>et al.</i> , 1999; Swartz <i>et al.</i> , 2006; Godfrey <i>et al.</i> , 2007; Phillips <i>et al.</i> , 2015
Adjacent to surface waters	Writer <i>et al.</i> , 2010; Subedi <i>et al.</i> , 2015
Densely-located onsite treatment	Rudel <i>et al.</i> , 1998; Miller and Meek, 2006; Swartz <i>et al.</i> , 2006; Godfrey <i>et al.</i> , 2007; Phillips <i>et al.</i> , 2015; Subedi <i>et al.</i> , 2015
Unusually high consumption of pharmaceuticals	Phillips <i>et al.</i> , 2015

### 2.3.3 Enhanced Treatment Units (ETUs)

#### 2.3.3.1 Overview

ETUs improve the performance of a septic system by adding a treatment step between the septic tank and the septic field. In this configuration, the ETU acts as the secondary treatment step instead of the septic field. Often, the ETU will support increased microbial degradation processes via aeration or contact with some kind of media filter, like sand or peat. Both aerobic units and media filters (also called biofilters) are described in detail in the text by Jantrania and Gross (2006). Design configurations may vary widely between manufactured systems, but they do not include macrophytes and typically have residence times of a few days.

Compared with CS systems and CWs, there are fewer studies focused on the TORC-remediation capacity of ETUs, particularly for a given type. Conn and Siegrist (2009) considered TORC remediation by textile-media biofilters followed by a sandy loam septic field. Garcia *et al.* (2013) compared the treatment performance of conventional centralized WWTP with that of a septic tank and an aerobic ETU (ATUs) all treating the same influent. The ATU was essentially a miniaturized WWTP which contained chambers for pretreatment, aeration (analogous to activated sludge), and clarification. The two onsite treatments did not incorporate a septic field. Subedi *et al.* (2015) measured concentrations of TORCs in tap water, lake water, and STEs for four different ETUs at Skaneateles Lake in New York State.

### 2.3.3.2 Conventional Pollutants

ETUs supply a much cleaner stream of water to the septic field, so a smaller septic field can be used, or the lifetime of a normal-sized field can be extended (Siegrist, 1987). ETUs can also enhance the removal of nitrogen by providing opportunities for nitrification and denitrification before water is dispersed in the septic field. Significant phosphorus removal is only possible when ETUs supply a media that will sorb phosphorus.

Improved performance has been confirmed in the literature, as indicated in Table 2.3 below. It is important to note that ETUs treat STE, not raw sewage. Removal rates shown in the table below are calculated across the ETU only. In other words, they represent the difference between the ETU influent and the ETU effluent, not the difference between the septic tank influent and the ETU effluent.

**Table 2.3: Conventional pollutant removals - comparison between septic tanks and ETUs**

<sup>1</sup>Values were read off a chart and so are approximate. ETUs = enhanced treatment units; cBOD = carbonaceous biochemical oxygen demand; TSS = total suspended solids; TN = total nitrogen.

Reference	Removal Rates (%) or Effluent Concentrations (mg/L)	
	Septic System	ETU
Conn and Siegrist, 2009 <i>ETU: textile-media biofilter</i>	cBOD: 23±35%; 100-350 mg/L <sup>1</sup> TN: 48±11%	cBOD: 90±7%; 5-100 mg/L <sup>1</sup> TN: 9±15%
Garcia <i>et al.</i> , 2013 <i>ETU: aerobic treatment unit</i>	cBOD: 80 mg/L <sup>1</sup> TSS: 90 mg/L <sup>1</sup> TN: 27±7 mg/L	cBOD: <5 mg/L <sup>1</sup> TSS: 5 mg/L <sup>1</sup> TN: 20±7 mg/L
Stanford and Weinberg, 2010 <i>ETU: sand biofilter</i>	TN: 53 mg/L	TN: 30 mg/L

### 2.3.3.3 TOrC Removal Mechanisms

ETUs can mitigate TOrCs in a variety of other ways beyond those available in CS tanks and fields. Conn *et al.* (2006) collected data in a field survey of 30 operational onsite systems in Colorado and found that effluent from ETUs utilizing aerobic treatment in textile-media biofilters had significantly lower median concentrations of 24 TOrCs than their conventional counterparts ( $p < 0.05$ ). They also measured that TOrC loadings to dispersal systems downstream of ATUs were orders of magnitude lower than those downstream of CS tanks. Increased removal rates were

attributed to aerobic biodegradation, sorption, and volatilization, depending on chemical characteristics of the TOrC in question.

Garcia *et al.* (2013) also found that ATUs significantly improved effluent quality in terms of whole effluent toxicity and concentrations of specific hormones, including 17 $\alpha$ -ethinylestradiol and testosterone, relative to septic tanks alone. In fact, the performance of the ATU was not statistically different from that of the modeled conventional WWTP in terms of conventional pollutants or hormone removal rates, although the conventional WWTP outperformed the ATU in terms of *Daphnia magna* toxicity. It should also be noted that the septic tank in the study was not operating to standard regulatory limits, with an effluent CBOD near 80 mg/L and TSS near 90 mg/L. Unfortunately, removal mechanisms were not discussed.

Subedi *et al.* (2015) did not collect enough data to be able to quantitatively calculate TOrC removal rates in ETUs, or to compare performance of different ETUs. However, thanks to the sensitive analytical methods used in the study, they were able to demonstrate that ETUs can release TOrCs to downstream environments. They detected various TOrCs in the lake adjacent to the ETUs (median concentrations of 0.10 to 19.4 ng/L) and in the tap water sourced from the lake or nearby wells (median concentrations of 0.2 to 124 ng/L). Median TOrC concentrations in the lake adjacent to each ETU was one (triclocarban, carbamazepine, caffeine) to two (sulfamethoxazole, bisphenol A) orders of magnitude lower than those in STE. Trimethoprim, triclosan, and ibuprofen were detected in the STE, but were not detected in the lake, representing at least three-log removal for each. Atenolol and oxybenzone had higher median concentrations in the lake than they did in the STE, which could either indicate that some other source was contributing to lake levels (likely for oxybenzone, an active ingredient in sunscreen) or that metabolites were being converted back to the parent compound in the lake. The concentrations of PPCPs in tap water had roughly the same order of magnitude as lake water for all PPCPs detected, including sulfamethoxazole, triclocarban, atenolol, bisphenol A, oxybenzone, and caffeine. The one exception was carbamazepine, which was only detected at 0.10 ng/L in the lake water (at least eight times less than the next lowest concentration measured in the lake water) and was not detected in tap water. Since only parent compounds were monitored, carbamazepine metabolites could have been present at higher concentrations.

#### 2.3.3.4 Knowledge Gaps

Conn and Siegrist (2009) and Garcia *et al.* (2013) clearly demonstrated the improved TOrC remediation capacity of septic tanks plus ETUs versus septic tanks alone. But in many ways, the difference in resultant environmental concentrations is the most critical measure of performance. The question is, do septic fields provide the same TOrC removal rate (i.e. percent removal) regardless of influent concentration? In other words, will a lower TOrC concentration going into the septic field result in a lower TOrC concentration in the groundwater?

Unfortunately, only one study could be found in the literature to address this question. Conn and Siegrist (2009) considered subsequent treatment of septic tank and textile-media biofilter effluents in septic fields with sandy loam soils. The researchers could not detect a noticeable difference in septic field effluents between the two pretreatments, indicating that ultimately the ETU did not do more to prevent TOrC release into the environment. Most of the treatment in the septic fields occurred within the first 60 cm of depth, but removals continued through another 120 cm. The authors concluded that the addition of textile-media biofilters or similar technologies would not improve groundwater quality at sites with more than a 120-cm depth of aerobic soil between the septic field distribution lines and the groundwater table.

There are a couple potential explanations for their observations. First, there is some minimum concentration for TOrCs below which no additional removal can be attained by microbial degradation, sorption, or volatilization (the removal mechanisms available in the textile-media biofilter and the septic field). Second, microbial degradation of TOrCs in the STE was enhanced by the higher concentrations of organic matter and nutrients relative to the biofilter effluent, and this allowed for a higher overall mass removal rate in the septic field receiving STE.

The first explanation cannot be eliminated because most of the compounds considered were removed below analytical detection limits. However, the second explanation is probably the better one. In fact, biostimulation of native soil biota by addition of organic matter and nutrients is a common means of remediating soils that are contaminated with organic chemicals.

Although the findings have a strong theoretical explanation, there are a couple reasons why they should be viewed with a degree of skepticism. First, the reporting limit for this study was relatively high, 0.5 µg/L or more, so it may have been impossible to detect differences for some TOrC concentrations between wastewater sources. Second, the researchers used timed dosing to apply wastewater to the septic fields. This practice has been used to improve the performance and longevity of septic fields by promoting aerobic conditions (Hargett *et al.*, 1982). Aerobic microbial degradation is one of the most important removal mechanisms for TOrCs, and a field with less aerobic conditions may not have performed as well as the field in this study (Swartz *et al.*, 2006; Conn and Siegrist, 2009; Stanford and Weinberg, 2010). Third, the researchers only tested the performance of one type of ETU, and the biofilter they used did not include an anoxic treatment zone for denitrification. As noted by the authors, treatment systems that provide additional treatment strategies, such as cycling of redox conditions, may be more successful at removing TOrCs than either system considered in this study. Finally, septic field performance is site specific, and the removal obtained in the soils of the studied septic fields may not accurately represent the performance of other septic fields.

However, even if we take the authors' findings at face value, relying on the septic fields for all TOrC remediation may be undesirable, particularly in applications where soils are used for agricultural production. Persistent TOrCs with moderate Log  $K_{ow}$  values (1 to <4) could accumulate in soils and be taken up by plants. In applications where antibiotics, antimicrobials, or antifungals are used regularly, there is the potential for these chemicals to impact soil health and fertility or to build resistance genes in native microbial populations.

The conclusions that should be drawn from the Conn and Siegrist study (2009) are as follows:

1. ETUs have clear benefits when septic fields do not provide adequate treatment to protect downstream environments because: a) the groundwater table is less than 180 cm from the septic field distribution lines, b) the soil is not sufficiently aerobic, and c) the soil is too porous to retain TOrCs for remediation.
2. When septic fields do provide adequate treatment, it is only valuable to use ETUs that provide additional treatment mechanisms (including redox cycling to support microbial degradation) beyond those that will occur in the septic field.

3. TOrC degradation rates will decrease when organic matter and nutrient availabilities are limited.

Clearly, it will be important to gain a better understanding of TOrC removal rates in the septic fields downstream of septic tanks and ETUs before any conclusions can be drawn about their relative performance. There is also little data regarding the behavior of TOrCs in media-based ETUs. As in soils, sorption may preclude bioavailability and thus microbial degradation. If degradation does not occur, it is possible that TOrCs could saturate the media, resulting in a spike in effluent concentrations after some duration of use. Each type of media will have unique behavior.

### **2.3.4 Constructed Wetlands**

#### **2.3.4.1 Overview**

Studies across the globe have considered the potential of CWs for decentralized TOrC remediation (Matamoros and Bayona, 2006; Matamoros *et al.*, 2009; Hijosa-Valseiro *et al.* 2010a, b; Avila *et al.*, 2013; Verlicchi *et al.*, 2013; Jasper and Sedlak, 2013; Jasper *et al.*, 2013, 2014; Li *et al.*, 2014; Du *et al.*, 2014). Various reviews and comparative studies have found that CWs can be as or more effective than conventional WWTPs at TOrC removal (Hijosa-Valseiro *et al.*, 2010a, 2010b; Du *et al.*, 2014; Li *et al.*, 2014; Verlicchi and Zambello, 2014). They are also unarguably cheaper to build and operate. As such, they are considered a viable treatment option for small and/or decentralized communities, or institutions emitting relatively large quantities of pharmaceuticals, as either a secondary treatment step, or a final polishing step (Verlicchi and Zambello, 2014).

#### **2.3.4.2 Types of Wetlands**

Wetland design can vary widely, from flow regime, loading mode (continuous, intermittent), and hydraulic retention time (HRT), to the types of plants and media included, and the types of microorganisms that are encouraged to grow. Generally, wetlands are classified most broadly by their flow regime, either free water surface (FWS), surface flow (SF), horizontal subsurface flow (HSSF), or vertical subsurface flow (VSSF). Each type is known for promoting different removal mechanisms. For example, the FWS and SF wetlands allow for photodegradation of pollutants,

whereas subsurface flow (SSF) wetlands provide more opportunities for sorption. VSSF wetlands are commonly used with intermittent flow regimes, which promote aerobic microbial degradation and nitrification (Molle *et al.*, 2005). HSSF typically use continuous flow regimes and support anoxic/anaerobic conditions for denitrification and microbially-mediated reduction reactions.

TOrC removal efficiencies in VSSF wetlands has been evaluated in fewer studies than they have been for FWS, SF, or HSSF wetlands, but they seem to show promise because of their more aerobic conditions, greater space efficiency, and greater resistance to overloading conditions (Matamoros *et al.*, 2007). Matamoros *et al.* (2007) experimented with a pilot-scale (5 m<sup>2</sup>) VSSF wetlands using unsaturated flow (4- to 6-hour retention time) and achieved greater or equal removals for all 13 considered PPCPs than in referenced conventional WWTPs. The VSSF wetlands achieved at least 2-log removal for eight of 13 considered PPCPs (including salicylic acid, ibuprofen, and oxybenzone) and 1-log removal for four of the considered PPCPs (including naproxen, diclofenac, and galaxolide). Only 26% removal was achieved for carbamazepine, but this was greater than the cited removals in conventional WWTPs (<10%). A subsequent study by the same research group compared the performance of real onsite wastewater treatment wetlands, including four HSSF and four VSSF wetlands (Matamoros *et al.*, 2009). The onsite VSSF wetlands did not perform as well as the pilot-scale in terms of PPCP removal rates, despite expected lower loading rates. Compared to the HSSF wetlands, removals in the VSSF wetlands were higher for five of 10 considered PPCPs (including ibuprofen, naproxen, and caffeine), lower for four PPCPs (salicylic acid, oxybenzone, ketoprofen and ibuprofen metabolite CA-ibuprofen), and equal for methyl dihydrojasmonate. However, the VSSF wetlands occupied areas that were 270 to 30 times smaller than those of the HSSF wetlands, so they were much more space efficient. The BOD, TSS, and ammonia concentrations were also lower in effluents from the VSSF wetlands.

Hybrid wetlands, which combine different wetland types in series, have also been considered in various studies (Jasper *et al.*, 2013; Hijosa-Valsero *et al.*, 2010a; Avila *et al.*, 2014). As in conventional wastewater treatment, the order of redox conditions in different treatment cells may be used to target destruction of particular pollutants, such as nitrogen. For example, in the French system, intermittently operated VSSF wetlands are used to oxidize organic nitrogen and ammonia to nitrate (Molle *et al.*, 2005). Specific TOrCs may require analogous sequences of

aerobic/anoxic/anaerobic conditions in order to be mineralized. For example, Conn and Siegrist (2009) recommended a sequence of anaerobic/aerobic/anaerobic conditions with organic solids for remediation of nonylphenol and all metabolites. However, these degradation pathways would vary between chemicals and are not well understood for most compounds. Multiple researchers have hypothesized that one of the reasons why CWs are so effective for TOrC removal is due to the presence of microenvironments containing adjacent redox conditions (Imfeld *et al.*, 2009; Verlicchi and Zambello, 2014). Therefore, including macroscopic iterations of redox conditions in the overall design of a CW may: a) support the presence of microenvironments within the wetland, and b) maximize chances that TOrCs will be exposed to a viable degradation pathway.

So far, the potential of hybrid wetlands for TOrC removal has not been clearly demonstrated in the literature. Part of the reason for this is that there are so many potential design configurations for a hybrid wetland. Avila *et al.* (2014) tested a hybrid VSSF-HSSF-FWS wetland to remove 13 TOrCs using hydraulic loading rates of 60, 130, and 180 mm/d. Removal rates for non-antibiotics were slightly lower than a similar experiment using only VSSF wetlands (Matamoros *et al.*, 2007). However, it is difficult to contrast the studies because different TOrC mass loading rates were used in each – the study by Avila *et al.* (2014) injected TOrCs to ensure that the chemicals of interest were always present in the influent, and as a result the TOrC loading rates were higher. Also, although the overall system loading rates were similar (60 compared with 70 L m<sup>-2</sup> day<sup>-1</sup>), the loading to the VSSF wetlands were quite different (130 compared with 70 L m<sup>-2</sup> day<sup>-1</sup>).

Avila *et al.* (2014) and Hijosa-Valsero *et al.* (2010a) both noted that the highest removal percentages were achieved in the first treatment stage, with some exceptions. In the Avila *et al.* (2014) study, the first stage was an aerobic VSSF filter, and in the Hijosa-Valsero *et al.* (2010a) study the first stages were anaerobic or facultative ponds, so the redox conditions were strikingly different. However, this trend has been observed in other studies as well, which seems to indicate that degradation kinetics are dependent on influent concentration (Conkle *et al.*, 2008). A review by Verlicchi and Zambello stated that first order kinetics appeared to fit observed degradation of TOrCs in CWs, citing studies by Avila *et al.* (2013) and Dordio *et al.* (2010), among others. However, they qualified that first order reaction rates were dependent on influent concentrations. This suggests that a first order reaction rate is not appropriate – the same removal percentage will

not be obtained for a given kinetic constant and reaction time. A second order model, where removal percentages depend on the kinetic constant, the reaction time, *and* the influent concentration, may be more appropriate. Equations 1 and 2 below represent the first order kinetic model while equations 3 and 4 represent second order kinetics. [TOrC] is the concentration of a particular TOrC,  $k_1$  is the first order kinetic constant,  $k_2$  is the second order kinetic constant, and the ratio [TOrC] to [TOrC]<sub>o</sub> is the percent remaining at time t.

$$\frac{d[TOrC]}{dt} = -k_1[TOrC] \quad (1)$$

$$\frac{[TOrC]}{[TOrC]_o} = \exp(-k_1 t) \quad (2)$$

$$\frac{d[TOrC]}{dt} = -k_2[TOrC]^2 \quad (3)$$

$$\frac{[TOrC]}{[TOrC]_o} = \frac{1}{1+[TOrC]_o k_2 t} \quad (4)$$

### 2.3.4.3 Conventional Pollutants

CWs have variable performance in terms of conventional pollutants, depending on the design configuration, but in general perform better than septic fields and at a similar level to ETUs, as shown in Table 2.4 below. High performing wetlands, such as the hybrid wetland installed at the Vidaråsen Camphill community in Norway, have consistently generated effluents with BOD concentrations <20 mg/L (average of 2 mg/L), TSS concentrations <10 mg/L (average of 2.5 mg/L), and TN concentrations <15 mg/L (average of 4.7 mg/L) over an 11-year period (Pandey, 2016).

**Table 2.4: Conventional pollutant removals - comparison between ETUs and CWs**

<sup>1</sup>Values were read off a chart and so are approximate. <sup>2</sup>Median value is provided along with range, in parentheses. ETUs = enhanced treatment units; CW = constructed wetlands; HSSF = horizontal subsurface flow; VSSF = vertical subsurface flow; cBOD = carbonaceous biochemical oxygen demand; TSS = total suspended solids; TN = total nitrogen.

Reference	Removal Rates (%) or Effluent Concentrations (mg/L)	
	ETU	CW
Conn and Siegrist, 2009 <i>ETU: textile-media biofilter</i> <i>CW: HSSF</i>	cBOD: 90±7%; 5-100 mg/L <sup>1</sup> Ammonia: 59±23%; 5-100 mg/L <sup>1</sup>	cBOD: 56%; 80 mg/L <sup>1</sup> Ammonia: 33%; 50 mg/L

Reference	Removal Rates (%) or Effluent Concentrations (mg/L)	
	ETU	CW
Du <i>et al.</i> , 2014 <i>ETU: aerobic treatment unit</i> <i>CW: not specified</i>	cBOD: 97±2%; 4.8±2 mg/L TSS: 95±7%; 6±2 mg/L Ammonia: 9±5 mg/L	cBOD: 96±5%; 7±5 mg/L TSS: 96±4%; 4±1 mg/L Ammonia: 9±5 mg/L
Stanford and Weinberg, 2010 <i>ETU: sand biofilter</i> <i>CW: hybrid wetlands</i>	TN: 30 mg/L	TN: 0.9-9.9 mg/L
Matamoros <i>et al.</i> , 2009 <i>ETU: sand biofilter</i> <i>CW: HSSF and VSSF</i>	BOD: 96 (96-98) %; 11 (8-13) mg/L <sup>2</sup> TSS: 91 (78-96) %; 10 (7-37) mg/L <sup>2</sup> Ammonia: 80 (46-98) %; 48 (1-116) mg/L <sup>2</sup>	<i>HSSF:</i> BOD: 98 (96-99)%; 5 (5-12) mg/L <sup>2</sup> TSS: 94 (5-98)%; 8 (2-83) mg/L <sup>2</sup> Ammonia: 62 (33-98)%; 23 (38-74) mg/L <sup>2</sup>  <i>VSSF:</i> BOD: 99 (75-100) %; 1 (1-11) mg/L <sup>2</sup> TSS: 96 (12-97)%; 7 (0.7-61) mg/L <sup>2</sup> Ammonia: 98 (87-100)%; 0.4 (0.04-27) mg/L <sup>2</sup>

Temperature, or seasonal effects, warrants some focused discussion because of its implications for onsite treatment. Other factors can be considered during the system design process, but it is impossible to completely protect wetlands from the effects of seasonal variations in onsite applications for most parts of the United States. In the winter, biological activity is slower and plants are inactive, so the two methods for achieving more consistent performance during the winter include: a) maximizing wetland temperatures during the winter, and b) relying on bacteria instead of plants for nutrient removal. Insulation methods using straw or compost have been proposed to prevent SSF wetlands from freezing during the winter in states like Minnesota (Wallace, 2000). In Norway, a vertical biofilter (which can also be insulated in the winter) is first used to aerate the water via unsaturated flow, without relying on plants, and water is subsequently treated in a buried HSSF wetland (Jenssen, 2005). These measures should be sufficient to keep oxygen demand and nitrogen removal rates fairly consistent over colder months. A paper by Jenssen *et al* (2005) reported that a CW at Haugstein in Norway treating relatively strong wastewaters (influent TN concentrations of 60-130 mg/L) maintained TN removal rates and effluent concentrations throughout the winter, as summarized in Table 2.5 below. Results are based on nine years of monthly averaged data.

**Table 2.5: Summary of total nitrogen removal rates by Haugstein wetland (Jenssen, 2005)**

Time Range	Average Effluent Concentration (mg/L)	Average Removal Rate (%)
Overall	34	67
Winter (October-March)	33	66
Summer (April-September)	35	69

#### 2.3.4.4 TOrC Removal Mechanisms

There are many TOrC removal mechanisms at work within a CW, including phytoaccumulation, microbial degradation, sorption, photodegradation, and volatilization, and the overall ability of wetlands to remove TOrCs can be enhanced by optimizing system components, including plant species and media (Imfeld *et al.*, 2009; Dordio *et al.*, 2010; Verlicchi and Zambello, 2014). However, the efficiency of each removal mechanism is dependent on factors including redox conditions, pH, temperature, retention time, and loading mode (continuous or intermittent), as well as the particular TOrCs present and their initial concentration (Verlicchi and Zambello, 2014).

Like conventional pollutants, TOrC removal rates will likely be lower during the winter. Hijosa-Valsero *et al.* (2011a) found that temperature was one of the most important factors in removal rates for seven of the ten TOrCs considered, as shown in Table 2.6 below. The researchers considered HSSF wetlands with continuous and batch loading modes, with two different pretreatment strategies. Only results from planted wetlands are shown (unplanted wetlands performed very poorly). The temperature variation in the study was only between 8 and 23°C (46-73°F) or 3 and 19°C (37-66°F, depending on the system location, but none of the temperature control measures described previously were used. The authors also noted that higher evapotranspirative water losses would make summer removal rates artificially low, exacerbating the difference in performance between seasons.

**Table 2.6: Seasonal TOrC removal rates in Hijosa-Valsero et al. (2011a)**

TOrCs = trace organic contaminants.

TOrC	Maximum Winter Removal (%)	Maximum Summer Removal (%)
Caffeine	84±7	99±1
Methyl dihydrojasmonate	63±17	98±1
Salicylic acid	97±4	89±9
Naproxen	41±16	95±4
Ibuprofen	47±26	99±1

<b>TOrC</b>	<b>Maximum Winter Removal (%)</b>	<b>Maximum Summer Removal (%)</b>
Ketoprofen	27±14	37±32
Diclofenac	22±22	71±8
Carbamazepine	9±100	58±21
Galaxolide	0	87±5
Tonalide	0	76±12

#### 2.3.4.4.1 Microbes

Microbial degradation is a well-recognized removal mechanism for TOrCs in CWs (Imfeld *et al.*, 2009; Li *et al.*, 2014). However, metabolites are rarely analyzed in wetland studies, so degradation pathways are generally poorly understood.

Environmental conditions within the wetlands, particularly redox potential, strongly influence the microbial population composition and the available degradation pathways (Imfeld *et al.*, 2009; Hijosa-Valsero *et al.*, 2010a,b). For example, highly oxidized halogenated organics require low redox potentials to be degraded via reductive dehalogenation, while more reduced species like hormones and ibuprofen can be degraded under a variety of redox conditions (Imfeld *et al.*, 2009; Verlicchi and Zambello, 2014). Researchers have found that most TOrCs degrade more quickly under aerobic conditions, due to the higher energy of molecular oxygen as an electron acceptor (Matamoros *et al.*, 2005; Matamoros and Bayona, 2006; Hijosa-Valsero *et al.*, 2010a). HRT is another important factor in the effectiveness of microbial degradation (Hijosa-Valsero *et al.*, 2010b; Zhang *et al.*, 2015). A review by Li *et al.* (2014) found that HRTs used in the literature varied from 2 to 15 days, with the lowest HRTs used for VSSF wetlands (1-2 d) and the highest for hybrid wetlands (2-15 d). Finally, pH has important effects for microbial degradation because pH can impact sorption equilibrium, and sorbed TOrCs may not be accessible for microbial degradation (Imfeld *et al.*, 2009; Zhang *et al.*, 2012).

Algae appear to play an important role in some CWs. Hijosa-Valsero *et al.* (2010b) postulated that suspended algae in FWS wetlands contributed to higher removals of galaxolide and tonalide compared with SF wetlands even though the Log  $K_{ow}$  values of those species (5.90 and 5.70, respectively) would suggest sorption as a removal mechanism. It was not clear whether the researchers meant that algae had contributed to removals directly or indirectly. Jasper *et al.* (2014)

explored the use of a unique shallow wetland cell specifically designed to support a thick periphyton mat fixed to the bottom of the cell, so that photodegradation could occur simultaneously. The active algae population in the mat could raise pH from 8 to 10 and generate supersaturated dissolved oxygen (DO) concentrations over a 2-day retention time. More oxidized species (sulfamethoxazole and trimethoprim) were more efficiently removed when DO levels dropped overnight due to algal respiration, while metoprolol and propranolol were removed more quickly during the day.

#### 2.3.4.4.2 Plants

Plants play an important role in both temporary and permanent removal mechanisms in CWs. For example, The ANOVA analysis performed by Zhang *et al.* (2015) showed that the presence of plants in a wetland significantly improved removal rates for seven of eight TOrCs considered (including carbamazepine, although the overall regression for carbamazepine was not significant, with  $p = 0.0533$ ). Similarly, Dordio *et al.* (2010) found significantly higher removals of carbamazepine, clofibric acid, and ibuprofen in planted versus unplanted beds during summer conditions. Under winter conditions, only carbamazepine did not have significantly higher removals in planted wetlands. In studies where significantly higher removal rates could not be demonstrated in planted wetlands, there were clear explanations for why not. For example, Hijosa-Valsero *et al.* (2011b) found significantly lower removal rates in planted cells for two of 10 antibiotics in SF wetlands, but not in SSF wetlands. This could indicate that the relative contribution of phytodegradation for these two antibiotics made the effect of plants insignificant. As for the SSF wetlands, lower effluent concentrations were observed for most of the antibiotics in the planted wetlands, but only one of the differences was significant due to the low number of samples collected in the study (five) and high variability. The researchers also noted that their research was completed in the fall, when plants are less active. Similarly, Cardinal *et al.* (2014) did not find a significant difference in removal rates of six TOrCs after a 28-day retention time in planted and unplanted batch systems. In fact, removals were faster for unplanted systems for all TOrCs at some point during the experiment. However, the researchers noted that this was most likely because of the relative contributions of phytodegradation and unintended filamentous algae growth in unplanted, sunlight-penetrable cells.

In older reviews of CW treatment, authors have asserted that plants in CWs systems release oxygen through their roots, and this has a significant impact on the degradation of organic matter in treatment wetlands (Brix, 1997; Sundaravadivel and Vigneswaran, 2001). In more recently published literature, the ability of wetland plants to release oxygen to the rhizosphere is also cited as an important process that speeds microbial degradation of organic matter, as well as TOrCs (Li *et al.*, 2014). There is some evidence of the importance of this mechanism in the literature. For example, Matamoros *et al.* (2008) considered the mechanism in degradation studies with ibuprofen. The researchers found that hydroxy-ibuprofen was present in greater concentrations than carboxy-ibuprofen in the effluent of a microcosm scale HSSF CW. They posited that the difference was due to higher production rates of hydroxy-ibuprofen, rather than slower biodegradation rates, since hydroxy-ibuprofen is known to be readily biodegradable. Since hydroxy-ibuprofen is produced under aerobic conditions, the researchers concluded that the wetland plant roots must be generating aerobic microsites in the otherwise anaerobic system (effluent DO of 0.05 mg/L).

Despite these reports, a review by Nivala *et al.* (2013) concluded that plant-mediated oxygen transfer in SSF CWs cannot meet the oxygen demand of primary treated wastewaters. Nivala *et al.* (2013) did note that oxygen transfer rates by plants can vary based on the plants and other environmental conditions, and the small amounts of oxygen released by the roots may be enough to affect the composition of the microbial community and have other indirect effects on treatment performance. Other functions of plant roots, including releases of exudates and serving as a support media, may have greater impacts on TOrC removal efficiency (Brix *et al.*, 1997). Since BOD concentrations are reduced much more rapidly than most TOrCs by microorganisms, a consistent supply of degradable organic matter would stimulate the microbial community in CWs, and increase opportunities for co-metabolism. The environmental complexity sustained by wetland plants may be more important than any single resource contribution. The wetland rhizosphere has been described as an energy-rich web of nutrient, pH, carbon, and electron acceptor and donor gradients (Imfeld *et al.*, 2009).

Finally, wetland macrophytes have been observed to take up TOrCs through their roots, and other reviews have covered this removal mechanism in wetlands in detail (Carvalho *et al.*, 2014; Li *et*

*al.*, 2014). Compounds with Log  $K_{ow}$  values between 1 and 3.5 are considered most susceptible to plant uptake (Dietz and Schnoor, 2001). Although TOrCs can be transformed within plants by metabolic processes or phytovolatilized, they may also remain untransformed or not fully mineralized. Most plants cannot degrade a very wide array of chemicals, and most TOrCs that make it into domestic wastewater treatment systems are not volatile (Imfeld *et al.*, 2009). Plant trimmings from CWs must be managed appropriately so that they do not represent a pollutant source in the future (Carvalho *et al.*, 2014).

#### 2.3.4.4.3 Fungi

The ability of white rot fungi (WRF) to degrade TOrCs in wastewater byproducts has been widely reported (Marco-Urrea *et al.*, 2009; Rodriguez-Rodriguez *et al.*, 2010; Jelic *et al.*, 2012; Aydin, 2016;). WRF are members of the phyla Basidiomycota and have adapted to live in wood and degrade lignin and even cellulose, some of nature's most durable organic products, using nonspecific extracellular enzymes such as laccase, manganese peroxidase, lignin peroxidase, and versatile peroxidases as well as intracellular mechanisms. Harms *et al.* (2011) reviewed the known extracellular and intracellular fungal enzymes responsible for contaminant degradation. Extracellular enzymes generate organic radicals in the presence of molecular oxygen, peroxide, and/or manganese (II) and usually require low pHs (around 5) to be effective. Diverse intracellular cytochrome P450 oxidases are known to be able to degrade contaminants and/or their metabolites which have already been generated by extracellular enzymes (Harms *et al.*, 2011). Marco-Urrea *et al.* (2009) found that the cytochrome P450 system in *T. versicolor* was more active in the initial degradation step of carbamazepine and clofibric acid any extracellular enzymes.

Fungi are good candidates for the remediation of TOrCs because they can tolerate inhospitable growing environments (i.e. extreme temperatures, pH); transport water, nutrients, and electron acceptors to the contaminated site; mineralize even highly oxidized pollutants; and operate without depending on contaminants as any type of input (Harms *et al.*, 2011). The greatest challenge in mycoremediation is how to interface fungi with dissolved TOrCs, given that WRF typically grow in wood, require oxygen (although they have only one-third the oxygen requirement of bacteria), and cannot tolerate intense mechanical disturbance (More *et al.*, 2010; Harms *et al.*, 2011). It has been well-documented that WRF can degrade TOrCs and thereby reduce ecotoxicological impacts

of sterilized sewage sludge in solid phase treatment (Rodriguez-Rodriguez *et al.*, 2010; Aydin, 2016). In solid phase treatment, sewage sludge is combined with a lignocellulosic substrate, with periodic hand-mixing and watering, so that fungi can directly colonize the sewage sludge (Aydin, 2016). Researchers have also tried using a variety of different reactors, including bioslurries and air-pulsed fluidized beds (Rodriguez-Rodriguez *et al.*, 2010; Jelic *et al.*, 2012).

In bioslurry reactors, sludge is combined with water or a growth medium, inoculated, and stirred. The pH of the fluid medium should be kept below 5 to ensure the highest enzyme activity, and pH control is required to maintain these low levels (Rodriguez-Rodriguez *et al.*, 2010). Researchers have observed the formation of mycelial pellets as a result of mixing (Rodriguez-Rodriguez *et al.*, 2010). There is evidence to suggest that WRF can live off of the dissolved organic carbon in the sewage sludge without another source of carbon (Rodriguez-Rodriguez *et al.*, 2010).

In an experiment where the efficiency of bioslurry and solid-state treatments were compared, Rodriguez-Rodriguez *et al.* (2010) found that naproxen and carbamazepine could be degraded by 47% and 57%, respectively (0.067 mg/g dry solid of each initially) within 24 hours in a bioslurry system, after which degradation levelled off. In a solid state system in the same experiment, naproxen was degraded at the same rate for the first 24 hours (0.077 mg/g dry solid initially), but degradation continued until naproxen could no longer be detected after 72 hours. Carbamazepine degradation did not begin until the second day of solid state treatment, but then degraded to a concentration of 48% after 72 hours of total treatment (0.096 mg/g dry solid initially). The results of this experiment seem to indicate high potential for the bioslurry system, but perhaps higher stability for solid-state.

Removing dissolved TOrCs from the wastewater phase may be even more difficult than treating sewage solids. Marco-Urrea *et al.* (2009) demonstrated the potential for WRF to degrade dissolved pharmaceuticals by combining pelletized *T versicolor* mycelia with dissolved ibuprofen, clofibric acid, and carbamazepine in an air-saturated growth medium (no additional air provided) for an incubation period of seven days, during which ibuprofen was removed below detected limits, and clofibric acid and carbamazepine were removed by 91% and 58%, respectively.

SSF wetlands are potentially a good application of mycoremediation because they are largely solid-state treatment systems. In a VSSF, woody debris could be spread over the surface of the wetland and inoculated (naturally or intentionally) with WRF. Applied wastewater would trickle through the unsaturated mulch layer where it could be slowed by sorptive interactions with the organic substrate and degraded by fungal enzymes. The wastewater would need to be relatively free of suspended solids to prevent clogging, and fresh woody debris would have to be applied periodically. Perhaps the most promising application of mycoremediation for CWs application would be for remediation of the TOrCs retained in plant trimmings. Plant trimmings could be blended with inoculated woody debris and managed as a compost pile. Care would have to be taken to prevent leaching, but ideally TOrC levels would be significantly reduced to the extent that the wetland plant trimmings could serve as a fertilizer with minimal releases of TOrCs to the environment.

It is worth noting that the TOrC metabolites from WRF degradation should be researched in more detail. Marco-Urrea *et al.* (2009) observed an accumulation of 1,2-dihydroxy ibuprofen in reactors designed to degrade dissolved pharmaceuticals. This metabolite has not been observed in other bioremediation treatments previously, and was found to be significantly more toxic than the parent compound, as measured by Microtox tests.

#### 2.3.4.4.4 Media

The media in CWs must support biological growth of macrophytes and microorganisms, and can also play a role in TOrC removal via sorption, both to the media itself and to biofilms coating the media (Matamoros *et al.*, 2005; Dordio *et al.*, 2010). Verlicchi and Zambello (2014) reported that CWs in the literature typically use a gravel media with particle size of 8 to 15 mm and porosity of 30 to 40%. Relatively coarse, porous materials are preferable for wetlands because they allow for consistent hydraulic conductivity throughout the wetland, which prevents short-circuiting. However, a balance in particle size must be struck to maximize the surface area for biofilm growth (Dordio and Carvalho, 2013). The gravel media that is typically chosen for wastewater treatment CWs has relatively low affinities for TOrCs, but may provide limited modulation in effluent TOrC concentrations on a seasonal basis (Dordio and Carvalho, 2013).

Additional media types may be included in the wetland to provide a carbon source (e.g. organic soils or compost), or enhance sorption of specific TOrCs (Dordio and Carvalho, 2013; Li *et al.*, 2014). Choice of an appropriate media is complicated by the fact that, in onsite applications, TOrCs are present in extremely low concentrations and in unique combinations of chemicals and environmental conditions (e.g. pH, temperature) that may not be accurately simulated under the laboratory conditions (Dordio and Carvalho, 2013; Verlicchi and Zambello, 2014; Li *et al.*, 2014). It is also important to consider that reliance on sorption as a major removal mechanism in wetlands would mean generating a huge amount of contaminated waste which may have limited and expensive disposal options. Therefore, in small onsite applications, it may be more desirable to choose a durable, low-cost media, like gravel, which supports the other functions of the CWs and promotes TOrC biosorption to support biodegradation as a permanent removal mechanism.

#### 2.3.4.4.5 Photodegradation

Several authors have considered photodegradation as a major removal mechanism in surface waters and CWs (Matamoros *et al.*, 2005; Jasper and Sedlak, 2013; Cardinal *et al.*, 2014). Many TOrCs contain functional groups, such as aromatic rings and amide groups, that can absorb solar radiation or react with other irradiated dissolved species to initiate degradation (Verlicchi and Zambello, 2014). Jasper and Sedlak (2013) modeled the surface area required to achieve 1-log removal of a host of TOrCs commonly found in wastewater in shallow, plant-free, FWS wetlands termed “open-water” cells. Assuming this degree of removal was sufficient, and that environmental conditions were similar to those used in the model, a family of four would require a 46 m<sup>2</sup> open-water cell, in addition to the space required to nitrify and denitrify the water before and after the open-water cell, respectively. The space requirement is reasonable, but the use of photodegradation requires careful maintenance to prevent: a) growth of plants that would block sunlight, and b) stagnant conditions that would support mosquito populations or other unwanted pests. For these reasons, photodegradation may be an untenable removal strategy for many onsite applications, particularly small ones.

#### 2.3.4.5 Knowledge Gaps

Multiple researchers have remarked that the interplay of removal mechanisms and environmental conditions results in variable and unpredictable performance between wetlands and even within a single wetland (Li *et al.*, 2014; Zhang *et al.*, 2015). It is possible that removals would be predictable if there was a holistic model for CW design. The variability in wetland design parameters and the lack of design data specified in the literature make it difficult to develop such a model based on previous work. Hijosa-Valsero *et al.* (2011c) attempted to generate multiple regression equation models for removal efficiencies for 10 PPCPs based on data collected from eight 1-m<sup>2</sup> mesocosm wetlands of different types. The variables considered were temperature, pH, conductivity, DO concentration, redox potential at different locations in the wetland, presence of plants, and the influent concentrations of COD, TSS, volatile suspended solids (VSS), ammonia, and orthophosphate. The resulting equations had R<sup>2</sup> values over 0.50 for only four of the pollutants (ketoprofen, caffeine, galaxolide, and tonalide), and none of those were greater than 0.70. Given the small size and relative homogeneity of the considered results, the exercise indicated that removal efficiency does not have a linear relationship with the considered parameters. Therefore, although many researchers have considered TOrC removal by CWs, significantly more targeted research will be required before engineers will be able to accurately predict removal rates of specific TOrCs in a particular CW configuration.

As with CS systems and ETUs, metabolites have not been extensively considered, so it is difficult to assess whether permanent or temporary removal has been achieved in most of the reviewed literature. Also, no studies could be found which considered the behavior of wetland effluents in septic fields. However, CWs could be characterized as an enhanced septic field themselves, with additional removal mechanisms such as phytoremediation and potentially photodegradation and mycoremediation. Microbial degradation reactions within the wetland are supplied with a ready source of stimulants from plant exudates during seasons when plants are active, and sorption reactions can occur within the wetland, instead of in agricultural fields. In order to accurately mimic septic field conditions, it is recommended that CWs use HRTs on the order of 20 days. This figure was chosen because the retention time was 22 to 32 days in the first 60 cm of the septic fields tested by Conn and Siegrist (2009), where most microbial degradation took place.

### 2.3.5 Comparison of Onsite Treatment Systems

Several studies in the literature have compared the TOrC remediation capacity of one or more onsite treatment systems (Hinkle *et al.*, 2005; Conn and Siegrist, 2009; Matamoros *et al.*, 2009; Stanford and Weinberg, 2010; Du *et al.*, 2014). Instead of relating performance of different treatment systems between studies, this review will focus on directed comparisons where analytical methods were consistent throughout the study.

Conn and Siegrist (2009) and Hinkle *et al.* (2005) compared the performance of various types of ETUs (including textile-media and sand biofilters, aerated treatment units, and packed bed filters) with septic tanks. Conn and Siegrist (2009) also considered the performance of HSSF wetlands. However, neither study will be reviewed here. Major findings from Conn and Siegrist (2009) have already been reviewed, and Hinkle *et al.* (2005) had similarly high reporting limits as well as low detection of TOrCs across the board. Consequently, it is difficult to draw conclusions from either about the comparative effectiveness of different ETUs, wetlands, and their performance relative to CS tanks. Instead, findings from studies with lower reporting limits and higher detection rates will be used.

A comparative study by Matamoros *et al.* (2009) measured the performance of 13 operating onsite treatment systems in Denmark. The treatment technologies included two compact biofilters (using light weight aggregate, LWA, media), two biological sand filters, five HSSF wetlands, and four VSSF wetlands. LWA has been successfully applied in CWs treatment for enhanced sorption of phosphorus and TOrCs, including carbamazepine (Jenssen *et al.*, 2010; Dordio *et al.*, 2010). One biofilter, one sand filter, and three VSSF wetlands included recirculation. The HRTs for the biofilters (not including the effluent filter), sand filters, and VSSF wetlands were on the order of hours, while the HRT of the HSSF wetlands was on the order of days. The biofilters had the largest normalized space requirement (median of 10.5 m<sup>2</sup>/PE) as a result of the large underground effluent filter. Without the effluent filter, they had the smallest normalized space requirement (1.5 m<sup>2</sup>/PE), however results showed that the effluent filter was essential to achieving sufficient BOD and TSS removals. The normalized space requirements for the sand filters, HSSF wetlands, and VSSF wetlands were similar (median of 4.5, 5.9, and 4.0 m<sup>2</sup>/PE, respectively) with the HSSF occupying

the most space. All of the treatment systems used a sedimentation tanks as pretreatment and discharged directly to surface water.

Removal of conventional pollutants was effective for each of the considered systems, and most of the 13 TOrCs considered had removal rates exceeding 80%. No statistical differences in performance could be identified, but the authors concluded that the VSSF wetland consistently performed the best. Unfortunately, this conclusion comes with significant caveats. First, the three most recalcitrant compounds – carbamazepine, diclofenac, and ketoprofen – were not detected in the influent to any of the VSSF wetlands or biofilters. Only the influent to the HSSF wetlands contained all of the recalcitrant compounds, and carbamazepine, diclofenac, and ketoprofen exhibited 38, 21, and 90% removals, respectively. The removal rate of ketoprofen was extremely high relative to other studies in the literature. The sand filters were able to achieve 82% removal for diclofenac, but ketoprofen and carbamazepine were not detected in the sand filter influent. The other major limitation of the study was that all samples were gathered in either May or July, when wetlands would be performing at their peak. Perhaps the most valuable finding from the study was that the operating onsite treatment systems performed as well or better than more controlled pilot studies.

Stanford and Weinberg (2010) performed a similar study of five operating onsite treatment systems which focused on removal of EDCs and estrogenic activity. Three of the systems (systems 1-3) included a vegetated aerobic sand filter or wetland, and two of those also included an anaerobic wetland (systems 1 and 3). The fourth system used a spray irrigation sand filter (aerobic), and the fifth system was CS tank. Estradiol equivalency could be reduced to or below 0.5 ng/L, representing a 2- to 3-log removal rate, in the systems that included aerobic units (systems 1-4). Conversely, estradiol equivalency actually increased from 93 to 140 ng/L in the CS system due to conversion between metabolic forms. The vegetated aerobic units achieved nonylphenol removal rates of 1 to 3-log, so that effluent concentrations in most cases were below the 10 µg/L levels known to have chronic effects on aquatic organisms (Balch and Metcalfe, 2006). Conversely, the CS system had very low removals of nonylphenol with effluent concentrations of 310 to 320 µg/L. The spray irrigation sand filter had effluent nonylphenol concentrations of 20 to 32 µg/L, representing 1-log removal. Although these concentrations were higher than those observed from

vegetated systems (systems 1-3), no statistical significance can be inferred due to the small sample size of the study.

Stanford and Weinberg (2010) primarily attributed high removals of nonylphenols and estrogens to aerobic biodegradation, since removals in initial anaerobic treatment units were so small compared with removals in subsequent aerobic tanks. Since estrogens have relatively high Log  $K_{ow}$  values ( $>3.5$ ), they could be transported into effluent streams by organic colloids, but the researchers believed they could differentiate removal by degradation from sorption because both aerobic and anaerobic treatment units removed solids. However, the data shows that the systems with aerated units had lower effluent TOC concentrations and turbidity than the CS system ( $<10$  mg/L and  $<20$  NTU compared with about 40 mg/L and 50 NTU). Therefore, it is difficult to differentiate between microbial degradation and sorption mechanisms, especially given the small data samples in this study.

Regardless of the removal mechanism, it is clear from the Stanford and Weinberg study (2010) that aerobic treatment is highly effective for the removal of EDCs and estradiol equivalency. Additionally, the removal of solids in ATUs prevents transport of sorbed EDCs into the septic field, as well as hydraulic failure of the field which could lead to uncontrolled environmental releases (Siegrist, 1987).

Du *et al.* (2014) used the same experimental set-up as Garcia *et al.* (2013) except that STE was routed to a SSF wetland. The flow configuration of the CW was not specified. The researchers were able to compare the performance of a WWTP with that of a CS tank, a CS tank plus wetland, and an ATU, all treating the same influent. Septic fields were not included in the experimental set-up. The WWTP had a retention time of approximately six hours and each of the other treatment systems had a retention time of about two days, so the septic tank plus wetland system had a total retention time of about four days.

Despite the shorter retention time, the WWTP performed the best of the three systems, followed by the wetland, followed by the ATU. The CS tank had the worst performance for all measured TOxCs for both sampling periods. The researchers collected samples in October (mean temperature of  $18.3^{\circ}\text{C}$ ) and January (mean temperature of  $10.8^{\circ}\text{C}$ ), and surprisingly the wetland performed

better during the colder sampling period. The researchers did not comment on removal mechanisms; however, the longer retention time of the tank plus wetland system may have contributed. The WWTP had the best removal rates for the most hydrophobic compounds: diclofenac ( $\log K_{ow}= 4.5$ ), gemfibrozil ( $\log K_{ow}= 4.3$ ), and diltiazem ( $\log K_{ow}= 4.7$ ).

## **2.4 Conclusion**

### **2.4.1 Potential Impacts from Release of TOrCs via Onsite Treatment**

Based on environmental fate and toxicity studies, it seems that the most serious potential impacts from onsite treatment systems are for aquatic macro- and microorganisms in surface and ground waters. Impacts to plants and terrestrial microorganisms living above septic fields have not been observed at environmentally-relevant concentrations resulting from biosolids application, and therefore are not expected to result from subsurface dispersal in septic fields.

Onsite TOrC releases have the potential to leach into groundwater, be taken up by plants, or bioaccumulate in livestock located near septic fields, and it is possible for humans to consume TOrCs via these pathways. Based on an analysis of TOrC concentrations observed in groundwaters impacted by onsite wastewater systems, it is possible that human health risks could result from consuming EDCs and carcinogens that have leached into groundwaters from via onsite treatment systems. However, it is unlikely that consuming contaminated plants grown over septic fields, or livestock raised on plants growing above septic fields, would have significant impacts to human health (Prosser and Sibley, 2015).

Both humans and environmental receptors are likely to be more sensitive to TOrCs present in the aqueous phase. As a result, onsite wastewater treatment systems should prioritize the prevention of TOrC releases to groundwater and surface water resources. Discharges to soils should be minimized when TOrC travel times or redox conditions through the septic field do not allow for sufficient remediation to protect downstream groundwater and surface water resources (Standley *et al.*, 2008). In cases where anti-infectives are regularly used, septic field distribution lines are close to the soil surface, and/or agricultural applications, it would be prudent to minimize TOrC discharges to the soil.

Based on findings in the literature in which groundwater contamination by TOrCs has been identified, it is likely that sites with one or more of the following characteristics are susceptible to contamination of ground and surface water resources:

- Sandy or porous soils
- Shallow aquifers and wells
- Location in area of dense onsite treatment systems
- Unusually high pharmaceutical consumption rates

Sites with these characteristics should consider whether alternative treatment strategies are necessary for TOrC remediation. If existing CS systems do not contribute to elevated concentrations of TOrCs in adjacent surface waters, are not located on or near agricultural land, and are not present at concentrations that pose health risks for humans consuming groundwater and plants on the property, then there is no need to consider alternative treatment strategies. Unfortunately, it is expensive and analytically challenging to determine whether TOrCs are having environmental effects. Nitrate and chloride can be used as indicators of wastewater contamination in groundwater wells, but they do not directly indicate the presence of TOrCs (Hinkle *et al.*, 2005; Verstraeten *et al.*, 2005). Furthermore, results of TOrC analyses can be misleading if they do not consider metabolites, or if reporting levels are too high. Detailed knowledge of aquifer characteristics and expected dilution ratios may allow for the approximation of the maximum possible concentration of TOrCs in the groundwater, given pharmaceutical consumption and wastewater discharge rates. This may be a valuable tool for risk assessment at sites where septic fields have not reached steady-state in terms of sorption.

#### **2.4.2 Options for Alternative Onsite Treatment and Limitations**

Findings from the literature clearly indicate that both wetlands and ETUs have a greater capacity for TOrC remediation than CS tanks alone (Matamoros *et al.*, 2009; Stanford and Weinberg, 2010; Du *et al.*, 2014). Furthermore, wetlands and ETUs that include aeration, either forced or through the passive use of unsaturated filters, have been found to be more effective for the removal of TOrCs, particularly EDCs (Stanford and Weinberg, 2010). Because aerobic treatments often produce water with lower suspended solids concentrations, it is difficult to determine whether

higher removal rates are a result of faster microbial degradation or reduced transport of colloid-sorbed TOrCs (Conn *et al.*, 2006; Stanford and Weinberg, 2010). Either way, treatment systems that generate effluents with lower concentrations of oxygen demand and solids are less likely to speed hydraulic failure of receiving septic fields, thereby reducing the occurrence of uncontrolled environmental releases (Siegrist, 1987).

All of the reviewed comparison studies indicated slightly better performance in vegetated systems relative to ETUs, but statistical significance was not demonstrated in any of them (Matamoros *et al.*, 2009; Stanford and Weinberg, 2010; Du *et al.*, 2014). Because wetlands can provide phytodegradation and plant uptake removal mechanisms, as well as redox cycling and biostimulation for microbial degradation, they could be expected to achieve higher removal rates than ETUs during seasons when plants are active. None of the comparison studies considered SF or FWS wetlands, although these types of wetlands could allow for permanent removal via photodegradation, which would enhance the performance of wetlands compared to ETUs.

Before choosing an alternative treatment system, consideration must be made regarding byproduct generation for the considered alternative treatment systems. ATUs generate a sludge that must be disposed of periodically. While this could be considered an additional expense to the treatment system owner, it is not expected to be significantly more troublesome than managing CS tank waste. Other ETUs which rely more on sorption processes, such as LWA or peat biofilters, may generate additional TOrC-contaminated solids. Wetlands will also generate contaminated plant trimmings, often on a yearly basis. If not for TOrCs, these streams could be considered valuable sources of nutrients and organic matter (Jenssen, 2010). Disposal options would depend on the loads and environmental toxicity of TOrCs. Solid-phase composting or mycoremediation could be considered as a viable remediation strategy.

### **2.4.3 Qualitative Metrics for Assessing Successful Onsite Remediation**

Because of the limits on infrastructure costs and available land, it will not be possible to completely remove all TOrCs using onsite wastewater treatment systems. However, the aim of such systems should be to minimize release of TOrCs with wide varieties of chemical properties, and to prevent accumulation of potentially persistent TOrCs in the natural environment. Septic fields are typically

considered part of the CS system, which implies that a properly-functioning septic field and its discharges do not have significant negative effects on the surrounding environment. Based on the proven accessibility of TOrCs released in the septic field to downstream environments, as well as demonstrated impacts to aquatic organisms at environmentally-relevant concentrations, one could make the argument that, in some cases, wastewater treatment in subsurface drain fields constitutes environmental release of TOrCs. In these cases, septic fields should not be considered part of the treatment process when it comes to TOrC remediation.

Since CS tanks only provide opportunities for anaerobic or facultative degradation and limited sorption to largely organic solids, additional treatment units should provide different removal mechanisms, including aerobic degradation, photodegradation, or enhanced sorption to organic and inorganic solids. When adequate septic fields are available, additional treatment units should provide removal mechanisms beyond those available in the septic field, including redox cycling to support microbial degradation (Conn and Siegrist, 2009). It may also be valuable to provide a confined, solid-state matrix, analogous to the septic field but physically separated from downstream environments, to prevent TOrC accumulation in soils and impacts to terrestrial environments, especially in agricultural applications.

Treatment units that provide permanent removal of TOrCs and potentially bioactive byproducts should be valued more highly than those that only provide temporary removal mechanisms. However, it is also undesirable for treatments to generate large, concentrated waste streams that will be costly or difficult to dispose of, or that will represent a new source of TOrCs in the environment. Thus ideal sorption would not irreversibly bind TOrCs, but instead slow their movement through the treatment unit to allow time for degradation into innocuous byproducts. Of course, the inherent limitations to onsite wastewater treatment must also be considered when choosing onsite systems. Therefore, advanced treatment options like advanced oxidation or reverse osmosis may not be economically feasible and would require enhanced treatment of STEs.

#### **2.4.4 Summary of Knowledge Gaps and Research Needs**

There are some limitations to the findings on TOrC removal capacities of different treatment technologies which should be noted. First, only one study could be found which considered the

net impact of additional treatment units at sites with adequate septic fields (Conn and Siegrist, 2009). Second, metabolites and their environmental implications are not often known and are rarely measured. This makes it very difficult to understand whether TOrCs have been temporarily or permanently removed, or whether more toxic byproducts have been generated during the treatment process. Third, most studies use removal rates (or percent removal) as a relative measure of success for TOrC remediation, when final concentrations are ultimately more important in terms of environmental effects. In addition, studies have indicated that removal rates are a function of initial concentration. For example, in hybrid wetlands, the highest removal rates are often seen in the first cell, regardless of its configuration (Conkle *et al.*, 2008; Hijosa-Valsero *et al.*, 2010a; Avila *et al.*, 2014). This would suggest that first order degradation is not a good kinetic model for TOrC remediation.

Because metabolites and metabolic processes are rarely understood, and because it is often difficult to detect and quantify all of the potentially toxic chemicals in a wastewater matrix, it may be more valuable to compare treatment systems using biological assays of effluent water, as in Garcia *et al.* (2013). Effluent water chemistry, as measured by conventional pollutants and endocrine active compound concentrations, was not significantly different between WWTP and ATU treatments. However, the whole effluent toxicity, as measured by *Daphnia magna* reproductive responses, was slightly higher for the ATU effluent. These results suggest that simply measuring water contaminant concentrations can yield misleading results.

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## 3 Pilot Study

### Abstract

Three pilot-scale treatment systems processing septic tank effluent from a house with high pharmaceutical usage were operated for a period of seven months. The treated effluent from each system was compared via ANOVA analyses based on removals of conventional pollutants as well as one analgesic (acetaminophen), four anticonvulsants (carbamazepine, lamotrigine, levetiracetam, topiramate), one antidepressant (venlafaxine), one antidiabetic (metformin), and one antimicrobial (triclosan). The pilot-scale systems included a constructed wetland with a mycoremediation filter and two proprietary treatment systems, including an aerobic styrene media biofilter (E-Z Treat) and an aerobic treatment unit followed by peat biofilters (Anua). All treated effluents contained biochemical oxygen demand and total suspended solids concentrations below 10 mg/L. The constructed wetland had the best nutrient removal with total nitrogen concentrations below 10 mg/L throughout the study. The mycoremediation filters and Anua systems exhibited excellent removals of all monitored PPCPs including 100.0% for acetaminophen, >80% for carbamazepine, >95% for lamotrigine, 99.8% for levetiracetam, >73.5% for metformin, >93.5% for triclosan, and >99.0% for venlafaxine. Only parent compounds were monitored in this study, but removal of metabolites was considered via a literature review.

**Keywords:** pharmaceuticals and personal care products (PPCPs), acetaminophen, carbamazepine, lamotrigine, levetiracetam, metformin, topiramate, triclosan, constructed wetlands, enhanced treatment units (ETUs), septic systems

### 3.1 Introduction

Pharmaceuticals and personal care products (PPCPs) encompass a diverse group of chemicals that are not currently monitored or regulated in US drinking water or wastewater. Researchers have found low levels of PPCPs in aquatic and terrestrial environments all over the globe (Ternes, 1998; Kolpin *et al.*, 2002; Barnes *et al.*, 2008; Schlusener *et al.*, 2015), and observed negative effects on impacted biota (Writer *et al.*, 2010).

The primary source of PPCPs in the environment is domestic wastewater discharges, including those of centralized and decentralized origin (Conn *et al.*, 2006; Li *et al.*, 2014). On a global scale, TOrCs discharged in effluents from centralized wastewater treatment plants (WWTPs) can have greater impacts for sensitive environmental receptors because they process such large volumes of wastewater and often discharge directly to surface water resources. However, on a local scale, onsite treatment systems may present more potent impacts to valuable groundwater, surface water, or soil resources, which residents may use for drinking water, food production, and/or recreation.

Conventional septic (CS) systems are the most commonly used onsite treatment systems in the U.S. (US EPA, 2002). They treat wastewater to primary standards in an anaerobic tank, followed by secondary treatment via subsurface soil application in a septic field. Unfortunately, they provide limited mechanisms for PPCP removal during primary treatment; Conn *et al.* (2006) found that less than half of most PPCPs measured were removed in the septic tank. Septic fields are typically considered part of the CS system, which implies that a properly-functioning septic field and its discharges do not have significant negative effects on the surrounding environment. Yet multiple studies have shown that PPCPs released in the septic field can be detected in adjacent ground and surface water resources (Godfrey *et al.*, 2007; Phillips *et al.*, 2015; Subedi *et al.*, 2015). Since impacts of PPCPs at environmentally-relevant concentrations have been thoroughly documented, one could make the argument that, in some cases, wastewater treatment in subsurface drain fields constitutes environmental release of PPCPs (Lange *et al.*, 2001; Fenske *et al.*, 2005; Kidd *et al.*, 2007; Palace *et al.*, 2009).

Enhanced treatment units (ETUs) improve the performance of a septic system by adding a treatment step between the septic tank and the septic field. Often, that step involves aeration or contact with some kind of media, like sand or peat. Both aerobic treatment units (ATUs) and media filters are described in detail in the text by Jantrania and Gross (2006) and multiple researchers have considered remediation of PPCPs using these systems (Hinkle *et al.*, 2005; Conn and Siegrist, 2009; Stanford and Weinberg, 2010; Du *et al.*, 2014; Subedi *et al.*, 2015). Design configurations may vary widely between manufactured systems, but they do not include macrophytes and typically have residence times of a few days. Constructed wetlands (CW) have also been used as a secondary treatment step for decentralized treatment or as an alternative to conventional WWTPs

(Conkle *et al.*, 2008; Conn and Siegrist, 2009; Matamoros *et al.*, 2009; Stanford and Weinberg, 2010; Du *et al.*, 2014). Wetland design can vary widely as well, from flow regime, loading mode (continuous, intermittent), and hydraulic retention time (HRT), to the types of plants and media included, and the types of microorganisms that are encouraged to grow. Generally, wetlands are classified most broadly by their flow regime, either free water surface (FWS), surface flow (SF), horizontal subsurface flow (HSSF), or vertical subsurface flow (VSSF). Each type is known for promoting different removal mechanisms. The use of CWs for the remediation of PPCPs and other trace organic contaminants has been reviewed by several researchers (Imfeld *et al.*, 2009; Carvalho *et al.*, 2014; Li *et al.*, 2014; Verlicchi and Zambello, 2014).

Findings from the literature clearly indicate that both wetlands and ETUs have a greater capacity for PPCP remediation than CS tanks alone (Matamoros *et al.*, 2009; Conn and Siegrist, 2009; Stanford and Weinberg, 2010; Du *et al.*, 2014). Furthermore, CWs and ETUs that include aeration, either forced or through the passive use of unsaturated filters, have been found to be more effective for the removal of TOCs, particularly endocrine disrupting compounds (EDCs) (Stanford and Weinberg, 2010). In this study, the PPCP remediation capacities of a hybrid constructed wetland and two different ETUs were compared as they treated real STE over a period of seven months. The site of the project was an 80-person community for intellectually-disabled adults in rural Virginia. The community was interested in considering alternative onsite treatment options for their wastewater because septic fields on the property are sited over groundwater resources and under agricultural land. Eight PPCPs were chosen for monitoring throughout the study based on relatively high consumption rates in the community: acetaminophen (ACE), carbamazepine (CBZ), lamotrigine (LAM), levetiracetam (LEV), metformin (MET), topiramate (TOP), triclosan (TCS), and venlafaxine (VEN).

A few studies in the literature have compared grab samples from ETUs and CWs processing real septic tank or primary treated effluents (Conn and Siegrist, 2009; Matamoros *et al.*, 2009; Stanford and Weinberg, 2010). One study compared the performance of an ATU that included a settling basin with that of a CW treating STE and the septic tank alone (Du *et al.*, 2014). However, this study is unique in the following respects:

- The ETUs and CW were processing the same STE

- The ETUs and CW were operated and samples collected over a relatively long duration (seven months, ten sampling events)
- The consumption rates for most of the monitored PPCPs were known and consistent from day to day
- Mycoremediation of CW effluents was considered
- Some of the monitored PPCPs, including LEV, LAM, MET, and TOP, have received little prior attention in the decentralized treatment literature

## **3.2 Materials and Methods**

### **3.2.1 Pilot System Description**

A schematic of the pilot system, which was located outdoors at a small community in rural Virginia, is shown in Figure 3.1. A brief description of the system follows and a more detailed explanation is provided in Appendix C. All three treatment systems were fed domestic STE from a single house with nine residents and had at least a one-month start-up period (2.5 months for the CW). The nine residents of the house consume 500 mg ACE, 1,200 mg CBZ, 500 mg LAM, 1,500 LEV, and 75 mg VEN (combined) on a daily basis. The estimated average daily wastewater generation rate from the house is 700 gpd. In the community as a whole, residents consume >500 mg ACE, 3,600 mg CBZ, 800 mg LAM, 3,500 mg LEV, 1,500 mg MET, 300 mg TOP, and 300 mg VEN (combined) daily and generate an estimated average 6,000 gpd of wastewater. Daily use of TCS is unknown. Data collected from quarterly sampling of the five of the community's largest septic systems are included in Appendix B.

The Anua treatment system included an ATU with a settling chamber, aerated attached growth filter, and clarifier, followed by two peat filters in series, with a recycle line to return effluent from the first peat filter to the ATU. Well Basin 2 was filled with seashells to add alkalinity and promote denitrification. Both Anua Well Basins had submerged pumps which dosed the peat filters once approximately every 6 hours. The E-Z Treat system sprayed a batch of water from the holding tank onto an unsaturated, styrene bead biofilter every 15 minutes. The hybrid CW was comprised of four variable-sized tanks (1-4) with different wetland plants, media, and flow regimes, followed by two tanks (5-6) which were unplanted and filled with fungi-inoculated wood chips and mulch

for mycoremediation. The flow regimes of the first four tanks were VSSF (half unsaturated), HSSF, VSSF/FWS hybrid, VSSF (one-third unsaturated). Tanks 3 and 4 had a two-inch layer of inoculated mulch on the surface of the gravel. Most of the CW system was gravity fed, with the exception of the pumped line between Tanks 2 and 3.

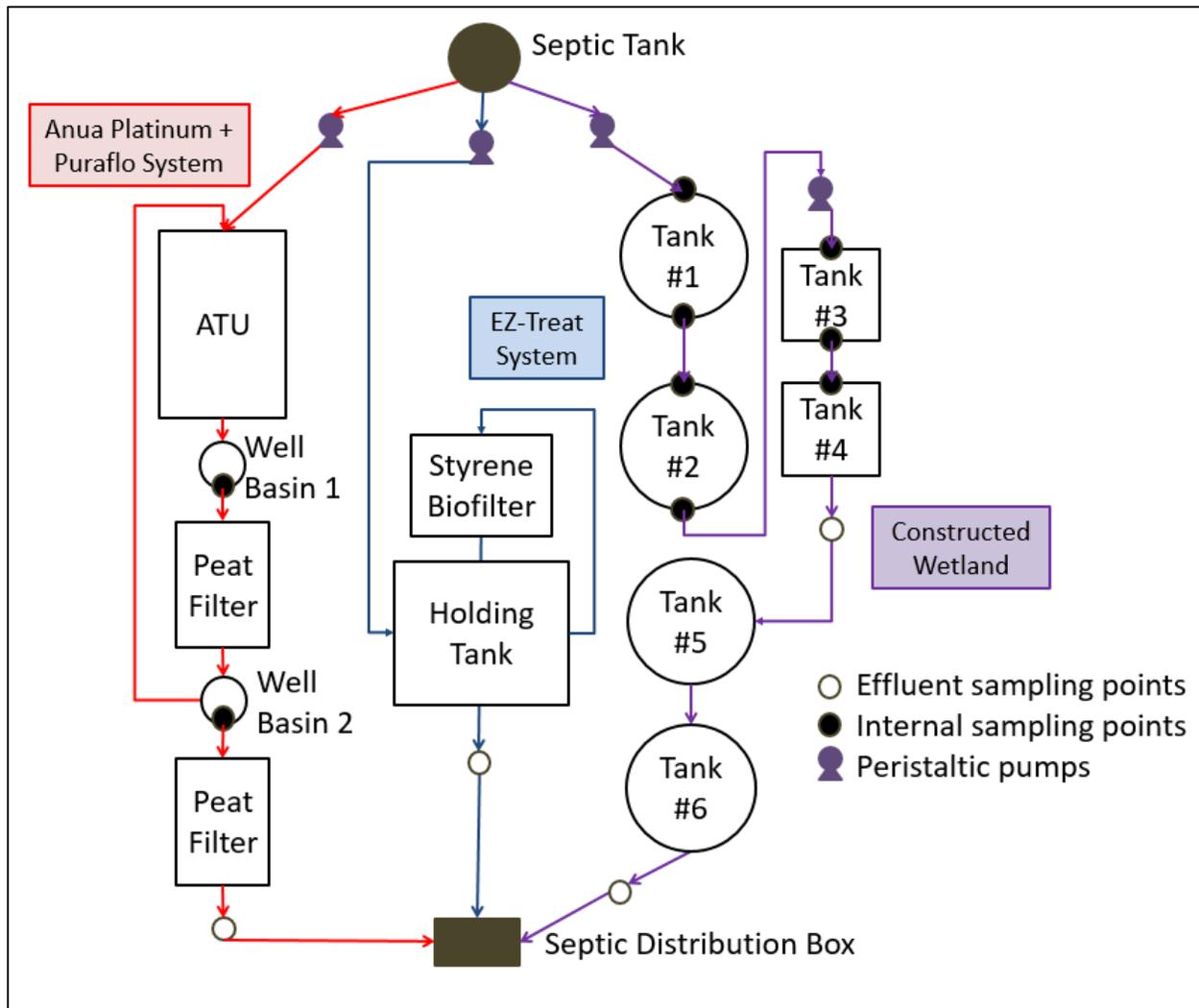


Figure 3.1: Pilot system schematic

All treatment units were placed at grade, with the exception of the Anua Well Basin 2, which was buried with the top of the basin at grade, and the E-Z Treat filter, which was elevated approximately 3 ft off the ground to allow for gravity drainage through the holding tank. For each system, water was pumped from a 2-inch PVC pipe pump casing in the septic tank using peristaltic

pumps (Pump 1, 3, and 4). The casing was perforated (1/2-inch holes) to collect water from the lower one-third to one-half of the septic tank. STE was pumped into Tank 1 for the CW system, the holding tank for the E-Z Treat system, and the ATU settling chamber for the Anua system.

Only the CW was open to the air, and therefore subject to precipitation and evapotranspirative losses. Weather data were collected by a Weather Underground station at Mount Fair Farm (KVACROZE8), which is 2.1 miles (3.4 km) away and at an elevation 220 ft (67 m) lower than the pilot system. Weather at the site was slightly different than that measured at the weather station, but the recorded data are still an excellent approximation of conditions at the site. The weather data (temperature, wind speed, relative humidity, and cloud cover) were used to calculate evapotranspiration rates at the pilot system site using the Penman Monteith equation, as described in Appendix C. Calculated evapotranspiration rates were high throughout the sampling period, with an average rate of 0.18 in or 9.1 gpd. Therefore, except on weeks with significant rain events, the effluent flow rates from the wetland were lower than the influent flow rates, artificially concentrating the effluent pollutant concentration. Negligible evapotranspirative losses were assumed for the mycoremediation tanks based on observation. Even during the hottest days of the summer, moisture was retained below a thick, dry crust of wood chips formed on the top of each tank.

Flow rates in the system were measured manually throughout the sampling period. Theoretical hydraulic residence times (HRTs) were calculated by dividing the effective Anua and E-Z Treat system volumes by their average flow rates. The residence time in the Anua peat modules was determined by a manufacturer study. Flow rates and HRTs were calculated for each tank in the CW on a weekly basis using influent flow rates; empty, saturated tank volumes; precipitation data; and evapotranspiration calculations, as explained in Appendix C. Table 3.1 below shows the average theoretical HRTs for each system over the sampling period.

**Table 3.1: Pilot system theoretical hydraulic residence times in days**

<b>Time Period</b>	<b>Anua</b>	<b>E-Z Treat</b>	<b>CW Tanks 1-4</b>	<b>CW Tanks 5-6</b>
June 18 – Aug 22	9.8	5.5	23.1	5.9
Aug 23– Oct 3	10.3	5.8		
Oct 4 – Nov 16	15.1			

## 3.2.2 Experimental Design

### 3.2.2.1 Sampling Strategy

Grab samples were collected at the locations indicated in Figure 3.1. It should be noted that STE samples were collected at the influent to the CW system throughout the experiment. It was expected that samples collected at this location would represent influent samples to each system because water traveled through the same length of pipe to get to each system; however, some of the results suggest otherwise (see subsection 3.4.2). Samples were collected at each of the effluent sampling locations over the course of the sampling period (June 19 – November 16; August 7 – November 16 for the E-Z Treat system). On four sampling dates, additional grab samples were collected at the CW internal sampling locations, and on six sampling dates, additional grab samples were collected at the Anua internal sampling locations. All wetland samples were collected from sampling ports on the drainage end of tanks or from the influent piping and were gravity fed, with the exception of Tank 2 effluent and Tank 3 influent. All ETU samples were collected from sampling ports while system pumps were running (every 15 minutes for E-Z Treat tanks or approximately every 6 hours from the Anua system). All samples for PPCP analyses were collected and stored in 1-L or 250-mL amber glass bottles with Teflon® liners, cleaned for organic analysis. Samples for conventional analyses were collected and stored in high density polyethylene (HDPE) bottles. After collection, PPCP samples were placed in a cooler to protect them from sunlight.

### 3.2.2.2 Conventional Pollutants

Temperature, dissolved oxygen (DO), and pH were measured in the field after collection using an Oakton 5+ pH meter and an Oakton DO 6+ meter for temperature and DO concentration. Samples were transported on ice to the lab and stored at  $\leq 6^{\circ}\text{C}$ . Samples for conventional analyses, including alkalinity (2320 B), biochemical oxygen demand (BOD, 5210 B), total suspended solids (TSS, 2540 D), and ammonium ( $\text{NH}_4$ , 4500- $\text{NH}_3$  B, C) were collected and analyzed per Standard Methods within three days (APHA, 1998). Chloride, fluoride (F), nitrate ( $\text{NO}_3$ ), nitrite ( $\text{NO}_2$ ), phosphate ( $\text{PO}_4$ ), and sulfate ( $\text{SO}_4$ ) were measured via ion chromatography (Dionex ICS-1600) within 48 hours. Data were analyzed using the Dionex workstation with Chromeleon SE software (Version 7.0). Chemical oxygen demand (COD), total nitrogen (TN), and total phosphorus (TP)

were measured using Hach kits (COD HR and LR with USEPA Reactor Digestion Method 8000; TN HR with Persulfate Digestion Method 10072; TP HR with Molybdovanadate Method with Acid Persulfate Digestion Method 10127) within 48 hours if unpreserved or within two weeks if preserved. At least one duplicate was run for each test in a sampling set (excluding field measurements).

### **3.2.2.3 Pharmaceuticals and Personal Care Products (PPCPs)**

#### **3.2.2.3.1 General**

All plastic containers were rinsed with glass-distilled ethanol (95% v/v) and dried before use. Unless indicated otherwise, all solvents and vials used were LC-MS grade. Labeled standards were purchased from two sources: Cerilliant Laboratories and CDN Isotopes. The Cerilliant standards (acetaminophen-D<sub>4</sub>, carbamazepine-D<sub>10</sub>, lamotrigine-<sup>13</sup>C-<sup>15</sup>N<sub>4</sub>, levetiracetam-D<sub>6</sub>, topiramate-D<sub>12</sub> and venlafaxine-D<sub>6</sub>) were purchased at known concentrations (Cerilliant Certified Spiking Solutions). These solutions were aliquoted into primary standard vials, freeze-dried to dryness, and stored at -80 °C until use. CDN standard metformin-D<sub>6</sub> was purchased as a solid, dissolved in known concentrations, and processed to dryness as was done for the Cerilliant standards. TCS was purchased from Sigma-Aldrich at the highest purity available.

#### **3.2.2.3.2 Sample Preparation**

All samples were processed within a week of collection. Solutions were centrifuged (3600 x g, 10 min, Avanti J-E Centrifuge, Beckman-Coulter) to remove solids and then passed sequentially through filter paper (Whatman Qualitative 5, 70 mm) and a 0.45 µm nitrocellulose filter (Millipore MF membrane) to remove small particulates and microbes. Two aliquots (30 mL) for each sample were freeze-dried in centrifuge tubes (50 mL). The freeze-dried extracts were reconstituted with LC-MS grade methanol (300 µL), then centrifuged (12,000 x g, 5 min). An aliquot (190 µL) was transferred to clean tube, frozen in liquid nitrogen and dried on a high-vacuum line. The dried extract was reconstituted with 300 µL of 0.1% formic acid:acetonitrile (9:1, v/v) which contained all labeled internal standards (ISs) at concentrations of 10 ng/mL. The sonicated samples were then centrifuged (12,000 x g, 5 min) and transferred to LC-MS vials for analysis.

### 3.2.2.3.3 Sample Analysis

Analyses were performed in duplicate using UPLC (H-Class, Waters) interfaced with an autosampler and a triple quadrupole mass spectrometer (Xevo TQ-S, Waters). The mass spectrometer was operated in multiple reaction monitoring mode (MRM) with parameters determined by direct infusion (see Appendix A). A gradient separation was performed on a reversed-phase column (BEH C18 1.7 $\mu$ m, Waters) maintained at 35°C. Solvent A was water and solvent B was acetonitrile, with both solvents containing formic acid (0.1% v/v). A 15-minute separation at a flow rate of 200  $\mu$ L/min began with 0.5 minutes of elution with 95% A. Next, the proportion of A was decreased to 5% over 7.5 minutes, ending at the 8-minute time point. Elution with 5% A continued for 0.5 minutes, followed by a rapid linear gradient to 95% A over 0.5 minutes, ending at the 9-minute time point. The 95% A solvent ratio was held for 6 minutes, until a total of 15 minutes had elapsed. Calibration curves and statistical analyses were performed by TargetLynx software (Waters) and transferred to Excel for further processing and analysis.

### 3.2.2.4 Plant Nutrient Analyses

CW plants were cut on November 2<sup>nd</sup> and analyzed for total dry mass (dried at 60°C until constant weight was obtained) and nutrient composition. Nutrient analyses were performed by A&L Eastern Laboratory in Richmond, VA using a microwave digestion with nitric and hydrochloric acid, followed by analysis via ion chromatography (Method 3051A). Nitrogen was analyzed via LECO combustion analysis.

## 3.3 Results

### 3.3.1 Data Processing and Statistical Analyses

Because effluent flow rates from the CW were lower than influent flow rates for almost the entire sampling period, CW4 and 6 (CW4 and CW6, respectively) pollutant concentrations were artificially high compared to Anua and E-Z Treat effluents. Using effluent flow rates estimated for the week of or before each sampling event based on evapotranspiration data, an adjustment factor was calculated by dividing the effluent flow rate by the influent flow rate (25.0 gpd throughout the sampling period). Values ranged from 0.48 to 1.0 for CW4 and from 0.48 to 1.2 for CW6.

TN was only measured directly on two sampling dates, so TN had to be estimated on other sampling dates. Total inorganic nitrogen (TIN) could be calculated for each sample as the sum of NH<sub>4</sub>, NO<sub>3</sub>, and NO<sub>2</sub>. For treated samples, organic nitrogen concentrations were expected to be negligible based on the low effluent TSS concentrations. To test this theory, TN concentrations were estimated for all sampling dates on which TSS was measured by assuming that all suspended solids were microbial biomass (a conservative assumption) with stoichiometry C<sub>5</sub>H<sub>7</sub>O<sub>2</sub>N (Grady et al, 2011). The hypothesis that the estimated TN and measured TIN values were equal was rejected (p<0.05) by constructing a paired data set of differences between estimated TN and measured TIN and finding that the confidence interval did not include zero (see Appendix C for R code). However, the average difference was 0.48 mg N/L, which is less than the standard deviation (SD) of all TIN concentrations for all treated systems except CW6, and less than 5% of the average TIN concentration for all treated systems except CW4 and CW6. Therefore, it is reasonable to say that the organic nitrogen fraction is negligible in treated effluents from the Anua and E-Z Treat systems. Because the average TIN of the wetland effluents was lower, the organic nitrogen fraction could not be neglected.

Next, estimated TN values for all treated systems and the STE were compared to TN values measured using Hach kits, and the two were found to be statistically equivalent (p>0.05). Therefore, the method of estimating TN values based on TSS and TIN was deemed acceptable and used to estimate TN values when TSS measurements were available, but Hach TN measurements were not. Using this method, the percent (average ± SD) of NH<sub>4</sub>-N comprising TN was found to be 87.3±5.7% in STE samples.

Parametric and non-parametric analysis of variance (ANOVA) tests were performed at 95% confidence levels using R software to statistically differentiate between the performance of each treatment system for each pollutant. ANOVA tests were run on removal rates calculated relative to the average STE concentration as follows:

$$RR_{i,j} = 1 - \frac{C_{i,j}}{\bar{C}_{i,STE}}$$

$RR_{i,j}$  is the removal rate for pollutant  $i$  from treatment system  $j$ ;  $C_{i,j}$  is the effluent concentration for pollutant  $i$  from treatment system  $j$ ; and  $\bar{C}_{i,STE}$  is the average concentration of pollutant  $i$  in the STE. Samples below the limit of detection for  $NH_4$  were set to 0.005 mg N/L and PPCP samples below the limit of quantification (LOQ) were set to half of the value of the LOQ prior to analysis (0.5  $\mu\text{g/L}$  for TCS and 0.015  $\mu\text{g/L}$  for all other PPCPs). See Appendix C for the R code.

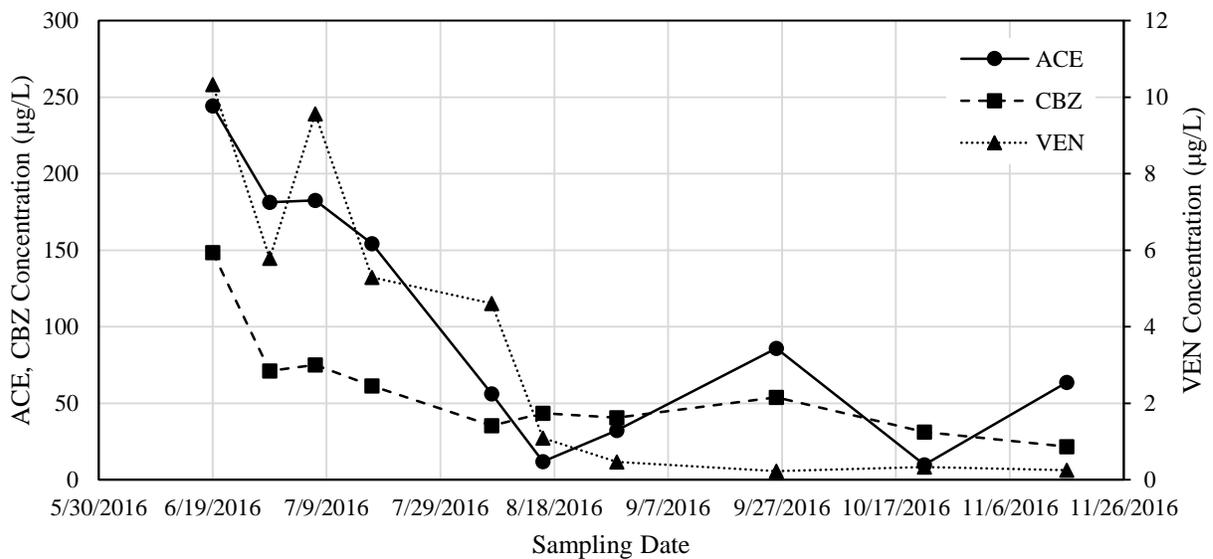
The use of this method assumes that STE samples were relatively consistent and independent of temporal changes. The assumption was checked by measuring RSDs for each STE parameter and performing the Durbin-Watson test for serial autocorrelation. Results are shown in Table 3.2 below. As reflected by the RSDs, variability was much higher in general for the monitored PPCPs, especially ACE, TOP, TRI, and VEN. The first sampling event was eliminated from statistical analyses for all PPCPs because the PPCP concentrations in the STE on the first sampling date were three to 15 times greater than the average concentration on all other dates. This could reflect evolution of microbial degradation capability for the biology living inside the pipes between the septic tank and the treatment systems. This issue will be discussed further in subsection 3.4.2 below.

**Table 3.2: Analysis of consistency and serial autocorrelation of STE samples**

Results based on samples 2-10 for all PPCP parameters. STE = septic tank effluent; RSD = relative standard deviation.

Parameter	RSD	Durbin-Watson Test Results
Alkalinity	16.6%	P=0.166
Biochemical oxygen demand	21.9%	P=0.558
Chemical oxygen demand	26.3%	P=0.798
Total suspended solids	64.7%	P=0.436
Ammonia-N	15.7%	P=0.498
Total nitrogen	32.3%	P=0.422
Acetaminophen	80.4%	P=0.056
Carbamazepine	38.0%	P=0.254
Lamotrigine	14.8%	P=0.576
Levetiracetam	24.7%	P=0.16
Metformin	60.5%	P=0.972
Topiramate	98.2%	P=0.836
Triclosan	107.6%	P=0.542
Venlafaxine	110.0%	P=0.176

After the first sampling event was removed, the STE concentrations for most of the PPCPs did not have any apparent trends when graphed over time, indicating that a steady state was reached by the second sampling date. However, STE concentrations continued to decrease over time for ACE, CBZ, and VEN, as shown in Figure 3.2 below. Note that the first sampling date is not eliminated in the figure. For these data sets, ANOVA analyses were run on both unpaired (described above) and paired removal rate data sets. For the paired data sets, percent removals were calculated based on the treated effluent concentrations and the STE concentration collected on the same day, instead of an average STE concentration. The approach was meant to eliminate trends in effluent concentrations caused by varying influent concentrations. It would not have been valid to use this approach if STE concentrations had not decreased consistently over time, since the HRTs were on the order of days and different for each treatment system.



**Figure 3.2: STE concentrations of ACE, CBZ, and VEN over time**  
 STE concentrations from samples 1-10. STE = septic tank effluent; ACE = acetaminophen; CBZ = carbamazepine; VEN = venlafaxine.

Before using the ANOVA test, it was necessary to prove that the treated effluent concentrations were not serially correlated. This was done using the Durbin-Watson test for serial autocorrelation. The following data sets were correlated: CW6 COD, CW4 LAM, E-Z Treat MET, Anua TOP, and E-Z Treat TCS. For CW4 LAM, E-Z Treat MET, and Anua TOP sequential sampling results were composited until results were no longer serially correlated. CW6 could not be composited in any

way to remove serial correlation for COD. When E-Z Treat TCS was composited, the data became more highly correlated, but this was probably because almost all of the data points were below the LOQ. Therefore, the original data set was used despite failing the Durbin-Watson test.

Next, the distribution of the data set was evaluated using the Shapiro-Wilk test of normality. Non-parametric tests were used when not all of the system effluents were normal for a given parameter. Equality of variances was verified for parametric data sets using the Bartlett test for homogeneity of variances. Equality of means was checked using the standard ANOVA test for parametric sets with equal variance, the one-way test for parametric sets with unequal variance, or the Kruskal-Wallis rank sum test for non-parametric tests. As a multiple comparison check, the parametric Tukey Honest Significant Differences (HSD) test and the non-parametric Kruskal-Wallis multiple comparison test were used. Then, confidence intervals were constructed for the groupings with the lowest percent removals to check whether those systems performed significantly better than the septic tank. Table 3.3 below summarizes the tests used. Results of the multiple comparison test will be discussed in the Results section.

**Table 3.3: Summary of ANOVA preparation**

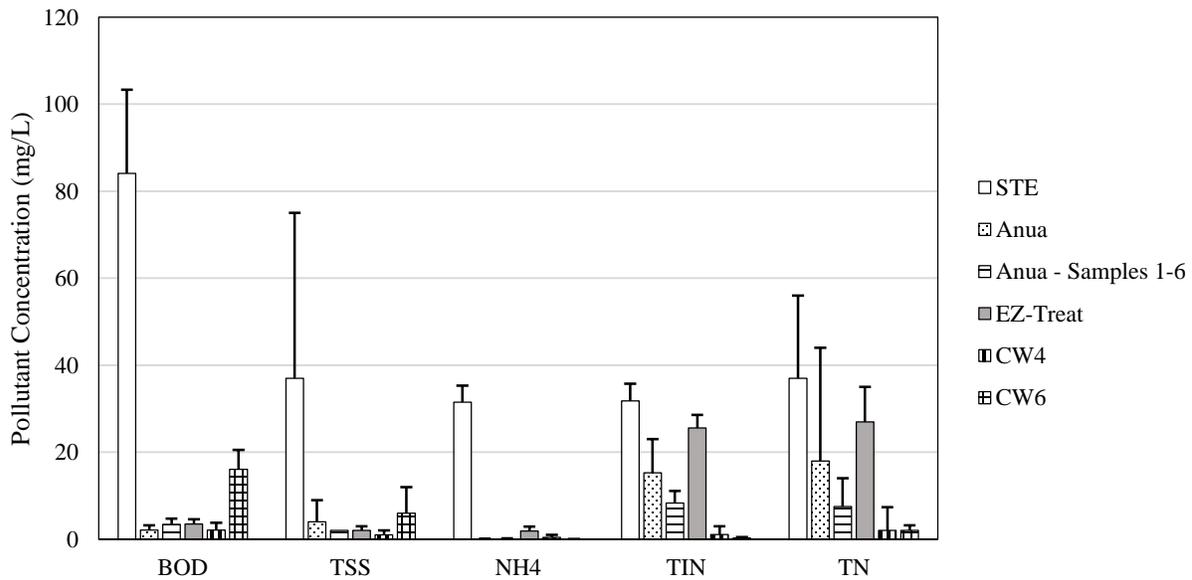
BOD = biochemical oxygen demand; COD = chemical oxygen demand; TSS = total suspended solids; NH<sub>4</sub> = ammonia-N; TN = total nitrogen; ACE = acetaminophen; CBZ = carbamazepine; LAM = lamotrigine; LEV = levetiracetam; MET = metformin; TOP = topiramate; TCS = triclosan; VEN = venlafaxine; CW4 = effluent from constructed wetland tank 4; CW6 = effluent from constructed wetland tank 6.

<b>Parameter</b>	<b>Distribution</b>	<b>Serial Correlation</b>	<b>Equality of Variance and Means</b>	<b>Multiple Comparison</b>
Alkalinity	Parametric – normal	No serial correlation	Bartlett test – variances equal AOV – means not equal	Tukey HSD
BOD	Non-parametric – CW4 not normal	No serial correlation	Kruskal-Wallis – means not equal	Kruskal Wallis multiple comparison
COD	Parametric – normal	CW6 serially correlated – use original data	Bartlett test – variances not equal One-way test -	Tukey HSD
TSS	Parametric – normal	No serial correlation	Bartlett test – variances not equal One-way – means equal	N/A
NH <sub>4</sub>	Non-parametric – CW4, CW6 not normal	No serial correlation	Kruskal-Wallis – means not equal	Kruskal Wallis multiple comparison

<b>Parameter</b>	<b>Distribution</b>	<b>Serial Correlation</b>	<b>Equality of Variance and Means</b>	<b>Multiple Comparison</b>
TIN	Non-parametric – CW4 not normal	No serial correlation	Kruskal-Wallace – means not equal	Kruskal Wallace multiple comparison
TN	Parametric – normal	No serial correlation	Bartlett test – variances not equal One-way test – means not equal	Tukey HSD
ACE	Non-parametric – Anua, CW4, CW6 not normal	No serial correlation	Kruskal-Wallace – means not equal	Kruskal Wallace multiple comparison
ACE – paired	Non-parametric – Anua, CW6 not normal	No serial correlation	Kruskal-Wallace – means not equal	Kruskal Wallace multiple comparison
CBZ	Parametric – normal	No serial correlation	Bartlett test – variances equal AOV – means not equal	Tukey HSD
CBZ – paired	Non-parametric – CW6 not normal	No serial correlation	Kruskal-Wallace – means not equal	Kruskal Wallace multiple comparison
LAM	Non-parametric – Anua not normal	CW4 serially correlated – use composited data	Kruskal-Wallace – means not equal	Kruskal Wallace multiple comparison
LEV	Non-parametric – Anua, CW4, CW6 not normal	Anua (pre-8/22) serially correlated – use composited data	Kruskal-Wallace – means not equal	Kruskal Wallace multiple comparison
MET	Non-parametric – none normal	E-Z Treat serially correlated – use composited data	Kruskal-Wallace – means equal	N/A
TOP	Non-parametric – none normal	Anua serially correlated – use composited data	Kruskal-Wallace – means equal	N/A
TCS	Non-parametric – Anua, CW4, CW6 not normal	E-Z Treat serially correlated – use original data	Kruskal-Wallace – means equal	N/A
VEN	Non-parametric – none normal	No serial correlation	Kruskal-Wallace – means unequal	Kruskal Wallace multiple comparison
VEN – paired	Non-parametric – Anua not normal	No serial correlation	Kruskal-Wallace – means unequal	Kruskal Wallace multiple comparison

### 3.3.2 Conventional Pollutants

Conventional pollutant parameters are summarized in Figure 3.3 and Table 3.4 for each of the treatment systems. On August 22<sup>nd</sup>, after six sampling events were completed, new pumps were installed for the Anua and E-Z Treat systems which had more consistent performance (less variation), but lower average flow rates. The drop in BOD loading had an obvious deleterious effect on denitrification processes in the Anua system. Dog food was added to the Anua ATU on October 3<sup>rd</sup>, and as a result, effluent TIN concentrations fell below 20 mg/L again on the last sampling event. Figure 3.3 summarizes all of the sampling results from the Anua system as well as the results from before August 22<sup>nd</sup> separately to illustrate the differences in performance when only the first half of the data set is considered. In addition, for parameters where there was sufficient data, ANOVA analyses were performed including the full set of Anua data and the Anua results prior to the pump replacement.



**Figure 3.3: Conventional pollutant concentrations in effluent samples**

Columns represent mean concentration and error bars represent 95% confidence intervals. Mean concentrations are calculated over the full sampling range for each treatment system unless otherwise noted. BOD = biochemical oxygen demand; TSS = total suspended solids; NH4 = ammonia-N; TIN = total inorganic nitrogen; TN = total nitrogen; STE = septic tank effluent; CW4 = effluent from constructed wetland tank 4; CW6 = effluent from constructed wetland tank 6.

**Table 3.4: Temperature, pH, DO, and alkalinity of effluent and internal samples**

DO = dissolved oxygen; SD = standard deviation; n = number of sampling points used to calculate mean.

Collection Point	Temp (°C)			pH			DO (mg/L)			Alkalinity (mg/L)		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	N
Anua System												
Well Basin 1 Effluent	26.8	7.7	6	7.08	0.98	6	7.53	1.44	6	51.6	47.1	4
Well Basin 2 Effluent	25.9	7.7	6	7.32	0.21	6	4.11	2.32	6	75.5	28.2	4
Anua Effluent	25.6	5.1	10	6.23	0.53	10	6.43	1.12	10	47.5	26.6	4
E-Z Treat Effluent	25.9	5.7	10	6.94	0.30	10	7.16	0.95	10	26.8	12.9	4
Constructed Wetland												
Tank 1 Effluent	20.0	4.7	4	6.93	0.26	4	0.87	0.16	4	275.1	26.4	2
Tank 2 Influent	21.0	5.2	4	7.00	0.27	4	2.90	1.03	4	268.6	20.6	2
Tank 2 Effluent	19.1	0.1	2	6.96	0.49	2	5.41	1.47	2	284.0	N/A	1
Tank 3 Influent	19.3	0.9	3	7.22	0.48	3	6.18	3.18	3	259.7	53.0	2
Tank 3 Effluent	22.5	9.2	2	7.71	0.33	2	6.73	3.36	2	260.7	N/A	1
Tank 4 Influent	23.5	8.9	2	8.06	0.34	2	9.94	1.73	2	261.6	N/A	1
CW4 (Tank 4 Effluent)	24.3	6.0	9	7.40	1.19	9	3.26	2.13	8	228.8	13.4	4
CW6 (Tank 6 Effluent)	24.3	5.3	10	6.30	0.19	10	1.62	0.45	10	312.1	24.0	4
Septic Tank Effluent	25.2	4.6	10	6.79	0.30	10	0.77	0.36	10	224.9	37.3	6

BOD and TSS concentrations were consistently under 10 mg/L for all treatments beyond the septic tank, with the exception of CW6. BOD, COD, and TSS concentrations increased from CW4 to CW6 throughout the sampling period, likely as a result of the wood chip media in Tanks 5 and 6. The high COD to BOD ratios (5.3 to 7.9) indicate that the organic matter in the Tank 6 was relatively stable, which would be typical of woody debris. The Anua system also had high effluent COD with even higher COD to BOD ratios (20 to 64), indicating stable organic matter. Peat particles were observed in filter waste produced during TSS analyses confirmed these findings.

The Anua and E-Z Treat systems consistently nitrified STEs, generating NH<sub>4</sub> concentrations below 2 mg-N/L throughout the study. The E-Z Treat system relied on septic field treatment for denitrification due to the lack of an anoxic zone downstream from the biofilter. The Anua system was able to sustain denitrification in the clarifier zone of the ATU and in the peat filters prior to August 22<sup>nd</sup>. The CW consistently achieved nitrification and denitrification, however NH<sub>4</sub> concentrations spiked from below 1 to above 7 mg N/L on the last sampling date. The wetland

plants were cut back on November 2<sup>nd</sup> and the first frost occurred on November 13<sup>th</sup>. Either or both of these factors could have been responsible for the change in effluent nutrient concentrations.

The CW plants were analyzed for nitrogen content by tank and the nutrient contents of the plants were found to decrease through the wetland, as indicated in Table 3.5 below. Assuming a constant flow rate of 25.0 gpd into the CW prior to flow measurement began on April 2<sup>nd</sup>, and an average influent TN concentration of 37 mg N/L (the average STE TN concentration during the sampling period), the total load of nitrogen to the CW when the plants were cut was 1.5 kg. Thus the nitrogen removed via plant uptake was approximately 10% of the total load delivered. Similarly, the average TP of the STE was 4.1 mg P/L (see Appendix C) so the total load of phosphorus to the CW was 83 g and 40% of that was taken up by plants. Crites and Tchobanoglous (1998) suggest a phosphorus uptake rate of 0.5 lb/ac-d for wetland plants, and the uptake rate seen in this wetland was only 0.17 lb/ac-d, which indicates that the plants may have been phosphorus-limited. Plants in Tank 4 began to die back in the late summer, followed by the plants in Tank 3 in early fall. This could have been an indication of nutrient shortage. Plants in Tanks 1 and 2 stayed green until they were cut in November.

**Table 3.5: Nutrient composition and weights of constructed wetland plants by tank**

Tank	Dry Weight (kg)	Percent Composition (%)			Mass Composition (g)		
		N	P	S	N	P	S
1	3.37	1.68	0.29	0.36	56.6	9.8	12
2	2.67	1.36	0.28	0.27	45.8	9.4	9.1
3	1.47	1.14	0.21	0.20	38.4	7.1	6.7
4	0.70	0.92	0.16	0.16	31	5.4	5.4
<i>Total</i>	<i>8.20</i>				<i>172</i>	<i>31.6</i>	<i>33.3</i>

Table 3.6 summarizes the findings from the ANOVA analyses. The CW4 was the only treatment system in the highest performing group for all analyzed pollutants. Considering only Anua data from before August 22<sup>nd</sup>, the Anua system was also in the highest group for all analyzed pollutants. The Anua, E-Z Treat, and CW4 systems had the highest oxygen demand removal rates, while the CW4 and 6 systems had the highest nitrogen removal rates. Performance between systems could not be differentiated for TSS so this parameter is not shown in the table.

In order to determine whether each system performed significantly better than the septic tank for a given parameter, 95% confidence intervals were calculated around the removal rates for each system. When the lower confidence limit (LCL) was greater than zero, this indicated that the system performed significantly better than the septic tank. When the upper confidence limit (UCL) was less than zero, this indicated that the treatment system effectively added pollutants to the STE. When zero was included in the confidence interval, this indicated that the system did not perform significantly differently than the septic tank. Confidence intervals were calculated using bootstrapping for nonparametric data sets (see Appendix C for R code).

Aside from alkalinity, all of the compared removal rates from the E-Z Treat, CW4, and CW6 systems were significantly greater than zero, indicating improved performance relative to the septic tank. The confidence intervals on alkalinity removal included zero for CW4 and was entirely negative for CW6, indicating that these systems had no significant change in alkalinity, and increased alkalinity, respectively. The Anua system did not have significant TN removal rates when data from the entire sampling period were considered. There were only two TN measurements for the Anua system before August 22<sup>nd</sup>, so a separate ANOVA test could not be run. However, the confidence interval for the removal rates based on those two data points did not include zero. Analyses were run for both the entire Anua data set and only the Anua data from before August 22<sup>nd</sup> for COD, NH<sub>4</sub>, and TIN. There were not enough Anua data points pre-August 22<sup>nd</sup> to run the analyses for the other parameters. Use of the abridged Anua data set only changed groupings for NH<sub>4</sub>.

**Table 3.6: ANOVA analysis findings for conventional pollutants**

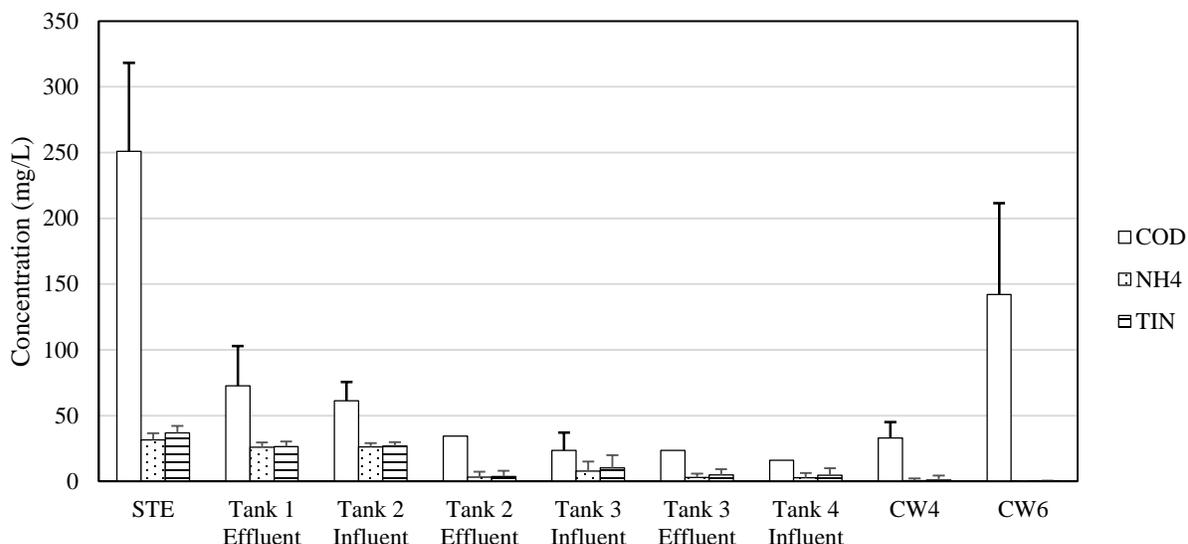
ANOVA tests compared data from full sampling ranges of each system unless otherwise noted. Removal rates for TIN were calculated relative to average TN concentrations of septic tank effluent. CW4 = effluent from constructed wetland tank 4; CW6 = effluent from constructed wetland tank 6; BOD = biochemical oxygen demand; COD = chemical oxygen demand; NH<sub>4</sub> = ammonia-N; TIN = total inorganic nitrogen; TN = total nitrogen.

Parameter	Significantly Distinct Systems			
	Anua	E-Z Treat	CW4	CW6
Alkalinity	A	A	B	C
BOD	A	AB	A	B
COD	B	AB	A	C
COD – Anua samples 1-6	AB	A	A	B
NH <sub>4</sub>	AB	B	A	A
NH <sub>4</sub> – Anua samples 1-6	AB	B	A	A
TIN	B	B	A	A

Parameter	Significantly Distinct Systems			
	Anua	E-Z Treat	CW4	CW6
TIN – Anua samples 1-6	AB	B	A	A
TN	AB	B	A	A

Throughout the sampling period, microbial growth was observed in the flexible PVC tubing between the CW Tanks. Additional samples were taken throughout the wetland cells to quantify the effects of the biofilms and each tank on overall CW performance. Table 3.4 shows that pH and DO generally increased across the biofilms, which could indicate the presence of algae in the PVC tubing. However, almost all of the tubing was buried under a thick layer of grass and it is unclear how algae would grow under such dark conditions.

Figure 3.4 illustrates the change in conventional pollutant concentrations across the wetland as measured by collecting samples at internal sampling locations on four dates. Error bars represent SDs. Not all locations were sampled on all four dates, and error bars are not shown when data was collected on only one date. STE TN concentrations are shown instead of STE TIN concentrations. In all cases,  $\text{NH}_4$  and TIN concentrations drop only slightly or increase across the biofilm (i.e. from a tank effluent to the influent of the next tank) indicating that nitrogen removals primarily occurred in the CW Tanks themselves. Based on the limited data set, the largest COD declines seem to occur in Tank 1, while the largest nitrogen declines occur in Tank 2. COD removals are negligible in Tanks 3 and 4, but these Tanks are important for  $\text{NH}_4$  removal, particularly during later sampling events.



**Figure 3.4: Conventional pollutants measured in internal wetland samples**

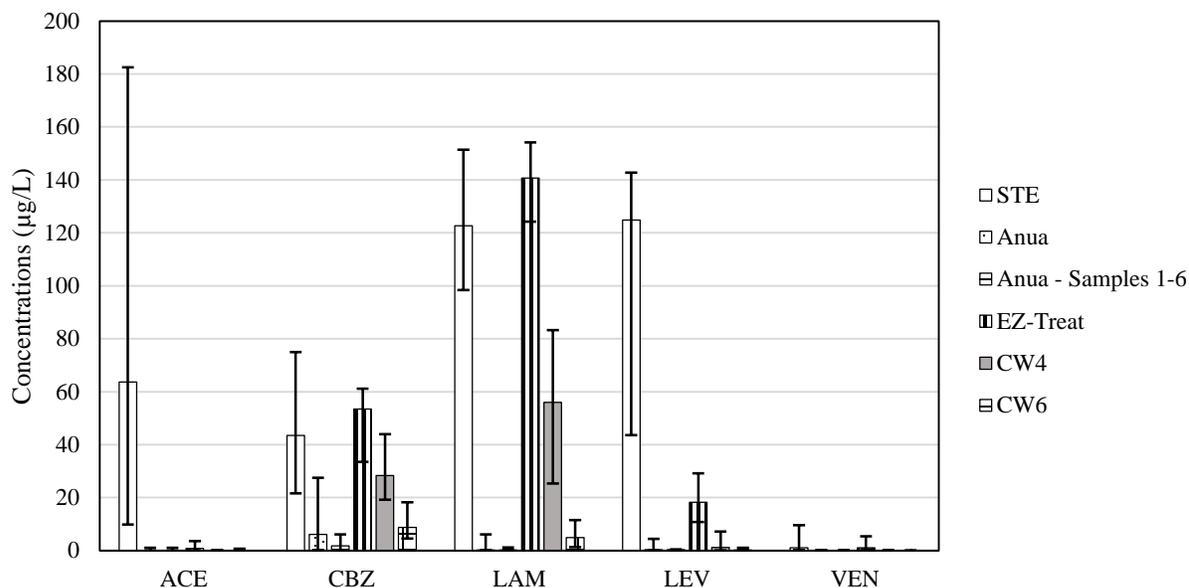
Columns represent mean concentration and error bars represent standard deviations. Error bars are not shown when data was collected on only one date. STE TN concentrations are shown instead of STE TIN concentrations. COD = chemical oxygen demand; NH4 = ammonia-N; TIN = total inorganic nitrogen; STE = septic tank effluent; CW4 = effluent from constructed wetland tank 4; CW6 = effluent from constructed wetland tank 6.

### 3.3.3 Pharmaceuticals and Personal Care Products (PPCPs)

Measured PPCP concentrations are summarized in Figure 3.5 for each of the treatment systems. The columns in the figure represent median values and the error bars represent the range. Results for MET, TOP, and TCS are shown in Table 3.7. Most of the MET and TOP measurements were below the LOQ. A labeled IS was not available for TCS so TCS measurements are only valid as a relative value.

It should be noted that because no metabolites were monitored, the calculated removal rates represent removal of the parent compound only. Removal of metabolites will be considered by analyzing the literature in section 3.4 below.

The entire range of removal rates was above 75% for ACE, LEV, and TCS in all treatment systems, and for VEN in all treatment systems except E-Z Treat. Median removal rates for CBZ varied widely, from -10.9% for E-Z Treat to 87.3% for the Anua system over the entire sampling period and 96.4% for the Anua system prior to August 22<sup>nd</sup>. Median removals for LAM had a similar range: from -13.8% for E-Z Treat to 99.8% for the Anua system (both sampling ranges).



**Figure 3.5: PPCP concentrations in effluent samples**

Columns represent median concentration and error bars represent range. Median concentrations are taken from the full sampling range for each treatment system unless otherwise noted. PPCP = pharmaceuticals and personal care products; ACE = acetaminophen; CBZ = carbamazepine; LAM = lamotrigine; LEV = levetiractam; VEN = venlafaxine; STE = septic tank effluent; CW4 = effluent from constructed wetland tank 4; CW6 = effluent from constructed wetland tank 6.

**Table 3.7: MET, TOP, and TCS results for effluent samples**

Median concentrations are taken from the full sampling range for each treatment system unless otherwise noted. n = the number of samples used to determine the mean; LOQ = 0.03 µg/L for MET and TOP. MET = metformin; TOP = topiramate; TCS = triclosan; n = number of samples; CW4 = effluent from constructed wetland tank 4; CW6 = effluent from constructed wetland tank 6; LOQ = limit of quantification.

System	Concentration (µg/L)			Removal Rate (%)			n
	Median	Min	Max	Median	Min	Max	
<b>MET</b>							
Anua	<LOQ	<LOQ	1.121	>73.5	-893.8	>73.5	9
Anua - Samples 1-6	<LOQ	<LOQ	<LOQ	>73.5	86.7	>73.5	5
E-Z Treat	0.032	<LOQ	0.686	71.6	-508.2	>73.5	6
CW4	<LOQ	<LOQ	0.029	>73.5	>73.5	>73.5	9
CW6	<LOQ	<LOQ	0.060	>73.5	46.8	>73.5	9
STE	0.096	0.052	0.268				9
<b>TOP</b>							
Anua	<LOQ	<LOQ	0.154				9
Anua - Samples 1-6	<LOQ	<LOQ	<LOQ				5
E-Z Treat	<LOQ	<LOQ	0.069				6
CW4	<LOQ	<LOQ	0.055				9
CW6	<LOQ	<LOQ	0.079				9
STE	<LOQ	<LOQ	0.081				9

System	Concentration ( $\mu\text{g/L}$ )			Removal Rate (%)			n
	Median	Min	Max	Median	Min	Max	
	<b>TCS</b>						
Anua				>93.5	83.9	>93.5	9
Anua - Samples 1-6				90.5	83.9	>93.5	5
E-Z Treat				>93.5	88.7	>93.5	6
CW4				>93.5	81.1	>93.5	9
CW6				>93.5	86.2	>93.5	9
STE							9

Table 3.6 summarizes the findings from the ANOVA analyses. Anua and CW6 were in the highest performing group for all analyzed PPCPs. Average effluent concentrations were lower for the Anua samples collected prior to August 22<sup>nd</sup> for all monitored PPCPs except TCS; however, no significant differences could be proved between the abridged and complete data sets.

As described in subsection 3.3.1, paired data sets were used to run ANOVA analyses for PPCPs whose STE concentrations decreased consistently over time. The use of paired data for ACE, CBZ, and VEN generated very similar results to the unpaired data. There were two differences. First, all removal rates, but particularly those of the E-Z Treat system, were pushed down by use of the paired data. Second, groupings changed for CBZ; less distinction could be made using the paired data versus the unpaired data, although order was preserved. The likely reason for the second observation is that the paired data sets were not normally distributed, so the more conservative, nonparametric Kruskal-Wallis rank sum test had to be used.

MET, TOP, and TCS are not shown in the table because there no significant differences between system for these compounds. For ACE, LEV, TCS, and paired VEN data, all treatment systems performed significantly better than the septic tank. This was determined using the same confidence interval method as for conventional pollutants. The following treatment systems did not perform significantly better than the septic tank:

- CBZ: E-Z Treat confidence interval included zero
- CBZ – paired data: E-Z Treat confidence interval below zero
- LAM: E-Z Treat confidence interval below zero

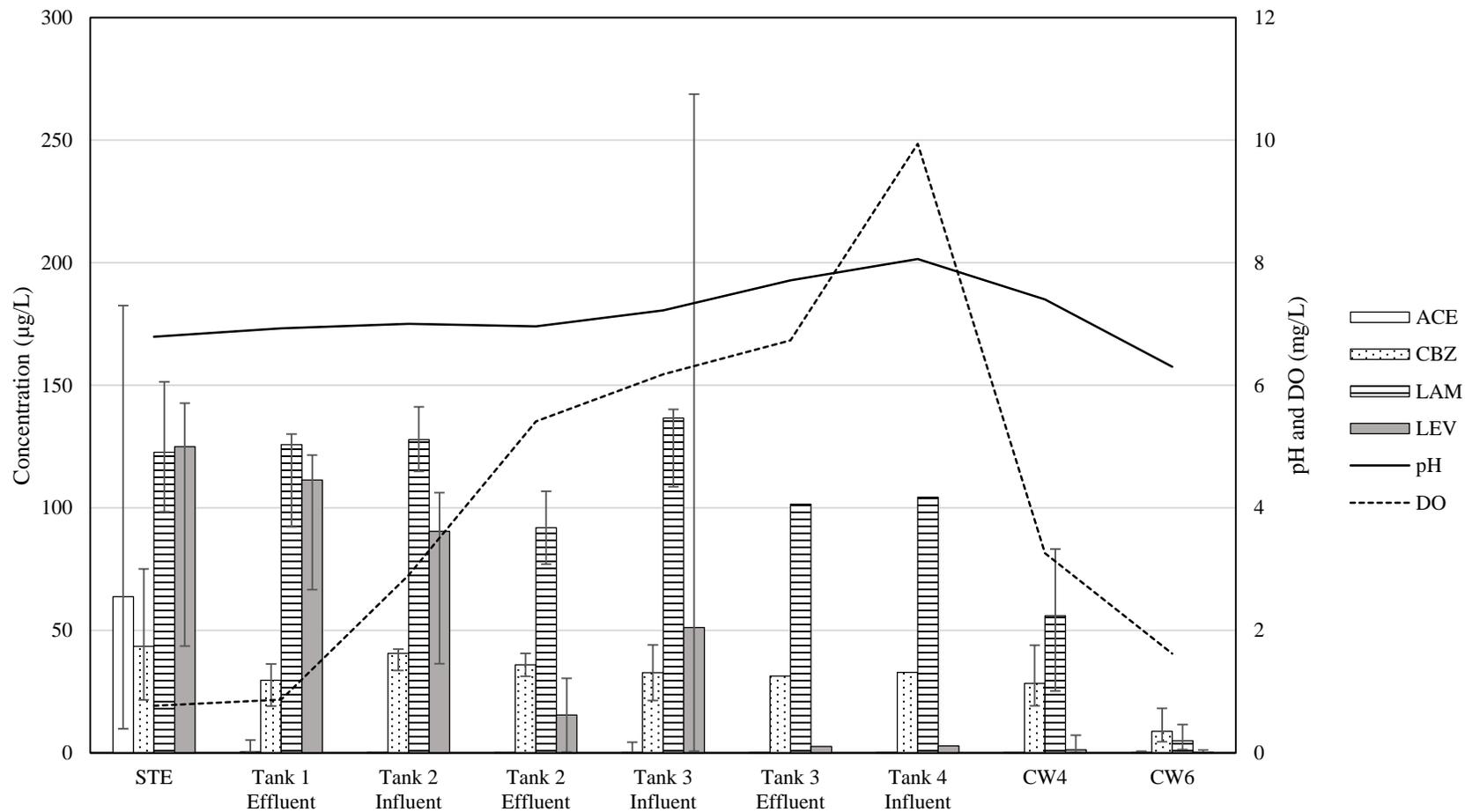
- MET: Anua and E-Z Treat confidence intervals below zero
- TOP: All confidence intervals included zero
- VEN: E-Z Treat confidence interval included zero
- VEN – paired data: E-Z Treat confidence interval below zero

**Table 3.8: ANOVA analysis findings for PPCPs**

ANOVA tests compared data from full sampling ranges of each system unless otherwise noted. For paired data sets, removal rates were calculated based on septic tank effluent concentrations collected on the same sampling date, otherwise removal rates were calculated based on average septic tank effluent concentrations. PPCPs = pharmaceuticals and personal care products; CW4 = effluent from constructed wetland tank 4; CW6 = effluent from constructed wetland tank 6; ACE = acetaminophen; CBZ = carbamazepine; LAM = lamotrigine; LEV = levetiracetam; VEN = venlafaxine.

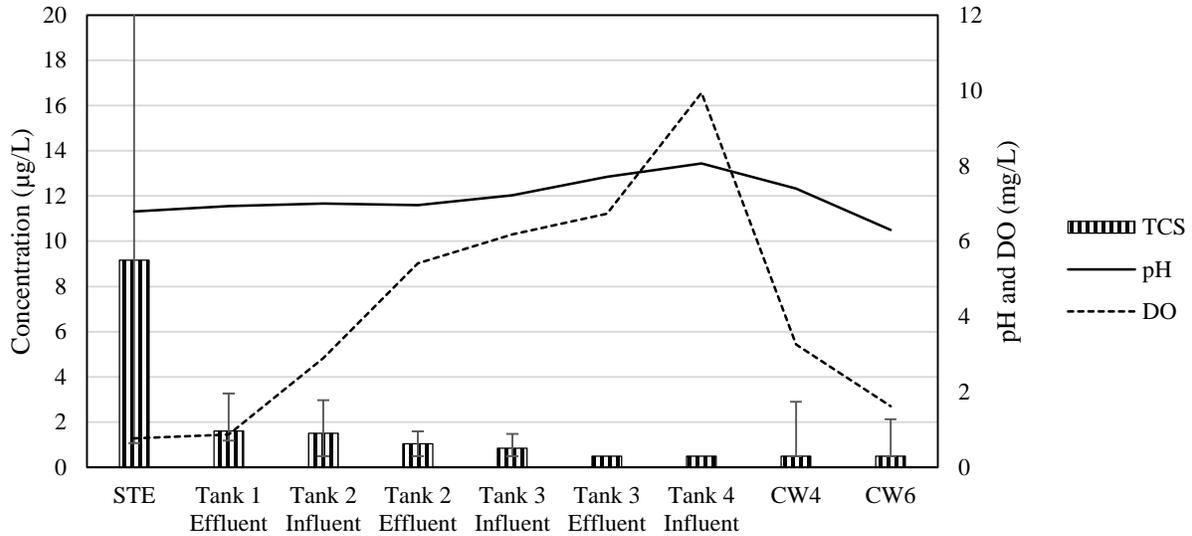
Parameter	Significantly Distinct Systems			
	Anua	E-Z Treat	CW4	CW6
ACE	AB	B	A	AB
ACE – paired	AB	B	A	AB
CBZ	A	C	B	A
CBZ – paired	A	B	AB	A
CBZ – paired, Anua samples 1-6	A	C	BC	AB
LAM	A	C	BC	AB
LEV	A	B	AB	A
VEN	A	B	A	A
VEN – paired	A	B	A	A

Internal wetland samples were analyzed for PPCPs on 1 to 3 sampling dates to assess the contribution of the biofilms and each wetland tank to PPCP removal. Results can be seen in Figure 3.6, Figure 3.7, and Figure 3.8 below. As in Figure 3.5, the columns represent the median concentration and the error bars represent the observed range. ACE and TCS were primarily removed in Tank 1 while LEV and VEN were primarily removed in Tanks 2 and 3. This suggests that ACE and TCS are more readily biodegradable and degradable under anoxic conditions, while LEV and VEN require aerobic conditions to degrade. It also appears that LEV metabolites may have been converted back to the parent compound within Tank 3. CBZ and LAM had negligible removals until they reached the CW mycoremediation Tanks 5 and 6. This suggests they are less biodegradable and were only removed by fungal degradation or sorption. It is difficult to draw any conclusions about MET or TOP due to low levels in the STEs, but MET appeared to be largely removed by the time it passed through Tank 2.



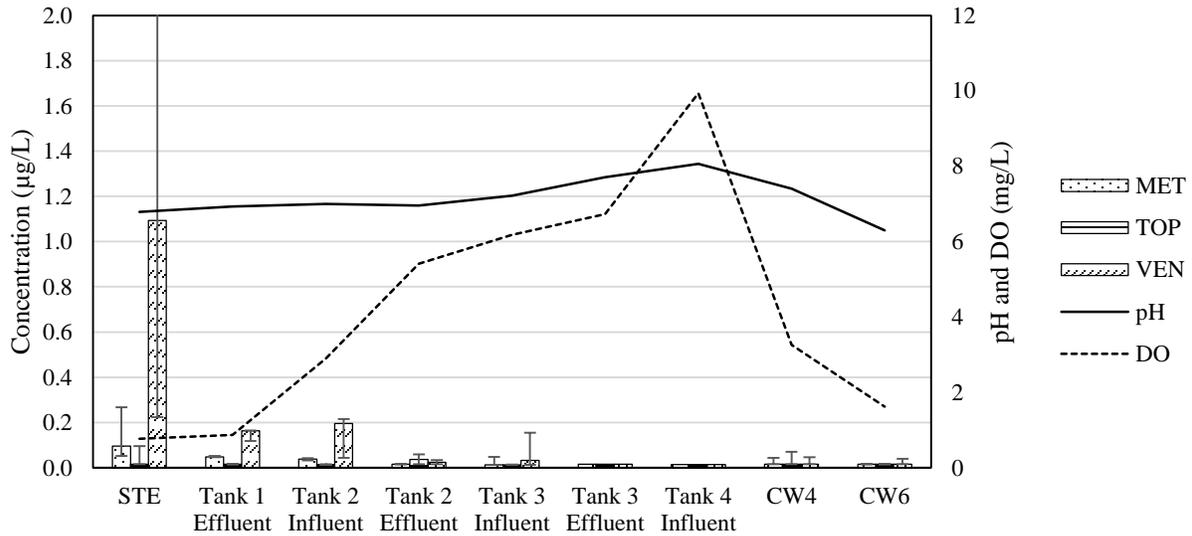
**Figure 3.6: ACE, CBZ, LAM, and LEV concentrations measured in internal wetland samples**

Columns represent median concentration and error bars represent range. ACE = acetaminophen; CBZ = carbamazepine; LAM = lamotrigine; LEV = levetiracetam; DO = dissolved oxygen; STE = septic tank effluent; CW4 = effluent from constructed wetland tank 4; CW6 = effluent from constructed wetland tank 6.



**Figure 3.7 TCS concentrations measured in internal wetland samples**

Columns represent median concentration and error bars represent range. The maximum observed STE concentration of 53 µg/L is not shown for TCS. TCS = triclosan; DO = dissolved oxygen; STE = septic tank effluent; CW4 = effluent from constructed wetland tank 4; CW6 = effluent from constructed wetland tank 6.

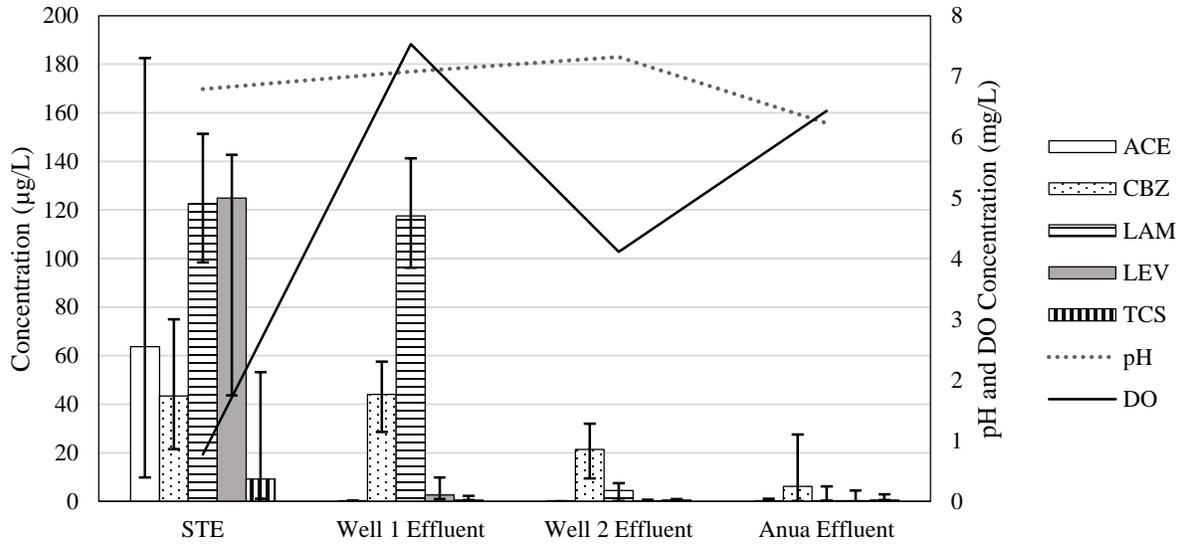


**Figure 3.8: MET, TOP, and VEN concentrations measured in internal wetland samples**

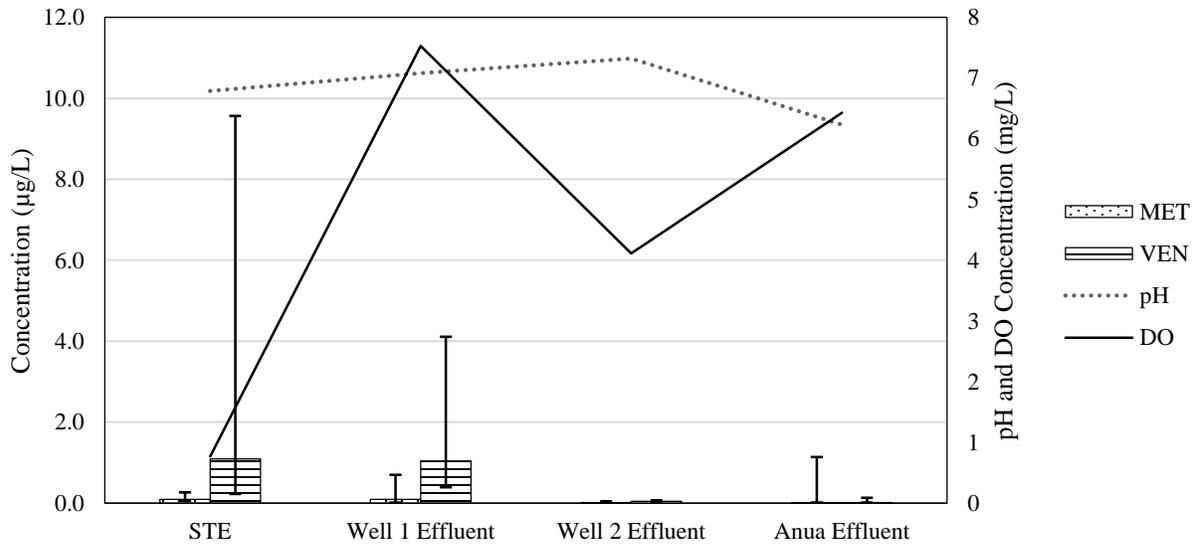
Columns represent median concentration and error bars represent range. The maximum observed STE concentration of 9.6 µg/L is not shown for MET. MET = metformin; TOP = topiramate; VEN = venlafaxine; DO = dissolved oxygen; STE = septic tank effluent; CW4 = effluent from constructed wetland tank 4; CW6 = effluent from constructed wetland tank 6.

Internal samples for the Anua system also yielded interesting results, as shown in Figure 3.9 and Figure 3.10 below. Again, columns represent the median concentration and the error bars represent the observed concentration range. ACE, LEV, MET, and TCS were removed in the ATU, while

CBZ, LAM, and VEN were removed in the peat filters. A greater proportion of LAM and VEN appear to be removed in the first peat filter than for CBZ.

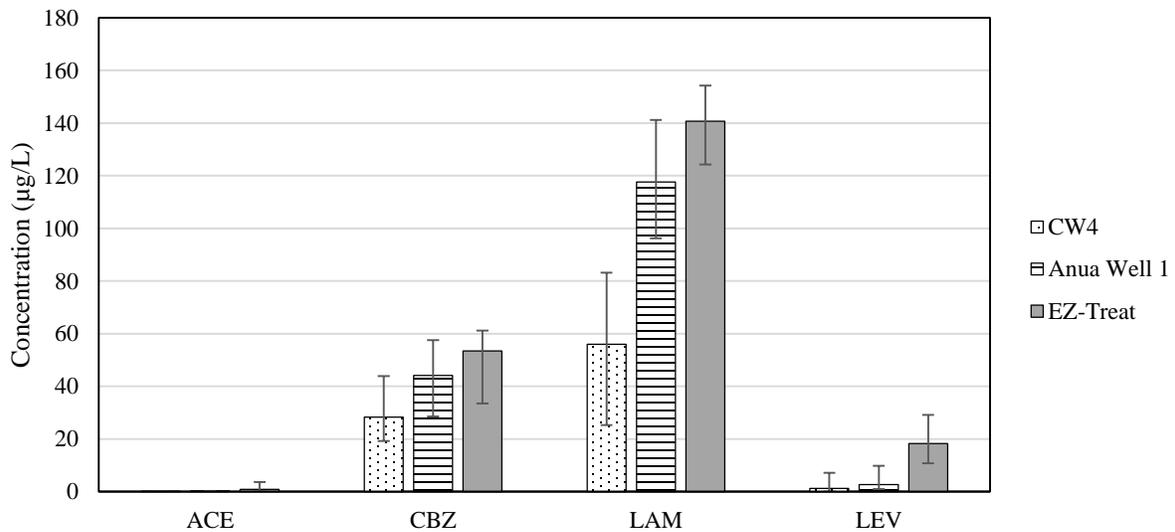


**Figure 3.9: ACE, CBZ, LAM, LEV, and TCS concentrations measured in internal Anua samples**  
Columns represent median concentration and error bars represent range. ACE = acetaminophen; CBZ = carbamazepine; LAM = lamotrigine; LEV = levetiracetam; TCS = triclosan; DO = dissolved oxygen; STE = septic tank effluent.

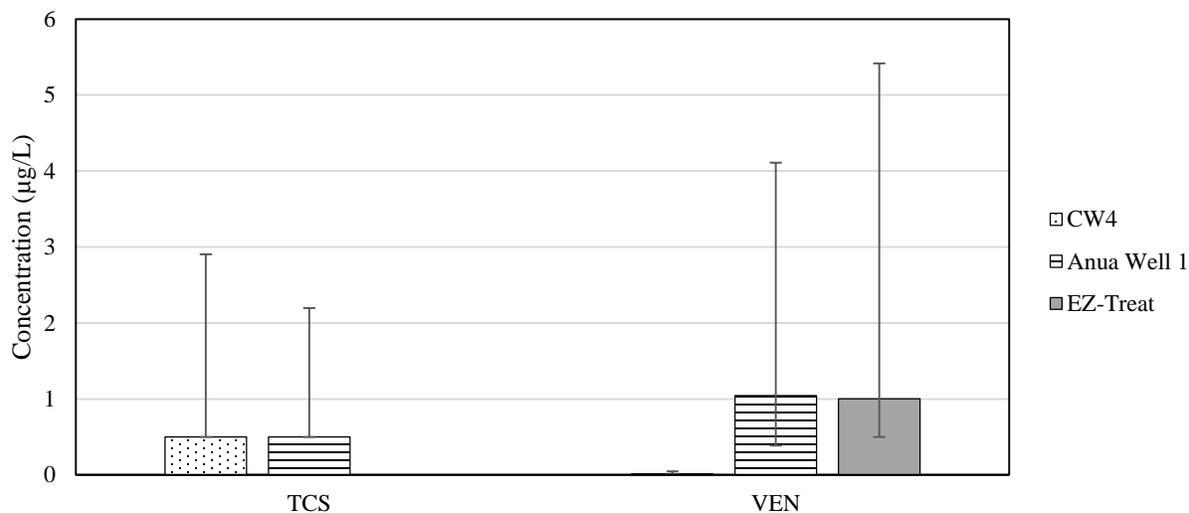


**Figure 3.10: MET and VEN concentrations measured in internal Anua samples**  
Columns represent median concentration and error bars represent range. MET = metformin; VEN = venlafaxine; DO = dissolved oxygen; STE = septic tank effluent.

It is also instructive to compare the performance of the treatment units in which sorption was not the primary removal mechanism. As shown in Figure 3.11 and Figure 3.12 below, the CW4 effluent had noticeably lower concentrations of CBZ, LAM, LEV, and VEN than the Anua ATU or E-Z Treat system. Additional removal mechanisms in the CW4 tanks included plant uptake, phytodegradation, and enhanced microbial degradation as a result of redox cycling and a longer HRT. There were some limited opportunities for photodegradation in the small pond in CW Tank 3 and in clear waterfalls (designed for aeration) at the influents to Tanks 2 and 3. However, sorption could have been a significant removal mechanism for the first four CW tanks as well given the low surface loading of the system and the mulch layers on the surface of Tanks 3 and 4.



**Figure 3.11: ACE, CBZ, LAM, and LEV concentrations in CW4, Anua Well 1, and E-Z Treat effluents**  
 Columns represent median concentration and error bars represent range. ACE = acetaminophen; CBZ = carbamazepine; LAM = lamotrigine; LEV = levetiracetam; CW4 = effluent from constructed wetland tank 4.



**Figure 3.12: TCS and VEN concentrations in CW4, Anua Well 1, and E-Z Treat effluents**

Columns represent median concentration and error bars represent range. TCS = triclosan; VEN = venlafaxine; CW4 = effluent from constructed wetland tank 4.

## 3.4 Discussion

### 3.4.1 Literature Overview

Table 3.9 and Table 3.10 below summarize findings from the literature and online sources regarding physical and chemical properties of the monitored PPCPs, as well as known pharmacokinetic data and removals in different treatment systems. These data were used to inform the discussion of removal mechanisms, which follows in subsection 3.4.3.

**Table 3.9: Physical and chemical properties of PPCPs**

Experimental or estimated properties sourced from PubChem or ChemSpider unless otherwise labeled. PPCPs = pharmaceuticals and personal care products;  $pK_a$  = acid dissociation constant;  $\text{Log } K_{ow}$  = logarithm of the octanol-water partition coefficient;  $\text{Log } K_{oc}$  = logarithm of the soil organic carbon-water partition coefficient; ACE = acetaminophen; CBZ = carbamazepine; LAM = lamotrigine; LEV = levetiracetam; MET = metformin; TOP = topiramate; TCS = triclosan; VEN = venlafaxine.

PPCP, Type	$pK_a$	Water Solubility (mg/L)	Vapor Pressure (mm Hg)	$\text{Log } K_{ow}$	$\text{Log } K_{oc}$
ACE, analgesic	9.38	14,000	6.29E-05	0.46	1.79
CBZ, anticonvulsant	13.9	17.7	1.84E-07	2.45	3.59
LAM, anticonvulsant	5.7	170	9.4E-09	0.99	3.13

<b>PPCP, Type</b>	<b>pK<sub>a</sub></b>	<b>Water Solubility (mg/L)</b>	<b>Vapor Pressure (mm Hg)</b>	<b>Log K<sub>ow</sub></b>	<b>Log K<sub>oc</sub></b>
LEV, anticonvulsant		1.04E+06	3.5E-06	-0.49	1.76
MET, antidiabetic	10.2, 12.4 (Scheurer <i>et al.</i> , 2009)	1.00E+06	0.0119	-1.4	2.15
TOP, anticonvulsant		9,800	7.0E-08	-0.33	1.0
TCS, antimicrobial	7.9	10	4.6E-06	4.8	4.27
VEN, antidepressant	10.09	267	2.46E-07	3.28	3.17

**Table 3.10: Literature review of pharmacokinetics and removal rates**

WWTPs = wastewater treatment plants; ETUs = enhanced treatment units; CWs = constructed wetlands; ACE = acetaminophen; CBZ = carbamazepine; LAM = lamotrigine; LEV = levetiracetam; MET = metformin; TOP = topiramate; TCS = triclosan; VEN = venlafaxine; ATU = aerobic treatment unit; HSSF = horizontal subsurface flow; VSSF = vertical subsurface flow; SF = surface flow; LECA = lightweight expanded clay aggregate; DOC = dissolved organic carbon; GAC = granular activated carbon.

PPCP, Type	Pharmacokinetics & Major Species in WWTPs	Observed Removal Rates				
		WWTPs	ETUs	CWs	Septic Systems	Other
ACE analgesic	<p><i>Pharmacokinetics:</i> &lt;5% excreted unchanged</p> <p>Major metabolites: glucuronide (52-57%), sulfate (30-44%)</p> <p>Other metabolites: N-acetyl-p-benzoquinone imine (NAPQI)-glutathione (5-10%)</p> <p><a href="http://www.pharmagkb.org/pathway">www.pharmagkb.org/pathway</a></p> <p><i>Major Species in WWTPs:</i> ACE</p>	<p>Removal to below detection limits in WWTP (De la Cruz <i>et al.</i>, 2012)</p> <p>&gt;99% removal in WWTP (Du <i>et al.</i>, 2014)</p>	<p>&gt;99% removal in ATU (Du <i>et al.</i>, 2014)</p>	<p>&gt;95% removal in SSF wetlands (Avila <i>et al.</i>, 2013)</p> <p>&gt;99% removal in hybrid wetlands (Avila <i>et al.</i>, 2014)</p> <p>&gt;99% removal in septic tank + wetland (unspecified type) (Du <i>et al.</i>, 2014)</p> <p>&gt;99% removal in hybrid wetlands (Conkle <i>et al.</i>, 2008)</p>	<p>&gt;5-log removal between septic tank and groundwater (Phillips <i>et al.</i>, 2015)</p> <p>Removal from 20 µg/L to below detection between septic tank and groundwater (Godfrey <i>et al.</i>, 2007)</p> <p>28-65% removal in septic tank alone (Du <i>et al.</i>, 2014)</p>	

PPCP, Type	Pharmacokinetics & Major Species in WWTPs	Observed Removal Rates				
		WWTPs	ETUs	CWs	Septic Systems	Other
CBZ, anti-convulsant	<p><i>Pharmacokinetics:</i> &lt;5% excreted unchanged/as pharmacologically active CBZ-10,11-epoxide (CBZ-E)</p> <p>Major metabolite: trans-10,11-dihydroxy-10,11-dihydro-CBZ (DiOHCBZ)</p> <p>Other metabolites: 2-hydroxy-CBZ (2-OH-CBZ); 3-hydroxy-CBZ (3-OH-CBZ); N-glucuronide</p> <p>(Eichelbaum <i>et al.</i>, 1985)</p> <p><i>Major Species in WWTPs:</i> CBZ, DiOHCBZ, 10,11-dihydro-10-hydroxy-CBZ (MHD)</p> <p>(Kaiser <i>et al.</i>, 2014; Gurke <i>et al.</i>, 2015)</p>	<p>Insignificant removal of CBZ in biological WWTP (Hollender <i>et al.</i>, 2009)</p> <p>Negligible removal of CBZ, 15% removal of MHD in biological WWTP with chemical P removal (Gurke <i>et al.</i>, 2015)</p> <p>Negligible removal of CBZ in biological WWTP (Joss <i>et al.</i>, 2006)</p> <p>3-6% removal in WWTP (Du <i>et al.</i>, 2014)</p>	6-8% removal in ATU (Du <i>et al.</i> , 2014)	<p>50% removal of CBZ in lagoon wetland (Conkle <i>et al.</i>, 2008)</p> <p>6-17% removal in septic tank + wetland (unspecified type) (Du <i>et al.</i>, 2014)</p> <p>0-25% removal of CBZ; 5-28% removal of DiOHCBZ; 28-93% removal of MHD; 0-88% removal of 2- and 3-OH-CBZ in SSF and 2 pond wetlands (Ruhmland <i>et al.</i>, 2015)</p> <p>27-28% removal of CBZ in continuous, planted HSSF wetlands; 20-26% removal in VSSF wetlands; 0-58% removal in hybrid wetlands; 0-50% removal in SF wetlands (multiple studies, reported in Li <i>et al.</i>, 2014)</p>	<p>10% increase in CBZ seen between septic tank and groundwater (Phillips <i>et al.</i>, 2015)</p> <p>6-7% removal in septic tank (Du <i>et al.</i>, 2014)</p>	<p>95% removal by LECA with initial concentration of 1 mg/L (Dordio <i>et al.</i>, 2009)</p> <p>CBZ effectively removed via activated carbon adsorption (Yu <i>et al.</i>, 2008)</p> <p>&gt;99% removal via post-treatment ozonation with ~0.6 g O<sub>3</sub> g<sup>-1</sup> DOC (Hollender <i>et al.</i>, 2009)</p> <p>Removal to below detection limits via exposure to UV<sub>254</sub> and H<sub>2</sub>O<sub>2</sub> at 50 mg/L for 30 minutes (De la Cruz <i>et al.</i>, 2012)</p>

PPCP, Type	Pharmacokinetics & Major Species in WWTPs	Observed Removal Rates				
		WWTPs	ETUs	CWs	Septic Systems	Other
LAM, anti-convulsant	<p><i>Pharmacokinetics:</i> &lt;10% excreted unchanged in urine; &lt;2% excreted unchanged in feces</p> <p>Major metabolites: N-2 glucuronide (70%)</p> <p>(Garnett, 1997)</p> <p><i>Major Species in WWTPs:</i> LAM, OXO-LAM (synthetic impurity), LMG-N2-G-TP430</p> <p>(Zonja <i>et al.</i>, 2016)</p>	Concentrations of LAM and OXO-LAM increase in WWTP (Zonja <i>et al.</i> , 2016)				<p>Limited removal via post-treatment ozonation with ~0.6 g O<sub>3</sub> g<sup>-1</sup> DOC (Hollender <i>et al.</i>, 2009)</p> <p>Not removed via UV or O<sub>3</sub> disinfection applications, but sensitive to advanced oxidation with hydroxyl radicals (Keen <i>et al.</i>, 2014)</p>
LEV, anti-convulsant	<p><i>Pharmacokinetics:</i> 66% excreted unchanged in urine</p> <p>Major metabolites: acid metabolite UCB L057 (24%)</p> <p>Minor metabolites: 2 metabolites (~2%)</p> <p>(Patsalos <i>et al.</i>, 2000; Radtke, 2001)</p> <p><i>Major Species in WWTPs:</i> LEV</p>	98% removal in biological WWTP with chemical P removal (Gurke <i>et al.</i> , 2015)				Limited removal via post-treatment ozonation with ~0.6 g O <sub>3</sub> g <sup>-1</sup> DOC (Hollender <i>et al.</i> , 2009)

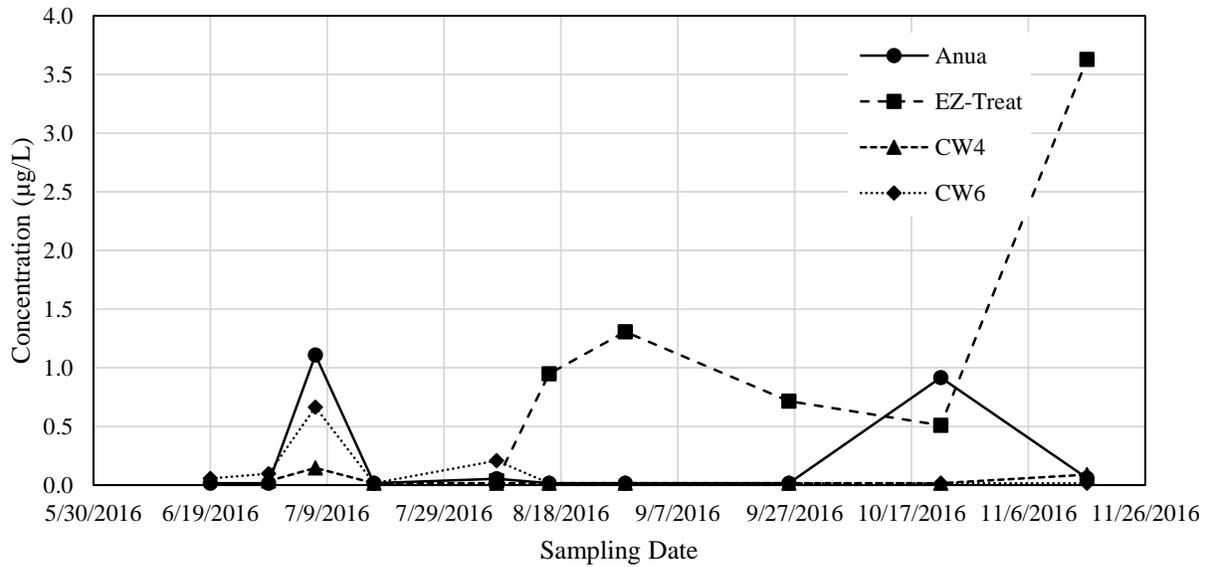
PPCP, Type	Pharmacokinetics & Major Species in WWTPs	Observed Removal Rates				
		WWTPs	ETUs	CWs	Septic Systems	Other
MET, antidiabetic	<p><i>Pharmacokinetics:</i> Excrete unchanged in urine  (Graham <i>et al.</i>, 2011)</p> <p><i>Major species in WWTPs:</i> MET, guanylurea  (Trautwein and Kummerer, 2011)</p>	<p>79-91% removal of MET in WWTP (Scheurer <i>et al.</i>, 2009)</p> <p>Metformin may be primarily degraded to guanylurea in WWTPs. Guanylurea is polar and resistant to photo- and UV-degradation (Trautwein and Kummerer, 2011)</p>			>3-log removal of MET seen between septic tank and groundwater (Phillips <i>et al.</i> , 2015)	11% removal via exposure to UV254 and H <sub>2</sub> O <sub>2</sub> at 50 mg/L for 30 minutes (De la Cruz <i>et al.</i> , 2012)
TOP, anti-convulsant	<p><i>Pharmacokinetics:</i> &gt;80% excreted unchanged in urine</p> <p>Minor metabolites: six inactive metabolites formed via glucuronidation, hydroxylation, and hydrolysis  (Garnett, 2000)</p> <p><i>Major species in WWTPs:</i> TOP</p>	<p>&lt;1-log removal in biological WWTP with chemical P removal (Gurke <i>et al.</i>, 2015)</p>				

PPCP, Type	Pharmacokinetics & Major Species in WWTPs	Observed Removal Rates				
		WWTPs	ETUs	CWs	Septic Systems	Other
TCS, anti-microbial	<p><i>Pharmacokinetics:</i> N/A</p> <p><i>Major species in WWTPs:</i> TCS</p>	>98.5% removal in conventional activated sludge, including 80-90% mineralization to CO <sub>2</sub> or incorporation by biomass and <5% sorption to settled solids (Federle, 2002)		>90% removal in hybrid wetlands (Avila <i>et al.</i> , 2014) 7-48% removal in hybrid wetlands (48)	>1-log removal seen between septic tank and groundwater (Phillips <i>et al.</i> , 2015)	Removal to below detection limits via exposure to UV254 and H <sub>2</sub> O <sub>2</sub> at 50 mg/L for 10 minutes (De la Cruz <i>et al.</i> , 2012)
VEN, anti-depressant	<p><i>Pharmacokinetics:</i> 5% excreted unchanged in urine</p> <p>Major metabolites: O-desmethyl-VEN (ODV) (30%), VEN-glucuronide (25%), N,O-didesmethyl-VEN (N,O-DDM-VEN) (10%)</p> <p>Minor metabolites: N,O-didesmethyl-VEN-glucuronide, N-desmethyl-venlafaxine (NDV)</p> <p>(Howell <i>et al.</i>, 1993)</p> <p><i>Major species in WWTPs:</i> VEN, ODV, V2 [C<sub>17</sub>H<sub>24</sub>NO]<sup>+</sup></p> <p>(Boix <i>et al.</i>, 2016)</p>	<p>Negligible removal of VEN and 30% increase in ODV in biological WWTP with chemical P removal (Gurke <i>et al.</i>, 2015)</p> <p>37-56% removal of VEN and 29-41% removal of ODV by conventional activated sludge treatment (Rua-Gomez <i>et al.</i>, 2012)</p>		25-81% removal of VEN; 9-83% removal of ODV; 13-72% removal of NDV; 6-75% removal of N,O-DDM-VEN in SSF and 2 pond wetlands (Ruhmland <i>et al.</i> , 2015)	>2-log removal of VEN seen between septic tank and groundwater (Phillips <i>et al.</i> , 2015)	<p>Effective removal via post-treatment ozonation with ~0.6 g O<sub>3</sub> g<sup>-1</sup> DOC (Hollender <i>et al.</i>, 2009)</p> <p>&gt;80% removal VEN and &gt;95% removal ODV via ozonation; &gt;70% removal VEN and &gt;92% removal ODV via GAC columns (Rua-Gomez <i>et al.</i>, 2012)</p>

### 3.4.2 Removal in Septic Tank and Influent Piping

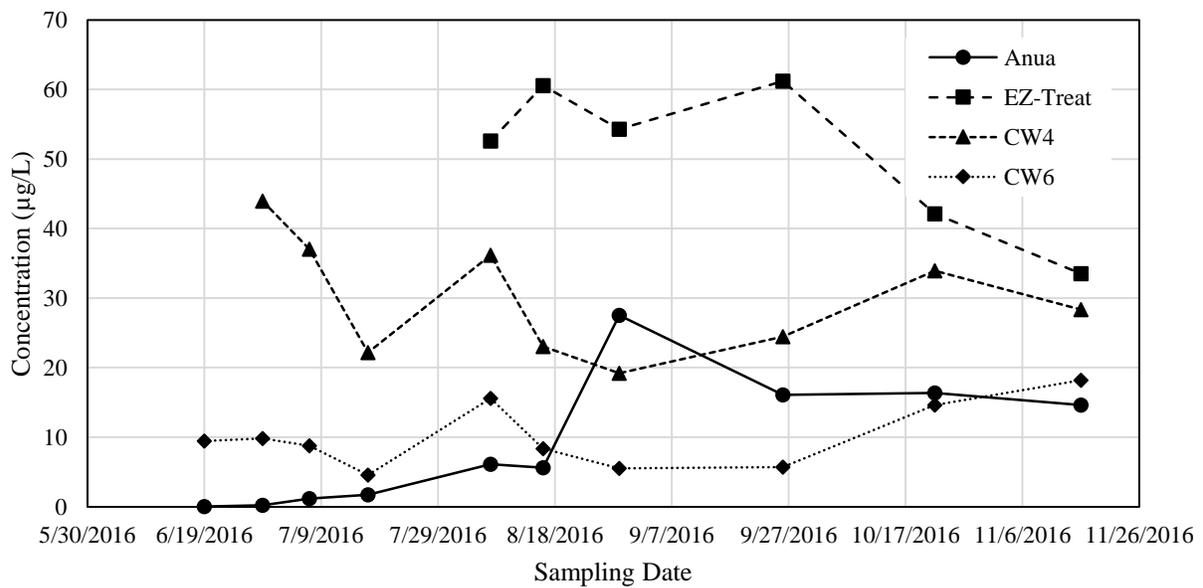
STE samples were collected at the influent pipe to the CW Tank 1. It was expected that samples collected at this location would represent influent samples to each system because water traveled through the same length of pipe to get to each system; however, some of the results suggest otherwise. The PPCP concentrations in the STE on the first sampling date were three to 15 times greater than the average concentration on all other dates. This could reflect evolution of microbial degradation capability for the biology living inside the pipes between the septic tank and the treatment systems. For most of the PPCPs, STE concentrations showed no trends over times after the first sampling date was removed. However, ACE, CBZ, and VEN concentrations continued to decrease in the STE during the first couple months of operation, as shown in Figure 3.2. The CW had been receiving STE for much longer than the E-Z Treat, and especially the Anua systems prior to sampling (2.5 months instead of 1.75 and 1, respectively). Therefore, if significant PPCP degradation was occurring in the piping between the septic tank and the treatment systems, the Anua and E-Z Treat systems could have been receiving higher loadings of PPCPs than the CW. This should be taken into consideration when looking at the results.

Figure 3.13, Figure 3.14, and Figure 3.15 plot the ACE, CBZ, and VEN concentrations, respectively, for the treated effluents over time and demonstrate that there were no obvious downward trends for the Anua or CW effluent concentrations. However, E-Z Treat concentrations decreased with time for all three PPCPs, as well as for MET and TCS (not shown). The trend of decreasing effluent concentrations could be a result of microbial adaption to degrade those PPCPs either in the E-Z Treat influent or within the E-Z Treat module itself. In either case, there is reason to expect that the E-Z Treat performance would improve over time. The Anua Well 1 effluent, reflecting the performance of the Anua ATU, also had decreasing PPCP concentrations over time for CBZ, MET, TCS, and VEN, although no decreasing trends were seen for the Anua system effluent. These observations suggest that the treatments relying on microbial degradation may show adaptation to wastewater-borne PPCPs and increased removal rates over time. For CBZ concentrations in the Anua ATU effluents, the trends were apparent even after 6 months of operation.



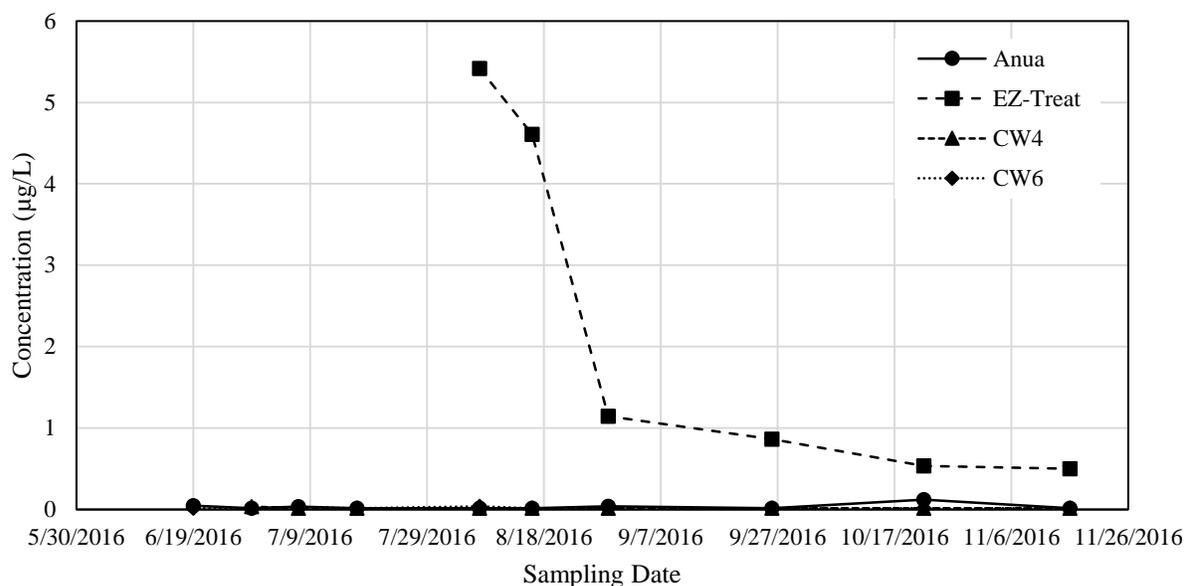
**Figure 3.13: ACE concentrations in treated effluent over time**

ACE = acetaminophen; CW4 = effluent from constructed wetland tank 4; CW6 = effluent from constructed wetland tank 6.



**Figure 3.14: CBZ concentrations in treated effluent over time**

CBZ = carbamazepine; CW4 = effluent from constructed wetland tank 4; CW6 = effluent from constructed wetland tank 6.



**Figure 3.15: VEN concentrations in treated effluent over time**  
 VEN = venlafaxine; CW4 = effluent from constructed wetland tank 4; CW6 = effluent from constructed wetland tank 6.

### 3.4.3 Removal Mechanisms in Treatment Systems

#### 3.4.3.1 Acetaminophen (ACE) and Triclosan (TCS)

There is a significant body of research suggesting that both ACE and TCS are readily degraded and even mineralized in wastewater treatment (Federle, 2002; Godfrey *et al.*, 2007; Conkle *et al.*, 2008; Avila *et al.*, 2013; Avila *et al.*, 2014; Du *et al.*, 2014; Phillips *et al.*, 2015). ACE can be degraded under both aerobic and anaerobic conditions, while TCS reportedly requires aerobic conditions for degradation (McAvoy, 2002; Avila *et al.*, 2013; Du *et al.*, 2014). The results of this study were somewhat consistent with previous findings. ACE had median removal rates of 99% or more in the Anua ATU and the aerobic E-Z Treat biofilter, as well as the CW Tank 1, which had an influent and effluent DO below 1 mg/L. TCS had removals over 90% for the aerobic treatment units of Anua and E-Z Treat. Despite low oxygen availability in CW Tank 1, 82% removal of TCS were achieved. Overall removals in the CW increased to 89% and >94% at the effluent of Tanks 2 and 3, respectively.

### 3.4.3.2 *Levetiracetam (LEV)*

The research on removal of LEV in wastewater treatment is limited, but suggests that at least the parent compound is removed by conventional wastewater treatment (Gurke *et al.*, 2015). It is unknown whether any LEV metabolites persist through wastewater treatment. The Anua and both CW effluents showed removal rates of >99% for this compound, while E-Z Treat removals ranged from 75 to 91%. The Anua ATU alone achieved 98% removal of LEV, but within the CW, only 11% removal was achieved in Tank 1 compared with overall removals of 60% and 98% in the Tank 2 and 3 effluents, respectively. The findings suggest that LEV requires aerobic conditions to be significantly degraded.

### 3.4.3.3 *Carbamazepine (CBZ) and Lamotrigine (LAM)*

CBZ is notoriously difficult to remove from wastewater except by sorption and advanced oxidation processes; however, there is a relatively large body of research available for this chemical (Joss *et al.*, 2006; Yu *et al.*, 2008; Dordio *et al.*, 2009; Hollender *et al.*, 2009; De la Cruz *et al.*, 2012; Du *et al.*, 2014). CBZ metabolites like trans-10,11-dihydroxy-10,11-dihydro-CBZ (DiOHCBZ) and 10,11-dihydro-10-hydroxy CBZ (MHD) are likely to be present in raw and treated wastewater at similar, if not higher, concentrations as the parent compound. Many other metabolites may be generated in the wastewater treatment process, and most of them have been found to have similar microbial toxicity as the parent compound (Kaiser *et al.*, 2014). Although LAM has received less attention in the literature, it has been shown to be just as persistent as CBZ, if not more so, given its resistance to degradation via ozonation and UV light (Hollender *et al.*, 2009; Keen *et al.*, 2014; Zonja *et al.*, 2016). LAM metabolites found in WWTPs are likely generated from the primary human metabolite, N-2 glucuronide, and not from the parent compound (Zonja *et al.*, 2016).

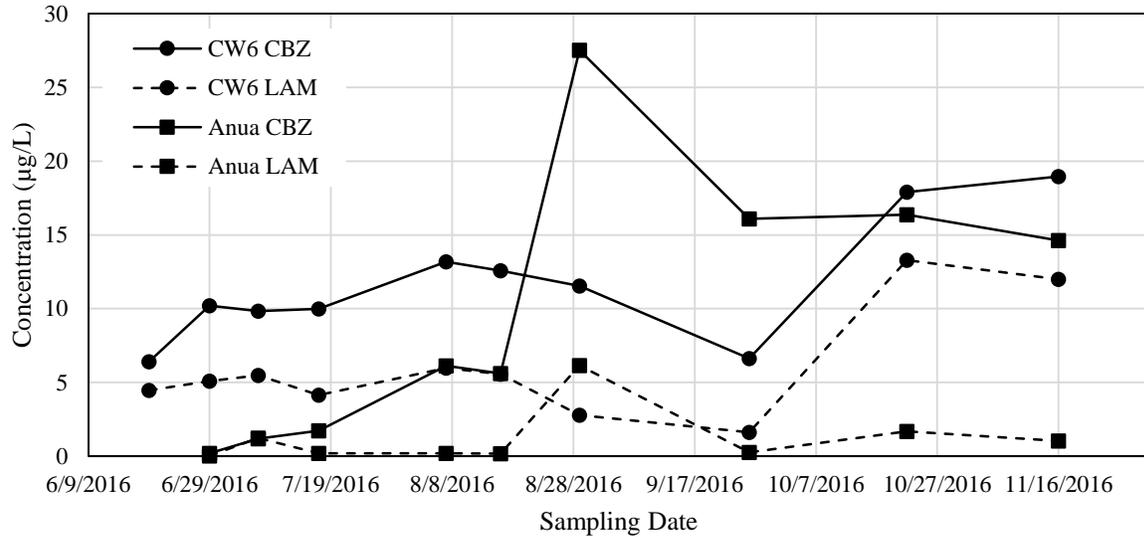
In this study, CBZ was found to resist removal in the E-Z Treat biofilter (-11%), Anua ATU (-1.5%), and CW between Tanks 1 and 4 (41%). Like, CBZ, LAM resisted removal in the E-Z Treat biofilter (-14%), Anua ATU (-4%), and CW between Tanks 1 and 4 (55%). However, much higher removal rates were seen for the Anua peat filters (99.8% overall) and the CW mycoremediation Tanks 5 and 6 (96% overall). Both compounds have been shown to be very susceptible to sorption, so the observed removal in these organic-rich tanks is not surprising (Yu *et al.*, 2008; Dordio *et*

*al.*, 2009; Paz *et al.*, 2016). The relatively high removals seen in CW4 could have been as a result of sorption to the thin mulch layers on the surface of Tanks 3 and 4. Although the pH and point of net zero charge (PNZC) is not known for the peat used in this study, peats typically have low pHs and net negative charge at neutral pH (Delicato, 1996). The pH and PNZC of the wood chip/mulch media in the mycoremediation tanks is not known, but the pH of wood chips is generally low (less than 6) with lower values observed as moisture content increases (Sitholé, 2005).

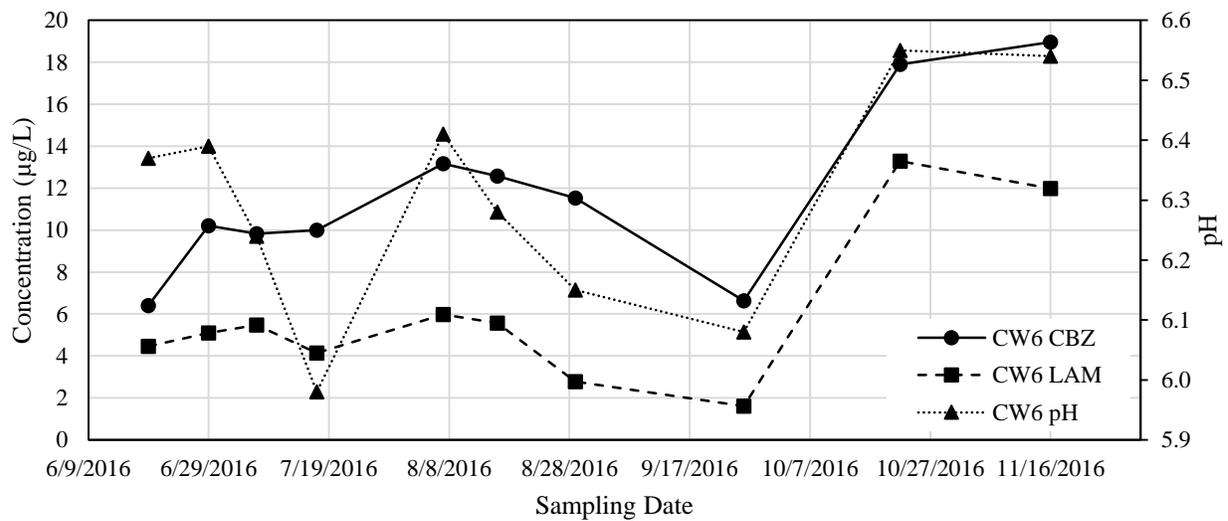
CBZ is uncharged at neutral pH ranges ( $pK_a = 13.9$ ), but its structure is highly polarizable so the chemical likely has extensive van der Waals interactions with charged surfaces (Dordio *et al.*, 2009). LAM has a  $pK_a$  of 5.7, so the fraction of positively charged LAM in solution would increase as the solution pH decreased. It has been suggested that the presence of a triazine ring and amino groups on its structure would allow for hydrogen-bonding with functional groups on soil organic matter (Paz *et al.*, 2016). Although LAM has a relatively low  $\text{Log } K_{ow}$  value (0.99), the ability to form hydrogen bonds with functional groups on soil organic matter increases its sorptive ability relative to other compounds with similar  $\text{Log } K_{ow}$  values. Paz *et al.* (2016) found that LAM and CBZ were strongly sorbed by soils and that sorption increased with soil organic matter content. CBZ metabolites CBZ-E and DiOHCBZ had weaker interactions with soil, which was predicted based on their lower  $\text{Log } K_{ow}$  values compared with CBZ (1.97 and 0.81, respectively).

There were also some notable trends for the effluent concentrations of CBZ and LAM which further support sorption as a removal mechanism in the CW mycoremediation tanks and the Anua peat filters. First, effluent concentrations of CBZ and LAM increased with time in the effluents from both systems even as they decreased in the STE and E-Z Treat effluents. See Figure 3.16 below. Significant linear correlations with positive slopes were found for CW4 with CBZ and LAM and for the Anua system with CBZ only ( $p < 0.1$ ). This could indicate that sorption capacity was being reduced as the media adsorbed greater quantities of PPCPs and other wastewater constituents. Alternatively, the sorption patterns could have been tracking pH trends, as pH increased for both systems on the last two sampling dates. Since pH affects surface charge on media and ionization states of dissolved chemicals, it can influence the degree of sorption. In addition, CBZ and LAM concentrations in the CW6 effluent, but not the Anua effluent, showed a strong correlation with pH ( $p < 0.01$ ). See Figure 3.17 below. Note that unadjusted concentration

values were used for CW6 effluents because sorption behavior would be affected by the actual dissolved concentration in the tank, not the mass loading. The implications of this pH correlation will be discussed with respect to mycoremediation, below.



**Figure 3.16: CBZ and LAM concentrations in CW6 and Anua effluents over time**  
 CBZ = carbamazepine; LAM = lamotrigine; CW6 = effluent from constructed wetland tank 6.



**Figure 3.17: Relationship between CW6 effluent pH CBZ and LAM concentrations**  
 CBZ = carbamazepine; LAM = lamotrigine; CW6 = effluent from constructed wetland tank 6.

It is also possible that fungal degradation played a role in removal of CBZ and LAM in the mycoremediation tanks. The literature on this removal mechanism will be reviewed in greater

detail below, along with observations of fungal activity during the course of the study. However, it should be noted that no clear indication of mycoremediation exists due to lack of monitoring data on fungal metabolites or enzymes and the lack of an appropriate control trial.

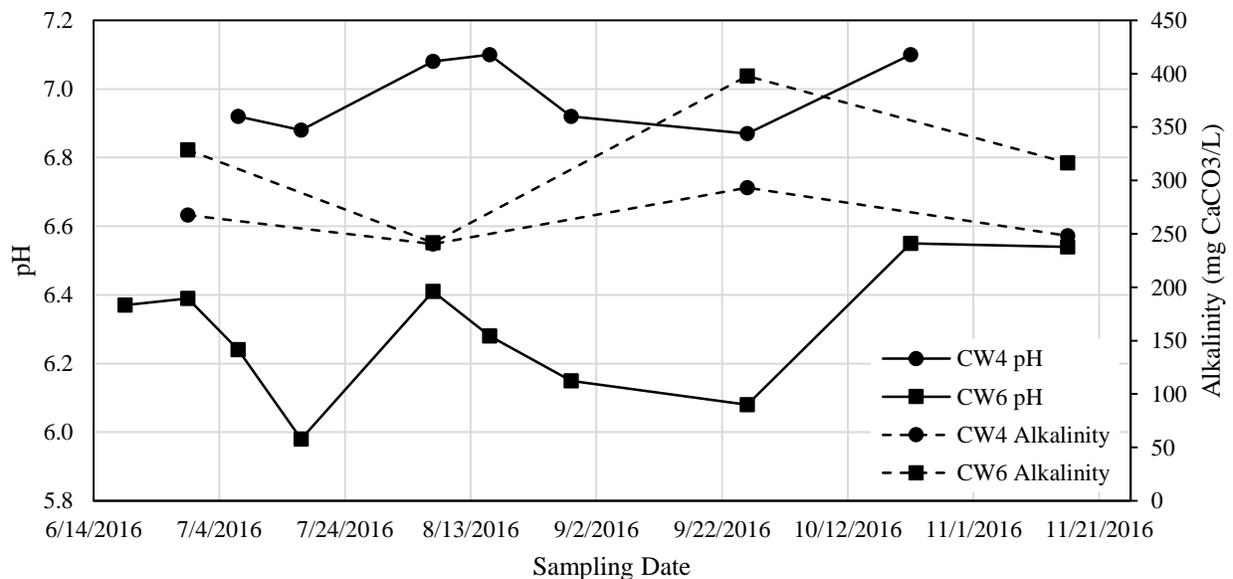
The ability of white rot fungi (WRF) to degrade TOrCs in wastewater byproducts has been widely reported (Marco-Urrea *et al.*, 2009; Rodriguez-Rodriguez *et al.*, 2010; Jelic *et al.*, 2012; Aydin, 2016;). WRF are members of the phyla Basidiomycota and have adapted to live in wood and degrade lignin and even cellulose, some of nature's most durable organic products, using nonspecific extracellular enzymes such as laccase, manganese peroxidase, lignin peroxidase, and versatile peroxidases as well as intracellular mechanisms. Harms *et al.* (2011) reviewed the known extracellular and intracellular fungal enzymes responsible for contaminant degradation. Extracellular enzymes generate organic radicals in the presence of molecular oxygen, peroxide, and/or manganese (II) and usually require low pHs (around 5) to be effective. Diverse intracellular cytochrome P450 oxidases are known to be able to degrade contaminants and/or their metabolites which have already been generated by extracellular enzymes (Harms *et al.*, 2011). Marco-Urrea *et al.* (2009) found that the cytochrome P450 system in *Trametes versicolor* was more active in the initial degradation step of carbamazepine and clofibric acid any extracellular enzymes.

Multiple studies have shown that CBZ can be degraded by mycoremediation (Marco-Urrea *et al.*, 2009; Rodriguez-Rodriguez *et al.*, 2010; Jelic *et al.*, 2012). This might be expected given the sensitivity of CBZ to advanced oxidation processes (Hollender *et al.*, 2009; De la Cruz *et al.*, 2012). Although no studies could be found demonstrating LAM degradation by fungi, LAM is somewhat sensitive to hydroxyl radicals, so theoretically it could be degraded under biological advanced oxidation processes (Keen *et al.*, 2014). Keen *et al.* (2014) found that 40% of LAM could be removed via UV-H<sub>2</sub>O<sub>2</sub> advanced oxidation with 10 mg/L H<sub>2</sub>O<sub>2</sub> and 500 mJ/cm<sup>2</sup> with a low-pressure UV lamp, representing a hydroxyl radical reaction rate of  $2.1 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ . The reaction rate for CBZ is estimated to be four times faster, at  $8.02 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  (Wols and Hofman-Caris, 2012).

CW Tanks 5 and 6 were originally inoculated with local strains of two different WRF species: *T. versicolor* and *Pleurotus ostreatus*. However, the dominant mushrooms within the tanks evolved to other species in July and August, including *Coprinus comatus*, which is a WRF species believed

to prefer colonizing woody media that has already been degraded by more aggressive WRF (such as *T. versicolor* and *P. ostreatus*) or brown rot fungi (Oliver *et al.*, 2010). Fungal blooms were observed in the mycoremediation tanks between May and August, but no later. Very small amounts of mycelium were observed in the first 3 inches of the tanks in the fall. CW Tanks 3 and 4 were covered with a thin layer of mulch inoculated with *P. ostreatus*, and similar patterns of blooms were seen in these tanks, but not after July.

Oxalic, formic, and citric acids have been identified as fungal metabolites, and each of these organic acids would increase alkalinity by buffering solutions at the titration endpoint for alkalinity determination (pK<sub>a</sub>s of 4.27, 3.75, and 4.76) (Gadd, 1999; Schilling, 2006; Harms *et al.*, 2011). Figure 3.18 shows the relationship between the effluent pH and alkalinities of CW Tanks 4 and 6. It is possible that the increased alkalinity and decreased pH of CW6 relative to CW4 indicates the presence of fungal metabolites such as these organic acids. It should also be noted that the CW4 and CW6 effluent pHs were highly correlated ( $p < 0.05$ ), and this could reflect mirrored fungal activity in CW Tanks 3 and 4 and Tanks 5 and 6.



**Figure 3.18: Relationship between CW4 and CW6 effluent pH and alkalinity**  
 CW4 = effluent from constructed wetland tank4; CW6 = effluent from constructed wetland tank 6.

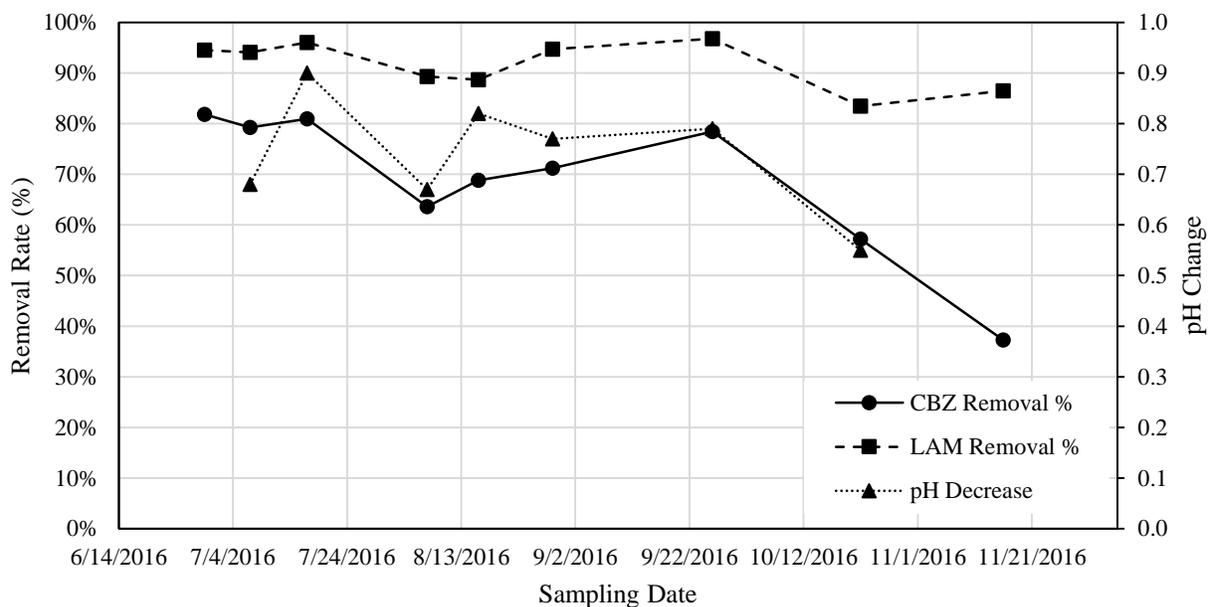
A significant correlation was also found between the change in pH and the removal rate (%) of both CBZ and LAM between CW Tanks 4 and 6 ( $p < 0.1$ ). See Figure 3.19 below. It was already

demonstrated that the CW6 effluent pH was significantly correlated with effluent CBZ and LAM concentrations (see Figure 3.17). However, the *change* in pH between CW Tanks 4 and 6 could be an indicator of fungal activity, and increased removal rates between Tanks 4 and 6 could indicate increased removal by mycoremediation. Unadjusted concentration values were used because the ratio of estimated CW Tank 5 influent to Tank 6 effluent flows were close to 1 throughout the sampling period. The slopes of the linear correlations were found to be significant ( $p < 0.1$ ) for each compound and were equal to 5.5% CBZ removal per pH unit and 3.0% LAM removal pH unit. Since CBZ is more sensitive to hydroxyl radicals, it is logical that the slope should be steeper for CBZ than for LAM (Wols and Hofman-Caris, 2012; Keen *et al.*, 2014). Intercepts were not significant for either compound. R code is included in Appendix C.

Analogous correlations were not found for Anua Well 1 and the Anua effluent. Although the peat does not serve as a perfect control for fungi-inoculated wood chips, this observation has some value since both materials have high organic matter content and are expected to have similar pHs.

To conclude, the following observations are potential indications of fungal activity:

1. Observation of inky caps, which usually can only survive on woody debris that has already been significantly degraded by more aggressive mushrooms
2. Increased alkalinity and reduced pH in CW6 relative to CW4
3. CW6 effluent pHs showing a negative correlation with alkalinity, and
4. Significant correlation between pH change and effluent removal rates of CBZ and LAM between CW Tanks 4 and 6.



**Figure 3.19: Correlation between CBZ and LAM removal rates and pH change between CW4 and CW6**  
 CBZ = carbamazepine; LAM = lamotrigine; CW4 = effluent from constructed wetland tank 4; CW6 = effluent from constructed wetland tank 6.

#### 3.4.3.4 Metformin (MET) and Topiramate (TOP)

Both MET and TOP were detected at very low concentrations in the STE, making it difficult to assess the performance of any of the treatment systems with regard to their removals. MET was detected in all STE samples but always at concentrations less than 0.150 µg/L, while TOP was only detected in two samples. Low concentrations were expected because none of the residents in the house serving the septic tank consume these pharmaceuticals regularly. The presence of these chemicals in the septic tank therefore indicated contribution by visitors.

MET is readily removed in WWTPs, however removal is expected to represent conversion to guanylurea, which is persistent and polar (Scheurer *et al.*, 2009; Trautwein and Kummerer, 2011; Scheurer *et al.*, 2012). Although researchers have found both MET and guanylurea to be partially removed by chlorination and ozonation (byproducts not assessed), the most effective remediation strategy found was riverbank filtration or artificial groundwater recharge (Scheurer *et al.*, 2012). Metabolites were not investigated in the filtrate. Researchers postulated that removal during this treatment process was via biological degradation, since MET and guanylurea were not

significantly sorbed to any of three soils tested. However, both MET and guanylurea are cations at neutral pH and have the ability to form hydrogen bonds with functional groups on organic matter, so both could be subject to sorption via net negatively charged peat.

There has been very little research into TOP behavior in WWTPs in the literature. TOP is largely unchanged during human metabolism and it is likely that glucuronide metabolites are converted back to the parent compound in WWTPs via hydrolysis, as has been observed for other pharmaceuticals (Zonja *et al.*, 2016). Gurke *et al.* (2015) found less than 1-log removal of the compound in a biological WWTP with chemical phosphorus removal, however the researchers did not look for metabolites.

#### **3.4.3.5 Venlafaxine (VEN)**

Studies of VEN and metabolites in WWTP samples indicate that ODV is typically present at much higher concentrations than VEN – up to six times as high as the corresponding ODV concentration (Gasser *et al.*, 2012; Boix *et al.*, 2016). Gasser *et al.* (2012) found that VEN was converted stoichiometrically to ODV under anaerobic conditions, and that ODV was not substantially degraded further under these conditions. Under aerobic conditions, ODV was the primary degradation product of VEN and vice versa, but a mass balance was not obtained. It is possible that a VEN metabolite, V2, found by Boix *et al.* (2016) in 30 of 30 WWTP influents (in which VEN and ODV were also detected), was the missing metabolite in the Gasser *et al.* (2012) study.

Based on the reviewed research it can be inferred that the total mass loading of VEN and all metabolites was likely much higher than what was measured in the current study, because VEN was probably converted to ODV under the anaerobic conditions in the piping between the septic tank and the treatment systems. The removals of VEN seen in CW Tank 2 could have represented conversion to metabolites ODV or V2. The increase in VEN concentration seen in the Anua ATU could have been conversion of ODV to VEN, with the removals seen in the peat filters representing conversion back to a metabolite or removal via sorption. Both VEN and ODV have relatively high Log  $K_{ow}$  values (3.20 and 2.72, respectively) so sorption would be a viable removal mechanism for either compound.

### 3.5 Summary and Conclusions

In this study, the performance of three alternative onsite treatment systems was compared as they treated STE over the course of seven months. The treatment systems included a hybrid CW (CW4, HRT = 23 days) with mycoremediation filters (CW6, HRT = 6 days), an ATU with peat fiber biofilters (Anua, HRT = 10-15 days), and a styrene bead biofilter (E-Z Treat, HRT = 5-6 days).

As determined by ANOVA analyses, the CW4 was the only treatment system in the highest performing group for all analyzed conventional pollutants. The Anua system was also in the highest group for all conventional pollutants when ANOVA analyses were performed using an abridged data set collected prior to operational changes in August. The Anua, E-Z Treat, and CW4 systems had the highest removal rates for oxygen demand, while the CW4 and CW6 systems had the highest removal rates for nitrogen. BOD and TSS concentrations were consistently under 10 mg/L for all treatments beyond the septic tank, with the exception of CW6 as a result of its wood chip media. Average NH<sub>4</sub> concentrations were below 0.5 mg N/L for Anua, CW4, and CW6, and below 2 mg N/L for E-Z Treat.

The Anua system experienced an operational disruption in late August after its feed pumps were changed and BOD loading dropped. After the change, denitrification ceased and BOD increased slightly but performance remained consistent in terms of TSS and NH<sub>4</sub> removal. The system recommenced denitrification by the last sampling date in November. The CW consistently nitrified and denitrified throughout the sampling period with average TN of 2 mg N/L for both CW4 and CW6. The CW4 TN concentration jumped to 7 mg N/L on the last sampling date in November, indicating that a drop in nitrogen removals could be expected over the winter. The E-Z Treat system was not equipped for denitrification but achieved nitrification consistently throughout the sampling period.

Performance was also compared with regard to removal of eight PPCPs. Removal of metabolites was not monitored. Per ANOVA analyses, Anua and CW6 were in the highest performing group for all analyzed PPCPs. Average effluent concentrations were lower for the Anua samples collected prior to the operational disruption in all cases except for TCS, however no significant differences could be proved between the abridged and complete data sets.

The entire range of removal rates was above 75% for ACE, LEV, and TCS in all treatment systems, and for VEN in all treatment systems except for E-Z Treat. Median removal rates for CBZ varied widely from -10.9% for E-Z Treat to 87.3% for the Anua system over the entire sampling period and 96.4% for the Anua system prior to August 22<sup>nd</sup>. Median removals for LAM had a similar range, from -13.8% for E-Z Treat to 99.8% for the Anua system (both sampling ranges). Both MET and TOP were detected at very low concentrations in the STE, making it difficult to assess the performance of any of the treatment systems with regard to their removals. MET was detected in all STE samples but always at concentrations less than 0.150 µg/L, while TOP was only detected in two samples. This was expected because none of the residents in the house serving the septic tank consume these pharmaceuticals regularly. MET had average removal rates above 70% for all treatment systems; however, Anua and E-Z Treat removals could not be significantly distinguished from zero.

Although it was in the lowest performing group for all PPCPs where performance could be significantly differentiated, E-Z Treat system showed improved removal over time for ACE, CBZ, MET, TCS, and VEN. The observed trends could have been a result of microbial adaption to degrade those PPCPs either in the E-Z Treat influent piping or within the E-Z Treat module itself. For either case, there is reason to expect that the E-Z Treat performance would have improved over time. The Anua Well 1 effluent, reflecting the performance of the Anua ATU, also had decreasing PPCP concentrations over time for CBZ, MET, TCS, and VEN, although analogous trends were not seen for the Anua effluent. These observations suggest that treatments relying on microbial degradation may have increased removal rates the longer they are operated. For CBZ concentrations in the Anua ATU effluents, the trends were apparent even after 6 months of operation.

Removal mechanisms for each PPCP could be inferred based on where each was removed. Internal sampling of the CW and the Anua system made this exercise possible. ACE was removed at average rates > 99% in all systems and was well-removed in both the first tank of the CW (average effluent DO below 1 mg/L) and the ATU of the Anua system (average effluent DO above 7 mg/L), indicating removal under anaerobic/anoxic as well as aerobic conditions. TCS was removed by over 90% for the aerobic biological units of Anua and E-Z Treat. Despite low oxygen availability

in CW Tank 1, 82% removal of TCS were achieved. Overall removals in the CW increased to 89% and >94% at the effluent of Tanks 2 and 3 (average effluent DO above 6 mg/L), respectively. Results for ACE and TCS reflected findings from other studies in the literature, although TCS showed surprisingly high removal rates under low oxygen conditions (McAvoy, 2002; Avila *et al.*, 2013; Du *et al.*, 2014). LEV appeared to be degraded only under aerobic conditions, achieving 98% removal in the Anua ATU, compared with 11% removal in CW Tank 1. This was consistent with other findings the literature, but unfortunately, no studies considering LEV metabolites from wastewater treatment could be found (Gurke *et al.*, 2015).

CBZ and LAM were primarily removed in the CW mycoremediation tanks and the Anua peat filters. Expected removal mechanisms include sorption for both treatment systems and potentially fungal degradation for the CW mycoremediation tanks. Due to lack of monitoring data on fungal metabolites or enzymes and the lack of an appropriate control trial, it is not possible to definitively prove fungal degradation as a removal mechanism. However, patterns of pH and alkalinity changes between CW4 and CW6, observed fungal species succession, as well as a significant correlation between CBZ and LAM removal rates with pH change between these two tanks suggest fungal degradation as a mechanism.

The limited measurements of MET and TOP from the study make it difficult to theorize about removal mechanisms. However, it is likely that all MET removals represented generation of its persistent metabolite, guanylurea, although sorptive removal in the CW mycoremediation tanks or Anua peat filters is also possible (Scheurer *et al.*, 2009; Trautwein and Kummerer, 2011; Scheurer *et al.*, 2012). VEN removals were observed mainly in the CW Tank 2 and the Anua peat filters; however, findings from the literature suggest that observed removals represent conversion to similarly persistent compounds (Gasser *et al.*, 2012; Gurke *et al.*, 2015; Boix *et al.*, 2016).

The results from this study indicate that the Anua and CW treatment systems show promise for onsite remediation of PPCPs. Although it is likely that PPCP remediation capacity of the E-Z Treat system would improve with time, it is doubtful that this system would be able to achieve the high removal rates seen in the other two systems, particularly for persistent PPCPs CBZ and LAM. However, the E-Z Treat system may be a good secondary treatment system to install for communities considering advanced tertiary treatment like advanced oxidation or activated carbon

filtration, because of its consistent performance, aerobic treatment for EDC removal, low effluent BOD and TSS concentrations, and lack of solid waste production. If recirculation through an anoxic zone was included in this treatment option, denitrification and potentially even enhanced PPCP removal could be achieved.

It would be extremely useful to conduct additional experiments with these two systems in which:

- The Anua system received a higher and more consistent organic matter loading
- Effluent from CW mycoremediation tanks was polished in gravel media wetland filter to remove high BOD (for example, move the CW Tank 4 from this study after the mycoremediation tanks)
- The CW was operated through the winter to quantify the effect of lower temperatures of PPCP and nitrogen removals
- Fungal activity was quantified by monitoring effluents or tank contents for fungal metabolites

Finally, before considering implementation of either the Anua peat fiber biofilters or the CW, disposal options must be considered for solid waste products generated during treatment, including peat for the Anua system and plant trimmings and wood chip media for the CW. It would be valuable to measure whether there is any degradation of sorbed PPCPs in either media over time.

### **3.6 Acknowledgements**

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## 4 Conclusions

This report consisted of two articles. Findings from both articles indicated that it will not be possible to completely degrade or even remove all TOrCs using onsite wastewater treatment systems because of the limits on infrastructure costs and available land. However, the aim of such systems should be to minimize release of TOrCs with wide varieties of chemical properties, and to prevent accumulation of potentially persistent TOrCs in the natural environment.

It is important to note that alternative treatment may be unnecessary in many onsite applications. The TOrC remediation capacity of septic fields is not well understood, likely because it relies on highly site-specific factors, including soil composition, soil porosity, groundwater depth, aquifer characteristics, and surface water proximity. Based on findings in the literature in which groundwater contamination by TOrCs has been identified, it is likely that sites with one or more of the following characteristics are susceptible to contamination of ground and surface water resources:

- Sandy or porous soils
- Shallow aquifers and wells
- Location in area of dense onsite treatment systems
- Unusually high pharmaceutical consumption rates

Sites with these characteristics should consider whether alternative treatment strategies are necessary for TOrC remediation. However, one study in the literature has called into question the utility of certain alternative treatment options at sites with moderately-drained septic field soils and groundwater tables more than 180 cm (6 ft) below septic field distribution lines (Conn and Siegrist, 2009). Alternative treatment is unnecessary at sites that have large enough groundwater flow rates to sufficiently dilute TOrCs transported through the septic field before they reach drinking water wells. For aquatic organisms and humans, the concentration of a chemical within water dictates the rate of consumption of that chemical, and therefore the toxicity. When TOrCs cross the lower boundary of the septic field into groundwater, they are dissolved in a relatively large body of water compared to original volume of water discharged to the septic field. This dilution may be enough to eliminate toxicity concerns, depending on the flow rate of groundwater

below the septic field and the degree of mixing in groundwater between the point of entrance and the receptor. Detailed knowledge of aquifer characteristics may allow for the approximation of the maximum possible concentration of TOrCs in the groundwater, given septic tank effluent concentrations and wastewater discharge rates. This may be a valuable tool for risk assessment at sites where septic fields have not reached steady-state in terms of sorption.

Since CS tanks only provide opportunities for anaerobic or facultative degradation and limited sorption to largely organic solids, additional treatment units should provide different removal mechanisms, including aerobic degradation, photodegradation, or enhanced sorption to organic and inorganic solids. When adequate septic fields are available, additional treatment units should provide removal mechanisms beyond those available in the septic field, including redox cycling to support microbial degradation (Conn and Siegrist, 2009). It may also be valuable to provide a confined, solid-state matrix, analogous to the septic field but physically separated from downstream environments, to prevent TOrC accumulation in soils and impacts to terrestrial environments, especially in agricultural applications.

Treatment units that provide permanent removal of TOrCs and potentially bioactive byproducts should be valued more highly than those that only provide temporary removal mechanisms. However, it is also undesirable for treatments to generate large, concentrated waste streams that will be costly or difficult to dispose of, or that will represent a new source of TOrCs in the environment. Thus ideal sorption would not irreversibly bind TOrCs, but instead slow their movement through the treatment unit to allow time for degradation into innocuous byproducts. Of course, the inherent limitations to onsite wastewater treatment must also be considered when choosing onsite systems. Therefore, advanced treatment options like advanced oxidation or reverse osmosis may not be economically feasible and would require enhanced treatment of STEs.

Findings from the literature clearly indicate that both wetlands and ETUs have a greater capacity for TOrC remediation than CS tanks alone (Matamoros *et al.*, 2009; Stanford and Weinberg, 2010; Du *et al.*, 2014). Furthermore, wetlands and ETUs that include aeration, either forced or through the passive use of unsaturated filters, have been found to be more effective for the removal of TOrCs, particularly EDCs (Stanford and Weinberg, 2010).

All of the reviewed comparison studies indicated slightly better performance in vegetated systems relative to ETUs, but statistical significance was not demonstrated in any of them (Matamoros *et al.*, 2009; Stanford and Weinberg, 2010; Du *et al.*, 2014). Similar conclusions were drawn in the pilot study described in Chapter 3. The considered treatment systems included a hybrid constructed wetland (CW4, HRT = 23 days) with mycoremediation post-treatment (CW6, HRT = 6 days), an ATU with peat biofilter post-treatment (Anua, HRT = 10-15 days), and a styrene bead biofilter (E-Z Treat, HRT = 5-6 days). No metabolites were monitored in the study, so removal rates do not necessarily represent permanent removal. As determined by ANOVA analyses, Anua and CW6 were in the highest performing group for all analyzed PPCPs. These systems exhibited excellent removal rates, including 100.0% for ACE, 99.8% for LEV, >73.5% for MET, >93.5% for TCS, and >99.0% for VEN (all reported values represent medians; MET, TCS, and VEN were removed below detection). Removals for CBZ and LAM were particularly notable: > 80% for CBZ and > 95% for LAM. It is believed these removals were primarily due to sorption in the Anua peat filters and the CW mycoremediation tanks. Due to lack of monitoring data on fungal metabolites or enzymes and the lack of an appropriate control trial, it is not possible to definitively prove fungal degradation as a removal mechanism. However, patterns of pH and alkalinity changes between CW4 and CW6, observed fungal species succession, as well as a significant correlation between CBZ and LAM removal rates with pH change between these two tanks suggest fungal degradation as a mechanism.

All of the treatment systems had excellent performance with regards to conventional pollutants. As determined by ANOVA analyses, the CW4 produced the best quality effluent in terms of BOD, COD, NH<sub>4</sub>, TIN, and TN. The Anua, E-Z Treat, and CW4 systems had the highest removal rates for oxygen demand, while the CW4 and CW6 systems had the highest removal rates for nitrogen. BOD and TSS concentrations were consistently under 10 mg/L for all treatments beyond the septic tank, with the exception of CW6 as a result of its wood chip media. Average NH<sub>4</sub> concentrations were below 0.5 mg N/L for Anua, CW4, and CW6 and below 2 mg N/L for E-Z Treat.

The results from this study indicate that the Anua and CW treatment systems show promise for onsite remediation of PPCPs. They meet many of the qualitative metrics established in the first article of this report, providing a confined solid state matrix to prevent accumulation in the

terrestrial environment, as well as redox cycling and opportunities for enhanced aerobic degradation relative to the septic field. Although it is likely that PPCP remediation capacity of the E-Z Treat system would improve with time, it is extremely doubtful that this system would be able to achieve the high removal rates seen in the other two systems, particularly for persistent PPCPs CBZ and LAM. However, the E-Z Treat system may be a good secondary treatment system for communities considering advanced tertiary treatment like advanced oxidation or activated carbon filtration, because of its consistent performance, aerobic treatment for EDC removal, low effluent BOD and TSS concentrations, and lack of solid waste production. If recirculation through an anoxic zone was included in this treatment option, denitrification and potentially even enhanced PPCP removal could be achieved.

It would be extremely useful to conduct additional experiments with these two systems in which:

- The Anua system received a higher and more consistent organic matter loading
- Effluent from CW mycoremediation tanks was polished in gravel media wetland filter to remove high BOD (for example, move the CW Tank 4 from this study after the mycoremediation tanks)
- The CW was operated through the winter to quantify the effect of lower temperatures of PPCP and nitrogen removals
- Fungal activity was quantified by monitoring effluents or tank contents for fungal metabolites

Finally, before considering implementation of either the Anua peat fiber biofilters or the CW, disposal options must be considered for solid waste products generated during treatment, including peat for the Anua system and plant trimmings and wood chip media for the CW. It would be valuable to measure whether there is any degradation of sorbed PPCPs in either media over time.

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## Appendix A: Analytical Methods

### A.1 Conventional Pollutants

#### Alkalinity

Use Standard Method 2320 Titration Method for alkalinity analysis. Samples should be refrigerated at or below 6°C and analyzed within 24 hours of collection. At least one sample should be analyzed in duplicate for each sampling event. Use a sample volume of 50 or 100 mL and titrate with 0.02 N standardized sulfuric acid.

Be aware that soaps, oily matter, suspended solids, or precipitates may coat the pH electrode so more time should be allowed for the electrode to come to equilibrium with the sample after each acid addition.

#### Apparatus

1. pH meter with glass electrode that can be read to the nearest 0.05 pH unit.
  - a. Oakton 5+ pH meter
2. Magnetic stirrer
3. Buret
4. Watch glasses
5. Hot plate

#### Reagents

1. Sodium carbonate solution, approximately 0.05 N (do not keep longer than 1 week) made up using a known weight of solid sodium carbonate.
2. Standardized sulfuric acid, approximately 0.02 N.

#### Procedure

1. Standardize the sulfuric acid against 10.00 mL (measured using a volumetric pipet) of the sodium carbonate solution. Titrate to a pH of about 5. Lift out electrodes, rinse into the same beaker, and boil gently for 3 to 5 minutes under a watch glass cover. Cool to room temperature, rinse cover glass into beaker, and finish titrating to pH 4.30. Calculate the

normality of the sulfuric acid using the formula below and find the average of at least duplicate trials. Do this at least once every 4 months of using the same acid, or at least when the acid is first made up and just before it runs out.

$$\text{Normality, eq/L} = \frac{\text{g Na}_2\text{CO}_3 \text{ dissolved in 1 L} \times \text{mL Na}_2\text{CO}_3 \text{ solution used}}{53.00 \times \text{mL of standard acid}}$$

2. Rinse electrodes and titration vessel well with distilled water before using. Adjust sample to room temperature and measure desired volume using a graduated cylinder.
3. Titrate to the end-point pH of 4.3 to 5.0, depending on the alkalinity of the sample, per equation in the calculation section below. Between pH 4.3 and 5.0, make smaller additions of acid. As the end point is approached, make smaller additions of acid and take careful notes. Stop the titration once the sample pH has dropped below 4.3.

#### *Calculations*

Determine the end point pH iteratively using the equations for the end-point pH (derived based on Table 2320:1 in APHA, 1998) and the alkalinity shown below.

$$\text{End Point pH} = \text{Log}(7.27 \times 10^{-11} \times \text{Alk}^2 + 1.19 \times 10^{-7} \times \text{Alk} + 8.79 \times 10^{-6})$$

$$\text{Alkalinity, mg CaCO}_3/\text{L} = \frac{\text{mL of standard acid} \times \text{acid normality} \times 50,000}{\text{mL sample}}$$

#### **Biochemical Oxygen Demand, 5-Day**

Use the Standard Method 5210 5-Day BOD Test. From Crites and Tchobanoglous, 1998, the typical range for BOD<sub>5</sub> in STE is 150 – 250 mg/L. Based on practice samples, the STE is expected to have a BOD<sub>5</sub> of 100 – 150 mg/L. The high and low dilution rates should ensure a DO change of at least 1 mg/L and a minimum final DO concentration of 1 mg/L during the BOD<sub>5</sub> analyses. Use the following formula:

$$BOD_5 = \frac{D_1 - D_2}{P}$$

where  $D_1$  is the initial DO concentration,  $D_2$  is the final DO concentration, and  $P$  is the dilution factor (or mL of sample required per 300 mL total sample size). Assume that  $D_1$  will be 7.8 mg/L based on practice sampling runs. For STE samples, use dilution levels of 0.020 and 0.040 (or 6.0 and 12.0 mL sample sizes, respectively). For treated effluent samples, use dilution levels of 0.03 and 0.20 (or 10.0 and 60.0 mL sample sizes). Three replicates from each dilution level should be used. At least three blanks should be used for each round of sampling.

Samples should be stored at or below 6 °C. If it is not possible to initiate the BOD<sub>5</sub> tests within 6-12 hours of collection, note the duration of storage with the results. In no case should tests be initiated more than 24 hours after collection. Samples and dilution water should be allowed to warm to room temperature before beginning the analysis.

#### *Apparatus*

1. 300 mL incubation bottles having a ground-glass stopper and a flared mouth. Clean bottles with a detergent, rinse thoroughly, and drain before use.
2. Air incubator thermostatically controlled at 20±1 °C.
3. Dissolved oxygen analyzer
  - a. YSI model 57 DO analyzer and 5905 BOD probe

#### *Reagents*

Prepare reagents in advance but discard them if there is any sign of precipitation or biological growth in the stock bottles.

1. Phosphate buffer solution
2. Magnesium sulfate solution, calcium chloride solution, ferric chloride solution
3. Distilled dilution water

#### *Procedure*

1. Measure out an appropriate volume of dilution water and place it in the incubator at least 48 hours before running the test so that its temperature is 20±3 °C before making up the BOD bottles. Protect water quality by using clean glassware, tubing, and bottles. Do not aerate dilution water as this can cause supersaturation.

2. Run a dilution water blank with all samples to ensure the cleanliness of incubation bottles and quality of source water. The DO uptake should not be more than 0.2 mg/L and preferably not more than 0.1 mg/L. Discard all dilution water having a DO uptake greater than 0.2 mg/L.
3. Using a graduated pipet or cylinder, measure three replicates of each dilution level into numbered, 300-mL BOD bottles. For dilutions greater than 1:100, make a primary dilution in a graduated cylinder before making final dilution in the bottle. Fill the reservoir with water and position the plastic cap on the bottle.
4. Add 1 mL each of phosphate buffer, magnesium sulfate, calcium chloride, and ferric chloride solutions per liter of dilution water to the dilution water bottle using a graduated pipet. Swirl to mix the dilution water.
5. Calibrate the DO analyzer. Be sure to apply the 0.93 correction for high elevation.
6. For each set of three replicates, add dilution water to the brim and then measure the initial DO of each before adding dilution water to the next set of replicates. After recording the initial DO of each, insert the bottle stoppers, fill the wells, and attach the plastic cap. Ensure there are no air bubbles in the bottles. Then record the time of completion for those three replicates.
7. Repeat step 6 for each group of three replicates. Rinse DO electrode between determinations to prevent cross-contamination of samples.
8. Place all BOD bottles in the incubator set to 20 °C and store for 5 days.
9. After 5 days, measure the DO in each BOD bottle in the same order as was used to measure the initial DO. Record the final DO reading. Discard any samples with air bubbles in the BOD bottles.

### *Calculations*

For each test bottle meeting the 1.0-mg/L minimum DO depletion and the 1.0-mg/L residual DO, calculate BOD<sub>5</sub> using the equation at the beginning of this section. If these conditions are not met, record the BOD<sub>5</sub> but note the discrepancy.

## **Total Suspended Solids**

Use Standard Method 2540D Total Suspended Solids Dried at 103-105 °C Method for TSS analysis. The sample size should be limited to that yielding between 2.5 and 200 mg residue. Per Crites and Tchobanoglous, 1998, residential STE is expected to have between 40 and 140 mg/L TSS, so use a 50 or 100 mL sample size for STEs. Use as large a sample size as possible (100 to 300 mL) for treated effluent samples.

Samples should be stored in glass bottles if possible. If this is not possible, any adhesion of solids to bottle walls should be noted. Samples should be refrigerated at or below 6 °C and analyzed within 24 hours of collection.

Duplicates of all samples should be analyzed. For samples high in dissolved solids thoroughly wash the filter to ensure removal of dissolved material.

### *Apparatus*

1. Drying oven for operation at 103 – 105 °C
2. Analytical balance capable of weighing to 0.1 mg
3. Magnetic stirrer with TFE stirring bar
4. Glass-fiber filter disks without organic binder
5. Filtration apparatus suitable for the filter disk selected
6. Suction flask
7. Aluminum weighing dishes

### *Procedure*

1. Insert disk with wrinkled side up in filtration apparatus. Apply vacuum and wash disk with three successive 20-mL portions of reagent-grade water. Continue suction to remove all traces of water, turn vacuum off, and discard washings. Remove filter from filtration apparatus and transfer to an inert aluminum weighing dish. Dry in an oven at 103 to 105 °C for 1 hour. Repeat cycle of drying or igniting, cooling, and weighing until a constant weight is obtained or until weight change is less than 4% of the previous weighing or 0.5

mg, whichever is less. Store in drying oven until needed. Record final weight of filter + aluminum weighing dish.

2. Choose sample volume to yield between 2.5 and 200 mg dried residue. If volume filtered fails to meet minimum yield, increase sample volume up to 1 L. If complete filtration takes more than 10 minutes, increase filter diameter or decrease sample volume.
3. Assemble filtering apparatus and filter and begin suction. Wet filter with a small volume of reagent-grade water to seat it. Measure out sample volume using a graduated cylinder, pour onto filter, and apply suction. Wash filter with three successive 10 mL volumes of reagent grade water, allowing complete drainage between washings, and continue suction for about 3 minutes after filtration is complete. Samples with high dissolved solids may require additional washings.
4. Carefully remove filter from filtration apparatus and transfer to an aluminum weighing dish as support. Dry for at least 1 hour at 103 to 105 °C in an oven, cool to balance temperature, and weigh. Repeat the cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained or until the weight change is less than 4% of the previous weight or 0.5 mg, whichever is less. Analyze at least 10% of all samples in duplicate. Duplicate determinations should agree within 5% of their average weight.

#### *Calculations*

$$mg\ TSS = \frac{(weight\ of\ filter + dried\ residue, mg - weight\ of\ filter, mg) \times 1,000}{weight\ of\ filter, mg}$$

#### **Ammonia**

Use Standard Method 4500-NH<sub>3</sub> B for preliminary distillation and 4500-NH<sub>3</sub> C for titration. Use a 100-mL sample for each replicate.

If the pH of the composite sample is greater than 8, then a drop of acid should be added to the ammonia sample prior to storage. Samples should be refrigerated at or below 6 °C, or frozen if unacidified. Ammonia analyses should be completed within 72 hours of sampling. At least one sample should be analyzed in duplicate for each sampling event.

### *Apparatus*

1. Distillation apparatus
2. 250-mL flask and 100-mL beaker for each sample
3. pH meter with glass electrode that can be read to the nearest 0.05 pH unit.
  - a. Oakton 5+ pH meter
4. Magnetic stirrer
5. Buret

### *Reagents*

1. Nanopure water (ammonia free)
2. Borate buffer solution
3. Sodium hydroxide 1 N
4. Mixed indicator solution
5. Indicating boric acid solution
6. Standard sulfuric acid titrant 0.02 N

### *Procedure*

1. Rinse glassware for ammonia analysis with nanopure water just before use. Carry a blank (nanopure water) through all steps of the procedure and apply the necessary correction to the results.
2. Rinse distillation equipment with nanopure water before running the blanks and samples to minimize contamination.
3. Transfer 100 mL of sample/standard/blank into a 250-mL distillation flask. Add 5 mL borate buffer solution and adjust to pH 9.5 with 1 N NaOH drop-wise and using a pH meter. **USE GOGGLES WHEN WORKING WITH SODIUM HYDROXIDE.**
4. Transfer sample flask to distillation apparatus. Using a graduated cylinder, measure 10 mL indicating boric acid solution and pour into a 100 mL beaker. Position the beaker beneath the condenser so that the condenser tip is below the surface of the indicating boric acid trapping solution.

5. Turn the burner on and adjust to the highest setting. Stop the distillation after about 60 mL of condensate has been collected in the boric acid beaker. Turn the burner off and lower the beaker so that the tip of the condenser is no longer below the surface of the liquid. Catch the last condensate drips in the beaker. TURN OFF THE COOLING WATER.
6. Titrate ammonia in distillate with standard 0.02 N H<sub>2</sub>SO<sub>4</sub> titrant to pH 4.30.

*Calculation*

$$\frac{\text{mg NH}_3 - N}{L} = \frac{(\text{vol. H}_2\text{SO}_4 \text{ for sample, mL} - \text{vol. H}_2\text{SO}_4 \text{ for blank, mL}) \times \text{acid normality, N} \times 14,000}{\text{mL sample}}$$

**Major Ions**

Use Standard Method 4110B for Ion Chromatography to determine the concentrations of fluoride, chloride, nitrate, nitrite, phosphate, and sulfate. 5 mL samples are required to perform this analysis.

Samples for ion chromatography should be stored at or below 6 °C in polyethylene plastic or glass and analyzed within 48 hours. Use standards and two replicates for quality control.

*Apparatus*

1. Ion chromatograph
  - a. Dionex ICS-1600 with conductivity detector, column heater and ASDV autosampler

*Reagents*

2. Nanopure water for blanks
3. Eluent solution of 0.0017 M NaHCO<sub>3</sub>-0.0018 Na<sub>2</sub>CO<sub>3</sub>
4. Regenerant solution of 0.025 N H<sub>2</sub>SO<sub>4</sub>
5. Standard anion solutions

*Procedure*

1. Follow instructions for use with the ion chromatograph.

2. Run blanks using nanopure water, plus a range of standards. Filter samples using 0.45  $\mu\text{m}$  syringe filters.
3. Analyze data using Dionex workstation with Chromeleon SE software (Version 7.0).

### **Total Kjeldahl Nitrogen**

Use Standard Method 4500-N<sub>org</sub> B to digest and distill sample, followed by Standard Method 4500-NH<sub>3</sub> C for titration. Per Crites and Tchobanoglous, 1998, the TKN concentration for residential STE is expected to be 50-90 mg N/L. Use a 10 mL sample size.

After compositing the sample, acidify to pH 1.5 to 2.0 with concentrated H<sub>2</sub>SO<sub>4</sub> and store at or below 4 °C in a glass container. Samples should be analyzed within two weeks, or one week if possible.

Run two to three replicates of the analyses along with a laboratory blank.

### *Apparatus*

1. Digestion apparatus – 100-mL Kjeldahl flasks and a heating devise adjusted so that 250 mL water at an initial temperature of 25 °C can be heated to a rolling boil in approximately 5 min. A heating devise meeting this specification should provide the temperature range of 375 to 385 °C for effective digestion.
2. Distillation apparatus and apparatus for ammonia determination (see Ammonia Apparatus section above)

### *Reagents*

Prepare all reagents and dilutions in ammonia-free water.

1. Digestion reagent
2. Sodium hydroxide-sodium thiosulfate reagent
3. Mixed indicator solution, indicating boric acid solution, and standard sulfuric acid titrant (see Ammonia Reagents section above)

### *Procedure*

1. Carry a blank through all steps of the procedure and apply the necessary correction to the results.
2. Place 10 mL of sample in a Kjeldahl digestion flask. Add boiling chips to the flask. This will help the samples boil evenly. Boiling chips should not be reused.
3. Pipet 10 mL of digestion solution into the flask. Use splash goggles. Mix the contents of the flask by swirling. Position the flask on the digestion rack. Turn the burner to a low setting to boil off the water.
4. When the sample volume has been reduced to less than 5 mL, copious white fumes will appear in the flask. The boiling point of the mixture will increase to above 350 °C. Turn the heat to a higher setting and begin the digestion. The flask must be heated at the highest setting for a minimum of 30 minutes.
5. When the flask has cooled, carefully transfer the contents to a distillation flask using several aliquots of ammonia-free water. The final volume should be approximately 100 mL.
6. Carefully pour 10 mL of sodium hydroxide/sodium thiosulfate solution into the distillation flask. Use splash goggles. Rinse the glass joint with a few milliliters of water to keep the glass from fusing.
7. Swirl the flask and position it on the distillation unit. Turn the heat setting to high. The tip of the condenser should be below the surface of 20 mL of indicating boric acid in the trapping beaker. Make sure that the cooling water is flowing at a moderate rate.
8. Continue distillation until about 60 mL of condensate has been collected. Disengage the trapping solution from the condenser. Turn off the cooling water.
9. Titrate the entire volume of trapping solution to pH 4.30 using standardized 0.02 N H<sub>2</sub>SO<sub>4</sub>.

### *Calculation*

See ammonia calculation section.

## Chemical Oxygen Demand

Use the Hach USEPA Reactor Digestion Method 8000 for high range (HR) COD concentrations of 20 to 1,500 mg/L, or low range (LR) concentrations of 3 – 150 mg/L. Per Crites and Tchobanoglous, 1998, the typical range for COD in STE is 250 – 500 mg/L. The required sample volume for the Hach test is 2.0 mL.

To preserve samples for later analysis, adjust the sample pH to less than 2 with concentrated sulfuric acid (approximately 2 mL per liter). Keep the preserved samples at or below 6 °C for a maximum of 28 days, or preferably one week. At least one sample should be analyzed in duplicate for each sampling event.

Note that Hach COD kits contain mercury and must be disposed of carefully.

### *Apparatus*

1. Hach COD digestion reagent vials
2. Hach DRB200 Reactor
3. Light shield or adapter
4. Test tube rack
5. Hach Spectrophotometer, model DR 2800

### *Procedure*

1. Run one blank with each set of samples. If measuring samples using two different ranges, make one blank for each COD range.
2. Set the DRB200 Reactor power to on. Preheat to 150 °C. Refer to the DRB200 User Manual for selecting pre-programmed temperature applications.
3. To prepare the samples, remove the cap from a vial for the selected COD range. Hold the vial at an angle of 45 degrees. Use a clean pipet to add 2.0 mL of sample to the vial. To prepare the blank, remove the cap from another vial of the selected COD range. Hold the vial at an angle of 45 degrees. Use a clean pipet to add 2.0 mL of deionized water to the vial. Close the vials tightly. Rinse the vials with water and wipe with a clean paper towel.

Hold the vials by the cap over a sink. Invert gently several times to mix. The vials get very hot during mixing.

4. Put the vials in the preheated DRB200 reactor and close the lid, then heat the vials for 2 hours. After 2 hours, set the reactor power to off. Let the vials cool in the reactor for approximately 20 minutes to 120 °C or less. Invert each vial several times while it is still warm, and then put the vials in a tube rack to cool them to room temperature.
5. On the spectrophotometer, start program 435 COD HR. Clean the blank sample cell and insert it into the cell holder. Push ZERO. The display should show 0 or 0.0 mg/L COD. Then clean the prepared sample cell. Insert the prepared sample into the cell holder and push READ. Results will be shown in mg/L COD.

### **Total Phosphorus**

Use Hach Molybdovanadate Method with Acid Persulfate Digestion Method 10127 to analyze for total phosphorus. Per Crites and Tchobanoglous, 1998, total phosphorus in residential STE is expected to be between 12 and 20 mg P/L. Use a 5 mL sample size if using the high rate Hach kit.

Samples for TP analysis should be stored in plastic or glass bottles rinsed with HCl (followed by distilled water) and never washed with phosphate detergent. Analyze samples immediately after collection or preserve the sample for up to 28 days by adjusting the pH to 2 or less with concentrated H<sub>2</sub>SO<sub>4</sub> and storing at 6 °C. Preserved tests should be completed within 28 days of sampling – preferably 7 days. At least one sample should be analyzed in duplicate for each sampling event.

#### *Apparatus*

1. Hach Total High Range Phosphorus Test N' Tube™ Reagent Set
2. Hach DRB200 Reactor
3. Light Shield
4. Test tube rack
5. Hach DR2800 Spectrophotometer

#### *Procedure*

1. Turn on the digester. Heat to 150 °C.
2. Select the test on the DR2800 spectrophotometer. Install the light shield in cell compartment #2.
3. Add 5.0 mL of sample or distilled water to a TP vial. Use one blank for every set of tests.
4. Use a funnel to add the contents of one potassium persulfate powder pillow to each vial. Cap tightly and shake to dissolve.
5. Insert the vials in the digester and close the protective cover. Start the timer on the spectrophotometer and a 30-minute heating period will begin. After the timer expires, carefully remove the hot vials from the reactor. Insert them in a test tube rack and to cool to room temperature.
6. Add 2.0 mL of 1.54 N sodium hydroxide to each vial. Cap and invert to mix.
7. Use a polyethylene dropper to add 0.5 mL of molybdovanadate reagent to each vial. Cap and invert to mix.
8. Start the next timer on the spectrophotometer and a 7-minute reaction period will begin. Read the sample within seven to nine minutes after adding the molybdovanadate reagent. Before inserting vials into the spectrophotometer, wipe them with a damp towel and then a dry one to remove smudges.
9. When the timer expires, insert the blank into the 16-mm cell holder. Press zero. Then insert the prepared samples into the 16-mm cell holder one at a time. Press read and record the measurements, which are in mg/L PO<sub>4</sub><sup>3-</sup>.

### **Total Nitrogen**

Use Hach Persulfate Digestion Method 10072 to analyze for total nitrogen. Use a 0.5 mL sample size if using the high rate Hach kit.

Analyze samples immediately after collection or preserve the sample for up to 28 days by adjusting the pH to 2 or less with concentrated H<sub>2</sub>SO<sub>4</sub> and storing at 6 °C. Preserved tests should be

completed within 28 days of sampling – preferably 7 days. At least one sample should be analyzed in duplicate for each sampling event.

### *Apparatus*

1. Hach Total High Range Nitrogen Test N' Tube™ Reagent Set
2. Oven capable of heating to 105°C
3. Light Shield
4. Test tube rack
5. Hach DR2800 Spectrophotometer

### *Procedure*

1. Turn on the oven. Heat to 105°C.
2. Use a funnel to add the contents of one Total Nitrogen Persulfate Reagent Powder Pillow to each of two HR Total Nitrogen Hydroxide Digestion Reagent vials. Make sure to clean any reagent that gets on the lip of the vials or on the vial threads.
3. Add 0.5 mL of sample or distilled water to a TN vial. Use one blank for every set of tests.
4. Put the caps on all vials. Shake vigorously for at least 30 seconds to mix. Undissolved powder will not affect the accuracy of the test.
5. Put the vials in the oven. Leave the vials in the oven for exactly 30 minutes.
6. At 30 minutes, remove the vials from the reactor and let the vials cool to room temperature.
7. Add the contents of one TN Reagent A Powder Pillow to each vial.
8. Put the caps on both vials. Shake for 30 seconds.
9. Start the instrument timer. A 3-minute reaction time starts.
10. After the time expires, remove the caps from the vials. Add one TN Reagent B Powder Pillow to each vial.
11. Put the caps on all vials. Shake vigorously for 15 seconds to mix. The reagent will not dissolve completely. Undissolved powder will not affect the accuracy of the test. The solution will start to turn yellow. Wait 2 minutes.

12. When the time expires, use a pipet to put 2 mL of each digested, treated prepared sample (including the blank) into one TN Reagent C vial.
13. Put the caps on the vials. Invert 10 times to mix. Use slow, deliberate inversions for complete recovery. The vials will be warm to the touch. Wait 5 minutes.
14. When the timer expires, insert the blank into the 16-mm cell holder. Press zero. Then insert the prepared samples into the 16-mm cell holder one at a time. Press read and record the measurements, which are in mg/L N.

### **Total Coliforms and E. Coli**

Use the IDEXX Colilert Quanti-Tray/2000 trays with snap pack reagents. Use a 10<sup>-4</sup>-mL sample size for STE samples and a 50-, 10-, or 1-mL sample size for treated effluents.

Analyze samples within 24 hours of collection. Run all samples in duplicates at least.

#### *Apparatus*

1. Colilert Quanti-Tray/2000
2. Quanti-Tray sealer and rubber support.
3. Colilert snap packs
4. Shrinkbanded dilution vessels

#### *Procedure*

1. For each sample, measure the appropriate volume into a dilution vessel and add sterile water to the mark.
2. Add the contents of one snap pack to each dilution vessel. Shake well to dissolve the powdered reagent.
3. Pour the contents of the dilution vessel into a tray and seal using a Quanti-Tray sealer and rubber support.
4. Incubate the tray at 35°C for 24 hours. After 24 hours, count the number of yellow cells and the number of cells that fluoresce under a UV lamp. Use the IDEXX MPN Generator software to estimate the Total Coliform and E. Coli concentrations indicated by the test.

## A.2 PPCPs

The table below specifies mass spectrometer multiple reaction monitoring (MRM) parameters. All data acquisition was in positive ion mode except triclosan, which required negative ion mode. For triclosan, poor fragmentation led to quantification via isotope monitoring in MS1 mode. An example of all standards at 75 ng/mL is shown in Figure A.2.1.

**Table A.2.1 Mass spectrometer MRM parameters**

PPCP	Parent ion (m/z)	Fragment ion (m/z)	Dwell (secs)	Cone Voltage (V)	Collision Energy
Metformin (Scan time: 0.0-2.0 min, 568 scans)					
Nat. Isotope	129.94	60.06	0.025	22.0	12.0
Nat. Isotope	129.94	71.11	0.025	22.0	16.0
Labeled IS	135.99	60.15	0.025	22.0	12.0
Labeled IS	135.99	77.13	0.025	22.0	18.0
Acetaminophen (Scan time: 2.0-4.2 min, 596 scans)					
Nat. Isotope	151.97	92.86	0.025	26.0	22.0
Nat. Isotope	151.97	110.02	0.025	26.0	16.0
Labeled IS	155.98	96.83	0.025	28.0	24.0
Labeled IS	155.98	114.12	0.025	28.0	14.0
Levetiracetam (Scan time: 6.5-8.0 min, 293 scans)					
Nat. Isotope	171.01	126.11	0.025	14.0	16.0
Nat. Isotope	171.01	154.05	0.025	14.0	8.0
Labeled IS	177.03	132.12	0.025	12.0	16.0
Labeled IS	177.03	160.14	0.025	12.0	8.0
Carbamazepine (Scan time: 5.0-6.5 min, 293 scans)					
Nat. Isotope	237.00	178.96	0.025	30.0	30.0
Nat. Isotope	237.00	194.05	0.025	30.0	22.0
Labeled IS	247.06	187.00	0.025	32.0	32.0
Labeled IS	247.06	204.16	0.025	32.0	22.0
Lamotrigine (Scan time: 5.0-6.0 min, 183 scans)					
Nat. Isotope	255.91	144.96	0.025	44.0	36.0
Nat. Isotope	255.91	211.01	0.025	44.0	26.0
Labeled IS	260.89	144.98	0.025	38.0	34.0
Labeled IS	260.89	213.91	0.025	38.0	26.0
Venlafaxine (Scan time: 5.5-6.5 min, 146 scans)					
Nat. Isotope	278.17	58.17	0.025	24.0	18.0
Nat. Isotope	278.17	260.18	0.025	24.0	12.0
Labeled IS	284.15	64.23	0.025	22.0	18.0
Labeled IS	284.15	121.11	0.025	22.0	32.0
Topiramate (Scan time: 5.5-6.5 min, 227 scans)					
Nat. Isotope	340.07	264.01	0.025	24.0	8.0

<b>PPCP</b>	<b>Parent ion (m/z)</b>	<b>Fragment ion (m/z)</b>	<b>Dwell (secs)</b>	<b>Cone Voltage (V)</b>	<b>Collision Energy</b>
Nat. Isotope	362.07	321.00	0.025	34.0	16.0
Labeled IS	352.08	270.05	0.025	24.0	8.0
Triclosan <sup>1</sup> (Scan time: 8.0-15.0 min, 2745 scans, negative ion mode)					
Nat. Isotope	286.92		0.025	16.0	
Nat. Isotope	288.92		0.025	18.0	

<sup>1</sup>No labeled internal standard available.

### UPLC Details

Column Type: ACQUITY UPLC<sub>r</sub> BEH C18 1.7 $\mu$ m (reversed-phase)

Column Serial Number: 02123231415770

Autosampler Temperature: 10.0

Column Temperature: 35.0

Minimum System Pressure: 2237.0 psi

Maximum System Pressure: 4364.0 psi

Average System Pressure: 3827.0 psi

### UPLC Separation Conditions

Solvent A: Water + 0.1% formic acid

Solvent B: Acetonitrile + 0.1% formic acid

Table A.2.2 UPLC separation conditions

<b>Time (min)</b>	<b>Flow Rate (mL/min)</b>	<b>%A</b>	<b>%B</b>
0.00	0.200	95.0	5.0
0.50	0.200	95.0	5.0
8.00	0.200	5.0	95.0
8.50	0.200	5.0	95.0
9.00	0.200	95.0	5.0
15.00	0.200	95.0	5.0

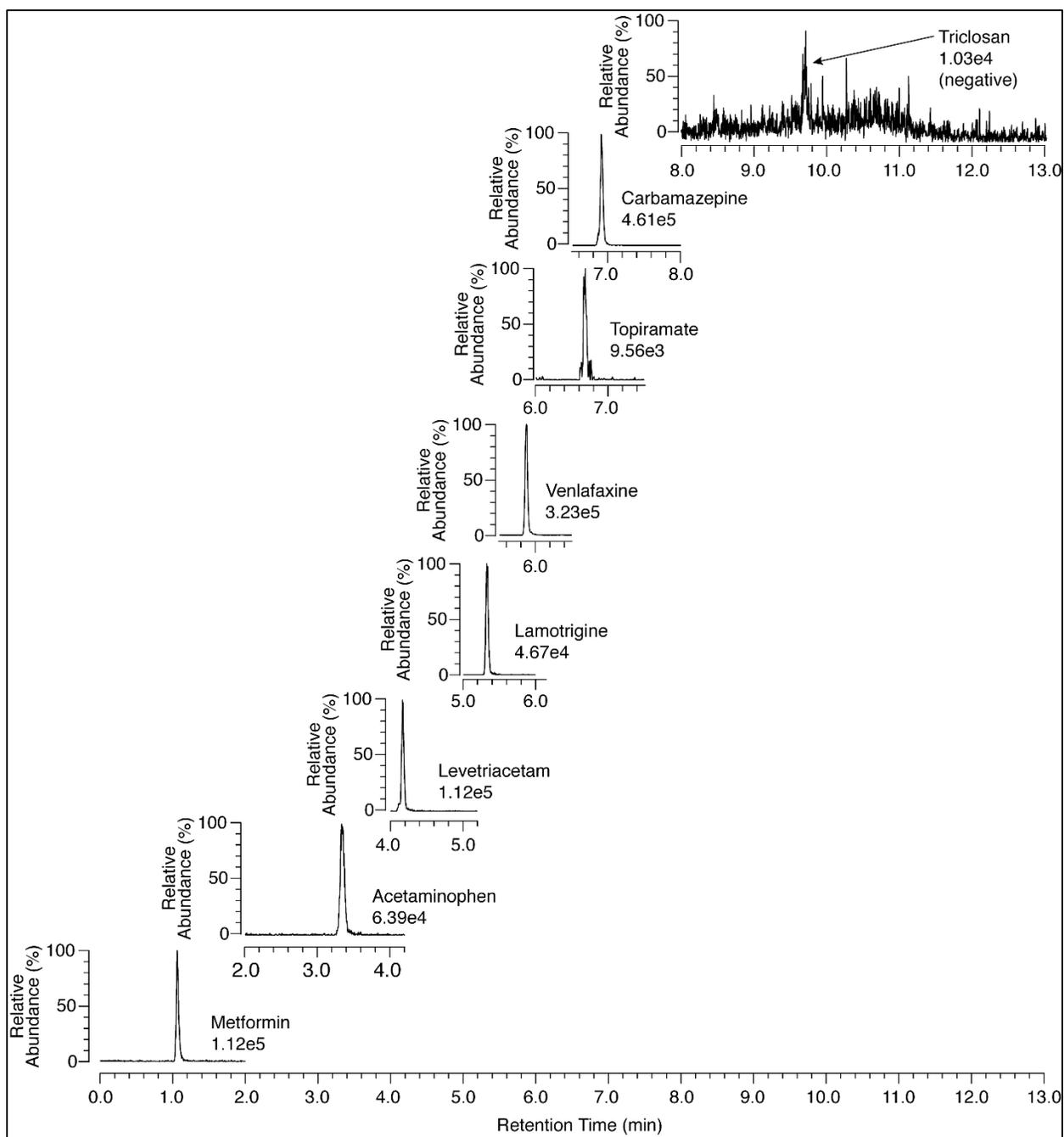


Figure A.2.1: Chromatogram of all standards at 75 ng/mL

## Appendix B: Quarterly Sampling Data

### B.1 Sampling Procedures and Compositing Ratios

1. Calibrate the field probes before starting to collect samples, and as needed during the process. Rinse probes with distilled water between measurements.
2. Collect wastewater from each of the septic tanks at approximately 11:00 am and again at approximately 3:00 pm. At each sampling time, two samples will be collected from each tank – one for non-organic analyses and another for organic analyses. Use a siphon pump to collect the samples. Insert the suction tubing just below the scum layer at the top of the septic tank. Pump sample through the siphon pump before collecting a sample.
3. The samples should be stored in appropriately-cleaned bottles. Table B.1 below shows how much sample should be collected for non-organic and organic analysis at each collection time. Non-organic analysis bottles should be filled to the brim to minimize headspace. Also at each sampling time, a field blank should be made from distilled water for one non-organic analysis and one organic analysis bottle as indicated in Table B.1.1 below.

**Table B.1.1 Volumes of bottles to be filled at septic tanks during each sampling event**

Septic Tank	Average Day Percent of Flow	Non-Organic Analysis Sample Bottles to be Filled	Organic Analysis Sample Bottles to be Filled
Laurel	30%	1 x 1000-mL	1 x 1-L
Dogwood	20%	1 x 500-mL	1 x 1-L
Sunflower	20%	1 x 500-mL	1 x 1-L
Amity	20%	1 x 500-mL	1 x 1-L
Community Center	10%	1 x 250-mL	1 x 1-L
Blank	N/A	1 x 250-mL	1 x 1-L

4. Immediately after collecting from each septic tank, measure and record the pH, temperature, and dissolved oxygen in each septic tank non-organic analysis sample using the field probes. After making the measurements, store the samples in a cooler and ensure they are protected from sunlight.
5. After collecting samples from all septic fields, combine the non-organic analysis samples volumetrically in a 2-L jug as indicated in Table B.1.2 below. Invert the samples to ensure that solids are well distributed, and then quickly pour the desired volume into a 1000-mL

or 250-mL graduated cylinder before the solids re-settle. Pour approximately 2 mL more than the desired volume into the cylinder, and then use a pipet to remove sample until the exact volume is obtained. Rinse the cylinders with distilled water between uses. The organic analysis samples will not be composited until returning to the lab.

**Table B.1.2: Summary of composite volumes for non-organic analyses**

Septic Tank	Average Day Percent of Flow	Volume for 2 L Composite Sample (mL)
Laurel	30%	600.0
Dogwood	20%	400.0
Sunflower	20%	400.0
Amity	20%	400.0
Community Center	10%	200.0

- Mix the composite for 1 minute by inversion. Then, distribute the combined samples among the different storage vessels using the graduated cylinders as indicated in Table B.1.3 below. Use the same procedure as described in step 4 above. Note that the jugs used for compositing can be used to store the largest bottle type.

**Table B.1.3: Bottles for non-organic analyses storage**

Bottle Type	Tests	Volume (L)	Preservation at Site	Preservation in Lab	No. Bottles
HDPE	Alkalinity	0.600	Refrigerate at or below 6°C. Fill bottles completely and cap tightly.	Analyze within 24 hours.	1 x 500-mL
HDPE	Ammonia	0.300	Add dilute H <sub>2</sub> SO <sub>4</sub> if composite pH is over 8 to reduce it to approx. 7. Fill bottles completely and cap tightly. Refrigerate at or below 6°C.	Analyze within 72 hours. Continue to refrigerate at or below 6°C or freeze if unacidified.	1 x 500-mL
HDPE	TKN	0.075	Refrigerate at or below 6°C.	Distribute into 1 x 100-mL glass bottle, acidify to pH 1.5-2.0 with approx. 0.2-mL concentrated H <sub>2</sub> SO <sub>4</sub> and store at or below 6°C.	1 x 2-L
	COD	0.006			
	TP	0.015		Distribute into 1 x 50-mL glass bottle rinsed with HCl, acidify to pH 1.5-2.0 with approx. 0.2-mL concentrated H <sub>2</sub> SO <sub>4</sub> and store at or below 4 °C.	
	TSS	0.300		Analyze within 24 hours.	
	Major Ions	0.015		Analyze within 48 hours.	

Bottle Type	Tests	Volume (L)	Preservation at Site	Preservation in Lab	No. Bottles
	BOD <sub>5</sub>	0.060		Analyze within 24 hours.	

7. Transport samples to the lab in coolers with ice packs. Immediately after returning to the lab, preserve the TKN, COD, and TP samples as indicated in Table B.1.3 above. Then, composite the samples for organic analyses as indicated in Table B.1.4 below using appropriately cleaned glassware. Separate TOC samples into 40-mL borosilicate glass bottles. Put 15-20 mL of sample from each organic analyses field blank into a 40-mL borosilicate glass bottle as well. Preserve them as indicated in Table B.1.5. Then, set up the BOD tests. If time allows, perform the alkalinity analyses as well.

**Table B.1.4: Summary of composite volumes for organic analyses**

Septic Tank	Average Day Percent of Flow	Volume for 1-L Composite Sample (mL)
Laurel	30%	300
Dogwood	20%	200
Sunflower	20%	200
Amity	20%	200
Community Center	10%	100

**Table B.1.5: Bottles for organic analyses**

Bottle Type	Tests	Volume (L)	Preservation in Lab	No. Bottles
Borosilicate Glass, Amber, Treated for TOC	TOC	0.050	Acidify to pH 4.0 or less with HCl and store at or below 6°C. Refrigerate until analysis is completed.	2 x 40 mL
Glass, Amber, Treated for TOrcs	TOrcs	2.000	Refrigerate at or below 6°C until analysis is completed.	2 x 1 L

## B.2 Quarterly Sampling Conventional Pollutant Results

**Table B.2.1: Quarterly sampling on-site measurements**

Sampling Date	House	pH	DO (mg/L)	Temp (°C)
02_20_16 AM	Dogwood	6.93	1.39	13.5
	Sunflower	6.74	0.96	17.1
	Laurel	6.53	1.24	15.8
	Amity	6.95	0.90	20.3
	Community Center	7.04	2.28	12.0
02_20_16 PM	Dogwood	6.34	1.27	14.7

<b>Sampling Date</b>	<b>House</b>	<b>pH</b>	<b>DO (mg/L)</b>	<b>Temp (°C)</b>
	Sunflower	6.24	1.00	18.9
	Laurel	6.08	1.13	16.9
	Amity	6.56	1.54	21.9
	Community Center	6.45	1.00	13.7
05_19_16 AM	Dogwood	7.00	1.27	19.4
	Sunflower	6.58	1.88	18.2
	Laurel	6.47	1.30	
	Amity	6.55	1.10	21.4
	Community Center	6.94	3.78	19.5
05_19_16 PM	Dogwood	6.96	1.79	21.1
	Sunflower	6.60	2.45	19.9
	Laurel	6.11	1.96	20.5
	Amity	6.50	1.62	22.0
	Community Center	6.80	1.28	19.3
08_12_16 AM	Dogwood	6.26	2.34	27.9
	Sunflower	6.57	2.02	28.2
	Laurel	6.42	1.67	25.4
	Amity	6.65	1.00	32.0
	Community Center	6.84	1.41	29.7
08_12_16 PM	Dogwood	5.71	0.97	26.7
	Sunflower	6.05	1.10	27.2
	Laurel	6.01	1.25	24.7
	Amity	6.58	0.70	30.1
	Community Center	6.94	0.76	27.7
10_16_16 AM	Dogwood	6.96	0.77	21.1
	Sunflower	6.67	1.43	22.6
	Laurel	6.30	3.10	17.5
	Amity	6.77	1.27	26.9
	Community Center	7.15	1.33	22.0
10_16_16 PM	Dogwood	6.91	0.69	22.0
	Sunflower	6.62	0.78	23.7

<b>Sampling Date</b>	<b>House</b>	<b>pH</b>	<b>DO (mg/L)</b>	<b>Temp (°C)</b>
	Laurel	6.34	3.77	18.2
	Amity	6.65	1.08	27.8
	Community Center	7.10	1.33	23.0

Table B.2.2: Quarterly sampling conventional pollutant results

Sample	DOT		Alkalinity (mg CaCO <sub>3</sub> /L)		TSS (mg/L)		BOD (mg/L)		COD (mg/L)		TOC (mg/L)		Ammonia (mg N/L)		NO <sub>3</sub> <sup>-</sup> (mg N/L)		NO <sub>2</sub> <sup>-</sup> (mg N/L)	
	W	Time	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
Quarterly #1 02_20_16	Sat	AM	206	5	125	4	150	6	434	20	75.53	0.24	29.70	1.00	nd	N/A	nd	N/A
	Sat	PM	204	0	212	7	173	2	517	11	62.90	0.54	31.00	0.60	nd	N/A	nd	N/A
Quarterly #2 05_19_16	Thurs	AM	294.7	2.4	270	10	>280	N/A	1515	153	52.19	0.91	44.53	0.21	0.32	0.02	0.36	0.01
	Thurs	PM	274.9	8.2	290	0	197	6	645	20	56.97	1.77	41.90	2.07	0.32	0.01	0.36	0.01
Quarterly #3 08_12_16	Fri	AM	202.5	1.1	83	7	148	5	389	16	63.38	0.37	30.32	0.28	0.58	N/A	0.11	0.03
	Fri	PM	217.4	2.1	444	N/A	251	13	870	25	57.94	0.98	30.87	1.13	0.58	0.01	0.07	0.00
Quarterly #4 10_16_16	Sun	AM	N/A	N/A	120	10	114	34	240	4			25.1	0.8	0.11	0.00	0.10	0.00
	Sun	PM	179.6	N/A	190	10	143	19	350	4			25.5	0.5	0.14	0.00	0.05	0.01

- No duplicates in sampling set
- Value above analytical test range
- RSD > 10%
- RSD ≥ 100%

Table B.2.2: Quarterly

Sample	DOT		TKN (mg N/L)		TIN (mg N/L)		PO <sub>4</sub> <sup>3-</sup> (mg P/L)		TP (mg P/L)		SO <sub>4</sub> <sup>2-</sup> (mg/L)		Cl <sup>-</sup> (mg/L)	
	W	Time	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
Quarterly #1 02_20_16	Sat	AM	41.1	N/A	29.70	1.00	3.31	0.09	5.04	0.16	6.49	0.00	27.65	0.32
	Sat	PM	40.2	0.2	31.00	0.60	3.02	0.01	5.77	0.09	7.23	0.07	24.17	0.09
Quarterly #2 05_19_16	Thurs	AM	55.2	7.2	45.21	0.21	5.53	0.75	14.8	0.2	11.35	0.58	32.02	0.01
	Thurs	PM	51.2	1.5	42.58	2.07	5.50	0.87	8.77	0.09	11.15	0.29	31.54	0.64
Quarterly #3 08_12_16	Fri	AM	38.9	0.8	31.00	0.28	4.01	0.04			6.99	0.05	24.54	0.36
	Fri	PM	49.9	0.3	31.52	1.13	4.55	0.03			1.54	0.05	24.94	0.04
Quarterly #4 10_16_16	Sun	AM	29.2	1.6	25.3	0.8	3.46	0.02	3.98	0.05	4.72	0.02	17.84	0.01
	Sun	PM	33.1	0.2	25.7	0.5	3.97	0.00	4.79	0.05	3.85	0.04	18.02	0.02

## B.3 Quarterly Sampling PPCP Results

Table B.3.1: Quarterly sampling PPCP results

Sample	DOTW	Time	Concentration (µg/L)							
			ACE	CBZ	LAM	LEV	MET	TOP	TCS	VEN
Quarterly #1 02_20_16	Sat	AM	203.621	10.512	52.876	106.687	54.191	27.214	5.733	18.640
	Sat	PM	159.637	18.455	39.765	117.599	69.260	34.699	4.231	22.273
	Sat	Field Blank	<LOQ	<LOQ	<LOQ	0.113	0.875	<LOQ	0.889	<LOQ
Quarterly #2 05_19_16	Thurs	AM	56.284	19.019	58.597	124.635	25.271	28.224	5.053	16.865
	Thurs	PM	64.180	20.193	55.775	117.554	27.298	25.383	1.655	13.475
	Thurs	Field Blank	<LOQ	0.062	0.035	0.159	<LOQ	<LOQ	1.852	0.057
Quarterly #3 08_12_16	Fri	AM	28.736	14.744	28.041	57.695	49.941	17.942	14.469	13.145
	Fri	PM	27.082	14.392	25.845	71.977	51.196	20.176	12.565	13.568
	Fri	Field Blank	0.221	0.106	0.342	0.246	<LOQ	<LOQ	<LOQ	<LOQ
Quarterly #4 10_16_16	Sun	AM	36.173	20.991	42.998	128.015	49.248	10.715	3.837	4.620
	Sun	PM	32.783	19.482	32.360	113.018	38.644	9.483	3.483	3.500
	Sun	Field Blank	<LOQ	<LOQ	0.057	0.056	<LOQ	<LOQ	<LOQ	<LOQ

## Appendix C: Pilot System Data

### C. 1 Pilot System Set-up

#### Anua System

Water from the septic tank was pumped into the 890 gallon ATU where it underwent treatment in a settling chamber, submerged attached growth filter, and clarifier. The filter was aerated by a compressor housed within the unit. After moving through the filter, the water drained into Well Basin 1, and was then pumped into Peat Filter 1, an unsaturated peat biofilter. After moving through the peat, the water drained into Well Basin 2, which was filled with bivalve shells to add alkalinity to the water, allowing for enhanced nitrogen removal. From Well Basin 2, half of the influent water was recycled to the ATU during the time period from installation to October 3<sup>rd</sup>, when the recycle line was shut off. Water that was not recycled from Well Basin 2 was pumped to Peat Filter 2, which was identical to Filter 1. After draining out of Peat Filter 1, water was drained out of the system to the septic field via the septic distribution box.

The Anua system was installed on May 16<sup>th</sup>-May 18<sup>th</sup> and started receiving STE within the week. A bag of dog food was added immediately following installation to jump start the system, and then again on October 3<sup>rd</sup> after effluent TN concentrations spiked. Baking powder was also added to the system on October 3<sup>rd</sup> to counteract a drop in alkalinity. The following table shows the feed pumps used, average flow rates, and theoretical HRTs throughout the experimental period for the Anua system.

Table C.1.1: Anua system operation summary

Date Range	Pump	Recycle?	Flow Rate (gpd)		Theor. HRT (days)
			Avg	SD	
6/18 – 8/22	Cole Parmer Economy Drive	Yes	77.8	16.0	11.6
8/23 – 10/3	Dolphin Mec-O-Matic	Yes	72.7	2.9	12.2
10/4 – 11/16	Dolphin Mec-O-Matic	No	69.0	8.0	16.9

## E-Z Treat System

Water from the septic tank was pumped into the 550 gallon E-Z Treat holding tank. Every 15 minutes, water from the holding tank was pumped to the elevated filter module, where it was sprayed at high pressure onto a biofilter with styrene bead media. After draining through the filter, a small amount of water was recycled to the holding tank and the rest was drained out of the system to the septic field via the septic distribution box.

The E-Z Treat system was installed on May 11<sup>th</sup> and started receiving STE during the week of May 18-24. From installation to just before the fourth sampling date (July 17<sup>th</sup>), the effluent piping from the E-Z Treat system was configured such that water sat stagnant in the effluent pipes, promoting bacterial growth. After the piping was reconfigured, COD and BOD measurements dropped. The following table shows the feed pumps used, average flow rates, and theoretical HRTs throughout the experimental period for the E-Z Treat system.

Table C.1.2 E-Z Treat system operation summary

Date Range	Pump	Flow Rate (gpd)		Theor. HRT (days)
		Avg	SD	
6/18 – 8/22	Cole Parmer Economy Drive	99.7	11.9	5.5
8/23 – 11/16	Dolphin Mec-O-Matic	94.6	3.9	5.8

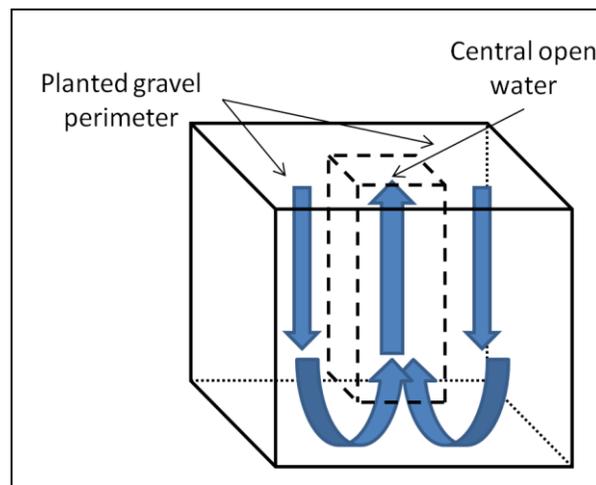
## Constructed Wetland System

The constructed wetland was a series of six tanks, each with a unique combination of flow pattern, media, and biology. A pump transported Tank 2 effluent to Tank 3, otherwise water flowed by gravity through the wetland. The first four tanks were wetland cells, and the last two were mycofilters. The first four tanks were primarily comprised of granitic gravel (with feldspar, quartz, and biotite mica), with 3/8- to 1/2-inch particle size and 44% porosity. Collection piping at the bottom of all six tanks was buried in approximately 4 inches of 1- to 1½-inch particle sized gravel of the same composition and 42% porosity. Each tank is described in more detail below.

- Tank 1: *Cylindrical tank, 290-gallon volume, 6-foot diameter, 2-foot height.* Wastewater moved vertically through this planted gravel filter. Influent was added intermittently (five

minutes on, five minutes off) and the water depth was kept 10 inches below the surface. Plants in this tank included *Elymus virginicus* (Virginia wildrye), *Juncus effuses* (common rush), *Panicum virgatum* (switchgrass), and *Scirpus cyperinus* (woolgrass).

- Tank 2: *Cylindrical tank, 290-gallon volume, 6-foot diameter, 2-foot height.* Water entered this tank after falling through an 18-inch waterfall constructed from recycled plastic bottles and bamboo supports. Baffles prevented water from short-circuiting the tank. The water elevation was approximately two inches below the surface. Plants in this tank included *Carex crinita* (fringed sedge), *Iris versicolor* (blue flag), and *Pontederia cordata* (pickerelweed).
- Tank 3: *Rectangular tank, 270-gallon volume, approximately 3-foot by 3.5-foot, 2.5 height.* There was another waterfall at the inlet to this tank. Incoming water was distributed around the perimeter of the tank, which had a planted gravel media covered with approximately two inches of mulch. After flowing downward around the perimeter, water flowed upward into an open water surface in the center of the tank, inside a ring of stacked and cemented chimney stones. Water exited through a PVC pipe at the surface of the water. Plants included *Sagittaria latifolia* (duck potato) and *Juncus effusus*.



**Figure C.1.1: Constructed wetland tank 3 schematic**

- Tank 4: *Rectangular tank, 270-gallon volume, approximately 3-foot by 3.5-foot, 2.5 height.* Water flowed vertically through this tank as in the first tank, but the water was only three

inches below the surface. Like Tank #3, this tank had a planted gravel media covered with two inches of mulch. Plants in this tank included *Sagittaria latifolia* and *Juncus effusus*.

- Tank 5: *Cylindrical tank, 290-gallon volume, 6-foot diameter, 2-foot height.* Water flows vertically through this tank, which had an unplanted mulch/compost media originally inoculated with *Trametes versicolor* (turkey tail) mushrooms. Three mechanisms aerated the water in this tank: a waterfall at the influent, water depth 10 inches below the surface, and vertical perforated PVC pipes that allowed air to access lower levels of the filter. This tank was covered with a shade cloth in mid-May to protect it from direct sunlight.
- Tank 6: *Elliptical tank, 130-gallon volume, approximately 3-foot by 4.5-foot, 2-foot height.* This tank was just like Tank #5 except that it was originally inoculated with *Pleurotus ostreatus* (oyster) mushrooms and there was no waterfall at the influent. The effluent from Tank #6 was drained to the septic field via the septic distribution box. This tank was covered with a shade cloth in mid-May to protect it from direct sunlight.

The wetland plants were planted on March 13<sup>th</sup> into a small pocket of compost within the gravel bed media. The wetland tanks were filled with well water and covered with translucent tarps. The mycofilters were inoculated on March 26<sup>th</sup>. The wetland began receiving STE at an unquantified rate (approximately 25 gpd) on April 3<sup>rd</sup>, and the tarps were removed at that time. There was one late freeze after the tarps were removed. None of the plants seemed to be harmed by the freeze except for the *Pontederia cordata*. After dying back for about a month, they recovered as well.

The inoculated mushrooms in Tanks 5 and 6 bloomed several times during the months of May and June. In July and August, blooms of other species of mushrooms were sited in Tank 6 and in the thin mulch layers on Tanks 3 and 4. After August, no more blooms were sited, and very little mycelium could be found by digging into the tanks. Despite the shade clothes, it may have been too hot and dry for the mushrooms to prosper.

Grasses sprouted in Tanks 1 and 2, particularly 2, over the course of the summer, but they were not cut back. On November 2<sup>nd</sup>, all of the wetland plants (including the weeds) were trimmed, dried, weighed, and analyzed for nutrient composition and PPCPs. Results from these analyses are shown in sections C.2 and C.3 below.

The flow rate into the wetland were consistent throughout the sampling period – the time-weighted average flow rate was 25.0 gpd with a standard deviation of 0.3 gpd. The peristaltic pumps used for the wetlands were Dolphin Mec-O-Matics.

Since the CW was open to the atmosphere, precipitation and evapotranspiration rates had to be accounted for in order to accurately estimate HRTs. Evapotranspiration rates were calculated as described in section C.5 based on weather data from a local weather station (see section C.5). Daily precipitation rates were included in the weather data. Change in tank volume was calculated using a mass balance approach:

$$\frac{dV}{dt} = Q_{in} - Q_{out} + A_p P - A_{ET} ET_c$$

Where V is the tank volume,  $Q_{in}$  is the influent flow rate [ $L^3/T$ ],  $Q_{out}$  is the effluent flow rate [ $L^3/T$ ], P is the precipitation rate [ $L/T$ ], ET is the evapotranspiration rate [ $L/T$ ], and A is the area subject to precipitation and evapotranspiration [ $L^2$ ].

On a daily basis, the volumes of the tanks changed due to restricted effluent flow rates. On a weekly basis, however, the system approached steady state, with no change in tank volume over time. Therefore, a weekly time frame was used to estimate HRTs in each tank. Weekly precipitation and evapotranspiration were calculated as follows:

$$P_{wk} = \sum_i^{i+6} P_d$$

$$ET_{c,wk} = \sum_i^{i+6} ET_{c,d}$$

Where the subscript d indicates a rate in units of in/day and the subscript wk indicates a rate in units of in/week. Then for each tank, starting with Tank 1, effluent flow rates and HRTs were calculated using the following equations:

$$Q_{out,i} = Q_{in,i} + A_{p,i} P_{wk} - A_{ET,i} ET_{c,wk}$$

$$HRT_i = \frac{V_i}{Q_{out,i}}$$

Where the subscript *i* represents the tank number. Once  $Q_{out,1}$  is calculated,  $Q_{in,2}$  is set equal to  $Q_{out,1}$  and the process repeats. The HRT for Tanks 1 through *j* is calculated by summing  $HRT_1$  through  $HRT_j$ . The table below shows the calculation procedure for flow rates and HRT for each tank. Unplanted Tanks 5 and 6 were assumed to have negligible evapotranspiration rates.

Table C.1.3: Weekly summary of constructed wetlands inter-tank flow rates and HRTs

Week	P <sub>wk</sub> (in/wk)	E <sub>t,wk</sub>	Tank 1			Tank 2			Tank 3			Tank 4			HRT Tank 1-4 (day)	
			A (sf)	V (gal)	HRT (d)	A (sf)	V (gal)	HRT (d)	A (sf)	V (gal)	HRT (d)	A (sf)	V (gal)	HRT (d)		
6/1	6/7	1.07	1.23	175	172	3.48	172	169	6.72	169	168	4.30	168	166	4.59	19.1
6/8	6/14	0.00	1.63	175	143	4.18	143	111	10.18	111	99	7.25	99	88	8.71	30.3
6/15	6/21	1.93	1.36	175	186	3.22	186	197	5.75	197	201	3.58	201	205	3.72	16.3
6/22	6/28	0.77	1.31	175	164	3.64	164	154	7.36	154	150	4.81	150	146	5.22	21.0
6/29	7/5	0.68	1.39	175	161	3.71	161	147	7.70	147	142	5.08	142	137	5.58	22.1
7/6	7/12	0.22	1.97	175	141	4.25	141	107	10.62	107	94	7.67	94	81	9.39	31.9
7/13	7/19	0.20	2.09	175	138	4.33	138	102	11.17	102	88	8.22	88	74	10.32	34.0
7/20	7/26	0.00	2.16	175	133	4.50	133	91	12.46	91	75	9.58	75	60	12.82	39.4
7/27	8/2	2.51	1.66	175	192	3.12	192	208	5.45	208	214	3.36	214	221	3.46	15.4
8/3	8/9	1.11	1.11	175	175	3.42	175	175	6.47	175	175	4.11	175	175	4.35	18.4
8/10	8/16	0.57	1.98	175	148	4.05	148	120	9.43	120	110	6.56	110	100	7.66	27.7
8/17	8/23	0.43	1.58	175	153	3.92	153	130	8.71	130	122	5.92	122	113	6.73	25.3
8/24	8/30	0.01	1.72	175	142	4.22	142	109	10.44	109	96	7.50	96	84	9.12	31.3
8/31	9/6	0.11	1.48	175	148	4.03	148	122	9.30	122	112	6.44	112	102	7.48	27.3
9/7	9/13	0.37	1.64	175	150	3.98	150	126	9.03	126	116	6.20	116	107	7.13	26.3
9/14	9/20	0.57	1.18	175	163	3.67	163	152	7.48	152	147	4.90	147	143	5.34	21.4
9/21	9/27	0.40	1.07	175	162	3.69	162	149	7.60	149	144	5.00	144	139	5.47	21.8
9/28	10/4	2.27	0.57	175	208	2.88	208	241	4.70	241	254	2.84	254	266	2.87	13.3
10/5	10/11	0.66	0.46	175	179	3.34	179	183	6.20	183	184	3.91	184	186	4.11	17.6
10/12	10/18	0.00	0.56	175	164	3.65	164	153	7.39	153	149	4.83	149	145	5.25	21.1
10/19	10/25	0.03	0.64	175	163	3.67	163	151	7.51	151	147	4.92	147	142	5.37	21.5
10/26	11/1	0.01	0.51	175	165	3.62	165	156	7.29	156	152	4.74	152	148	5.14	20.8
11/2	11/8	0.04	0.46	175	167	3.59	167	159	7.15	159	156	4.63	156	153	5.00	20.4
11/9	11/15	0.16	0.37	175	171	3.50	171	167	6.79	167	165	4.36	165	164	4.66	19.3
11/16	11/22	0.00	0.40	175	167	3.58	167	159	7.12	159	156	4.61	156	153	4.97	20.3
11/23	11/30	1.29	0.41	175	192	3.56	192	209	6.19	209	216	3.82	216	222	3.92	17.5
<i>Average</i>															23.1	
<i>SD</i>															6.3	

Table C.1.3: Weekly summary of consi

Week	P <sub>wk</sub> (in/wk)	E <sub>t,wk</sub>	Tank 5			Tank 6			HRT Tank 1-6 (d)	
			Q <sub>in</sub> (gpw)	Q <sub>out</sub>	HRT (d)	Q <sub>in</sub> (gpw)	Q <sub>out</sub>	HRT (d)		
6/1	6/7	1.07	1.23	166	187	2.91	187	196	1.22	23.22
6/8	6/14	0.00	1.63	88	88	6.21	88	88	2.74	39.27
6/15	6/21	1.93	1.36	205	243	2.24	243	259	0.93	19.44
6/22	6/28	0.77	1.31	146	161	3.38	161	168	1.43	25.84
6/29	7/5	0.68	1.39	137	150	3.62	150	156	1.54	27.23
7/6	7/12	0.22	1.97	81	85	6.36	85	87	2.75	41.04
7/13	7/19	0.20	2.09	74	78	6.98	78	80	3.01	44.03
7/20	7/26	0.00	2.16	60	60	9.13	60	60	4.03	52.51
7/27	8/2	2.51	1.66	221	270	2.02	270	291	0.82	18.23
8/3	8/9	1.11	1.11	175	197	2.76	197	207	1.16	22.28
8/10	8/16	0.57	1.98	100	111	4.91	111	116	2.08	34.69
8/17	8/23	0.43	1.58	113	122	4.47	122	125	1.92	31.68
8/24	8/30	0.01	1.72	84	84	6.49	84	84	2.86	40.64
8/31	9/6	0.11	1.48	102	104	5.22	104	105	2.28	34.75
9/7	9/13	0.37	1.64	107	114	4.76	114	117	2.05	33.15
9/14	9/20	0.57	1.18	143	154	3.53	154	159	1.51	26.44
9/21	9/27	0.40	1.07	139	147	3.69	147	151	1.59	27.05
9/28	10/4	2.27	0.57	266	310	1.75	310	330	0.73	15.77
10/5	10/11	0.66	0.46	186	199	2.74	199	204	1.18	21.48
10/12	10/18	0.00	0.56	145	145	3.74	145	145	1.65	26.52
10/19	10/25	0.03	0.64	142	143	3.81	143	143	1.68	26.95
10/26	11/1	0.01	0.51	148	149	3.66	149	149	1.61	26.07
11/2	11/8	0.04	0.46	153	153	3.55	153	154	1.56	25.48
11/9	11/15	0.16	0.37	164	167	3.26	167	168	1.43	24.00
11/16	11/22	0.00	0.40	153	153	3.54	153	153	1.56	25.39
11/23	11/30	1.29	0.41	222	247	2.51	247	258	1.06	21.07
									<i>Average</i>	29.0
									<i>SD</i>	8.8

## **C.2 Pilot System Sampling Procedures**

1. Calibrate the field probes before starting to collect samples, and as needed during the process. Rinse probes with distilled water between measurements.
2. Collect treated samples from all four effluent sampling points, as well as biofilm sampling points as needed. At each sampling point, two samples will be collected – one for conventional pollutant analyses and another for PPCP analyses. The samples should be stored in appropriately-cleaned bottles. The volume of conventional pollutant sampling bottles will depend on the analyses to be run. Also at each sampling time, a field blank should be made from distilled water for one conventional pollutant and one PPCP analysis bottle.
3. Immediately after collecting from each sampling point, measure and record the pH, temperature, and dissolved oxygen in each septic tank conventional pollutant analysis sample using the field probes. After making the measurements, store the samples in a cooler and ensure they are protected from sunlight.
4. Next, pour samples from the conventional pollutant sampling bottles into glass 50-mL bottles and preserve by adding concentrated H<sub>2</sub>SO<sub>4</sub> dropwise to reduce the pH below 2.0.
5. Transport samples to the lab in coolers with ice packs and refrigerate at or below 6°C. Set up BOD<sub>5</sub> tests.

## **C.3 Pilot System Conventional Pollutant Results**

Table C.3.1 Pilot system conventional pollutant results

Sample	DOTW System		Onsite Measurements			Alkalinity (mg CaCO <sub>3</sub> /L)		Oxygen Demand (mg/L)				TSS (mg/L)		Nitrogen (mg N/L)									
			Temp (°C)	pH	DO (mg/L)	Avg	SD	BOD		COD		Avg	SD	NH <sub>4</sub>		NO <sub>3</sub> <sup>-</sup>		NO <sub>2</sub> <sup>-</sup>		TIN		TN	
								Avg	SD	Avg	SD			Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
Sample #1 06_19_16	Sat	Anua	25.5	5.20	6.64					91	1												
	Sat	EZ Treat	26.0	7.22	7.38					47	4												
	Sat	Wetland 6	26.5	6.37	1.66					204	1												
	Sat	Septic Tank	26.7	6.66	0.40					316	7												
Sample #2 06_29_16	Wed	Anua	27.9	6.03	5.70	16	1			79	3			0.1	0.2	10.64	0.21	0.06	0.00	10.8	0.3		
	Wed	EZ Treat	27.8	7.14	6.80	56.8	0.6			47	3			5.63	0.05	14.83	0.12	1.97	0.02	22.43	0.13		
	Wed	Wetland 4	28.8	7.18	9.81	267.5	0.3			34	4			0.1	0.0	0.06	N/A	nd	N/A	0.2	0.0		
	Wed	Wetland 6	25.4	6.39	1.67	328.6	1.9			204	1			nd	N/A	0.31	N/A	0.06	N/A	0.37	N/A		
	Wed	Septic Tank	27.6	6.48	0.66	212.1	0.2			291	3			31.87	0.42	0.43	0.05	0.10	0.00	32.39	0.42		
Sample #3 07_07_16	Thurs	Anua	25.8	5.93	6.09			≤3.5	0.0	82	4	3	0	nd	N/A	7.45	0.32	0.06	0.01	7.5	0.3		
	Thurs	EZ Treat	31.8	6.91	6.32			21	1	47	2	11	1	4.81	N/A	19.79	0.14	1.61	0.04	26.21	0.14		
	Thurs	Wetland 4	26.5	6.92	2.16			≤3.1	0.2	38	1	1	0	nd	N/A	0.65	0.00	nd	N/A	0.65	0.00		
	Thurs	Wetland 6	26.3	6.24	1.62			25.6	1.0	173	1	8.0	0.5	nd	N/A	0.65	0.00	nd	N/A	0.65	0.00		
	Thurs	Septic Tank	26.4	6.45	0.82			112	6	319	0	68	1	41.02	N/A	0.65	0.00	0.08	0.01	41.75	N/A		
Sample #3B 07_07_16	Thurs	Tank 1 Eff	26.7	6.63	0.66									28.06	N/A	0.66	0.01	0.07	0.01	28.79	N/A		
	Thurs	Tank 2 Inf	28.6	6.73	3.25									25.3	N/A	0.70	0.00	N/A	N/A	26.05	N/A		
	Thurs	Tank 3 Eff	29.0	7.48	4.35									1.2	N/A	0.65	0.01	0.38	0.02	2.24	N/A		
	Thurs	Tank 4 Inf	29.8	8.30	11.16									0.41	N/A	0.65	0.00	0.22	0.03	1.28	0.03		
Sample #4 07_17_16	Sun	Anua	31.0	5.89	5.46					105	1			0.03	0.04	5.67	0.03	0.06	0.01	5.76	0.04		
	Sun	EZ Treat	30.3	6.56	6.56					57	0			3.91	0.01	20.72	0.08	2.31	0.03	26.94	0.09		
	Sun	Wetland 4	28.2	6.88	2.15					42	1			0.04	0.06	N/A	N/A	N/A	N/A	0.04	0.06		
	Sun	Wetland 6	28.0	5.98	1.13					190	1			0.08	0.11	0.31	0.00	N/A	N/A	0.31	0.11		
	Sun	Septic Tank	28.4	6.28	1.28					335	1			33.71	0.51	0.31	0.00	0.11	0.03	34.14	0.51		
Sample #5 08_07_16	Sun	Anua	28.3	6.40	5.68	63.3	0.0							0.2	0.1	10.23	0.10	0.07	0.01	10.5	0.1		
	Sun	Anua Well 1	30.7	7.48	6.57	59.8	0.0							0.3	0.1	15.81	0.15	0.08	0.02	16.2	0.2		
	Sun	Anua Well 2	29.5	7.30	2.75	87.7	0.2							0.1	0.0	12.24	0.10	nd	N/A	12.4	0.1		
	Sun	EZ Treat	28.5	7.14	6.72	33.8	0.2							0.74	0.11	23.57	0.29	0.43	0.00	24.74	0.31		
	Sun	Wetland 4	26.9	7.08	1.34	240.3	0.1							0.02	0.02	N/A	N/A	N/A	N/A	0.02	0.02		
	Sun	Wetland 6	26.9	6.41	1.21	241.7	0.5							0.03	0.04	N/A	N/A	N/A	N/A	0.03	0.04		
	Sun	Septic Tank	27.2	7.19	0.39	284.9	0.2							29.56	0.53	N/A	N/A	0.11	0.00	29.67	0.53		
	Fri	EZ Treat	30.0	7.27	6.53	39.4	1.7	4.8	0.1	33	1	2	N/A	0.94	N/A	20.58	0.08	0.73	0.02	22.25	0.09		
Sample #6 08_16_16	Tues	Anua	30.8	6.40	5.80			≤3.3	0.2	67	5	1	0	0.04	0.04	6.91	0.56	0.08	0.00	7.03	0.56		
	Tues	Anua Well 1	33.4	7.62	6.38			≤2.8	0.1	29	3	1	0	0.03	0.04	16.63	0.04	0.07	0.02	16.72	0.06		
	Tues	Anua Well 2	32.7	7.33	1.82			5.3	0.3	153	3	1	0	0.81	0.11	11.51	0.02	0.12	0.02	12.45	0.11		
	Tues	EZ Treat	30.2	7.13	6.37			≤3.4	0.1	27	2	1	0	1.3	0.1	20.09	0.52	0.71	0.00	22.1	0.5		
	Tues	Wetland 4	30.0	7.10	3.03			≤1.7	0.0	34	2	≤1	0	0.1	0.1	0.23	N/A	0.06	N/A	0.4	N/A		
	Tues	Wetland 6	29.9	6.28	1.72			19.7	0.3	156	1	5	1	nd	N/A	0.24	N/A	0.07	N/A	0.31	N/A		
	Tues	Septic Tank	29.4	6.80	0.57	173.2	1.0	72.2	11.9	189	5	14	4	22.0	0.5	nd	N/A	0.09	N/A	22.1	0.5		

Table C.3.1 Pilot system conventi

Sample	DOTW	System	Phosphorous (mg P/L)				Other Ions (mg/L)				Total Col (MPN/100 mL) Geo Mean	E Coli (MPN/ 100 mL) Geo Mean
			PO <sub>4</sub> <sup>3-</sup>		TP		SO <sub>4</sub> <sup>2-</sup>		Cl <sup>-</sup>			
			Avg	SD	Avg	SD	Avg	SD	Avg	SD		
Sample #1 06_19_16	Sat	Anua										
	Sat	EZ Treat										
	Sat	Wetland 6										
	Sat	Septic Tank										
Sample #2 06_29_16	Wed	Anua	7.26	0.09	6.03	N/A	24.73	0.37	27.67	0.79		
	Wed	EZ Treat	3.82	0.06	2.6	N/A	20.74	0.01	23.59	0.22		
	Wed	Wetland 4	0.47	0.06	nd	N/A	3.83	0.12	19.55	0.68		
	Wed	Wetland 6	nd	N/A	nd	N/A	nd	N/A	11.89	11.53		
	Wed	Septic Tank	3.88	0.04	3.85	N/A	10.28	1.05	24.13	0.37		
Sample #3 07_07_16	Thurs	Anua	5.96	0.15			25.54	0.85	23.56	0.75		
	Thurs	EZ Treat	4.42	0.08			23.89	0.29	23.28	0.70		
	Thurs	Wetland 4	0.41	0.08			3.33	0.13	15.71	1.11		
	Thurs	Wetland 6	0.43	0.11			nd	N/A	16.03	0.77		
	Thurs	Septic Tank	4.47	0.04			14.50	0.08	26.67	0.27		
Sample #3B 07_07_16	Thurs	Tank 1 Eff	3.58	0.01			12.72	0.14	20.81	0.27		
	Thurs	Tank 2 Inf	3.10	0.03			17.83	0.09	20.55	0.20		
	Thurs	Tank 3 Eff	0.99	0.06			6.81	0.04	15.93	0.17		
	Thurs	Tank 4 Inf	0.59	0.06			6.11	0.16	15.67	0.30		
Sample #4 07_17_16	Sun	Anua	5.15	0.11			25.73	0.34	24.41	0.30	432.4	14.1
	Sun	EZ Treat	4.87	0.04			27.18	0.02	25.08	0.42	18552.8	134.0
	Sun	Wetland 4	0.05	0.02			5.37	0.03	16.62	0.20	72720.5	<10.0
	Sun	Wetland 6	N/A	N/A			N/A	N/A	16.22	0.07	9850.3	<10.0
	Sun	Septic Tank	3.86	0.05			3.18	0.02	27.85	0.85	3256692.8	57236.4
Sample #5 08_07_16	Sun	Anua	3.44	0.03			29.70	0.10	20.34	0.10		
	Sun	Anua Well 1	4.39	0.11			31.38	0.16	22.18	0.06		
	Sun	Anua Well 2	3.60	0.05			29.88	0.04	19.77	0.01		
	Sun	EZ Treat	3.26	0.02			27.90	0.29	20.18	0.14		
	Sun	Wetland 4	0.10	0.01			5.84	0.09	13.32	0.06		
	Sun	Wetland 6	N/A	N/A			N/A	N/A	10.08	1.61		
	Sun	Septic Tank	2.97	0.03			8.85	0.04	25.62	0.05		
	Fri	EZ Treat	3.68	0.01			26.54	0.09	19.27	0.16		
Sample #6 08_16_16	Tues	Anua	2.99	0.18			28.81	2.04	18.53	1.35		
	Tues	Anua Well 1	4.51	0.07			30.64	0.11	20.24	0.13		
	Tues	Anua Well 2	3.81	0.04			34.41	0.02	20.93	0.17		
	Tues	EZ Treat	3.72	0.04			24.58	0.49	17.91	0.19		
	Tues	Wetland 4	0.00	N/A			8.23	0.11	11.94	0.25		
	Tues	Wetland 6	nd	N/A			nd	N/A	9.18	0.25		
	Tues	Septic Tank	2.78	0.06			9.86	0.00	16.20	0.04		

Sample	DOTW System		Onsite Measurements			Alkalinity (mg CaCO <sub>3</sub> /L)		Oxygen Demand (mg/L)				TSS (mg/L)		Nitrogen (mg N/L)									
			Temp (°C)	pH	DO (mg/L)	Avg	SD	BOD		COD		Avg	SD	NH <sub>4</sub>		NO <sub>3</sub> <sup>-</sup>		NO <sub>2</sub> <sup>-</sup>		TIN		TN	
								Avg	SD	Avg	SD			Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
Sample #7 08_29_16	Mon	Anua	30.4	6.38	5.61			≤1.6	0.2	85	14			0.1	0.1	18.82	0.16	0.07	0.02	19.0	0.2		
	Mon	Anua Well 1	33.0	7.23	6.80			≤1.2	0.3	20	4			0.2	N/A	25.31	0.13	0.09	0.01	25.6	0.1		
	Mon	Anua Well 2	32.0	7.22	2.90			≤2	0.0	56	4			0.1	N/A	21.30	0.40	0.10	N/A	21.5	0.4		
	Mon	EZ Treat	30.2	6.83	6.32			≤4.6	3.2	20	1			2.0	0.1	22.65	0.24	0.69	0.01	25.3	0.3		
	Mon	Wetland 4	28.7	6.92	2.60			≤0.58	0.00	26	1			nd	N/A	0.23	N/A	0.09	N/A	0.31	N/A		
	Mon	Wetland 6	28.8	6.15	0.94			23.6	0.1	167	11			nd	N/A	0.22	N/A	0.10	N/A	0.33	N/A		
	Mon	Septic Tank	29.4	7.04	0.45	232.9	0.1	75.1	2.6	195	21			32.45	1.38	0.24	0.01	0.10	0.01	32.55	1.38		

Sample	DOTW System		Phosphorous (mg P/L)				Other Ions (mg/L)				Total Col (MPN/100 mL) Geo Mean	E Coli (MPN/ 100 mL) Geo Mean
			PO <sub>4</sub> <sup>3-</sup>		TP		SO <sub>4</sub> <sup>2-</sup>		Cl <sup>-</sup>			
			Avg	SD	Avg	SD	Avg	SD	Avg	SD		
Sample #7 08_29_16	Mon	Anua	3.60	0.06			29.42	0.07	19.04	0.17	1094.8	<2.0
	Mon	Anua Well 1	4.35	0.01			29.47	0.01	19.19	0.12		
	Mon	Anua Well 2	3.78	0.05			29.92	0.04	18.91	0.15		
	Mon	EZ Treat	3.81	0.05			29.26	0.14	19.05	0.08	1684.2	49.0
	Mon	Wetland 4	nd	N/A			13.52	0.34	12.12	0.32	618.0	<2.0
	Mon	Wetland 6	nd	N/A			nd	N/A	9.15	3.35	1753.9	<2.0
	Mon	Septic Tank	3.68	0.12			8.06	0.11	21.94	0.09	1675037.3	57500.0

Sample	Onsite Measurements		Alkalinity (mg CaCO <sub>3</sub> /L)		Oxygen Demand (mg/L)				TSS (mg/L)		Nitrogen (mg N/L)																
											Temp (°C)	pH	DO (mg/L)	BOD		COD		NH <sub>4</sub>		NO <sub>3</sub> <sup>-</sup>		NO <sub>2</sub> <sup>-</sup>		TIN		TN	
														Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
DOTW	System																										
Sample #8 09_26_16	Mon	Anua	20.9	6.09	6.84	35.5	1.8	≤1.0	0.1	64	1			0.08	N/A	25.41	3.55	0.04	N/A	25.53	3.55						
	Mon	Anua Well 1	19.6	5.36	8.08	0.9	0.0	≤1.9	0.2	41	1	1	0	2.90	N/A	31.38	0.80	0.08	0.02	34.36	0.80	34	N/A				
	Mon	Anua Well 2	19.5	7.09	5.78	43.2	1.0	≤1.1	0.1	51	4	0.5	0	0.00	N/A	32.45	0.35	0.04	N/A	32.49	0.35	33	N/A				
	Mon	EZ Treat	20.3	6.30	7.72	10	1	≤3.0	0.1	40	1			4.12	N/A	24.68	0.16	0.25	0.02	29.05	0.16						
	Mon	Wetland 4	18.5	6.87	2.83	293.3	1.8	≤1.8	0.3	45	7			0.06	N/A	0.34	N/A	0.04	N/A	0.44	N/A						
	Mon	Wetland 6	19.5	6.08	1.78	397.7	0.8	21.4	0.7	155	2			nd	N/A	0.69	0.01	0.05	N/A	0.73	0.01						
	Mon	Septic Tank	20.7	6.93	0.66	208.1	2.2	96.9	7.4	231	6			30.35	N/A	0.34	N/A	0.09	0.02	30.44	0.02						
Sample #8B 09_29_16	Mon	Tank 1 Eff	19.6	6.92	0.96									25.3	0.1	0.69	0.00	0.04	N/A	26.0	0.1						
	Mon	Tank 2 Inf	18.4	6.93	4.20									26.6	0.1	0.69	0.00	0.19	0.16	27.5	0.2						
	Mon	Tank 2 Eff	19.0	6.61	4.37									0.77	0.08	0.34	N/A	0.05	N/A	1.17	N/A						
	Mon	Tank 3 Inf	18.8	6.79	2.52									1.0	0.0	0.70	0.01	0.04	N/A	1.7	N/A						
Sample #9 10_22_16	Sat	Anua	18.5	6.98	7.37	75.4	0.5	≤1.4	0.1	78	2	4	1	0.05	N/A	35.78	8.35	0.02	0.01	35.85	8.35	42	0				
	Sat	Anua Well 1	18.0	7.70	5.96	273.7	1.0	10.0	0.0	88	0	1	N/A	23.60	0.08	0.99	0.02	5.28	0.08	29.86	0.12	38	7				
	Sat	Anua Well 2	18.9	7.36	5.08	93.1	0.3	≤1.04	0.3	65	1	1	N/A	0.20	0.10	41.29	0.09	0.09	0.00	41.58	0.13	44	N/A				
	Sat	EZ Treat	17.4	7.21	8.32			≤2.2	1.7	26	0	4	0	2.0	N/A	22.63	0.62	0.44	0.01	25.1	0.6	32	4				
	Sat	Wetland 4	16.7	7.10	3.77			≤1.3	0.1	30	1	1	0	nd	N/A	0.71	0.01	nd	N/A	0.71	0.01	2	0				
	Sat	Wetland 6	16.7	6.55	1.94			18.0	0.1	20	25	1	0	nd	N/A	0.35	N/A	nd	N/A	0.35	N/A	3	3				
	Sat	Septic Tank	15.7	6.97	1.32	238.2	N/A	86.7	9.7	155	7	23	7	32.73	0.15	0.35	N/A	0.02	N/A	32.75	0.15	43	N/A				
Sample #9B 10_22_16	Sat	Tank 1 Eff	18.0	6.90	1.01	293.7	N/A			54	3			23.9	1.7	0.69	0.01	nd	N/A	24.6	1.7						
	Sat	Tank 2 Inf	17.2	6.96	2.12	283.2	N/A			54	1			25.3	N/A	0.34	N/A	0.14	N/A	25.8	N/A						
	Sat	Tank 2 Eff	19.2	7.31	6.45	284.0	N/A			40	5			6.66	N/A	0.72	0.00	0.03	0.00	7.41	N/A						
	Sat	Tank 3 Inf	18.8	7.14	7.84	297.1	N/A			36	1			9.02	N/A	1.00	0.03	0.07	0.00	10.1	N/A						
Sample #10 11_16_16	Wed	Anua	17.3	6.99	9.07			≤1.7	0.2	58	1	8	0	0.06	N/A	15.78	0.42	0.04	N/A	15.88	0.42	15	2				
	Wed	Anua Well 1	17.3	7.71	9.82	94.1	3.8	≤1.1	0.1	4	N/A	1	1	0.08	N/A	16.34	0.24	0.08	0.01	16.50	0.24	18	N/A				
	Wed	Anua Well 2	16.0	7.66	7.30	95.5	N/A	≤2.6	0.1	17	21	1	1	nd	N/A	16.60	0.54	0.03	N/A	16.63	0.54	15	N/A				
	Wed	EZ Treat	16.3	6.94	9.10	24.1	0.5	≤2.8	0.2	16	6	3	N/A	2.1	0.2	28.14	0.50	0.43	0.00	30.7	0.5	31	1				
	Wed	Wetland 4	14.5	10.55	8.22	248.2	N/A	6.6	0.3	17	3	1	1	3.2	0.2	3.54	0.12	0.48	0.01	7.2	0.2	7	7				
	Wed	Wetland 6	14.8	6.54	2.52	316.4	N/A	17.2	0.6	91	5	11	1	nd	N/A	0.67	N/A	0.04	N/A	0.71	N/A	11	22				
	Wed	Septic Tank	20.7	7.06	1.17			61.4	1.7	226	3	43	2	29.68	N/A	0.67	0.01	0.06	0.02	30.41	N/A	30	0				
Sample #10B 11_16_16	Wed	Tank 1 Eff	15.7	7.27	0.84	256.4	N/A			97	12			32.59	N/A	0.68	N/A	nd	N/A	33.27	N/A						
	Wed	Tank 2 Inf	19.7	7.38	2.01	254.0	N/A			74	8			31.71	N/A	0.67	0.02	0.06	N/A	32.44	N/A						
	Wed	Tank 3 Inf	20.3	7.74	8.19	222.2	N/A			17	3			15.4	N/A	5.12	0.16	0.77	0.02	21.3	N/A						
	Wed	Tank 3 Eff	16.0	7.94	9.10	260.7	N/A			25	3			5.32	N/A	2.84	N/A	0.29	N/A	8.45	N/A						
	Wed	Tank 4 Inf	17.2	7.82	8.71	261.6	N/A			17	0			5.5	0.2	2.85	0.07	0.33	0.00	8.7	0.2						

No duplicates in sampling set  
 Value above analytical test range  
 RSD > 10%  
 RSD ≥ 100%

Sample	DOTW	System	Phosphorous (mg P/L)				Other Ions (mg/L)				Total Col (MPN/100 mL) Geo Mean	E Coli (MPN/ 100 mL) Geo Mean
			PO <sub>4</sub> <sup>3-</sup>		TP		SO <sub>4</sub> <sup>2-</sup>		Cl <sup>-</sup>			
			Avg	SD	Avg	SD	Avg	SD	Avg	SD		
Sample #8 09_26_16	Mon	Anua	4.33	0.35	4.30	0.05	24.06	3.22	17.68	2.57		
	Mon	Anua Well 1	5.42	0.17	4.24	0.05	28.71	0.32	20.92	0.17		
	Mon	Anua Well 2	4.92	0.49	4.39	0.07	29.28	0.15	21.11	0.04		
	Mon	EZ Treat	4.69	0.09	3.73	0.07	29.81	0.22	20.35	0.15		
	Mon	Wetland 4	0.42	0.12	nd	N/A	14.81	0.04	16.66	0.02		
	Mon	Wetland 6	nd	N/A	nd	N/A	nd	N/A	11.35	0.50		
	Mon	Septic Tank	3.50	0.14	3.2	0.1	11.75	0.07	16.54	0.18		
Sample #8B 09_29_16	Mon	Tank 1 Eff	3.00	0.25	2.5	0.3	10.37	0.98	19.25	2.25		
	Mon	Tank 2 Inf	3.55	0.13	2.3	0.1	12.48	0.09	20.39	0.38		
	Mon	Tank 2 Eff	0.19	N/A	nd	N/A	1.99	0.00	21.71	0.20		
	Mon	Tank 3 Inf	0.70	0.01	nd	N/A	2.46	0.05	21.66	0.28		
Sample #9 10_22_16	Sat	Anua	11.19	1.89	13.09	1.13	33.16	7.14	29.25	6.75	4405.9	45.0
	Sat	Anua Well 1	13.25	0.42	13.13	0.02	37.66	0.13	31.84	0.10		
	Sat	Anua Well 2	13.04	0.33	12.31	0.12	38.48	0.13	33.28	0.15		
	Sat	EZ Treat	4.40	0.17	4.89	0.78	40.67	0.89	24.05	0.66	>4839.2	709.5
	Sat	Wetland 4	0.49	0.01	nd	N/A	10.47	0.04	24.23	0.13	>4839.2	61.5
	Sat	Wetland 6	nd	N/A	nd	N/A	2.66	0.06	20.62	0.38	>4839.2	10.3
	Sat	Septic Tank	4.25	0.10	4.0	0.0	2.44	0.03	20.96	0.06	3980000.0	120000.0
Sample #9B 10_22_16	Sat	Tank 1 Eff	4.17	0.10			4.61	0.13	25.75	0.92		
	Sat	Tank 2 Inf	3.68	0.46			15.18	2.80	22.02	4.67		
	Sat	Tank 2 Eff	1.41	0.03			13.41	0.37	28.70	0.54		
	Sat	Tank 3 Inf	2.07	0.05			11.70	0.66	28.66	1.51		
Sample #10 11_16_16	Wed	Anua	10.97	0.22	13.84	2.33	39.45	0.82	27.62	0.46	976.7	3.5
	Wed	Anua Well 1	10.38	0.13	11.58	0.14	36.88	0.42	26.50	0.38		
	Wed	Anua Well 2	10.83	0.39	12.03	0.05	39.09	1.51	27.12	0.91		
	Wed	EZ Treat	3.92	0.08	5.48	0.97	30.17	0.50	24.95	0.41	>4839.2	4384.5
	Wed	Wetland 4	1.41	0.05	1.45	0.07	22.24	0.90	28.40	1.12	>4839.2	63.2
	Wed	Wetland 6	nd	N/A	1.53	1.61	0.71	0.02	24.32	0.06	3281.1	<2.0
	Wed	Septic Tank	3.40	0.02	5.19	0.32	2.68	0.01	19.66	0.44	3077140.7	287499.6
Sample #10B 11_16_16	Wed	Tank 1 Eff	3.79	0.05	4.16	0.07	13.61	0.37	24.31	0.82		
	Wed	Tank 2 Inf	3.68	0.20	4.92	1.25	21.85	1.12	24.45	1.16		
	Wed	Tank 3 Inf	2.73	0.10	2.79	0.02	18.71	0.78	26.70	2.31		
	Wed	Tank 3 Eff	1.45	N/A	1.26	0.02	23.62	N/A	29.85	N/A		
	Wed	Tank 4 Inf	1.50	0.04	1.65	0.62	22.61	0.80	28.77	1.05		

## **C.4 Pilot System PPCP Results**

Table C.4.1: Pilot system PPCPs results

Sample	DOTW	System	Concentration (µg/L)							
			ACE	CBZ	LAM	LEV	MET	TOP	TCS	VEN
Sample #1 06_19_16	Sat	Anua	<LOQ	<LOQ	0.036	26.654	<LOQ	<LOQ	1.500	0.046
	Sat	EZ Treat	<LOQ	102.905	151.339	56.662	<LOQ	<LOQ	2.115	6.557
	Sat	Wetland 6	0.038	6.395	4.467	1.774	<LOQ	<LOQ	1.321	<LOQ
	Sat	Septic Tank	244.396	148.547	527.045	386.120	0.881	0.330	8.506	10.327
	Sat	Field Blank	0.093	0.048	0.102	0.185	<LOQ	<LOQ	2.600	<LOQ
Sample #2 06_29_16	Wed	Anua	<LOQ	0.201	<LOQ	0.342	<LOQ	<LOQ	2.477	<LOQ
	Wed	EZ Treat	0.044	88.010	138.263	42.655	0.035	<LOQ	2.926	8.676
	Wed	Wetland 4	0.045	56.126	93.387	7.291	0.037	<LOQ	3.708	0.041
	Wed	Wetland 6	0.101	10.207	5.089	0.484	<LOQ	<LOQ	2.186	<LOQ
	Wed	Septic Tank	181.318	71.083	125.761	131.988	0.074	<LOQ	9.167	5.791
	Wed	Field Blank	<LOQ	<LOQ	0.040	0.069	<LOQ	<LOQ	1.663	<LOQ
Sample #3 07_07_16	Thurs	Anua	1.108	1.177	1.218	0.481	<LOQ	<LOQ	1.722	0.034
	Thurs	EZ Treat	0.367	105.529	141.336	4.815	<LOQ	<LOQ	1.424	12.119
	Thurs	Wetland 4	0.186	47.317	92.318	1.794	<LOQ	<LOQ	<LOQ	<LOQ
	Thurs	Wetland 6	0.744	9.827	5.469	0.372	<LOQ	<LOQ	<LOQ	0.035
	Thurs	Septic Tank	182.549	74.995	147.034	43.620	0.056	<LOQ	4.186	9.566
	Thurs	Field Blank	0.236	0.131	0.472	0.285	<LOQ	<LOQ	<LOQ	<LOQ
Sample #4 07_17_16	Sun	Anua	<LOQ	1.717	0.196	0.147	<LOQ	<LOQ	1.004	<LOQ
	Sun	EZ Treat	0.100	87.185	139.516	30.334	<LOQ	<LOQ	2.479	7.176
	Sun	Wetland 4	<LOQ	52.468	104.580	0.274	<LOQ	<LOQ	0.957	<LOQ
	Sun	Wetland 6	0.046	9.991	4.143	0.081	<LOQ	<LOQ	<LOQ	<LOQ
	Sun	Septic Tank	154.193	61.308	151.390	142.704	0.063	<LOQ	53.276	5.288
	Sun	Field Blank	0.040	<LOQ	0.043	0.079	<LOQ	<LOQ	0.872	<LOQ
Sample #5 08_07_16	Sun	Anua	0.055	6.134	0.192	0.271	<LOQ	<LOQ	<LOQ	<LOQ
	Sun	Anua Well 1	0.245	50.049	115.850	3.147	0.694	<LOQ	1.695	4.108
	Sun	Anua Well 2	<LOQ	19.771	3.449	0.466	<LOQ	<LOQ	<LOQ	0.072
	Sun	EZ Treat	0.038	52.600	124.618	10.775	0.686	<LOQ	1.744	5.417
	Sun	Wetland 4	<LOQ	36.168	55.980	7.166	<LOQ	<LOQ	<LOQ	<LOQ
	Sun	Wetland 6	0.177	13.170	5.977	1.109	0.051	0.067	1.073	0.033
	Sun	Septic Tank	56.087	35.462	104.846	114.235	0.268	<LOQ	27.751	4.611
	Sun	Field Blank	0.091	<LOQ	0.059	0.087	<LOQ	<LOQ	<LOQ	<LOQ
Fri	EZ Treat	2.086	49.041	111.658	18.567	0.427	<LOQ	1.521	5.647	
Sample #6 08_16_16	Tues	Anua	<LOQ	5.614	0.176	0.056	<LOQ	<LOQ	1.460	<LOQ
	Tues	Anua Well 1	0.097	57.560	119.295	2.149	0.453	<LOQ	<LOQ	3.584
	Tues	Anua Well 2	<LOQ	31.941	7.446	0.092	0.034	<LOQ	<LOQ	0.035
	Tues	EZ Treat	0.950	60.579	134.396	29.167	0.339	<LOQ	1.246	4.606
	Tues	Wetland 4	<LOQ	40.313	49.284	0.508	<LOQ	<LOQ	<LOQ	<LOQ
	Tues	Wetland 6	<LOQ	12.573	5.568	0.057	<LOQ	<LOQ	<LOQ	<LOQ
	Tues	Septic Tank	11.894	43.416	98.415	118.730	0.125	<LOQ	14.636	1.093
	Tues	Field Blank	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Mon	Anua	<LOQ	27.518	6.138	0.137	<LOQ	0.113	<LOQ	0.039	
Mon	Anua Well 1	0.253	52.327	127.526	4.652	0.136	0.110	0.853	1.560	

Sample	DOTW	System	Concentration (µg/L)							
			ACE	CBZ	LAM	LEV	MET	TOP	TCS	VEN
Sample #7 08_29_16	Mon	Anua Well 2	<LOQ	9.483	0.216	0.070	<LOQ	0.076	<LOQ	<LOQ
	Mon	EZ Treat	1.307	54.278	149.963	17.115	0.049	0.069	0.792	1.145
	Mon	Wetland 4	<LOQ	40.010	52.713	0.078	<LOQ	<LOQ	<LOQ	<LOQ
	Mon	Wetland 6	<LOQ	11.531	2.772	0.056	<LOQ	<LOQ	0.754	<LOQ
	Mon	Septic Tank	32.186	40.613	134.277	129.845	0.052	0.081	18.184	0.464
	Mon	Field Blank	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Sample #8 09_26_16	Mon	Anua	<LOQ	16.082	0.247	0.479	<LOQ	<LOQ	<LOQ	<LOQ
	Mon	Anua Well 1	0.205	28.561	96.254	9.829	<LOQ	<LOQ	<LOQ	0.441
	Mon	Anua Well 2	<LOQ	21.369	2.404	0.636	<LOQ	<LOQ	<LOQ	0.067
	Mon	EZ Treat	0.715	61.178	154.207	20.905	<LOQ	<LOQ	<LOQ	0.862
	Mon	Wetland 4	<LOQ	30.775	49.997	0.311	<LOQ	0.070	0.384	<LOQ
	Mon	Wetland 6	<LOQ	6.630	1.611	0.201	<LOQ	<LOQ	2.468	<LOQ
	Mon	Septic Tank	85.878	53.845	122.681	124.888	0.148	<LOQ	8.265	0.224
	Mon	Field Blank	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.737	<LOQ
Sample #8B 09_29_16	Mon	Tank 1 Eff	0.174	32.036	99.567	71.870	0.056	<LOQ	3.524	0.178
	Mon	Tank 2 Inf	0.185	45.707	138.077	97.658	0.046	<LOQ	3.213	0.232
	Mon	Tank 2 Eff	<LOQ	36.595	90.354	0.387	<LOQ	0.069	1.873	<LOQ
	Mon	Tank 3 Inf	<LOQ	38.347	164.568	0.748	<LOQ	<LOQ	1.741	<LOQ
Sample #9 10_22_16	Sat	Anua	0.915	16.376	1.690	4.441	1.121	0.154	<LOQ	0.119
	Sat	Anua Well 1	<LOQ	30.900	104.109	1.007	0.036	<LOQ	<LOQ	0.388
	Sat	Anua Well 2	<LOQ	26.712	5.546	0.034	<LOQ	<LOQ	<LOQ	0.047
	Sat	EZ Treat	0.509	42.113	124.241	17.214	<LOQ	<LOQ	<LOQ	0.534
	Sat	Wetland 4	<LOQ	41.828	80.331	3.863	<LOQ	<LOQ	<LOQ	<LOQ
	Sat	Wetland 6	<LOQ	17.901	13.281	0.570	<LOQ	<LOQ	<LOQ	<LOQ
	Sat	Septic Tank	9.849	31.267	108.544	131.011	0.096	<LOQ	1.999	0.334
	Sat	Field Blank	<LOQ	<LOQ	0.098	0.087	<LOQ	<LOQ	<LOQ	<LOQ
Sample #9B 10_22_16	Sat	Tank 1 Eff	0.413	38.865	135.017	130.432	0.051	<LOQ	1.730	0.176
	Sat	Tank 2 Inf	0.274	43.534	151.540	113.944	0.041	<LOQ	1.616	0.210
	Sat	Tank 2 Eff	<LOQ	47.064	123.668	35.262	<LOQ	<LOQ	<LOQ	0.040
	Sat	Tank 3 Inf	<LOQ	51.016	125.791	59.307	<LOQ	<LOQ	<LOQ	0.038
Sample #10 11_16_16	Wed	Anua	0.060	14.630	1.031	0.400	0.106	0.129	<LOQ	<LOQ
	Wed	Anua Well 1	0.279	38.100	141.208	1.627	0.052	0.050	<LOQ	0.531
	Wed	Anua Well 2	<LOQ	21.487	7.142	<LOQ	<LOQ	0.052	<LOQ	0.051
	Wed	EZ Treat	3.629	33.503	147.013	19.227	<LOQ	<LOQ	<LOQ	0.499
	Wed	Wetland 4	0.095	30.235	88.782	1.291	<LOQ	<LOQ	<LOQ	<LOQ
	Wed	Wetland 6	<LOQ	18.967	11.986	0.056	<LOQ	<LOQ	<LOQ	<LOQ
	Wed	Septic Tank	63.659	21.573	119.454	117.303	0.132	<LOQ	1.070	0.253
	Wed	Field Blank	<LOQ	<LOQ	0.068	0.093	<LOQ	<LOQ	<LOQ	<LOQ
Sample #10B 11_16_16	Wed	Tank 1 Eff	5.320	19.490	133.089	113.898	0.048	<LOQ	1.215	0.121
	Wed	Tank 2 Inf	0.247	34.424	117.574	37.221	0.034	<LOQ	<LOQ	0.045
	Wed	Tank 3 Inf	4.560	22.452	143.147	281.710	0.050	<LOQ	0.893	0.163
	Wed	Tank 3 Eff	<LOQ	33.190	107.587	2.749	<LOQ	<LOQ	<LOQ	<LOQ
	Wed	Tank 4 Inf	0.071	34.806	110.695	3.047	<LOQ	<LOQ	<LOQ	<LOQ

## C.5 Evapotranspiration Calculations

Evapotranspiration rates were estimated using the Penman Monteith equation and weather data from the Mount Fair Farm Weather Underground station (KVACROZE8) located 2.1 miles (3.4 km) and at elevation 220 ft (67 m) lower than the project site. The calculation procedure, summarized below, was based on the FAO Guidelines for Computing Crop Water Requirements (Allen et al, 1998).

1. Site location was found using Google maps: (38.2, -78.7), elevation 1020 ft.
2. Weather data including maximum and minimum daily temperatures, maximum daily humidity, precipitation, and cloud cover was downloaded for dates encompassing the sampling period (June 1 – November 30).
3. Atmospheric pressure was calculated as follows:

$$P_{atm} = 101.3 \left[ \frac{293 - 0.0065z}{293} \right]^{5.26}$$

Where  $P_{atm}$  is in kPa and  $z$  is the elevation in m.

4. The psychrometric constant was calculated as follows:

$$\gamma = 0.000665P_{atm}$$

Where  $\gamma$  is in kPa/°C and  $P_{atm}$  is the atmospheric pressure in kPa.

5. Daily average temperatures ( $T_{mean}$ ) were calculated as an average of  $T_{min}$  and  $T_{max}$ .
6. Saturation vapor pressure was calculated as follows:

$$e^o(T) = 0.6108 \exp\left(\frac{17.27T}{T + 237.3}\right)$$

$$e_s = \frac{e^o(T_{max}) + e^o(T_{min})}{2}$$

Where  $e_s$  is in kPa and  $T$  is in °C.

7. Actual vapor pressure was calculated as follows:

$$e_a = e^o(T_{min}) \times \frac{RH_{max}}{100}$$

Where  $e_a$  is in kPa,  $RH_{max}$  is a percent, and T is in °C.

8. The slope of the vapor pressure curve is calculated as the derivative of the saturation vapor pressure equation:

$$\Delta(T) = \frac{4090 \times 0.6108 \exp\left(\frac{17.27T}{T + 237.3}\right)}{(T + 237.3)^2}$$

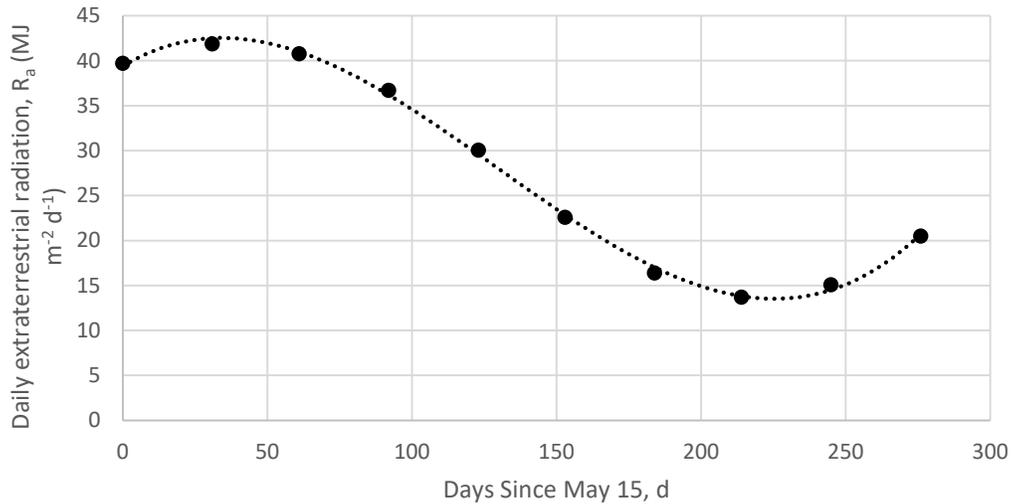
$$\Delta = \frac{\Delta(T_{max}) + \Delta(T_{min})}{2}$$

Where  $\Delta$  is in kPa/°C and T is in °C.

9. Extraterrestrial radiation ( $R_a$ ) values for different latitudes were provided in Annex 2 of the FAO report. The monthly average value at latitude 38.2 was interpolated between provided values at 38 and 40 degrees. Then, an equation was developed for the  $R_a$  value based on days past May 15<sup>th</sup>, assuming that the average value was the actual  $R_a$  value in the middle of the month. The table, graph, and equation ( $R^2 = 0.998$ ) for the relationship are shown below. This equation was used to calculate  $R_a$  values throughout the time period of interest.

**Table C.5.1: Monthly extraterrestrial radiation values as a function of latitude**

Date	Days Since 5/15	$R_A$ (MJ/m <sup>2</sup> d) Latitude Degree		
		40.0	38.2	38.0
5/15/2016	0	39.7	39.7	39.9
6/15/2016	31	41.9	41.9	41.8
7/15/2016	61	40.8	40.8	40.8
8/15/2016	92	36.7	36.7	37.0
9/15/2016	123	30.0	30.1	30.7
10/15/2016	153	22.5	22.6	23.6
11/15/2016	184	16.3	16.4	17.5
12/15/2016	214	13.6	13.7	14.8
1/15/2017	245	15.0	15.1	16.2
2/15/2017	276	20.4	20.5	21.5



**Figure C.5.1** Monthly extraterrestrial radiation values at latitude 38.2 degrees  
 $R_a = 8.32 \times 10^{-6}d^3 - 3.24 \times 10^{-3}d^2 + 0.193d + 39.40$

10. Solar radiation,  $R_s$  was calculated using the Angstrom formula:

$$R_s = \left( a_s + b_s \frac{n}{N} \right) R_a$$

Where  $R_s$  is the solar radiation in MJ/m<sup>2</sup>d,  $a_s$  and  $b_s$  are regression constants with value 0.25 and 0.50, respectively,  $n/N$  is the relative sunshine duration, and  $R_a$  is the extraterrestrial radiation in MJ/m<sup>2</sup>d. The relative sunshine duration was estimated based on cloud cover data from the weather station. Cloud cover values are integers from 0 to 8, representing the fraction of the sky covered by clouds. The relative sunshine duration was estimated using the following formula:

$$\frac{n}{N} = \frac{8 - CC}{8}$$

Where CC is the cloud cover.

11. The clear sky solar radiation,  $R_{s0}$  is the  $R_s$  value when  $n/N$  is equal to 1. However, if calibrated  $a_s$  and  $b_s$  values are not available, it is recommended that the following equation be used instead.

$$R_{so} = (0.75 + 2 \times 10^{-5}z)R_a$$

Where  $R_{so}$  is the clear sky solar radiation in MJ/m<sup>2</sup>d,  $z$  is the elevation in m, and  $R_a$  is the extraterrestrial radiation in MJ/m<sup>2</sup>d.

12. The net solar radiation,  $R_{ns}$  is calculated as follows:

$$R_{ns} = (1 - \alpha)R_s$$

Where  $R_{ns}$  is the net solar radiation in MJ/m<sup>2</sup>d,  $\alpha$  is the albedo, and  $R_s$  is the solar radiation in MJ/m<sup>2</sup>d. An  $\alpha$  value of 0.23 is recommended for a hypothetical grass reference crop.

13. The net long-wave radiation,  $R_{nl}$  is calculated as follows:

$$R_{nl} = 4.903 \times 10^{-9} \left[ \frac{T_{max,K}^4 + T_{min,K}^4}{2} \right] (0.34 - 0.14\sqrt{e_a}) \left( 1.35 \frac{R_s}{R_{so}} - 0.35 \right)$$

Where  $T_{max,K}$  and  $T_{min,K}$  are temperature in Kelvin,  $e_a$  is the actual vapor pressure in kPa,  $R_s$  is the solar radiation in MJ/m<sup>2</sup>d, and  $R_{so}$  is the clear sky solar radiation in MJ/m<sup>2</sup>d.

14. The net radiation,  $R_n$  is calculated as follows:

$$R_n = R_{ns} - R_{nl}$$

Where  $R_n$  is the net radiation,  $R_{ns}$  is the net solar radiation, and  $R_{nl}$  is the net long wave radiation, and all are in units of MJ/m<sup>2</sup>d.

15. The reference evapotranspiration is calculated based on weather and radiation using the following equation:

$$ET_o = \frac{1}{25.4} \left[ \frac{0.408\Delta(R_n - G) + \gamma \frac{900}{T + 273.2} u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34u_2)} \right]$$

Where  $ET_o$  is the reference evapotranspiration in in/day,  $R_n$  is the net radiation in MJ/m<sup>2</sup>d,  $G$  is the soil heat flux density (approximated as zero for daily time steps),  $\gamma$  is the psychrometric constant in kPa/°C,  $T$  is the mean temperature in °C,  $u_2$  is the wind speed at an elevation of 2 m in m/s, and  $e_s - e_a$  is the saturation vapor pressure deficit in kPa.

16. The crop coefficient,  $K_c$ , accounts for both crop transpiration and soil evaporation and is used to adjust the reference evapotranspiration to the crop evapotranspiration. Values of  $K_c$  for three growing stages (initial, mid, and end) were found in Table 12 using values for “Reed swamp, moist soil” in the group “Wetlands – temperature climate.” The  $K_{c,ini}$  value of 0.90 was used for the month of June. The  $K_{c,mid}$  value of 1.20 was used for the months July through September. The  $K_{c,end}$  value of 0.70 was used for the months of October and November.

17. The daily crop evapotranspiration rate,  $ET_c$  was calculated using the following equation:

$$ET_c = K_c ET_o$$

Where  $K_c$  is unitless and ET has value of in/day.

## C.6 Statistics Calculations – R Code

### Nitrogen Calculations

```
> N<-read.csv("nitrogen.csv")
> attach(N)
> #Determine whether measured treated TIN concentrations are
> #statistically different from estimated TN concentrations
> #(based on TSS measurements)
> TINVTN<-(esttTN-tTIN);TINVTN
 [1] 0.372 1.364 0.124 0.992 0.248 0.124 0.124 0.062 0.620 0.496 0.496 0.124
[13] 0.124 0.992 0.372 0.124 1.364
> shapiro.test(TINVTN)
      Shapiro-wilk normality test
data:  TINVTN
w = 0.81446, p-value = 0.003225
> lnTINVTN<-log(TINVTN)
> shapiro.test(lnTINVTN)
      Shapiro-wilk normality test
data:  lnTINVTN
w = 0.9046, p-value = 0.08113
> #Not parametric, so need to build CI using bootstrapping
> n<-100000
> bootTINVTN<-numeric(n)
> for (i in 1:n) {
+ bootTINVTN[i]<-mean(sample(TINVTN,replace=T))
+ }
> hist(bootTINVTN)
> quantile(bootTINVTN,probs=c(0.025,0.975))
      2.5%      97.5%
0.2881176 0.6929412
> mean(bootTINVTN)
[1] 0.4775729
```

```

> #Now find out if the estimated TN values are significantly different
> #from the TN values measured using Hach kits
> eTNvmTN<-measTN-estTN_short
> shapiro.test(eTNvmTN)
      Shapiro-wilk normality test
data:  eTNvmTN
W = 0.95914, p-value = 0.7715
> mu<-mean(eTNvmTN,na.rm=TRUE); mu
[1] 2.118667
> s<-sd(eTNvmTN,na.rm=TRUE); s
[1] 4.194994
> n<-length(which(eTNvmTN!="NA"));n
[1] 12
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] -0.5467054  2.1186667  4.7840388
> #Compare previous and current septic tank ammonia concentrations
> shapiro.test(stAMM)
      Shapiro-wilk normality test
data:  stAMM
W = 0.9055, p-value = 0.2856
> shapiro.test(qAMM)
      Shapiro-wilk normality test
data:  qAMM
W = 0.83548, p-value = 0.06767
> mu<-mean(stAMM,na.rm=TRUE); mu
[1] 31.48556
> s<-sd(stAMM,na.rm=TRUE); s
[1] 4.958748
> n<-length(which(stAMM!="NA"));n
[1] 9
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 27.67392 31.48556 35.29719
> mu<-mean(qAMM,na.rm=TRUE); mu
[1] 32.365
> s<-sd(qAMM,na.rm=TRUE); s
[1] 7.113311
> n<-length(which(qAMM!="NA"));n
[1] 8
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 26.41812 32.36500 38.31188

```

**Table C.6.1: Data from nitrogen.csv**

fTN	fTSS	esttTN	estTN_short	measTN	stAMM	stTN	stTSS	qAMM	qTN
7.5	3	7.872	29.962	38	31.87	50.18172	68	29.7	41.1
26.21	11	27.574	41.662	44	41.02	23.73973	14	31	40.2

0.65	2	0.898	36.346	42		33.71	35.597	23		44.53	55.9
0.65	8	1.642	25.596	32		29.56	35.7779	43		41.9	51.9
22.25	2	22.498	0.834	2		22				30.32	39.6
16.72	1	16.844	0.474	3		32.45				30.87	50.6
12.45	1	12.574	16.624	18		30.35				25.1	29.4
7.03	1	7.154	16.754	15		32.73				25.5	33.3
22.1	1	22.224	16.872	15		29.68					
0.4	1	0.524	31.072	31							
0.31	5	0.93	35.6	43							
34.36	1	34.484	35.78	30							
32.49	0.5	32.552									
29.9	0.5	29.962									
41.6	0.5	41.662									
35.85	4	36.346									
25.1	4	25.596									
0.71	1	0.834									
0.35	1	0.474									
16.5	1	16.624									
16.63	1	16.754									
15.88	8	16.872									
30.7	3	31.072									
7.2	1	7.324									
0.71	11	2.074									

## ANOVA Calculations – Conventional Pollutants

```
> library(car)
> library(pgirmess)
> dat<-read.csv("anova.csv")
> time<-dat$time
```

### *Alkalinity*

```
> ###set-up
> stAlk<-dat$stAlk
> avgstAlk<-mean(stAlk,na.rm=TRUE)
> sdstAlk<-sd(stAlk,na.rm=TRUE)
```

```

> RSDA1k<-sdstA1k/avgstA1k;RSDA1k
[1] 0.1658921
> dwt(lm(stA1k~time))
lag Autocorrelation D-W Statistic p-value
 1      -0.6441718      3.257586  0.118
Alternative hypothesis: rho != 0
> anuaA1k<-dat$anuaA1k
> rranuaA1k<-(avgstA1k-anuaA1k)/avgstA1k
> anua2A1k<-dat$anua2A1k
> rranua2A1k<-(avgstA1k-anua2A1k)/avgstA1k
> ezA1k<-dat$ezA1k
> rrezA1k<-(avgstA1k-ezA1k)/avgstA1k
> w4A1k<-dat$w4A1kN
> rrw4A1k<-(avgstA1k-w4A1k)/avgstA1k
> w6A1k<-dat$w6A1kN
> rrw6A1k<-(avgstA1k-w6A1k)/avgstA1k
> shapiro.test(rranuaA1k)
      Shapiro-wilk normality test
data:  rranuaA1k
W = 0.95029, p-value = 0.7179
> shapiro.test(rrezA1k)
      Shapiro-wilk normality test
data:  rrezA1k
W = 0.95733, p-value = 0.7621
> shapiro.test(rrw4A1k)
      Shapiro-wilk normality test
data:  rrw4A1k
W = 0.83944, p-value = 0.1938
> shapiro.test(rrw6A1k)
      Shapiro-wilk normality test
data:  rrw6A1k
W = 0.98473, p-value = 0.9291
> ###independent?
> #null hypothesis - no correlation
> #p>0.05 indicates independence
> dwt(lm(rranuaA1k~time))
lag Autocorrelation D-W Statistic p-value
 1      -0.7831793      3.409291  0.09
Alternative hypothesis: rho != 0
> dwt(lm(rrezA1k~time))
lag Autocorrelation D-W Statistic p-value
 1      -0.6356196      3.116247  0.41
Alternative hypothesis: rho != 0
> dwt(lm(rrw4A1k~time))
lag Autocorrelation D-W Statistic p-value
 1      -0.4071557      2.408215  0.854
Alternative hypothesis: rho != 0
> dwt(lm(rrw6A1k~time))
lag Autocorrelation D-W Statistic p-value
 1      -0.7485996      3.382346  0.242
Alternative hypothesis: rho != 0
> ###equal variance?
> #null hypothesis - equal variance
> #p>0.05 indicates equal variance
> rrA1k<-cbind(rranuaA1k,rrezA1k,rrw4A1k,rrw6A1k)
> rrA1kstack<-stack(data.frame(rrA1k))

```

```

> #use with parametric cases
> bartlett.test(rrAlkstack$values,rrAlkstack$ind)
      Bartlett test of homogeneity of variances
data:  rrAlkstack$values and rrAlkstack$ind
Bartlett's K-squared = 2.1482, df = 3, p-value = 0.5422
> ###are column means the same?
> #null hypothesis - column means are the same
> #p<0.05 indicates significantly different means
> #use with parametric cases when variances are equal
> summary(aov(rrAlkstack$values~rrAlkstack$ind))
              Df Sum Sq Mean Sq F value    Pr(>F)
rrAlkstack$ind  3  4.597   1.532   190.4 2.2e-10 ***
Residuals     12  0.097   0.008
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
48 observations deleted due to missingness
> ###if column means are different, which columns?
> #null hypothesis - column means are the same
> #p<0.05 indicates significantly different means
> TukeyHSD(aov(rrAlkstack$values~rrAlkstack$ind))
      Tukey multiple comparisons of means
      95% family-wise confidence level
Fit: aov(formula = rrAlkstack$values ~ rrAlkstack$ind)
`rrAlkstack$ind`
              diff          lwr          upr      p adj
rrezAlk-rranuaAlk  0.09115162 -0.09716872  0.2794720 0.5018206
rrw4Alk-rranuaAlk -0.80699994 -0.99532028 -0.6186796 0.0000001
rrw6Alk-rranuaAlk -1.17713968 -1.36546002 -0.9888193 0.0000000
rrw4Alk-rrrezAlk  -0.89815156 -1.08647190 -0.7098312 0.0000000
rrw6Alk-rrrezAlk  -1.26829130 -1.45661164 -1.0799710 0.0000000
rrw6Alk-rrw4Alk  -0.37013974 -0.55846008 -0.1818194 0.0004031
> #compare rrw4Alk and rrw6Alk to 0
> mu<-mean(rrw4Alk,na.rm=TRUE); mu
[1] -0.01742679
> s<-sd(rrw4Alk,na.rm=TRUE); s
[1] 0.05964531
> n<-length(which(rrw4Alk!="NA"));n
[1] 4
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] -0.11233578 -0.01742679  0.07748220
> mu<-mean(rrw6Alk,na.rm=TRUE); mu
[1] -0.3875665
> s<-sd(rrw6Alk,na.rm=TRUE); s
[1] 0.1068473
> n<-length(which(rrw6Alk!="NA"));n
[1] 4
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] -0.5575845 -0.3875665 -0.2175486
> #CI for anuaAlk
> mu<-mean(anuaAlk,na.rm=TRUE); mu

```

```

[1] 47.325
> s<-sd(anuaA1k,na.rm=TRUE); s
[1] 26.55038
> n<-length(which(anuaA1k!="NA"));n
[1] 4
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] 5.077426 47.325000 89.572574
> #CI for anua2A1k
> mu<-mean(anua2A1k,na.rm=TRUE); mu
[1] 39.65
> s<-sd(anua2A1k,na.rm=TRUE); s
[1] 33.44615
> n<-length(which(anua2A1k!="NA"));n
[1] 2
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] -260.8517 39.6500 340.1517
> #CI for ezA1k
> mu<-mean(ezA1k,na.rm=TRUE); mu
[1] 26.825
> s<-sd(ezA1k,na.rm=TRUE); s
[1] 12.87488
> n<-length(which(ezA1k!="NA"));n
[1] 4
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] 6.338195 26.825000 47.311805
> #CI for w4A1k
> mu<-mean(w4A1k,na.rm=TRUE); mu
[1] 228.8193
> s<-sd(w4A1k,na.rm=TRUE); s
[1] 13.41423
> n<-length(which(w4A1k!="NA"));n
[1] 4
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] 207.4743 228.8193 250.1643
> #CI for w6A1k
> mu<-mean(w6A1k,na.rm=TRUE); mu
[1] 312.0637
> s<-sd(w6A1k,na.rm=TRUE); s
[1] 24.02996
> n<-length(which(w6A1k!="NA"));n
[1] 4
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)

```

```

> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 273.8267 312.0637 350.3007
> #CI for stAlk
> mu<-mean(stAlk,na.rm=TRUE); mu
[1] 224.9
> s<-sd(stAlk,na.rm=TRUE); s
[1] 37.30914
> n<-length(which(stAlk!="NA"));n
[1] 6
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 185.7465 224.9000 264.0535

```

## **BOD**

```

> #set-up
> stBOD<-dat$stBOD
> avgstBOD<-mean(stBOD,na.rm=TRUE)
> sdstBOD<-sd(stBOD,na.rm=TRUE)
> RSDBOD<-sdstBOD/avgstBOD;RSDBOD
[1] 0.2185352
> dwt(lm(stBOD~time))
lag Autocorrelation D-W Statistic p-value
  1      -0.09614452      1.994222  0.618
Alternative hypothesis: rho != 0
> anuaBOD<-dat$anuaBOD
> rranuaBOD<-(avgstBOD-anuaBOD)/avgstBOD
> anua2BOD<-dat$anua2BOD
> rranua2BOD<-(avgstBOD-anua2BOD)/avgstBOD
> ezBOD<-dat$ezBOD
> rrezBOD<-(avgstBOD-ezBOD)/avgstBOD
> w4BOD<-dat$w4BODN
> rrw4BOD<-(avgstBOD-w4BOD)/avgstBOD
> w6BOD<-dat$w6BODN
> rrw6BOD<-(avgstBOD-w6BOD)/avgstBOD
> ###lognormally/normally distributed?
> #null hypothesis - normal distribution
> #p>0.05 indicates normality
> shapiro.test(rranuaBOD)
      Shapiro-wilk normality test
data:  rranuaBOD
w = 0.83888, p-value = 0.1276
> shapiro.test(rrezBOD)
      Shapiro-wilk normality test
data:  rrezBOD
w = 0.91768, p-value = 0.4888
> shapiro.test(rrw4BOD)
      Shapiro-wilk normality test
data:  rrw4BOD
w = 0.78425, p-value = 0.04221
> shapiro.test(rrw6BOD)
      Shapiro-wilk normality test
data:  rrw6BOD

```

```

w = 0.9577, p-value = 0.8018
> ###independent?
> #null hypothesis - no correlation
> #p>0.05 indicates independence
> dwt(lm(rranuaBOD~time))
lag Autocorrelation D-w Statistic p-value
1 0.1356161 1.514711 0.168
Alternative hypothesis: rho != 0
> dwt(lm(rrezBOD~time))
lag Autocorrelation D-w Statistic p-value
1 -0.61258 2.96566 0.462
Alternative hypothesis: rho != 0
> dwt(lm(rrw4BOD~time))
lag Autocorrelation D-w Statistic p-value
1 -0.1329665 1.633625 0.236
Alternative hypothesis: rho != 0
> dwt(lm(rrw6BOD~time))
lag Autocorrelation D-w Statistic p-value
1 -0.5727937 3.124321 0.252
Alternative hypothesis: rho != 0
> ###equal variance?
> rrBOD<-cbind(rranuaBOD,rrezBOD,rrw4BOD,rrw6BOD)
> rrBODstack<-stack(data.frame(rrBOD))
> #use with nonparametric cases
> leveneTest(rrBODstack$values,rrBODstack$ind)
Levene's Test for Homogeneity of Variance (center = median)
Df F value Pr(>F)
group 3 1.4199 0.2664
20
> ###are column means the same?
> #use with nonparametric cases
> kruskal.test(rrBODstack$values~rrBODstack$ind)
Kruskal-wallis rank sum test
data: rrBODstack$values by rrBODstack$ind
Kruskal-wallis chi-squared = 15.547, df = 3, p-value = 0.001404
> ###if column means are different, which columns?
> #use with nonparametric cases
> kruskalmc(rrBODstack$values~rrBODstack$ind)
Multiple comparison test after Kruskal-wallis
p.value: 0.05
Comparisons
      obs.dif critical.dif difference
rranuaBOD-rrezBOD 4.666667 10.77064 FALSE
rranuaBOD-rrw4BOD 1.666667 10.77064 FALSE
rranuaBOD-rrw6BOD 13.000000 10.77064 TRUE
rrezBOD-rrw4BOD 6.333333 10.77064 FALSE
rrezBOD-rrw6BOD 8.333333 10.77064 FALSE
rrw4BOD-rrw6BOD 14.666667 10.77064 TRUE
> #compare removal rate CIs for systems in lowest group(s) to zero
> mu<-mean(rrw6BOD,na.rm=TRUE); mu
[1] 0.8278825
> s<-sd(rrw6BOD,na.rm=TRUE); s
[1] 0.03151939
> n<-length(which(rrw6BOD!="NA"));n
[1] 6
> a<-0.05

```

```

> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.7948049 0.8278825 0.8609601
> #CI for anuaBOD
> mu<-mean(anuaBOD,na.rm=TRUE); mu
[1] 2.083333
> s<-sd(anuaBOD,na.rm=TRUE); s
[1] 1.049603
> n<-length(which(anuaBOD!="NA"));n
[1] 6
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.9818424 2.0833333 3.1848242
> #CI for anua2BOD
> mu<-mean(anua2BOD,na.rm=TRUE); mu
[1] 3.4
> s<-sd(anua2BOD,na.rm=TRUE); s
[1] 0.1414214
> n<-length(which(anua2BOD!="NA"));n
[1] 2
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 2.12938 3.40000 4.67062
> #CI for ezBOD
> mu<-mean(ezBOD,na.rm=TRUE); mu
[1] 3.466667
> s<-sd(ezBOD,na.rm=TRUE); s
[1] 1.032796
> n<-length(which(ezBOD!="NA"));n
[1] 6
> t<-qt(1-a/2,n-1)
> a<-0.05
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 2.382814 3.466667 4.550519
> #CI for w4BOD
> n<-100000
> w4BODbs<-w4BOD[!is.na(w4BOD)];w4BODbs
[1] 2.4268571 0.9714286 0.2784000 1.4297143 1.0548571 6.1851429
> bootw4BOD<-numeric(n)
> for (i in 1:n) {
+ bootw4BOD[i]<-mean(sample(w4BODbs,replace=T),na.rm=TRUE)
+ }
> hist(bootw4BOD)
> quantile(bootw4BOD,probs=c(0.025,0.975))
      2.5%      97.5%
0.8307048 3.8208571
> mean(bootw4BOD)
[1] 2.05481
> #CI for w6BOD
> mu<-mean(w6BOD,na.rm=TRUE); mu

```

```

[1] 14.46648
> s<-sd(w6BOD,na.rm=TRUE); s
[1] 2.649204
> n<-length(which(w6BOD!="NA"));n
[1] 6
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] 11.68631 14.46648 17.24665
> #CI for stBOD
> mu<-mean(stBOD,na.rm=TRUE); mu
[1] 84.05
> s<-sd(stBOD,na.rm=TRUE); s
[1] 18.36788
> n<-length(which(stBOD!="NA"));n
[1] 6
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] 64.77409 84.05000 103.32591

```

## ***COD***

```

> #set-up
> stCOD<-dat$stCOD
> avgstCOD<-mean(stCOD,na.rm=TRUE)
> sdstCOD<-sd(stCOD,na.rm=TRUE)
> RSDCOD<-sdstCOD/avgstCOD;RSDCOD
[1] 0.2627184
> dwt(lm(stCOD~time))
lag Autocorrelation D-W Statistic p-value
 1      -0.168857      2.118626  0.804
Alternative hypothesis: rho != 0
> anuaCOD<-dat$anuaCOD
> rranuaCOD<-(avgstCOD-anuaCOD)/avgstCOD
> anua2COD<-dat$anua2COD
> rranua2COD<-(avgstCOD-anua2COD)/avgstCOD
> ezCOD<-dat$ezCOD
> rrezCOD<-(avgstCOD-ezCOD)/avgstCOD
> w4COD<-dat$w4CODN
> rrw4COD<-(avgstCOD-w4COD)/avgstCOD
> w6COD<-dat$w6CODN
> rrw6COD<-(avgstCOD-w6COD)/avgstCOD
> ###lognormally/normally distributed?
> #null hypothesis - normal distribution
> #p>0.05 indicates normality
> shapiro.test(rranuaCOD)
      Shapiro-wilk normality test
data:  rranuaCOD
W = 0.97267, p-value = 0.9165
> shapiro.test(rranua2COD)
      Shapiro-wilk normality test
data:  rranua2COD

```

```

w = 0.9848, p-value = 0.9586
> shapiro.test(rrezCOD)
      Shapiro-wilk normality test
data:  rrezCOD
w = 0.97724, p-value = 0.9371
> shapiro.test(rrw4COD)
      Shapiro-wilk normality test
data:  rrw4COD
w = 0.97133, p-value = 0.9082
> shapiro.test(rrw6COD)
      Shapiro-wilk normality test
data:  rrw6COD
w = 0.74482, p-value = 0.007159
> ###independent?
> #null hypothesis - no correlation
> #p>0.05 indicates independence
> dwt(lm(rranuaCOD~time),max.lag=3)
  lag Autocorrelation D-W Statistic p-value
  1      -0.6568965      3.286971  0.056
  2       0.2702993      1.221206  0.264
  3      -0.2532636      2.173364  0.346
Alternative hypothesis: rho[lag] != 0
> #anuaCODuse<-dat$anuaCOD_use
> #rranuaCODuse<-(avgstCOD-anuaCODuse)/avgstCOD
> #shapiro.test(rranuaCODuse)
> #dwt(lm(rranuaCOD~time),max.lag=3)
> dwt(lm(rranua2COD~time))
  lag Autocorrelation D-W Statistic p-value
  1      -0.3305213      2.571565  0.868
Alternative hypothesis: rho != 0
> dwt(lm(rrezCOD~time))
  lag Autocorrelation D-W Statistic p-value
  1      -0.2756132      2.417324  0.926
Alternative hypothesis: rho != 0
> dwt(lm(rrw4COD~time))
  lag Autocorrelation D-W Statistic p-value
  1      -0.1858045      2.298373  0.95
Alternative hypothesis: rho != 0
> dwt(lm(rrw6COD~time))
  lag Autocorrelation D-W Statistic p-value
  1       0.2914714      0.8928461  0.01
Alternative hypothesis: rho != 0
> #w6CODuse<-dat$w6COD_use
> #rrw6CODuse<-(avgstCOD-w6CODuse)/avgstCOD
> #shapiro.test(rrw6CODuse)
> #dwt(lm(rrw6COD~time),max.lag=3)
> ###equal variance?
> rrcod<-cbind(rranuaCOD,rrezCOD,rrw4COD,rrw6COD)
> rrcodstack<-stack(data.frame(rrcod))
> rrcod2<-cbind(rranua2COD,rrezCOD,rrw4COD,rrw6COD)
> rrcodstack2<-stack(data.frame(rrcod2))
> #use with nonparametric cases
> leveneTest(rrcodstack$values,rrcodstack$ind)
Levene's Test for Homogeneity of Variance (center = median)
      Df F value Pr(>F)
group  3  2.5412 0.07735 .

```

```

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> leveneTest(rrCODstack2$values,rrCODstack2$ind)
Levene's Test for Homogeneity of Variance (center = median)
      Df F value Pr(>F)
group  3  2.1337 0.1236
      23
> ###are column means the same?
> #use with nonparametric cases
> kruskal.test(rrCODstack$values~rrCODstack$ind)
      Kruskal-wallis rank sum test
data:  rrCODstack$values by rrCODstack$ind
Kruskal-wallis chi-squared = 24.323, df = 3, p-value = 2.138e-05
> kruskal.test(rrCODstack2$values~rrCODstack2$ind)
      Kruskal-wallis rank sum test
data:  rrCODstack2$values by rrCODstack2$ind
Kruskal-wallis chi-squared = 20.312, df = 3, p-value = 0.0001462
> ###if column means are different, which columns?
> #use with nonparametric cases
> kruskalmc(rrCODstack$values~rrCODstack$ind)
Multiple comparison test after Kruskal-wallis
p.value: 0.05
Comparisons
              obs.dif critical.dif difference
rranuaCOD-rrezCOD 11.388889    12.64245    FALSE
rranuaCOD-rrw4COD 13.722222    11.65576     TRUE
rranuaCOD-rrw6COD  5.902778    11.65576    FALSE
rrezCOD-rrw4COD   2.333333    12.95465    FALSE
rrezCOD-rrw6COD  17.291667    12.95465     TRUE
rrw4COD-rrw6COD  19.625000    11.99368     TRUE
> kruskalmc(rrCODstack2$values~rrCODstack2$ind)
Multiple comparison test after Kruskal-wallis
p.value: 0.05
Comparisons
              obs.dif critical.dif difference
rranua2COD-rrezCOD 10.166667    12.68011    FALSE
rranua2COD-rrw4COD 12.500000    11.93793     TRUE
rranua2COD-rrw6COD  3.250000    11.93793    FALSE
rrezCOD-rrw4COD   2.333333    11.30917    FALSE
rrezCOD-rrw6COD  13.416667    11.30917     TRUE
rrw4COD-rrw6COD  15.750000    10.47026     TRUE
> #compare removal rate CIs for systems in lowest group(s) to zero
> n<-100000
> rrw6CODbs<-rrw6COD[!is.na(rrw6COD)];rrw6CODbs
[1] -0.2039344  0.2190696  0.6570441  0.6536490  0.5876600  0.6803545  0.4666878
[8]  0.6516438
> bootrrw6COD<-numeric(n)
> for (i in 1:n) {
+ bootrrw6COD[i]<-mean(sample(rrw6CODbs,replace=T),na.rm=TRUE)
+ }
> hist(bootrrw6COD)
> quantile(bootrrw6COD,probs=c(0.025,0.975))
      2.5%      97.5%
0.2378654 0.6350639
> mean(bootrrw6COD)

```

```

[1] 0.4640205
> #CI for anuaCOD
> mu<-mean(anuaCOD,na.rm=TRUE); mu
[1] 78.77778
> s<-sd(anuaCOD,na.rm=TRUE); s
[1] 14.4722
> n<-length(which(anuaCOD!="NA"));n
[1] 9
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] 67.65346 78.77778 89.90209
> #CI for anua2COD
> mu<-mean(anua2COD,na.rm=TRUE); mu
[1] 84.8
> s<-sd(anua2COD,na.rm=TRUE); s
[1] 14.1845
> n<-length(which(anua2COD!="NA"));n
[1] 5
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] 67.18762 84.80000 102.41238
> #CI for ezCOD
> mu<-mean(ezCOD,na.rm=TRUE); mu
[1] 27
> s<-sd(ezCOD,na.rm=TRUE); s
[1] 8.671793
> n<-length(which(ezCOD!="NA"));n
[1] 6
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] 17.89951 27.00000 36.10049
> #CI for w4COD
> mu<-mean(w4COD,na.rm=TRUE); mu
[1] 22.75643
> s<-sd(w4COD,na.rm=TRUE); s
[1] 7.77527
> n<-length(which(w4COD!="NA"));n
[1] 8
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] 16.25614 22.75643 29.25672
> #CI for w6COD
> n<-100000
> w6CODbs<-w6COD[!is.na(w6COD)];w6CODbs
[1] 301.92000 195.84000 86.00571 86.85714 103.40571 80.16000 133.74286
[8] 87.36000
> bootw6COD<-numeric(n)
> for (i in 1:n) {

```

```

+ bootw6COD[i]<-mean(sample(w6CODbs,replace=T),na.rm=TRUE)
+ }
> hist(bootw6COD)
> quantile(bootw6COD,probs=c(0.025,0.975))
      2.5%      97.5%
91.57714 191.04216
> mean(bootw6COD)
[1] 134.5158
> #CI for stCOD
> mu<-mean(stCOD,na.rm=TRUE); mu
[1] 250.7778
> s<-sd(stCOD,na.rm=TRUE); s
[1] 65.88395
> n<-length(which(stCOD!="NA"));n
[1] 9
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] 200.1349 250.7778 301.4207

```

## ***TSS***

```

> #set-up
> stTSS<-dat$stTSS
> avgstTSS<-mean(stTSS,na.rm=TRUE)
> sdstTSS<-sd(stTSS,na.rm=TRUE)
> RSDTSS<-sdstTSS/avgstTSS;RSDTSS
[1] 0.6475215
> dwt(lm(stTSS~time))
lag Autocorrelation D-W Statistic p-value
 1      -0.3126707      2.180431      0.48
Alternative hypothesis: rho != 0
> anuaTSS<-dat$anuaTSS
> rranuaTSS<-(avgstTSS-anuaTSS)/avgstTSS
> anua2TSS<-dat$anua2TSS
> rranua2TSS<-(avgstTSS-anua2TSS)/avgstTSS
> ezTSS<-dat$ezTSS
> rrezTSS<-(avgstTSS-ezTSS)/avgstTSS
> w4TSS<-dat$w4TSSN
> rrw4TSS<-(avgstTSS-w4TSS)/avgstTSS
> w6TSS<-dat$w6TSSN
> rrw6TSS<-(avgstTSS-w6TSS)/avgstTSS
> ###lognormally/normally distributed?
> #null hypothesis - normal distribution
> #p>0.05 indicates normality
> shapiro.test(rranuaTSS)
      Shapiro-wilk normality test
data:  rranuaTSS
W = 0.95278, p-value = 0.7335
> shapiro.test(rranua2TSS)
Error in shapiro.test(rranua2TSS) :
  sample size must be between 3 and 5000
> shapiro.test(rrezTSS)

```

```

      Shapiro-wilk normality test
data: rrezTSS
W = 0.99291, p-value = 0.9719
> shapiro.test(rrw4TSS)
      Shapiro-wilk normality test
data: rrw4TSS
W = 0.91854, p-value = 0.5288
> shapiro.test(rrw6TSS)
      Shapiro-wilk normality test
data: rrw6TSS
W = 0.84655, p-value = 0.2152
> ###independent?
> #null hypothesis - no correlation
> #p>0.05 indicates independence
> dwt(lm(rranuaTSS~time))
lag Autocorrelation D-W Statistic p-value
1 -0.2503323 2.006546 0.236
Alternative hypothesis: rho != 0
> dwt(lm(rrezTSS~time))
lag Autocorrelation D-W Statistic p-value
1 -0.7924291 3.271861 0.2
Alternative hypothesis: rho != 0
> dwt(lm(rrw4TSS~time))
lag Autocorrelation D-W Statistic p-value
1 -0.4084329 2.431475 0.79
Alternative hypothesis: rho != 0
> dwt(lm(rrw6TSS~time))
lag Autocorrelation D-W Statistic p-value
1 -0.4099404 2.376324 0.724
Alternative hypothesis: rho != 0
> ###equal variance?
> rrtSS<-cbind(rranuaTSS,rrezTSS,rrw4TSS,rrw6TSS)
> rrtSSstack<-stack(data.frame(rrTSS))
> #use with parametric cases
> bartlett.test(rrTSSstack$values,rrTSSstack$ind)
      Bartlett test of homogeneity of variances
data: rrtSSstack$values and rrtSSstack$ind
Bartlett's K-squared = 9.957, df = 3, p-value = 0.01893
> ###are column means the same?
> #use with nonparametric cases
> oneway.test(rrTSSstack$values~rrTSSstack$ind)
      One-way analysis of means (not assuming equal variances)
data: rrtSSstack$values and rrtSSstack$ind
F = 3.2997, num df = 3.0000, denom df = 5.3991, p-value = 0.1086
> ###if column means are different, which columns?
> #use with nonparametric cases
> TukeyHSD(aov(rrTSSstack$values~rrTSSstack$ind))
      Tukey multiple comparisons of means
      95% family-wise confidence level
Fit: aov(formula = rrtSSstack$values ~ rrtSSstack$ind)
`rrTSSstack$ind`
              diff          lwr          upr      p adj
rrezTSS-rranuaTSS 0.04054054 -0.10374691 0.18482799 0.8373271
rrw4TSS-rranuaTSS 0.08185328 -0.06243417 0.22614074 0.3727741
rrw6TSS-rranuaTSS -0.02355212 -0.16783958 0.12073533 0.9610613
rrw4TSS-rrezTSS 0.04131274 -0.10297471 0.18560019 0.8297563

```

```

rrw6TSS-rrrezTSS    -0.06409266 -0.20838012 0.08019479 0.5691565
rrw6TSS-rrw4TSS     -0.10540541 -0.24969286 0.03888205 0.1871795
> #compare removal rate CIs for systems in lowest group(s) to zero
> mu<-mean(rranuaTSS,na.rm=TRUE); mu
[1] 0.8918919
> s<-sd(rranuaTSS,na.rm=TRUE); s
[1] 0.07956541
> n<-length(which(rranuaTSS!="NA")); n
[1] 4
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] 0.7652856 0.8918919 1.0184982
> mu<-mean(rrezTSS,na.rm=TRUE); mu
[1] 0.9324324
> s<-sd(rrezTSS,na.rm=TRUE); s
[1] 0.03489174
> n<-length(which(rrezTSS!="NA")); n
[1] 4
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] 0.8769119 0.9324324 0.9879530
> mu<-mean(rrw4TSS,na.rm=TRUE); mu
[1] 0.9737452
> s<-sd(rrw4TSS,na.rm=TRUE); s
[1] 0.01146608
> n<-length(which(rrw4TSS!="NA")); n
[1] 4
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] 0.9555001 0.9737452 0.9919903
> mu<-mean(rrw6TSS,na.rm=TRUE); mu
[1] 0.8683398
> s<-sd(rrw6TSS,na.rm=TRUE); s
[1] 0.1059048
> n<-length(which(rrw6TSS!="NA")); n
[1] 4
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] 0.6998216 0.8683398 1.0368579
> #CI for anuaTSS
> mu<-mean(anuaTSS,na.rm=TRUE); mu
[1] 4
> s<-sd(anuaTSS,na.rm=TRUE); s
[1] 2.94392
> n<-length(which(anuaTSS!="NA")); n
[1] 4
> a<-0.05
> t<-qt(1-a/2,n-1)

```

```

> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] -0.6844341 4.0000000 8.6844341
> #CI for anua2TSS
> mu<-mean(anua2TSS,na.rm=TRUE); mu
[1] 2
> s<-sd(anua2TSS,na.rm=TRUE); s
[1] 1.414214
> n<-length(which(anua2TSS!="NA")); n
[1] 2
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] -10.7062 2.0000 14.7062
> #CI for ezTSS
> mu<-mean(ezTSS,na.rm=TRUE); mu
[1] 2.5
> s<-sd(ezTSS,na.rm=TRUE); s
[1] 1.290994
> n<-length(which(ezTSS!="NA")); n
[1] 4
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.4457397 2.5000000 4.5542603
> #CI for w4TSS
> mu<-mean(w4TSS,na.rm=TRUE); mu
[1] 0.9714286
> s<-sd(w4TSS,na.rm=TRUE); s
[1] 0.4242448
> n<-length(which(w4TSS!="NA")); n
[1] 4
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.2963604 0.9714286 1.6464968
> #CI for w6TSS
> mu<-mean(w6TSS,na.rm=TRUE); mu
[1] 4.871429
> s<-sd(w6TSS,na.rm=TRUE); s
[1] 3.918477
> n<-length(which(w6TSS!="NA")); n
[1] 4
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] -1.363742 4.871429 11.106599
> #CI for stTSS
> mu<-mean(stTSS,na.rm=TRUE); mu
[1] 37
> s<-sd(stTSS,na.rm=TRUE); s
[1] 23.9583

```

```

> n<-length(which(stTSS!="NA")); n
[1] 4
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] -1.122997 37.000000 75.122997

```

## *Ammonia*

```

> #set-up
> stAMM<-dat$stAMM
> avgstAMM<-mean(stAMM,na.rm=TRUE)
> sdstAMM<-sd(stAMM,na.rm=TRUE)
> RSDAMM<-sdstAMM/avgstAMM;RSDAMM
[1] 0.1574928
> dwt(lm(stAMM~time))
lag Autocorrelation D-w Statistic p-value
 1      0.05565039      1.876195 0.462
Alternative hypothesis: rho != 0
> anuaAMM<-dat$anuaAMM
> rranuaAMM<-(avgstAMM-anuaAMM)/avgstAMM
> anua2AMM<-dat$anua2AMM
> rranua2AMM<-(avgstAMM-anua2AMM)/avgstAMM
> ezAMM<-dat$ezAMM
> rrezAMM<-(avgstAMM-ezAMM)/avgstAMM
> w4AMM<-dat$w4AMMN
> rrw4AMM<-(avgstAMM-w4AMM)/avgstAMM
> w6AMM<-dat$w6AMMN
> rrw6AMM<-(avgstAMM-w6AMM)/avgstAMM
> ###lognormally/normally distributed?
> #null hypothesis - normal distribution
> #p>0.05 indicates normality
> shapiro.test(rranuaAMM)
      Shapiro-wilk normality test
data:  rranuaAMM
w = 0.89729, p-value = 0.2367

> shapiro.test(rranua2AMM)
      Shapiro-wilk normality test
data:  rranua2AMM
w = 0.88251, p-value = 0.3208
> shapiro.test(rrezAMM)
      Shapiro-wilk normality test
data:  rrezAMM
w = 0.85829, p-value = 0.1461
> shapiro.test(rrw4AMM)
      Shapiro-wilk normality test
data:  rrw4AMM
w = 0.41382, p-value = 6.174e-07
> shapiro.test(rrw6AMM)
      Shapiro-wilk normality test
data:  rrw6AMM
w = 0.58483, p-value = 6.349e-05
> ###independent?

```

```

> #null hypothesis - no correlation
> #p>0.05 indicates independence
> dwt(lm(rranuaAMM~time))
lag Autocorrelation D-W Statistic p-value
1 -0.3494611 2.670835 0.47
Alternative hypothesis: rho != 0
> dwt(lm(rranua2AMM~time))
lag Autocorrelation D-W Statistic p-value
1 -0.5138876 2.739348 0.758
Alternative hypothesis: rho != 0
> dwt(lm(rrezAMM~time))
lag Autocorrelation D-W Statistic p-value
1 0.07136651 1.703903 0.31
Alternative hypothesis: rho != 0
> dwt(lm(rrw4AMM~time))
lag Autocorrelation D-W Statistic p-value
1 -0.0866163 1.600447 0.278
Alternative hypothesis: rho != 0
> dwt(lm(rrw6AMM~time))
lag Autocorrelation D-W Statistic p-value
1 0.1903787 1.522047 0.226
Alternative hypothesis: rho != 0
> ###equal variance?
> rrAMM<-cbind(rranuaAMM,rrezAMM,rrw4AMM,rrw6AMM)
> rrAMMstack<-stack(data.frame(rrAMM))
> rrAMM2<-cbind(rranua2AMM,rrezAMM,rrw4AMM,rrw6AMM)
> rrAMMstack2<-stack(data.frame(rrAMM2))
> #use with nonparametric cases
> leveneTest(rrAMMstack$values,rrAMMstack$ind)
Levene's Test for Homogeneity of Variance (center = median)
      Df F value Pr(>F)
group 3  2.3809 0.08925 .
      30
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> leveneTest(rrAMMstack2$values,rrAMMstack2$ind)
Levene's Test for Homogeneity of Variance (center = median)
      Df F value Pr(>F)
group 3  1.8739 0.1588
      26
> ###are column means the same?
> #use with nonparametric cases
> kruskal.test(rrAMMstack$values~rrAMMstack$ind)
Kruskal-wallis rank sum test
data:  rrAMMstack$values by rrAMMstack$ind
Kruskal-wallis chi-squared = 20.585, df = 3, p-value = 0.0001283
> kruskal.test(rrAMMstack2$values~rrAMMstack2$ind)
Kruskal-wallis rank sum test
data:  rrAMMstack2$values by rrAMMstack2$ind
Kruskal-wallis chi-squared = 17, df = 3, p-value = 0.0007069
> ###if column means are different, which columns?
> #use with nonparametric cases
> kruskalmc(rrAMMstack$values,rrAMMstack$ind,probs=0.05)
Multiple comparison test after Kruskal-wallis
p.value: 0.05
Comparisons

```

	obs.dif	critical.dif	difference
rranuaAMM-rrezAMM	10.142857	13.24005	FALSE
rranuaAMM-rrw4AMM	5.500000	12.38494	FALSE
rranuaAMM-rrw6AMM	11.833333	12.38494	FALSE
rrezAMM-rrw4AMM	15.642857	13.24005	TRUE
rrezAMM-rrw6AMM	21.976190	13.24005	TRUE
rrw4AMM-rrw6AMM	6.333333	12.38494	FALSE

Warning message:

In `kruskalmc.default(rrAMMstack$values, rrAMMstack$ind, probs = 0.05)` :

50 lines including NA have been omitted

> `kruskalmc(rrAMMstack2$values, rrAMMstack2$ind, probs=0.05)`

Multiple comparison test after Kruskal-wallis

p.value: 0.05

Comparisons

	obs.dif	critical.dif	difference
rranua2AMM-rrezAMM	9.142857	13.59955	FALSE
rranua2AMM-rrw4AMM	3.277778	12.95465	FALSE
rranua2AMM-rrw6AMM	8.833333	12.95465	FALSE
rrezAMM-rrw4AMM	12.420635	11.70463	TRUE
rrezAMM-rrw6AMM	17.976190	11.70463	TRUE
rrw4AMM-rrw6AMM	5.555556	10.94868	FALSE

Warning message:

In `kruskalmc.default(rrAMMstack2$values, rrAMMstack2$ind, probs = 0.05)` :

54 lines including NA have been omitted

> #compare removal rate CIs for systems in lowest group(s) to zero

> `mu<-mean(rranuaAMM,na.rm=TRUE); mu`

[1] 0.9976568

> `s<-sd(rranuaAMM,na.rm=TRUE); s`

[1] 0.001811592

> `n<-length(which(rranuaAMM!="NA")); n`

[1] 9

> `a<-0.05`

> `t<-qt(1-a/2,n-1)`

> `se<-s/sqrt(n)`

> `ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu`

[1] 0.9962643 0.9976568 0.9990493

> `mu<-mean(rranua2AMM,na.rm=TRUE); mu`

[1] 0.9976243

> `s<-sd(rranua2AMM,na.rm=TRUE); s`

[1] 0.002487729

> `n<-length(which(rranua2AMM!="NA")); n`

[1] 5

> `a<-0.05`

> `t<-qt(1-a/2,n-1)`

> `se<-s/sqrt(n)`

> `ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu`

[1] 0.9945354 0.9976243 1.0007132

> `mu<-mean(rrezAMM,na.rm=TRUE); mu`

[1] 0.9401086

> `s<-sd(rrezAMM,na.rm=TRUE); s`

[1] 0.03577868

> `n<-length(which(rrezAMM!="NA")); n`

[1] 7

> `a<-0.05`

> `t<-qt(1-a/2,n-1)`

> `se<-s/sqrt(n)`

```

> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.9070188 0.9401086 0.9731984
> #CI for anuaAMM
> mu<-mean(anuaAMM,na.rm=TRUE); mu
[1] 0.07377778
> s<-sd(anuaAMM,na.rm=TRUE); s
[1] 0.05703897
> n<-length(which(anuaAMM!="NA")); n
[1] 9
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.02993374 0.07377778 0.11762181
> #CI for anua2AMM
> mu<-mean(anua2AMM,na.rm=TRUE); mu
[1] 0.0748
> s<-sd(anua2AMM,na.rm=TRUE); s
[1] 0.07832752
> n<-length(which(anua2AMM!="NA")); n
[1] 5
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] -0.02245646 0.07480000 0.17205646
> #CI for ezAMM
> mu<-mean(ezAMM,na.rm=TRUE); mu
[1] 1.885714
> s<-sd(ezAMM,na.rm=TRUE); s
[1] 1.126512
> n<-length(which(ezAMM!="NA")); n
[1] 7
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.8438647 1.8857143 2.9275638
> #CI for w4AMM
> shapiro.test(w4AMM)
      Shapiro-wilk normality test
data:  w4AMM
w = 0.41382, p-value = 6.174e-07
> n<-100000
> w4AMMbs<-w4AMM[!is.na(w4AMM)];w4AMMbs
[1] 0.078285714 0.003131429 0.016914286 0.020000000 0.057142857 0.001920000
[7] 0.047657143 0.003245714 2.998857143
> bootw4AMM<-numeric(n)
> for (i in 1:n) {
+ bootw4AMM[i]<-mean(sample(w4AMMbs,replace=T),na.rm=TRUE)
+ }
> hist(bootw4AMM)
> quantile(bootw4AMM,probs=c(0.025,0.975))
      2.5%      97.5%
0.01584508 1.02428705
> mean(bootw4AMM)

```

```

[1] 0.3595907
> #CI for w6AMM
> shapiro.test(w6AMM)
      Shapiro-wilk normality test
data:  w6AMM
W = 0.58483, p-value = 6.349e-05
> n<-100000
> w6AMMbs<-w6AMM[!is.na(w6AMM)];w6AMMbs
[1] 0.003840000 0.001988571 0.036571429 0.035485714 0.002651429 0.001920000
[7] 0.003451429 0.003268571 0.003840000
> bootw6AMM<-numeric(n)
> for (i in 1:n) {
+ bootw6AMM[i]<-mean(sample(w6AMMbs,replace=T),na.rm=TRUE)
+ }
> hist(bootw6AMM)
> quantile(bootw6AMM,probs=c(0.025,0.975))
      2.5%      97.5%
0.002821587 0.021168254
> mean(bootw6AMM)
[1] 0.01034588
> #CI for stAMM
> shapiro.test(stAMM)
      Shapiro-wilk normality test
data:  stAMM
W = 0.9055, p-value = 0.2856
> mu<-mean(stAMM,na.rm=TRUE); mu
[1] 31.48556
> s<-sd(stAMM,na.rm=TRUE); s
[1] 4.958748
> n<-length(which(stAMM!="NA")); n
[1] 9
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] 27.67392 31.48556 35.29719

```

## *TIN*

```

> #set-up
> stTN<-dat$stTN
> avgstTN<-mean(stTN,na.rm=TRUE)
> sdstTN<-sd(stTN,na.rm=TRUE)
> RSDTN<-sdstTN/avgstTN;RSDTN
[1] 0.3230159
> dwt(lm(stTN~time))
lag Autocorrelation D-w Statistic p-value
  1      -0.7436104      3.270712  0.448
Alternative hypothesis: rho != 0
> anuaTIN<-dat$anuaTIN
> rranuaTIN<-(avgstTN-anuaTIN)/avgstTN
> anua2TIN<-dat$anua2TIN
> rranua2TIN<-(avgstTN-anua2TIN)/avgstTN
> ezTIN<-dat$ezTIN
> rrezTIN<-(avgstTN-ezTIN)/avgstTN

```

```

> w4TIN<-dat$w4TINN
> rrw4TIN<-(avgstTN-w4TIN)/avgstTN
> w6TIN<-dat$w6TINN
> rrw6TIN<-(avgstTN-w6TIN)/avgstTN
> ###lognormally/normally distributed?
> #null hypothesis - normal distribution
> #p>0.05 indicates normality
> shapiro.test(rranuaTIN)
      Shapiro-wilk normality test
data:  rranuaTIN
w = 0.87307, p-value = 0.1326
> shapiro.test(rranua2TIN)
      Shapiro-wilk normality test
data:  rranua2TIN
w = 0.88585, p-value = 0.3367
> shapiro.test(rrezTIN)
      Shapiro-wilk normality test
data:  rrezTIN
w = 0.9001, p-value = 0.3316
> shapiro.test(rrw4TIN)
      Shapiro-wilk normality test
data:  rrw4TIN
w = 0.47346, p-value = 3.115e-06
> shapiro.test(rrw6TIN)
      Shapiro-wilk normality test
data:  rrw6TIN
w = 0.91076, p-value = 0.3212
> ###independent?
> #null hypothesis - no correlation
> #p>0.05 indicates independence
> dwt(lm(rranuaTIN~time))
lag Autocorrelation D-W Statistic p-value
1      -0.1306912      1.897104  0.524
Alternative hypothesis: rho != 0
> dwt(lm(rranua2TIN~time))
lag Autocorrelation D-W Statistic p-value
1      -0.4150037      2.568423  0.96
Alternative hypothesis: rho != 0
> dwt(lm(rrezTIN~time))
lag Autocorrelation D-W Statistic p-value
1      -0.4166215      2.691144  0.656
Alternative hypothesis: rho != 0
> dwt(lm(rrw4TIN~time))
lag Autocorrelation D-W Statistic p-value
1      -0.02786021      1.494356  0.21
Alternative hypothesis: rho != 0
> dwt(lm(rrw6TIN~time))
lag Autocorrelation D-W Statistic p-value
1      -0.1635367      2.090772  0.718
Alternative hypothesis: rho != 0
> ###equal variance?
> rrTIN<-cbind(rranuaTIN,rrezTIN,rrw4TIN,rrw6TIN)
> rrTINstack<-stack(data.frame(rrTIN))
> rrTIN2<-cbind(rranua2TIN,rrezTIN,rrw4TIN,rrw6TIN)
> rrTINstack2<-stack(data.frame(rrTIN2))
> #use with nonparametric cases

```

```

> leveneTest(rrTINstack$values,rrTINstack$ind)
Levene's Test for Homogeneity of Variance (center = median)
  Df F value Pr(>F)
group 3  4.8216 0.007409 **
    30
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> leveneTest(rrTINstack2$values,rrTINstack2$ind)
Levene's Test for Homogeneity of Variance (center = median)
  Df F value Pr(>F)
group 3  2.3794 0.09269 .
    26
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> ###are column means the same?
> #use with nonparametric cases
> kruskal.test(rrTINstack$values~rrTINstack$ind)
Kruskal-wallis rank sum test
data:  rrTINstack$values by rrTINstack$ind
Kruskal-wallis chi-squared = 25.382, df = 3, p-value = 1.284e-05
> kruskal.test(rrTINstack2$values~rrTINstack2$ind)
Kruskal-wallis rank sum test
data:  rrTINstack2$values by rrTINstack2$ind
Kruskal-wallis chi-squared = 21.99, df = 3, p-value = 6.553e-05
> ###if column means are different, which columns?
> kruskalmc(rrTINstack$values,rrTINstack$ind,probs=0.05)
Multiple comparison test after Kruskal-wallis
p.value: 0.05
Comparisons
      obs.dif critical.dif difference
rranuaTIN-rrezTIN  5.0634921    13.24005    FALSE
rranuaTIN-rrw4TIN 15.0000000    12.38494     TRUE
rranuaTIN-rrw6TIN 14.3333333    12.38494     TRUE
rrezTIN-rrw4TIN   20.0634921    13.24005     TRUE
rrezTIN-rrw6TIN   19.3968254    13.24005     TRUE
rrw4TIN-rrw6TIN   0.6666667    12.38494    FALSE
Warning message:
In kruskalmc.default(rrTINstack$values, rrTINstack$ind, probs = 0.05) :
  50 lines including NA have been omitted
> kruskalmc(rrTINstack2$values,rrTINstack2$ind,probs=0.05)
Multiple comparison test after Kruskal-wallis
p.value: 0.05
Comparisons
      obs.dif critical.dif difference
rranua2TIN-rrezTIN  6.2000000    13.59955    FALSE
rranua2TIN-rrw4TIN 11.5777778    12.95465    FALSE
rranua2TIN-rrw6TIN 10.9111111    12.95465    FALSE
rrezTIN-rrw4TIN   17.7777778    11.70463     TRUE
rrezTIN-rrw6TIN   17.1111111    11.70463     TRUE
rrw4TIN-rrw6TIN   0.6666667    10.94868    FALSE
Warning message:
In kruskalmc.default(rrTINstack2$values, rrTINstack2$ind, probs = 0.05) :
  54 lines including NA have been omitted
> #compare removal rate CIs for systems in lowest group(s) to zero
> mu<-mean(rranuaTIN,na.rm=TRUE); mu
[1] 0.58322

```

```

> s<-sd(rranuaTIN,na.rm=TRUE); s
[1] 0.2728754
> n<-length(which(rranuaTIN!="NA")); n
[1] 9
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.3734693 0.5832200 0.7929706
> mu<-mean(rranua2TIN,na.rm=TRUE); mu
[1] 0.7736599
> s<-sd(rranua2TIN,na.rm=TRUE); s
[1] 0.06052951
> n<-length(which(rranua2TIN!="NA")); n
[1] 5
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.6985026 0.7736599 0.8488172
> mu<-mean(rrezTIN,na.rm=TRUE); mu
[1] 0.3032459
> s<-sd(rrezTIN,na.rm=TRUE); s
[1] 0.08779772
> n<-length(which(rrezTIN!="NA")); n
[1] 7
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.2220465 0.3032459 0.3844452
> #CI for anuaTIN
> mu<-mean(anuaTIN,na.rm=TRUE); mu
[1] 15.31667
> s<-sd(anuaTIN,na.rm=TRUE); s
[1] 10.02817
> n<-length(which(anuaTIN!="NA")); n
[1] 9
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 7.608332 15.316667 23.025002
> #CI for anua2TIN
> mu<-mean(anua2TIN,na.rm=TRUE); mu
[1] 8.318
> s<-sd(anua2TIN,na.rm=TRUE); s
[1] 2.224459
> n<-length(which(anua2TIN!="NA")); n
[1] 5
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 5.555969 8.318000 11.080031
> #CI for ezTIN

```

```

> mu<-mean(ezTIN,na.rm=TRUE); mu
[1] 25.60571
> s<-sd(ezTIN,na.rm=TRUE); s
[1] 3.226566
> n<-length(which(ezTIN!="NA")); n
[1] 7
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 22.62164 25.60571 28.58979
> #CI for w4TIN
> mu<-mean(w4TIN,na.rm=TRUE); mu
[1] 0.972527
> s<-sd(w4TIN,na.rm=TRUE); s
[1] 2.174514
> n<-length(which(w4TIN!="NA")); n
[1] 9
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] -0.6989523 0.9725270 2.6440063
> #CI for w6TIN
> mu<-mean(w6TIN,na.rm=TRUE); mu
[1] 0.3129905
> n<-length(which(w6TIN!="NA")); n
[1] 9
> s<-sd(w6TIN,na.rm=TRUE); s
[1] 0.2181481
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.1453070 0.3129905 0.4806739
> #CI for stTIN
> stTIN<-dat$stTIN
> mu<-mean(stTIN,na.rm=TRUE); mu
[1] 31.8
> s<-sd(stTIN,na.rm=TRUE); s
[1] 5.10759
> n<-length(which(stTIN!="NA")); n
[1] 9
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 27.87396 31.80000 35.72604

```

## ***TN***

```

> #set-up
> stTN<-dat$stTN

```

```

> avgstTN<-mean(stTN,na.rm=TRUE)
> sdstTN<-sd(stTN,na.rm=TRUE)
> dwt(lm(stTN~time))
lag Autocorrelation D-W Statistic p-value
 1      -0.7436104      3.270712    0.38
Alternative hypothesis: rho != 0
> RSDTN<-sdstTN/avgstTN;RSDTN
[1] 0.3230159
> anuaTN<-dat$anuaTN
> rranuaTN<-(avgstTN-anuaTN)/avgstTN
> anua2TN<-dat$anua2TN
> rranua2TN<-(avgstTN-anua2TN)/avgstTN
> ezTN<-dat$ezTN
> rrezTN<-(avgstTN-ezTN)/avgstTN
> w4TN<-dat$w4TNN
> w6TN<-dat$w6TNN
> rrw4TN<-(avgstTN-w4TN)/avgstTN
> rrw6TN<-(avgstTN-w6TN)/avgstTN
> ###lognormally/normally distributed?
> #null hypothesis - normal distribution
> #p>0.05 indicates normality
> shapiro.test(rranuaTN)
      Shapiro-wilk normality test
data:  rranuaTN
w = 0.78905, p-value = 0.084
> shapiro.test(rranua2TN)
Error in shapiro.test(rranua2TN) : sample size must be between 3 and 5000
> shapiro.test(rrezTN)
      Shapiro-wilk normality tes
data:  rrezTN
w = 0.82067, p-value = 0.1447
> shapiro.test(rrw4TN)
      Shapiro-wilk normality test
data:  rrw4TN
w = 0.7875, p-value = 0.08162
> shapiro.test(rrw6TN)
      Shapiro-wilk normality test
data:  rrw6TN
w = 0.88301, p-value = 0.3517
> ###independent?
> #null hypothesis - no correlation
> #p>0.05 indicates independence
> dwt(lm(rranuaTN~time))
lag Autocorrelation D-W Statistic p-value
 1      -0.6612397      3.014143    0.632
Alternative hypothesis: rho != 0
> dwt(lm(rranua2TN~time))
lag Autocorrelation D-W Statistic p-value
 1          NaN          NaN      NA
Alternative hypothesis: rho != 0
> dwt(lm(rrezTN~time))
lag Autocorrelation D-W Statistic p-value
 1      -0.7242488      3.136602    0.396
Alternative hypothesis: rho != 0
> dwt(lm(rrw4TN~time))
lag Autocorrelation D-W Statistic p-value

```

```

1      -0.3151171      2.144425      0.408
Alternative hypothesis: rho != 0
> dwt(lm(rrw6TN~time))
lag Autocorrelation D-W Statistic p-value
1      -0.7923805      3.366311      0.21
Alternative hypothesis: rho != 0
> ###equal variance?
> rrTN<-cbind(rranuaTN,rrezTN,rrw4TN,rrw6TN)
> rrTNstack<-stack(data.frame(rrTN))
> #use with parametric cases
> bartlett.test(rrTNstack$values,rrTNstack$ind)
      Bartlett test of homogeneity of variances
data:  rrTNstack$values and rrTNstack$ind
Bartlett's K-squared = 17.325, df = 3, p-value = 0.0006058
> ###are column means the same?
> #use with parametric cases when variances are not equal
> oneway.test(rrTNstack$values~rrTNstack$ind)
      One-way analysis of means (not assuming equal variances)
data:  rrTNstack$values and rrTNstack$ind
F = 25.785, num df = 3.0000, denom df = 5.3485, p-value = 0.001347
> ###if column means are different, which columns?
> #use with parametric cases
> TukeyHSD(aov(rrTNstack$values~rrTNstack$ind))
      Tukey multiple comparisons of means
      95% family-wise confidence level
Fit: aov(formula = rrTNstack$values ~ rrTNstack$ind)
`rrTNstack$ind`

```

	diff	lwr	upr	p adj
rrezTN-rranuaTN	-0.24489796	-0.74391727	0.2541213	0.4907103
rrw4TN-rranuaTN	0.42792614	-0.07109317	0.9269454	0.1023947
rrw6TN-rranuaTN	0.45013800	-0.04888131	0.9491573	0.0821304
rrw4TN-rrezTN	0.67282410	0.17380479	1.1718434	0.0082194
rrw6TN-rrezTN	0.69503596	0.19601665	1.1940553	0.0065379
rrw6TN-rrw4TN	0.02221186	-0.47680745	0.5212312	0.9991277

```

> #compare removal rate CIs for systems in lowest group(s) to zero
> mu<-mean(rranuaTN,na.rm=TRUE); mu
[1] 0.5102041
> s<-sd(rranuaTN,na.rm=TRUE); s
[1] 0.4460151
> n<-length(which(rranuaTN!="NA")); n
[1] 4
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] -0.1995055 0.5102041 1.2199136
> mu<-mean(rranua2TN,na.rm=TRUE); mu
[1] 0.7959184
> s<-sd(rranua2TN,na.rm=TRUE); s
[1] 0.019241
> n<-length(which(rranua2TN!="NA")); n
[1] 2
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu

```

```

[1] 0.6230448 0.7959184 0.9687919
> mu<-mean(rrezTN,na.rm=TRUE); mu
[1] 0.2653061
> s<-sd(rrezTN,na.rm=TRUE); s
[1] 0.142262
> n<-length(which(rrezTN!="NA")); n
[1] 4
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] 0.03893551 0.26530612 0.49167673
> #CI for anuaTN
> mu<-mean(anuaTN,na.rm=TRUE); mu
[1] 18
> s<-sd(anuaTN,na.rm=TRUE); s
[1] 16.39105
> n<-length(which(anuaTN!="NA")); n
[1] 4
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] -8.081825 18.000000 44.081825
> #CI for anua2TN
> mu<-mean(anua2TN,na.rm=TRUE); mu
[1] 7.5
> s<-sd(anua2TN,na.rm=TRUE); s
[1] 0.7071068
> n<-length(which(anua2TN!="NA")); n
[1] 2
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] 1.146898 7.500000 13.853102
> #CI for ezTN
> mu<-mean(ezTN,na.rm=TRUE); mu
[1] 27
> s<-sd(ezTN,na.rm=TRUE); s
[1] 5.228129
> n<-length(which(ezTN!="NA")); n
[1] 4
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] 18.68088 27.00000 35.31912
> #CI for w4TN
> mu<-mean(w4TN,na.rm=TRUE); mu
[1] 2.273714
> s<-sd(w4TN,na.rm=TRUE); s
[1] 2.913304
> n<-length(which(w4TN!="NA")); n
[1] 4
> a<-0.05

```

```

> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] -2.362003 2.273714 6.909431
> #CI for w6TN
> mu<-mean(w6TN,na.rm=TRUE); mu
[1] 1.457429
> s<-sd(w6TN,na.rm=TRUE); s
[1] 0.8701716
> n<-length(which(w6TN!="NA")); n
[1] 4
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.0727913 1.4574286 2.8420658
> #CI for stTN
> mu<-mean(stTN,na.rm=TRUE); mu
[1] 36.75
> s<-sd(stTN,na.rm=TRUE); s
[1] 11.87083
> n<-length(which(stTN!="NA")); n
[1] 4
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 17.86086 36.75000 55.63914

```

## ANOVA Calculations – PPCPs

```

> library(car)
> library(pgirmess)
> dat<-read.csv("anovaPPCP.csv")
> time<-dat$time

```

### *ACE*

```

> ###set-up
> stACE<-dat$stACE
> avgstACE<-mean(stACE,na.rm=TRUE)
> sdstACE<-sd(stACE,na.rm=TRUE)
> RSDACE<-sdstACE/avgstACE;RSDACE
[1] 0.8043276
> dwt(lm(stACE~time))
lag Autocorrelation D-W Statistic p-value
1 0.3235067 1.129273 0.052
Alternative hypothesis: rho != 0

```

```

> anuaACE<-dat$anuaACE
> rranuaACE<-(avgstACE-anuaACE)/avgstACE
> rrpanuaACE<-(stACE-anuaACE)/stACE;rrpanuaACE
[1] 0.9999173      NA 0.9939321      NA 0.9999027      NA 0.9990278
[8]      NA 0.9987388      NA 0.9995340 0.9998253 0.9070600 0.9990526
> anua2ACE<-dat$anua2ACE
> rranua2ACE<-(avgstACE-anua2ACE)/avgstACE
> rrpanua2ACE<-(stACE-anua2ACE)/stACE;rrpanua2ACE
[1] 0.9999173      NA 0.9939321      NA 0.9999027      NA 0.9990278
[8]      NA 0.9987388      NA      NA      NA      NA      NA
> ezACE<-dat$ezACE
> rrezACE<-(avgstACE-ezACE)/avgstACE
> rrpezACE<-(stACE-ezACE)/stACE;rrpezACE
[1]      NA      NA      NA      NA      NA      NA 0.9993245
[8]      NA 0.9201600      NA 0.9594057 0.9916798 0.9482722 0.9429957
> w4ACE<-dat$w4ACEN
> rrw4ACE<-(avgstACE-w4ACE)/avgstACE
> rrpw4ACE<-(stACE-w4ACE)/stACE;rrpw4ACE
[1] 0.9998070      NA 0.9992036      NA 0.9999027      NA 0.9997326
[8]      NA 0.9987388      NA 0.9995340 0.9998253 0.9984770 0.9985960
> w6ACE<-dat$w6ACEN
> rrw6ACE<-(avgstACE-w6ACE)/avgstACE
> rrpw6ACE<-(stACE-w6ACE)/stACE;rrpw6ACE
[1] 0.9994633      NA 0.9979730      NA 0.9998633      NA 0.9962729
[8]      NA 0.9987388      NA 0.9995340 0.9998253 0.9984770 0.9997644
> ###lognormally/normally distributed?
> #null hypothesis - normal distribution
> #p>0.05 indicates normality
> shapiro.test(rranuaACE)
      Shapiro-wilk normality test
data:  rranuaACE
w = 0.58789, p-value = 6.897e-05
> shapiro.test(rrpanuaACE)
      Shapiro-wilk normality test
data:  rrpanuaACE
w = 0.43113, p-value = 9.879e-07
> shapiro.test(rranua2ACE)
      Shapiro-wilk normality test
data:  rranua2ACE
w = 0.57646, p-value = 0.000297
> shapiro.test(rrpanua2ACE)
      Shapiro-wilk normality test
data:  rrpanua2ACE
w = 0.72596, p-value = 0.01755
> shapiro.test(rrezACE)
      Shapiro-wilk normality test
data:  rrezACE
w = 0.81133, p-value = 0.07414
> shapiro.test(rrpezACE)
      Shapiro-wilk normality test
data:  rrpezACE
w = 0.94149, p-value = 0.6712
> shapiro.test(rrw4ACE)
      Shapiro-wilk normality test
data:  rrw4ACE
w = 0.63457, p-value = 0.0002434

```

```

> shapiro.test(rrpw4ACE)
      Shapiro-wilk normality test
data:  rrpw4ACE
W = 0.85418, p-value = 0.08283
> shapiro.test(rrw6ACE)
      Shapiro-wilk normality test
data:  rrw6ACE
W = 0.66537, p-value = 0.0005587
> shapiro.test(rrpw6ACE)
      Shapiro-wilk normality test
data:  rrpw6ACE
W = 0.82692, p-value = 0.04123
> ###independent?
> #null hypothesis - no correlation
> #p>0.05 indicates independence
> dwt(lm(rranuaACE~time))
lag Autocorrelation D-W Statistic p-value
 1      -0.3152519      2.573015  0.624
Alternative hypothesis: rho != 0
> dwt(lm(rrpanuaACE~time))
lag Autocorrelation D-W Statistic p-value
 1      -0.5000007      2.822536  0.32
Alternative hypothesis: rho != 0
> dwt(lm(rranua2ACE~time))
lag Autocorrelation D-W Statistic p-value
 1      -0.6335683      3.030021  0.406
Alternative hypothesis: rho != 0
> dwt(lm(rrpanua2ACE~time))
lag Autocorrelation D-W Statistic p-value
 1      -0.6363691      3.068258  0.454
Alternative hypothesis: rho != 0
> dwt(lm(rrezACE~time))
lag Autocorrelation D-W Statistic p-value
 1      -0.2232736      2.109583  0.644
Alternative hypothesis: rho != 0
> dwt(lm(rrpezACE~time))
lag Autocorrelation D-W Statistic p-value
 1      -0.3566762      2.478834  0.964
Alternative hypothesis: rho != 0
> dwt(lm(rrw4ACE~time))
lag Autocorrelation D-W Statistic p-value
 1      -0.109513      2.032312  0.612
Alternative hypothesis: rho != 0
> dwt(lm(rrpw4ACE~time))
lag Autocorrelation D-W Statistic p-value
 1      -0.3166343      2.622694  0.566
Alternative hypothesis: rho != 0
> dwt(lm(rrw6ACE~time))
lag Autocorrelation D-W Statistic p-value
 1      -0.5936025      3.113447  0.132
Alternative hypothesis: rho != 0
> dwt(lm(rrpw6ACE~time))
lag Autocorrelation D-W Statistic p-value
 1      -0.4277992      2.761795  0.39
Alternative hypothesis: rho != 0
> ###equal variance?

```

```

> #null hypothesis - equal variance
> #p>0.05 indicates equal variance
> rrACE<-cbind(rranuaACE,rrezACE,rrw4ACE,rrw6ACE)
> rrACEstack<-stack(data.frame(rrACE))
> rrACE2<-cbind(rranua2ACE,rrezACE,rrw4ACE,rrw6ACE)
> rrACEstack2<-stack(data.frame(rrACE2))
> rrpACE<-cbind(rrpanuaACE,rrpezACE,rrpw4ACE,rrpw6ACE)
> rrpACEstack<-stack(data.frame(rrpACE))
> rrpACE2<-cbind(rrpanua2ACE,rrpezACE,rrpw4ACE,rrpw6ACE)
> rrpACEstack2<-stack(data.frame(rrpACE2))
> #use with nonparametric cases
> leveneTest(rrACEstack$values,rrACEstack$ind)
Levene's Test for Homogeneity of Variance (center = median)
      Df F value Pr(>F)
group 3  3.2965 0.03432 *
      29
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> leveneTest(rrACEstack2$values,rrACEstack2$ind)
Levene's Test for Homogeneity of Variance (center = median)
      Df F value Pr(>F)
group 3  3.1064 0.04454 *
      25
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> leveneTest(rrpACEstack$values,rrpACEstack$ind)
Levene's Test for Homogeneity of Variance (center = median)
      Df F value Pr(>F)
group 3  2.624 0.06938 .
      29
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> leveneTest(rrpACEstack2$values,rrpACEstack2$ind)
Levene's Test for Homogeneity of Variance (center = median)
      Df F value Pr(>F)
group 3 12.219 4.08e-05 ***
      25
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> ###are column means the same?
> #null hypothesis - column means are the same
> #p<0.05 indicates significantly different means
> #use with nonparametric cases
> kruskal.test(rrACEstack$values~rrACEstack$ind)
      Kruskal-wallis rank sum test
data:  rrACEstack$values by rrACEstack$ind
Kruskal-wallis chi-squared = 11.594, df = 3, p-value = 0.00891
> kruskal.test(rrACEstack2$values~rrACEstack2$ind)
      Kruskal-wallis rank sum test
data:  rrACEstack2$values by rrACEstack2$ind
Kruskal-wallis chi-squared = 11.711, df = 3, p-value = 0.008442
> kruskal.test(rrpACEstack$values~rrpACEstack$ind)
      Kruskal-wallis rank sum test
data:  rrpACEstack$values by rrpACEstack$ind
Kruskal-wallis chi-squared = 8.93, df = 3, p-value = 0.03024
> kruskal.test(rrpACEstack2$values~rrpACEstack2$ind)

```

```

Kruskal-wallis rank sum test
data: rrpACEstack2$values by rrpACEstack2$ind
Kruskal-wallis chi-squared = 10.035, df = 3, p-value = 0.01827
> ###if column means are different, which columns?
> #null hypothesis - column means are the same
> #p<0.05 indicates significantly different means
> #use with nonparametric case
> kruskalmc(rrpACEstack2$values~rrpACEstack2$ind)
Multiple comparison test after Kruskal-wallis
p.value: 0.05
Comparisons
      obs.dif critical.dif difference
rranuaACE-rrezACE 12.222222   13.44534   FALSE
rranuaACE-rrw4ACE  3.166667   12.02588   FALSE
rranuaACE-rrw6ACE  1.111111   12.02588   FALSE
rrezACE-rrw4ACE   15.388889   13.44534   TRUE
rrezACE-rrw6ACE   13.333333   13.44534   FALSE
rrw4ACE-rrw6ACE   2.055556   12.02588   FALSE
> kruskalmc(rrpACEstack2$values~rrpACEstack2$ind)
Multiple comparison test after Kruskal-wallis
p.value: 0.05
Comparisons
      obs.dif critical.dif difference
rranua2ACE-rrezACE 11.3333333   13.60260   FALSE
rranua2ACE-rrw4ACE  2.2777778   12.52979   FALSE
rranua2ACE-rrw6ACE  0.4444444   12.52979   FALSE
rrezACE-rrw4ACE   13.6111111   11.83954   TRUE
rrezACE-rrw6ACE   11.7777778   11.83954   FALSE
rrw4ACE-rrw6ACE   1.8333333   10.58961   FALSE
> kruskalmc(rrpACEstack2$values~rrpACEstack2$ind)
Multiple comparison test after Kruskal-wallis
p.value: 0.05
Comparisons
      obs.dif critical.dif difference
rrpanuaACE-rrpezACE 12.3333333   13.44534   FALSE
rrpanuaACE-rrpw4ACE  1.8333333   12.02588   FALSE
rrpanuaACE-rrpw6ACE  0.3333333   12.02588   FALSE
rrpezACE-rrpw4ACE   14.1666667   13.44534   TRUE
rrpezACE-rrpw6ACE   12.0000000   13.44534   FALSE
rrpw4ACE-rrpw6ACE   2.1666667   12.02588   FALSE
> kruskalmc(rrpACEstack2$values~rrpACEstack2$ind)
Multiple comparison test after Kruskal-wallis
p.value: 0.05
Comparisons
      obs.dif critical.dif difference
rrpanua2ACE-rrpezACE 12.7666667   13.60260   FALSE
rrpanua2ACE-rrpw4ACE  0.2333333   12.52979   FALSE
rrpanua2ACE-rrpw6ACE  1.7111111   12.52979   FALSE
rrpezACE-rrpw4ACE   13.0000000   11.83954   TRUE
rrpezACE-rrpw6ACE   11.0555556   11.83954   FALSE
rrpw4ACE-rrpw6ACE   1.9444444   10.58961   FALSE
> #compare removal rate CIs for systems in lowest group(s) to zero
> mu<-mean(rrezACE,na.rm=TRUE); mu
[1] 0.9862137
> s<-sd(rrezACE,na.rm=TRUE); s
[1] 0.01467285

```

```

> n<-length(which(rrpezACE!="NA"));n
[1] 6
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> mu<-mean(rrpezACE,na.rm=TRUE); mu
[1] 0.9603063
> s<-sd(rrpezACE,na.rm=TRUE); s
[1] 0.0302117
> n<-length(which(rrpezACE!="NA"));n
[1] 6
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.9286011 0.9603063 0.9920116

```

## CBZ

```

> ###set-up
> stCBZ<-dat$stCBZ
> avgstCBZ<-mean(stCBZ,na.rm=TRUE)
> sdstCBZ<-sd(stCBZ,na.rm=TRUE)
> RSDCBZ<-sdstCBZ/avgstCBZ;RSDCBZ
[1] 0.3801992
> dwt(lm(stCBZ~time))
lag Autocorrelation D-W Statistic p-value
 1      0.1889356      1.592533 0.242
Alternative hypothesis: rho != 0
> anuaCBZ<-dat$anuaCBZ
> rranuaCBZ<-(avgstCBZ-anuaCBZ)/avgstCBZ
> rrpnanuaCBZ<-(stCBZ-anuaCBZ)/stCBZ;rrnanuaCBZ
[1] 0.9971754      NA 0.9843073      NA 0.9719931      NA 0.8270113
[8]      NA 0.8706987      NA 0.3224374 0.7013219 0.4762475 0.3218441
> anua2CBZ<-dat$anua2CBZ
> rranua2CBZ<-(avgstCBZ-anua2CBZ)/avgstCBZ
> rrpnanua2CBZ<-(stCBZ-anua2CBZ)/stCBZ;rrnanua2CBZ
[1] 0.9971754      NA 0.9843073      NA 0.9719931      NA 0.8270113
[8]      NA 0.8706987      NA      NA      NA      NA      NA
> ezCBZ<-dat$ezCBZ
> rrezCBZ<-(avgstCBZ-ezCBZ)/avgstCBZ
> rrpezCBZ<-(stCBZ-ezCBZ)/stCBZ;rrpezCBZ
[1]      NA      NA      NA      NA      NA      NA -0.4833010
[8]      NA -0.3953182      NA -0.3364586 -0.1361817 -0.3468637 -0.5529961
> w4CBZ<-dat$w4CBZN
> rrw4CBZ<-(avgstCBZ-w4CBZ)/avgstCBZ
> rrpw4CBZ<-(stCBZ-w4CBZ)/stCBZ;rrpw4CBZ
[1] 0.38186661      NA 0.50607136      NA 0.63811368      NA
[7] -0.01993263      NA 0.46941364      NA 0.52713271 0.54603507
[13] -0.08549120 -0.31342020
> w6CBZ<-dat$w6CBZN
> rrw6CBZ<-(avgstCBZ-w6CBZ)/avgstCBZ
> rrpw6CBZ<-(stCBZ-w6CBZ)/stCBZ;rrpw6CBZ
[1] 0.8621499      NA 0.9348562      NA 0.9254996      NA 0.5606913
[8]      NA 0.8080368      NA 0.8637234 0.8937516 0.5321854 0.1559872

```

```

> ###lognormally/normally distributed?
> #null hypothesis - normal distribution
> #p>0.05 indicates normality
> shapiro.test(rranuaCBZ)
      Shapiro-wilk normality test
data:  rranuaCBZ
W = 0.89478, p-value = 0.2233
> shapiro.test(rrpanuaCBZ)
      Shapiro-wilk normality test
data:  rrpanuaCBZ
W = 0.8504, p-value = 0.07529
> shapiro.test(rranua2CBZ)
      Shapiro-wilk normality test
data:  rranua2CBZ
W = 0.85367, p-value = 0.2064
> shapiro.test(rrpanua2CBZ)
      Shapiro-wilk normality test
data:  rrpanua2CBZ
W = 0.84569, p-value = 0.1813
> shapiro.test(rrezCBZ)
      Shapiro-wilk normality test
data:  rrezCBZ
W = 0.90146, p-value = 0.3826
> shapiro.test(rrpezCBZ)
      Shapiro-wilk normality test
data:  rrpezCBZ
W = 0.95292, p-value = 0.7638
> shapiro.test(rrw4CBZ)
      Shapiro-wilk normality test
data:  rrw4CBZ
W = 0.94033, p-value = 0.5853
> shapiro.test(rrpw4CBZ)
      Shapiro-wilk normality test
data:  rrpw4CBZ
W = 0.8413, p-value = 0.05971
> shapiro.test(rrw6CBZ)
      Shapiro-wilk normality test
data:  rrw6CBZ
W = 0.86808, p-value = 0.1172
> shapiro.test(rrpw6CBZ)
      Shapiro-wilk normality test
data:  rrpw6CBZ
W = 0.79195, p-value = 0.01652
> ###independent?
> #null hypothesis - no correlation
> #p>0.05 indicates independence
> dwt(lm(rranuaCBZ~time))
lag Autocorrelation D-W Statistic p-value
 1      0.02140585      1.845301  0.472
Alternative hypothesis: rho != 0
> dwt(lm(rrpanuaCBZ~time))
lag Autocorrelation D-W Statistic p-value
 1     -0.4551992      2.910389  0.154
Alternative hypothesis: rho != 0
> dwt(lm(rranua2CBZ~time))
lag Autocorrelation D-W Statistic p-value

```

```

1      -0.7122598      3.215965      0.298
Alternative hypothesis: rho != 0
> dwt(lm(rrpanua2CBZ~time))
lag Autocorrelation D-W Statistic p-value
1      -0.65163      3.069216      0.396
Alternative hypothesis: rho != 0
> dwt(lm(rrezCBZ~time))
lag Autocorrelation D-W Statistic p-value
1      -0.1891895      2.045536      0.55
Alternative hypothesis: rho != 0
> dwt(lm(rrpezCBZ~time))
lag Autocorrelation D-W Statistic p-value
1      0.1390417      1.33312      0.064
Alternative hypothesis: rho != 0
> dwt(lm(rrw4CBZ~time))
lag Autocorrelation D-W Statistic p-value
1      0.04693143      1.646343      0.29
Alternative hypothesis: rho != 0
> dwt(lm(rrpw4CBZ~time))
lag Autocorrelation D-W Statistic p-value
1      -0.006810674      1.854755      0.514
Alternative hypothesis: rho != 0
> dwt(lm(rrw6CBZ~time))
lag Autocorrelation D-W Statistic p-value
1      0.0006011698      1.822333      0.508
Alternative hypothesis: rho != 0
> dwt(lm(rrpw6CBZ~time))
lag Autocorrelation D-W Statistic p-value
1      0.1380738      1.447818      0.148
Alternative hypothesis: rho != 0
> ###equal variance?
> #null hypothesis - equal variance
> #p>0.05 indicates equal variance
> rrcbZ<-cbind(rranuaCBZ,rrezCBZ,rrw4CBZ,rrw6CBZ)
> rrcbZstack<-stack(data.frame(rrcbZ))
> rrcbZ2<-cbind(rranua2CBZ,rrezCBZ,rrw4CBZ,rrw6CBZ)
> rrcbZstack2<-stack(data.frame(rrcbZ2))
> rrpcbZ<-cbind(rrpanuaCBZ,rrpezCBZ,rrpw4CBZ,rrpw6CBZ)
> rrpcbZstack<-stack(data.frame(rrpcbZ))
> rrpcbZ2<-cbind(rrpanua2CBZ,rrpezCBZ,rrpw4CBZ,rrpw6CBZ)
> rrpcbZstack2<-stack(data.frame(rrpcbZ2))
> #use with parametric cases
> bartlett.test(rrcbZstack$values,rrcbZstack$ind)
      Bartlett test of homogeneity of variances
data:  rrcbZstack$values and rrcbZstack$ind
Bartlett's K-squared = 3.5044, df = 3, p-value = 0.3202
> bartlett.test(rrcbZstack2$values,rrcbZstack2$ind)
      Bartlett test of homogeneity of variances
data:  rrcbZstack2$values and rrcbZstack2$ind
Bartlett's K-squared = 7.8089, df = 3, p-value = 0.05013
> #use with nonparametric cases
> leveneTest(rrpcbZstack$values,rrpcbZstack$ind)
Levene's Test for Homogeneity of Variance (center = median)
      Df F value Pr(>F)
group 3  0.6236 0.6055
29

```

```

> leveneTest(rrCBZstack2$values,rrCBZstack2$ind)
Levene's Test for Homogeneity of Variance (center = median)
  Df F value Pr(>F)
group 3  1.0864  0.373
  25

> ###are column means the same?
> #null hypothesis - column means are the same
> #p<0.05 indicates significantly different means
> #use with parametric cases when variances are equal
> summary(aov(rrCBZstack2$values~rrCBZstack2$ind))
      Df Sum Sq Mean Sq F value    Pr(>F)
rrCBZstack2$ind  3  3.512  1.1706   38.46 3.1e-10 ***
Residuals      29  0.883  0.0304
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
23 observations deleted due to missingness
> summary(aov(rrCBZstack2$values~rrCBZstack2$ind))
      Df Sum Sq Mean Sq F value    Pr(>F)
rrCBZstack2$ind  3  3.715  1.2384   51.49 7.64e-11 ***
Residuals      25  0.601  0.0241
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
27 observations deleted due to missingness
> #use with nonparametric cases
> kruskal.test(rrCBZstack2$values~rrCBZstack2$ind)
Kruskal-wallis rank sum test
data:  rrCBZstack2$values by rrCBZstack2$ind
Kruskal-wallis chi-squared = 19.67, df = 3, p-value = 0.0001987
> kruskal.test(rrCBZstack2$values~rrCBZstack2$ind)
Kruskal-wallis rank sum test
data:  rrCBZstack2$values by rrCBZstack2$ind
Kruskal-wallis chi-squared = 22.258, df = 3, p-value = 5.765e-05
> ###if column means are different, which columns?
> #null hypothesis - column means are the same
> #p<0.05 indicates significantly different means
> #use with parametric case
> TukeyHSD(aov(rrCBZstack2$values~rrCBZstack2$ind))
Tukey multiple comparisons of means
95% family-wise confidence level
Fit: aov(formula = rrCBZstack2$values ~ rrCBZstack2$ind)
`rrCBZstack2$ind`
      diff      lwr      upr    p adj
rrezCBZ-rranuaCBZ -0.846309296 -1.0968353 -0.5957833 0.0000000
rrw4CBZ-rranuaCBZ -0.412502676 -0.6365799 -0.1884254 0.0001365
rrw6CBZ-rranuaCBZ  0.005060849 -0.2190164  0.2291381 0.9999143
rrw4CBZ-rrezCBZ   0.433806620  0.1832807  0.6843326 0.0003090
rrw6CBZ-rrezCBZ   0.851370145  0.6008442  1.1018961 0.0000000
rrw6CBZ-rrw4CBZ   0.417563525  0.1934863  0.6416408 0.0001152
> TukeyHSD(aov(rrCBZstack2$values~rrCBZstack2$ind))
Tukey multiple comparisons of means
95% family-wise confidence level

Fit: aov(formula = rrCBZstack2$values ~ rrCBZstack2$ind)
`rrCBZstack2$ind`
      diff      lwr      upr    p adj
rrezCBZ-rranua2CBZ -0.9910001 -1.2493106 -0.73268960 0.0000000

```

```

rrw4CBZ-rranua2CBZ -0.5571935 -0.7951316 -0.31925532 0.0000055
rrw6CBZ-rranua2CBZ -0.1396299 -0.3775681 0.09830821 0.3891724
rrw4CBZ-rrezCBZ 0.4338066 0.2089762 0.65863704 0.0000942
rrw6CBZ-rrezCBZ 0.8513701 0.6265397 1.07620056 0.0000000
rrw6CBZ-rrw4CBZ 0.4175635 0.2164691 0.61865796 0.0000338

```

```

> #use with nonparametric case
> kruskalmc(rrpCBZstack$values~rrpCBZstack$ind)

```

Multiple comparison test after Kruskal-wallis

p.value: 0.05

Comparisons

	obs.dif	critical.dif	difference
rrpanuaCBZ-rrpezCBZ	19.3333333	13.44534	TRUE
rrpanuaCBZ-rrpw4CBZ	9.3333333	12.02588	FALSE
rrpanuaCBZ-rrpw6CBZ	0.2222222	12.02588	FALSE
rrpezCBZ-rrpw4CBZ	10.0000000	13.44534	FALSE
rrpezCBZ-rrpw6CBZ	19.5555556	13.44534	TRUE
rrpw4CBZ-rrpw6CBZ	9.5555556	12.02588	FALSE

```

> kruskalmc(rrpCBZstack2$values~rrpCBZstack2$ind)

```

Multiple comparison test after Kruskal-wallis

p.value: 0.05

Comparisons

	obs.dif	critical.dif	difference
rrpanua2CBZ-rrpezCBZ	21.7333333	13.60260	TRUE
rrpanua2CBZ-rrpw4CBZ	13.5111111	12.52979	TRUE
rrpanua2CBZ-rrpw6CBZ	5.5111111	12.52979	FALSE
rrpezCBZ-rrpw4CBZ	8.2222222	11.83954	FALSE
rrpezCBZ-rrpw6CBZ	16.2222222	11.83954	TRUE
rrpw4CBZ-rrpw6CBZ	8.0000000	10.58961	FALSE

```

> #compare removal rate CIs for systems in lowest group(s) to zero

```

```

> mu<-mean(rranuaCBZ,na.rm=TRUE); mu

```

```

[1] 0.7936866

```

```

> s<-sd(rranuaCBZ,na.rm=TRUE); s

```

```

[1] 0.1917473

```

```

> n<-length(which(rranuaCBZ!="NA"));n

```

```

[1] 9

```

```

> a<-0.05

```

```

> t<-qt(1-a/2,n-1)

```

```

> se<-s/sqrt(n)

```

```

> ci.mu<-c(mu-t*se,mu,t*se);ci.mu

```

```

[1] 0.6462966 0.7936866 0.9410767

```

```

> mu<-mean(rrpanuaCBZ,na.rm=TRUE); mu

```

```

[1] 0.7192263

```

```

> s<-sd(rrpanuaCBZ,na.rm=TRUE); s

```

```

[1] 0.278543

```

```

> n<-length(which(rrpanuaCBZ!="NA"));n

```

```

[1] 9

```

```

> a<-0.05

```

```

> t<-qt(1-a/2,n-1)

```

```

> se<-s/sqrt(n)

```

```

> ci.mu<-c(mu-t*se,mu,t*se);ci.mu

```

```

[1] 0.5051192 0.7192263 0.9333334

```

```

> mu<-mean(rranua2CBZ,na.rm=TRUE); mu

```

```

[1] 0.9383774

```

```

> s<-sd(rranua2CBZ,na.rm=TRUE); s

```

```

[1] 0.05633213

```

```

> n<-length(which(rrpanua2CBZ!="NA"));n
[1] 5
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.8684318 0.9383774 1.0083230
> mu<-mean(rrpanua2CBZ,na.rm=TRUE); mu
[1] 0.9302372
> s<-sd(rrpanua2CBZ,na.rm=TRUE); s
[1] 0.07640072
> n<-length(which(rrpanua2CBZ!="NA"));n
[1] 5
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.8353732 0.9302372 1.0251012
> mu<-mean(rrezCBZ,na.rm=TRUE); mu
[1] -0.05262267
> s<-sd(rrezCBZ,na.rm=TRUE); s
[1] 0.2261195
> n<-length(which(rrezCBZ!="NA"));n
[1] 6
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] -0.28992054 -0.05262267 0.18467520
> mu<-mean(rrpezCBZ,na.rm=TRUE); mu
[1] -0.3751866
> s<-sd(rrpezCBZ,na.rm=TRUE); s
[1] 0.1436215
> n<-length(which(rrpezCBZ!="NA"));n
[1] 6
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] -0.5259080 -0.3751866 -0.2244651
> mu<-mean(rrw4CBZ,na.rm=TRUE); mu
[1] 0.3811839
> s<-sd(rrw4CBZ,na.rm=TRUE); s
[1] 0.1731893
> n<-length(which(rrw4CBZ!="NA"));n
[1] 9
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.2480588 0.3811839 0.5143091
> mu<-mean(rrpw4CBZ,na.rm=TRUE); mu
[1] 0.294421
> s<-sd(rrpw4CBZ,na.rm=TRUE); s
[1] 0.3411885
> n<-length(which(rrpw4CBZ!="NA"));n

```

```

[1] 9
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.03216035 0.29442101 0.55668167
> mu<-mean(rrw6CBZ,na.rm=TRUE); mu
[1] 0.7987475
> s<-sd(rrw6CBZ,na.rm=TRUE); s
[1] 0.1078137
> n<-length(which(rrw6CBZ!="NA"));n
[1] 9
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.7158745 0.7987475 0.8816204
> mu<-mean(rrpw6CBZ,na.rm=TRUE); mu
[1] 0.7263201
> s<-sd(rrpw6CBZ,na.rm=TRUE); s
[1] 0.2611515
> n<-length(which(rrpw6CBZ!="NA"));n
[1] 9
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.5255813 0.7263201 0.9270590

```

## **LAM**

```

> ###set-up
> stLAM<-dat$stLAM
> avgstLAM<-mean(stLAM,na.rm=TRUE)
> sdstLAM<-sd(stLAM,na.rm=TRUE)
> RSDLAM<-sdstLAM/avgstLAM;RSDLAM
[1] 0.1478286
> dwt(lm(stLAM~time))
lag Autocorrelation D-W Statistic p-value
1 0.00349483 1.934419 0.568
Alternative hypothesis: rho != 0
> anuaLAM<-dat$anuaLAM
> rranuaLAM<-(avgstLAM-anuaLAM)/avgstLAM
> anua2LAM<-dat$anua2LAM
> rranua2LAM<-(avgstLAM-anua2LAM)/avgstLAM
> ezLAM<-dat$ezLAM
> rrezLAM<-(avgstLAM-ezLAM)/avgstLAM
> w4LAM<-dat$w4LAMN
> rrw4LAM<-(avgstLAM-w4LAM)/avgstLAM
> w6LAM<-dat$w6LAMN
> rrw6LAM<-(avgstLAM-w6LAM)/avgstLAM
> ###lognormally/normally distributed?
> #null hypothesis - normal distribution

```

```

> #p>0.05 indicates normality
> shapiro.test(rranuaLAM)
      Shapiro-wilk normality test
data:  rranuaLAM
w = 0.63813, p-value = 0.000268
> shapiro.test(rranua2LAM)
      Shapiro-wilk normality test
data:  rranua2LAM
w = 0.68503, p-value = 0.006634
> shapiro.test(rrezLAM)
      Shapiro-wilk normality test
data:  rrezLAM
w = 0.87756, p-value = 0.258
> shapiro.test(rrw4LAM)
      Shapiro-wilk normality test
data:  rrw4LAM
w = 0.93935, p-value = 0.575
> shapiro.test(rrw6LAM)
      Shapiro-wilk normality test
data:  rrw6LAM
w = 0.85445, p-value = 0.0834
> ###independent?
> #null hypothesis - no correlation
> #p>0.05 indicates independence
> dwt(lm(rranuaLAM~time))
lag Autocorrelation D-W Statistic p-value
1      -0.3357955      2.629865  0.478
Alternative hypothesis: rho != 0
> dwt(lm(rranua2LAM~time))
lag Autocorrelation D-W Statistic p-value
1      -0.595738      2.903088  0.558
Alternative hypothesis: rho != 0
> dwt(lm(rrezLAM~time))
lag Autocorrelation D-W Statistic p-value
1      -0.1635293      2.162732  0.686
Alternative hypothesis: rho != 0
> dwt(lm(rrw4LAM~time))
lag Autocorrelation D-W Statistic p-value
1      0.4352629      0.8101197  0.006
Alternative hypothesis: rho != 0
> dwt(lm(rrw6LAM~time))
lag Autocorrelation D-W Statistic p-value
1      0.1276424      1.572595  0.262
Alternative hypothesis: rho != 0
> w4LAMuse<-dat$w4LAMuse
> rrw4LAMuse<-(avgstLAM-w4LAMuse)/avgstLAM
> dwt(lm(rrw4LAMuse~time), reps=10000)
lag Autocorrelation D-W Statistic p-value
1      -0.07415112      1.678914  0.1908
Alternative hypothesis: rho != 0
> shapiro.test(rrw4LAMuse)
      Shapiro-wilk normality test
data:  rrw4LAMuse
w = 0.96728, p-value = 0.8575
> ###equal variance?
> #null hypothesis - equal variance

```

```

> #p>0.05 indicates equal variance
> rrLAM<-cbind(rranuaLAM,rrezLAM,rrw4LAMuse,rrw6LAM)
> rrLAMstack<-stack(data.frame(rrLAM))
> rrLAM2<-cbind(rranua2LAM,rrezLAM,rrw4LAMuse,rrw6LAM)
> rrLAMstack2<-stack(data.frame(rrLAM2))
> #use with nonparametric cases
> leveneTest(rrLAMstack$values,rrLAMstack$ind)
Levene's Test for Homogeneity of Variance (center = median)
      Df F value    Pr(>F)
group 3  8.2547 0.0005499 ***
      25
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> leveneTest(rrLAMstack2$values,rrLAMstack2$ind)
Levene's Test for Homogeneity of Variance (center = median)
      Df F value    Pr(>F)
group 3  6.5557 0.002664 **
      21
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> ###are column means the same?
> #null hypothesis - column means are the same
> #p<0.05 indicates significantly different means
> #use with nonparametric cases
> kruskal.test(rrLAMstack$values~rrLAMstack$ind)
Kruskal-wallis rank sum test
data:  rrLAMstack$values by rrLAMstack$ind
Kruskal-wallis chi-squared = 24.175, df = 3, p-value = 2.296e-05
> kruskal.test(rrLAMstack2$values~rrLAMstack2$ind)
Kruskal-wallis rank sum test
data:  rrLAMstack2$values by rrLAMstack2$ind
Kruskal-wallis chi-squared = 22.2, df = 3, p-value = 5.927e-05
> ###if column means are different, which columns?
> #null hypothesis - column means are the same
> #p<0.05 indicates significantly different means
> #use with nonparametric case
> kruskalmc(rrLAMstack$values~rrLAMstack$ind)
Multiple comparison test after Kruskal-wallis
p.value: 0.05
Comparisons
      obs.dif critical.dif difference
rranuaLAM-rrezLAM      20.611111      11.83954      TRUE
rranuaLAM-rrw4LAMuse    15.111111      12.52979      TRUE
rranuaLAM-rrw6LAM       7.222222      10.58961      FALSE
rrezLAM-rrw4LAMuse      5.500000      13.60260      FALSE
rrezLAM-rrw6LAM        13.388889      11.83954      TRUE
rrw4LAMuse-rrw6LAM      7.888889      12.52979      FALSE
> kruskalmc(rrLAMstack2$values~rrLAMstack2$ind)
Multiple comparison test after Kruskal-wallis
p.value: 0.05
Comparisons
      obs.dif critical.dif difference
rranua2LAM-rrezLAM      19.5      11.75761      TRUE
rranua2LAM-rrw4LAMuse    14.0      12.28042      TRUE
rranua2LAM-rrw6LAM       7.0      10.83031      FALSE
rrezLAM-rrw4LAMuse      5.5      11.75761      FALSE

```

```

rrezLAM-rrw6LAM          12.5      10.23368      TRUE
rrw4LAMuse-rrw6LAM       7.0       10.83031      FALSE
> #compare removal rate CIs for systems in lowest group(s) to zero
> mu<-mean(rrezLAM,na.rm=TRUE); mu
[1] -0.1251848
> s<-sd(rrezLAM,na.rm=TRUE); s
[1] 0.1061957
> n<-length(which(rrezLAM!="NA"));n
[1] 6
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] -0.23663035 -0.12518476 -0.01373917
> mu<-mean(rrw4LAM,na.rm=TRUE); mu
[1] 0.562079
> s<-sd(rrw4LAM,na.rm=TRUE); s
[1] 0.1685356
> n<-length(which(rrw4LAM!="NA"));n
[1] 9
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.4325311 0.5620790 0.6916269
> mu<-mean(rrw6LAM,na.rm=TRUE); mu
[1] 0.9592412
> s<-sd(rrw6LAM,na.rm=TRUE); s
[1] 0.03184468
> n<-length(which(rrw6LAM!="NA"));n
[1] 9
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.9347632 0.9592412 0.9837192

```

## *LEV*

```

> ###set-up
> stLEV<-dat$stLEV
> avgstLEV<-mean(stLEV,na.rm=TRUE)
> sdstLEV<-sd(stLEV,na.rm=TRUE)
> RSDLEV<-sdstLEV/avgstLEV;RSDLEV
[1] 0.2471104
> dwt(lm(stLEV~time))
lag Autocorrelation D-W Statistic p-value
 1      -0.5799318      3.044526  0.136
Alternative hypothesis: rho != 0
> anuaLEV<-dat$anuaLEV
> rranuaLEV<-(avgstLEV-anuaLEV)/avgstLEV
> anua2LEV<-dat$anua2LEV
> rranua2LEV<-(avgstLEV-anua2LEV)/avgstLEV

```

```

> ezLEV<-dat$ezLEV
> rrezLEV<-(avgstLEV-ezLEV)/avgstLEV
> w4LEV<-dat$w4LEVN
> rrw4LEV<-(avgstLEV-w4LEV)/avgstLEV
> w6LEV<-dat$w6LEVN
> rrw6LEV<-(avgstLEV-w6LEV)/avgstLEV
> ###lognormally/normally distributed?
> #null hypothesis - normal distribution
> #p>0.05 indicates normality
> shapiro.test(rranuaLEV)
      Shapiro-wilk normality test
data:  rranuaLEV
w = 0.49513, p-value = 5.604e-06
> shapiro.test(rranua2LEV)
      Shapiro-wilk normality test
data:  rranua2LEV
w = 0.98659, p-value = 0.9664
> shapiro.test(rrezLEV)
      Shapiro-wilk normality test
data:  rrezLEV
w = 0.94121, p-value = 0.669
> shapiro.test(rrw4LEV)
      Shapiro-wilk normality test
data:  rrw4LEV
w = 0.80003, p-value = 0.02044
> shapiro.test(rrw6LEV)
      Shapiro-wilk normality test
data:  rrw6LEV
w = 0.72382, p-value = 0.00269
> ###independent?
> #null hypothesis - no correlation
> #p>0.05 indicates independence
> dwt(lm(rranuaLEV~time))
lag Autocorrelation D-W Statistic p-value
1      -0.4207224      2.662479  0.476
Alternative hypothesis: rho != 0
> dwt(lm(rranua2LEV~time))
lag Autocorrelation D-W Statistic p-value
1      -0.8445977      3.588703  0.012
Alternative hypothesis: rho != 0
> dwt(lm(rrezLEV~time))
lag Autocorrelation D-W Statistic p-value
1      -0.6062884      2.840949  0.504
Alternative hypothesis: rho != 0
> dwt(lm(rrw4LEV~time))
lag Autocorrelation D-W Statistic p-value
1      -0.32664      2.504007  0.728
Alternative hypothesis: rho != 0
> dwt(lm(rrw6LEV~time))
lag Autocorrelation D-W Statistic p-value
1      -0.4275562      2.851593  0.316
Alternative hypothesis: rho != 0
> anua2LEVuse<-dat$anua2LEVuse
> rranua2LEVuse<-(avgstLEV-anua2LEVuse)/avgstLEV
> dwt(lm(rranua2LEVuse~time))
lag Autocorrelation D-W Statistic p-value

```

```

1      -0.7930052      3.402699      0.094
Alternative hypothesis: rho != 0
> shapiro.test(rranua2LEVuse)
      Shapiro-wilk normality test
data:  rranua2LEVuse
W = 0.98185, p-value = 0.9128
> ###equal variance?
> #null hypothesis - equal variance
> #p>0.05 indicates equal variance
> rrLEV<-cbind(rranuaLEV,rrezLEV,rrw4LEV,rrw6LEV)
> rrLEVstack<-stack(data.frame(rrLEV))
> rrLEV2<-cbind(rranua2LEVuse,rrezLEV,rrw4LEV,rrw6LEV)
> rrLEVstack2<-stack(data.frame(rrLEV2))
> #use with nonparametric cases
> leveneTest(rrLEVstack$values,rrLEVstack$ind)
Levene's Test for Homogeneity of Variance (center = median)
      Df F value Pr(>F)
group 3  4.3541 0.01194 *
      29
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> leveneTest(rrLEVstack2$values,rrLEVstack2$ind)
Levene's Test for Homogeneity of Variance (center = median)
      Df F value Pr(>F)
group 3  4.0094 0.01906 *
      24
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> ###are column means the same?
> #null hypothesis - column means are the same
> #p<0.05 indicates significantly different means
> #use with nonparametric cases
> kruskal.test(rrLEVstack$values~rrLEVstack$ind)
      Kruskal-wallis rank sum test
data:  rrLEVstack$values by rrLEVstack$ind
Kruskal-wallis chi-squared = 16.821, df = 3, p-value = 0.0007694
> kruskal.test(rrLEVstack2$values~rrLEVstack2$ind)
      Kruskal-wallis rank sum test
data:  rrLEVstack2$values by rrLEVstack2$ind
Kruskal-wallis chi-squared = 16.124, df = 3, p-value = 0.00107
> ###if column means are different, which columns?
> #null hypothesis - column means are the same
> #p<0.05 indicates significantly different means
> #use with nonparametric case
> kruskalmc(rrLEVstack$values~rrLEVstack$ind)
Multiple comparison test after Kruskal-wallis
p.value: 0.05
Comparisons
      obs.dif critical.dif difference
rranuaLEV-rrezLEV 16.166667   13.44534      TRUE
rranuaLEV-rrw4LEV  3.111111   12.02588     FALSE
rranuaLEV-rrw6LEV  4.111111   12.02588     FALSE
rrezLEV-rrw4LEV   13.055556   13.44534     FALSE
rrezLEV-rrw6LEV   20.277778   13.44534      TRUE
rrw4LEV-rrw6LEV   7.222222   12.02588     FALSE
> kruskalmc(rrLEVstack2$values~rrLEVstack2$ind)

```

Multiple comparison test after kruskal-wallis  
 p.value: 0.05  
 Comparisons

	obs.dif	critical.dif	difference
rranua2LEVuse-rrezLEV	15.500000	14.00873	TRUE
rranua2LEVuse-rrw4LEV	4.777778	13.04142	FALSE
rranua2LEVuse-rrw6LEV	1.111111	13.04142	FALSE
rrezLEV-rrw4LEV	10.722222	11.43808	FALSE
rrezLEV-rrw6LEV	16.611111	11.43808	TRUE
rrw4LEV-rrw6LEV	5.888889	10.23053	FALSE

```
> #Do rr CIs include zero?
> mu<-mean(rrezLEV,na.rm=TRUE); mu
[1] 0.8372376
> s<-sd(rrezLEV,na.rm=TRUE); s
[1] 0.05141909
> n<-length(which(rrezLEV!="NA"));n
[1] 6
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,t*se);ci.mu
[1] 0.7832765 0.8372376 0.8911986
```

## ***MET***

```
> ###set-up
> stMET<-dat$stMET
> avgstMET<-mean(stMET,na.rm=TRUE)
> sdstMET<-sd(stMET,na.rm=TRUE)
> RSDMET<-sdstMET/avgstMET;RSDMET
[1] 0.605109
> dwt(lm(stMET~time))
lag Autocorrelation D-w Statistic p-value
1 -0.1250741 2.236556 0.916
Alternative hypothesis: rho != 0
> anuaMET<-dat$anuaMET
> rranuaMET<-(avgstMET-anuaMET)/avgstMET
> anua2MET<-dat$anua2MET
> rranua2MET<-(avgstMET-anua2MET)/avgstMET
> ezMET<-dat$ezMET
> rrezMET<-(avgstMET-ezMET)/avgstMET
> w4MET<-dat$w4METN
> rrw4MET<-(avgstMET-w4MET)/avgstMET
> w6MET<-dat$w6METN
> rrw6MET<-(avgstMET-w6MET)/avgstMET
> ###lognormally/normally distributed?
> #null hypothesis - normal distribution
> #p>0.05 indicates normality
> shapiro.test(rranuaMET)
```

Shapiro-wilk normality test

data: rranuaMET

```
w = 0.43213, p-value = 1.015e-06
```

```
> shapiro.test(rranua2MET)
```

```
Error in shapiro.test(rranua2MET) : all 'x' values are identical
```

```
> shapiro.test(rrezMET)
```

```
Shapiro-wilk normality test
```

```
data: rrezMET
```

```
w = 0.72667, p-value = 0.01166
```

```
> shapiro.test(rrw4MET)
```

```
Shapiro-wilk normality test
```

```
data: rrw4MET
```

```
w = 0.38984, p-value = 3.217e-07
```

```
> shapiro.test(rrw6MET)
```

```
Shapiro-wilk normality test
```

```
data: rrw6MET
```

```
w = 0.38984, p-value = 3.217e-07
```

```
> ###independent?
```

```
> #null hypothesis - no correlation
```

```
> #p>0.05 indicates independence
```

```
> dwt(lm(rranuaMET~time))
```

```
lag Autocorrelation D-W Statistic p-value  
1 -0.4914153 2.819928 0.342
```

```
Alternative hypothesis: rho != 0
```

```
> dwt(lm(rranua2MET~time))
```

```
lag Autocorrelation D-W Statistic p-value  
1 NaN NaN NA
```

```
Alternative hypothesis: rho != 0
```

```
warning message:
```

```
In summary.lm(mode1) : essentially perfect fit: summary may be unreliable
```

```
> dwt(lm(rrezMET~time))
```

```
lag Autocorrelation D-W Statistic p-value  
1 0.1886256 1.079414 0.016
```

```
Alternative hypothesis: rho != 0
```

```
> dwt(lm(rrw4MET~time))
```

```
lag Autocorrelation D-W Statistic p-value  
1 -0.05639898 1.371858 0.162
```

```
Alternative hypothesis: rho != 0
```

```
> dwt(lm(rrw6MET~time))
```

```
lag Autocorrelation D-W Statistic p-value  
1 -0.1804434 2.327542 0.96
```

```
Alternative hypothesis: rho != 0
```

```
> ezMETuse<-dat$ezMETuse
```

```
> rrezMETuse<-(avgstMET-ezMETuse)/avgstMET
```

```
> dwt(lm(rrezMETuse~time))
```

```
lag Autocorrelation D-W Statistic p-value  
1 -0.1888651 1.844626 0.32
```

```
Alternative hypothesis: rho != 0
```

```

> shapiro.test(rrezMETuse)

      Shapiro-wilk normality test

data:  rrezMETuse
W = 0.59751, p-value = 0.0005803

> ###equal variance?
> #null hypothesis - equal variance
> #p>0.05 indicates equal variance
> rrMET<-cbind(rranuaMET,rrezMETuse,rrw4MET,rrw6MET)
> rrMETstack<-stack(data.frame(rrMET))
> rrMET2<-cbind(rranua2MET,rrezMETuse,rrw4MET,rrw6MET)
> rrMETstack2<-stack(data.frame(rrMET2))
> #use with nonparametric cases
> leveneTest(rrMETstack$values,rrMETstack$ind)
Levene's Test for Homogeneity of Variance (center = median)
      Df F value Pr(>F)
group  3  0.8562 0.4752
      28
> leveneTest(rrMETstack2$values,rrMETstack2$ind)
Levene's Test for Homogeneity of Variance (center = median)
      Df F value Pr(>F)
group  3  1.8249 0.1696
      24
> ###are column means the same?
> #null hypothesis - column means are the same
> #p<0.05 indicates significantly different means
> #use with nonparametric cases
> kruskal.test(rrMETstack$values~rrMETstack$ind)

      Kruskal-wallis rank sum test

data:  rrMETstack$values by rrMETstack$ind
Kruskal-wallis chi-squared = 2.4461, df = 3, p-value = 0.4851

> kruskal.test(rrMETstack2$values~rrMETstack2$ind)

      Kruskal-wallis rank sum test

data:  rrMETstack2$values by rrMETstack2$ind
Kruskal-wallis chi-squared = 3.8654, df = 3, p-value = 0.2764

> ###if column means are different, which columns?
> #null hypothesis - column means are the same
> #p<0.05 indicates significantly different means
> #compare removal rate CIs for systems in lowest group(s) to zero
> #use bootstrapping to find confidence interval around rr
> n<-100000
> rranuaMETbs<-rranuaMET[!is.na(rranuaMET)];rranuaMETbs
[1] 0.86698525 0.86698525 0.86698525 0.86698525 0.86698525 0.86698525
[7] 0.86698525 -8.93819741 0.05652634
> bootrranuaMET<-numeric(n)
> for (i in 1:n) {
+ bootrranuaMET[i]<-mean(sample(rranuaMETbs,replace=T),na.rm=TRUE)
+ }

```

```

> hist(bootrranuaMET)
> quantile(bootrranuaMET, probs=c(0.025,0.975))
      2.5%      97.5%
-2.4914600  0.8669852
> mean(bootrranuaMET)
[1] -0.3141332
> rranua2METbs<-rranua2MET[!is.na(rranua2MET)]; rranua2METbs
[1] 0.8669852 0.8669852 0.8669852 0.8669852 0.8669852
> bootrranua2MET<-numeric(n)
> for (i in 1:n) {
+ bootrranua2MET[i]<-mean(sample(rranua2METbs, replace=T), na.rm=TRUE)
+ }
> hist(bootrranua2MET)
> quantile(bootrranua2MET, probs=c(0.025,0.975))
      2.5%      97.5%
0.8669852 0.8669852
> mean(bootrranua2MET)
[1] 0.8669852
> rrezMETbs<-rrezMETuse[!is.na(rrezMETuse)]; rrezMETbs
[1] -3.5457201 0.5657512 0.8669852 0.8669852 0.8669852
> bootrrezMET<-numeric(n)
> for (i in 1:n) {
+ bootrrezMET[i]<-mean(sample(rrezMETbs, replace=T), na.rm=TRUE)
+ }
> hist(bootrrezMET)
> quantile(bootrrezMET, probs=c(0.025,0.975))
      2.5%      97.5%
-1.8408848 0.8669852
> mean(bootrrezMET)
[1] -0.07814058
> rrw4METbs<-rrw4MET[!is.na(rrw4MET)]; rrw4METbs
[1] 0.7436050 0.8669852 0.8669852 0.8669852 0.8669852 0.8669852 0.8669852
[8] 0.8669852 0.8669852
> bootrrw4MET<-numeric(n)
> for (i in 1:n) {
+ bootrrw4MET[i]<-mean(sample(rrw4METbs, replace=T), na.rm=TRUE)
+ }
> hist(bootrrw4MET)
> quantile(bootrrw4MET, probs=c(0.025,0.975))
      2.5%      97.5%
0.8258585 0.8669852
> mean(bootrrw4MET)
[1] 0.8533446
> rrw6METbs<-rrw6MET[!is.na(rrw6MET)]; rrw6METbs
[1] 0.8669852 0.8669852 0.8669852 0.4675351 0.8669852 0.8669852 0.8669852
[8] 0.8669852 0.8669852
> bootrrw6MET<-numeric(n)
> for (i in 1:n) {
+ bootrrw6MET[i]<-mean(sample(rrw6METbs, replace=T), na.rm=TRUE)
+ }
> hist(bootrrw6MET)
> quantile(bootrrw6MET, probs=c(0.025,0.975))
      2.5%      97.5%
0.7338352 0.8669852
> mean(bootrrw6MET)
[1] 0.8225881

```

## TOP

```
> ###set-up
> stTOP<-dat$stTOP
> avgstTOP<-mean(stTOP,na.rm=TRUE)
> sdstTOP<-sd(stTOP,na.rm=TRUE)
> RSDTOP<-sdstTOP/avgstTOP;RSDTOP
[1] 0.9821633
> dwt(lm(stTOP~time))
lag Autocorrelation D-W Statistic p-value
  1      -0.1435915      2.257869  0.858
Alternative hypothesis: rho != 0
> anuaTOP<-dat$anuaTOP
> rranuaTOP<-(avgstTOP-anuaTOP)/avgstTOP
> anua2TOP<-dat$anua2TOP
> rranua2TOP<-(avgstTOP-anua2TOP)/avgstTOP
> ezTOP<-dat$ezTOP
> rrezTOP<-(avgstTOP-ezTOP)/avgstTOP
> w4TOP<-dat$w4TOPN
> rrw4TOP<-(avgstTOP-w4TOP)/avgstTOP
> w6TOP<-dat$w6TOPN
> rrw6TOP<-(avgstTOP-w6TOP)/avgstTOP
> ###lognormally/normally distributed?
> #null hypothesis - normal distribution
> #p>0.05 indicates normality
> shapiro.test(rranuaTOP)
      Shapiro-wilk normality test
data:  rranuaTOP
w = 0.67699, p-value = 0.0007641
> shapiro.test(rranua2TOP)
Error in shapiro.test(rranua2TOP) : all 'x' values are identical
> shapiro.test(rrezTOP)
      Shapiro-wilk normality test
data:  rrezTOP
w = 0.49609, p-value = 2.073e-05
> shapiro.test(rrw4TOP)
      Shapiro-wilk normality test
data:  rrw4TOP
w = 0.38984, p-value = 3.217e-07
> shapiro.test(rrw6TOP)
      Shapiro-wilk normality test
data:  rrw6TOP
w = 0.38984, p-value = 3.217e-07
> ###independent?
> #null hypothesis - no correlation
> #p>0.05 indicates independence
> dwt(lm(rranuaTOP~time))
lag Autocorrelation D-W Statistic p-value
  1      -0.6889641      3.357781  0.034
Alternative hypothesis: rho != 0
> dwt(lm(rranua2TOP~time))
lag Autocorrelation D-W Statistic p-value
```

```

1           NaN           NaN           NA
Alternative hypothesis: rho != 0
warning message:
In summary.lm(model) : essentially perfect fit: summary may be unreliable
> dwt(lm(rrezTOP~time))
lag Autocorrelation D-W Statistic p-value
1 -0.2957374 2.490251 0.896
Alternative hypothesis: rho != 0
> dwt(lm(rrw4TOP~time))
lag Autocorrelation D-W Statistic p-value
1 -0.2334385 2.392764 0.816
Alternative hypothesis: rho != 0
> dwt(lm(rrw6TOP~time))
lag Autocorrelation D-W Statistic p-value
1 -0.1804434 2.327542 0.954
Alternative hypothesis: rho != 0
> anuaTOPuse<-dat$anuaTOPuse
> rranuaTOPuse<-(avgstTOP-anuaTOPuse)/avgstTOP
> dwt(lm(rranuaTOPuse~time))
lag Autocorrelation D-W Statistic p-value
1 -0.5578832 3.086111 0.17
Alternative hypothesis: rho != 0
> shapiro.test(rranuaTOPuse)
Shapiro-wilk normality test
data: rranuaTOPuse
w = 0.71104, p-value = 0.003001
> ###equal variance?
> #null hypothesis - equal variance
> #p>0.05 indicates equal variance
> rrTOP<-cbind(rranuaTOPuse,rrezTOP,rrw4TOP,rrw6TOP)
> rrTOPstack<-stack(data.frame(rrTOP))
> rrTOP2<-cbind(rranua2TOP,rrezTOP,rrw4TOP,rrw6TOP)
> rrTOPstack2<-stack(data.frame(rrTOP2))
> #use with nonparametric cases
> leveneTest(rrTOPstack$values,rrTOPstack$ind)
Levene's Test for Homogeneity of Variance (center = median)
Df F value Pr(>F)
group 3 1.7821 0.1734
28
> leveneTest(rrTOPstack2$values,rrTOPstack2$ind)
Levene's Test for Homogeneity of Variance (center = median)
Df F value Pr(>F)
group 3 0.2834 0.8369
25
> ###are column means the same?
> #null hypothesis - column means are the same
> #p<0.05 indicates significantly different means
> #use with nonparametric cases
> kruskal.test(rrTOPstack$values~rrTOPstack$ind)
Kruskal-wallis rank sum test
data: rrTOPstack$values by rrTOPstack$ind
Kruskal-wallis chi-squared = 2.972, df = 3, p-value = 0.396
> kruskal.test(rrTOPstack2$values~rrTOPstack2$ind)
Kruskal-wallis rank sum test
data: rrTOPstack2$values by rrTOPstack2$ind
Kruskal-wallis chi-squared = 0.82575, df = 3, p-value = 0.8433

```

```

> #compare removal rate CIs for systems in lowest group(s) to zero
> #use bootstrapping to find confidence interval around rr
> n<-100000
> rranuaTOPpbs<-rranuaTOPuse[!is.na(rranuaTOPuse)];rranuaTOPpbs
[1] 0.3273878 0.3273878 0.3273878 0.3273878 -1.8651039 0.3273878 -5.9083254
[8] -4.7719097
> bootrranuaTOP<-numeric(n)
> for (i in 1:n) {
+ bootrranuaTOP[i]<-mean(sample(rranuaTOPpbs,replace=T),na.rm=TRUE)
+ }
> hist(bootrranuaTOP)
> quantile(bootrranuaTOP,probs=c(0.025,0.975))
      2.5%      97.5%
-3.14377709 0.05332632
> mean(bootrranuaTOP)
[1] -1.363798
> rranua2TOPpbs<-rranua2TOP[!is.na(rranua2TOP)];rranua2TOPpbs
[1] 0.3273878 0.3273878 0.3273878 0.3273878 0.3273878
> bootrranua2TOP<-numeric(n)
> for (i in 1:n) {
+ bootrranua2TOP[i]<-mean(sample(rranua2TOPpbs,replace=T),na.rm=TRUE)
+ }
> hist(bootrranua2TOP)
> quantile(bootrranua2TOP,probs=c(0.025,0.975))
      2.5%      97.5%
0.3273878 0.3273878
> mean(bootrranua2TOP)
[1] 0.3273878
> rrezTOPpbs<-rrezTOP[!is.na(rrezTOP)];rrezTOPpbs
[1] 0.3273878 0.3273878 -2.0774999 0.3273878 0.3273878 0.3273878
> bootrrrezTOP<-numeric(n)
> for (i in 1:n) {
+ bootrrrezTOP[i]<-mean(sample(rrezTOPpbs,replace=T),na.rm=TRUE)
+ }
> hist(bootrrrezTOP)
> quantile(bootrrrezTOP,probs=c(0.025,0.975))
      2.5%      97.5%
-0.8750561 0.3273878
> mean(bootrrrezTOP)
[1] -0.07448499
> rrw4TOPpbs<-rrw4TOP[!is.na(rrw4TOP)];rrw4TOPpbs
[1] 0.3273878 0.3273878 0.3273878 0.3273878 0.3273878 0.3273878 -1.4825831
[8] 0.3273878 0.3273878
> bootrrw4TOP<-numeric(n)
> for (i in 1:n) {
+ bootrrw4TOP[i]<-mean(sample(rrw4TOPpbs,replace=T),na.rm=TRUE)
+ }
> hist(bootrrw4TOP)
> quantile(bootrrw4TOP,probs=c(0.025,0.975))
      2.5%      97.5%
-0.2759359 0.3273878
> mean(bootrrw4TOP)
[1] 0.1258616
> rrw6TOPpbs<-rrw6TOP[!is.na(rrw6TOP)];rrw6TOPpbs
[1] 0.3273878 0.3273878 0.3273878 -2.5586491 0.3273878 0.3273878 0.3273878
[8] 0.3273878 0.3273878

```

```

> bootrrw6TOP<-numeric(n)
> for (i in 1:n) {
+ bootrrw6TOP[i]<-mean(sample(rrw6TOPbs,replace=T),na.rm=TRUE)
+ }
> hist(bootrrw6TOP)
> quantile(bootrrw6TOP,probs=c(0.025,0.975))
      2.5%      97.5%
-0.6346245  0.3273878
> mean(bootrrw6TOP)
[1] 0.007396837

```

## TCS

```

> ###set-up
> stTCS<-dat$stTCS
> avgstTCS<-mean(stTCS,na.rm=TRUE)
> sdstTCS<-sd(stTCS,na.rm=TRUE)
> RSDTCS<-sdstTCS/avgstTCS;RSDTCS
[1] 1.076317
> dwt(lm(stTCS~time))
lag Autocorrelation D-w Statistic p-value
1 -0.01926674 1.902175 0.56
Alternative hypothesis: rho != 0
> anuaTCS<-dat$anuaTCS
> rranuaTCS<-(avgstTCS-anuaTCS)/avgstTCS
> anua2TCS<-dat$anua2TCS
> ezTCS<-dat$ezTCS
> rranua2TCS<-(avgstTCS-anua2TCS)/avgstTCS
> rrezTCS<-(avgstTCS-ezTCS)/avgstTCS
> w4TCS<-dat$w4TCSN
> rrw4TCS<-(avgstTCS-w4TCS)/avgstTCS
> w6TCS<-dat$w6TCSN
> rrw6TCS<-(avgstTCS-w6TCS)/avgstTCS
> ###lognormally/normally distributed?
> #null hypothesis - normal distribution
> #p>0.05 indicates normality
> shapiro.test(rranuaTCS)
Shapiro-wilk normality test
data: rranuaTCS
w = 0.77702, p-value = 0.01113
> shapiro.test(rranua2TCS)
Shapiro-wilk normality test
data: rranua2TCS
w = 0.9925, p-value = 0.9877
> shapiro.test(rrezTCS)
Shapiro-wilk normality test
data: rrezTCS
w = 0.7054, p-value = 0.007043
> shapiro.test(rrw4TCS)
Shapiro-wilk normality test
data: rrw4TCS
w = 0.38984, p-value = 3.217e-07
> shapiro.test(rrw6TCS)

```

```

Shapiro-wilk normality test
data: rrw6TCS
w = 0.65985, p-value = 0.0004815
> ###independent?
> #null hypothesis - no correlation
> #p>0.05 indicates independence
> dwt(lm(rranuaTCS~time))
lag Autocorrelation D-W Statistic p-value
1 0.0422858 1.488373 0.186
Alternative hypothesis: rho != 0
> dwt(lm(rranua2TCS~time))
lag Autocorrelation D-W Statistic p-value
1 -0.04281631 1.553065 0.126
Alternative hypothesis: rho != 0
> dwt(lm(rrezTCS~time))
lag Autocorrelation D-W Statistic p-value
1 0.207811 1.138093 0.016
Alternative hypothesis: rho != 0
> dwt(lm(rrw4TCS~time))
lag Autocorrelation D-W Statistic p-value
1 -0.05639898 1.371858 0.134
Alternative hypothesis: rho != 0
> dwt(lm(rrw6TCS~time))
lag Autocorrelation D-W Statistic p-value
1 -0.3043859 2.346418 0.98
Alternative hypothesis: rho != 0
> ###equal variance?
> #null hypothesis - equal variance
> #p>0.05 indicates equal variance
> rrTCS<-cbind(rranuaTCS,rrezTCS,rrw4TCS,rrw6TCS)
> rrTCSstack<-stack(data.frame(rrTCS))
> rrTCS2<-cbind(rranua2TCS,rrezTCS,rrw4TCS,rrw6TCS)
> rrTCSstack2<-stack(data.frame(rrTCS2))
> #use with nonparametric cases
> leveneTest(rrTCSstack$values,rrTCSstack$ind)
Levene's Test for Homogeneity of Variance (center = median)
Df F value Pr(>F)
group 3 0.2159 0.8845
29
> leveneTest(rrTCSstack2$values,rrTCSstack2$ind)
Levene's Test for Homogeneity of Variance (center = median)
Df F value Pr(>F)
group 3 0.2141 0.8857
25
> ###are column means the same?
> #null hypothesis - column means are the same
> #p<0.05 indicates significantly different means
> #use with nonparametric cases
> kruskal.test(rrTCSstack$values~rrTCSstack$ind)
Kruskal-wallis rank sum test
data: rrTCSstack$values by rrTCSstack$ind
Kruskal-wallis chi-squared = 1.6808, df = 3, p-value = 0.6412
> kruskal.test(rrTCSstack2$values~rrTCSstack2$ind)
Kruskal-wallis rank sum test
data: rrTCSstack2$values by rrTCSstack2$ind
Kruskal-wallis chi-squared = 4.9437, df = 3, p-value = 0.176

```

```

> #compare removal rate CIs for systems in lowest group(s) to zero
> n<-100000
> rranuaTCSbs<-rranuaTCS[!is.na(rranuaTCS)];rranuaTCSbs
[1] 0.8390569 0.8881139 0.9347832 0.9675173 0.9051393 0.9675173 0.9675173
[8] 0.9675173 0.9675173
> bootrranuaTCS<-numeric(n)
> for (i in 1:n) {
+ bootrranuaTCS[i]<-mean(sample(rranuaTCSbs,replace=T),na.rm=TRUE)
+ }
> hist(bootrranuaTCS)
> quantile(bootrranuaTCS,probs=c(0.025,0.975))
      2.5%      97.5%
0.9024244 0.9602431
> mean(bootrranuaTCS)
[1] 0.9338616
> rranua2TCSbs<-rranua2TCS[!is.na(rranua2TCS)];rranua2TCSbs
[1] 0.8390569 0.8881139 0.9347832 0.9675173 0.9051393
> bootrranua2TCS<-numeric(n)
> for (i in 1:n) {
+ bootrranua2TCS[i]<-mean(sample(rranua2TCSbs,replace=T),na.rm=TRUE)
+ }
> hist(bootrranua2TCS)
> quantile(bootrranua2TCS,probs=c(0.025,0.975))
      2.5%      97.5%
0.8680136 0.9425661
> mean(bootrranua2TCS)
[1] 0.9069631
> rrezTCSbs<-rrezTCS[!is.na(rrezTCS)];rrezTCSbs
[1] 0.8867232 0.9190580 0.9675173 0.9675173 0.9675173 0.9675173
> bootrrrezTCS<-numeric(n)
> for (i in 1:n) {
+ bootrrrezTCS[i]<-mean(sample(rrezTCSbs,replace=T),na.rm=TRUE)
+ }
> hist(bootrrrezTCS)
> quantile(bootrrrezTCS,probs=c(0.025,0.975))
      2.5%      97.5%
0.9190437 0.9675173
> mean(bootrrrezTCS)
[1] 0.9459606
> rrw4TCSbs<-rrw4TCS[!is.na(rrw4TCS)];rrw4TCSbs
[1] 0.8114108 0.9675173 0.9675173 0.9675173 0.9675173 0.9675173 0.9675173
[8] 0.9675173 0.9675173
> bootrrw4TCS<-numeric(n)
> for (i in 1:n) {
+ bootrrw4TCS[i]<-mean(sample(rrw4TCSbs,replace=T),na.rm=TRUE)
+ }
> hist(bootrrw4TCS)
> quantile(bootrrw4TCS,probs=c(0.025,0.975))
      2.5%      97.5%
0.9154818 0.9675173
> mean(bootrrw4TCS)
[1] 0.9501385
> rrw6TCSbs<-rrw6TCS[!is.na(rrw6TCS)];rrw6TCSbs
[1] 0.8636901 0.9675173 0.9675173 0.9175820 0.9675173 0.9675173 0.8616306
[8] 0.9675173 0.9675173
> bootrrw6TCS<-numeric(n)

```

```

> for (i in 1:n) {
+ bootrrw6TCS[i]<-mean(sample(rrw6TCSbs,replace=T),na.rm=TRUE)
+ }
> hist(bootrrw6TCS)
> quantile(bootrrw6TCS,probs=c(0.025,0.975))
      2.5%      97.5%
0.9091491 0.9675173
> mean(bootrrw6TCS)
[1] 0.9386761

```

## VEN

```

> ###set-up
> stVEN<-dat$stVEN
> avgstVEN<-mean(stVEN,na.rm=TRUE)
> sdstVEN<-sd(stVEN,na.rm=TRUE)
> RSDVEN<-sdstVEN/avgstVEN;RSDVEN
[1] 1.099574
> dwt(lm(stVEN~time))
lag Autocorrelation D-W Statistic p-value
 1      0.1862342      1.510831      0.212
Alternative hypothesis: rho != 0
> anuaVEN<-dat$anuaVEN
> rranuaVEN<-(avgstVEN-anuaVEN)/avgstVEN
> rranuaVEN<-(stVEN-anuaVEN)/stVEN; rranuaVEN
[1] 0.9974097      NA 0.9964489      NA 0.9971634      NA 0.9967468
[8]      NA 0.9862739      NA 0.9151818 0.9330696 0.6437849 0.9405952
> anua2VEN<-dat$anua2VEN
> rranua2VEN<-(avgstVEN-anua2VEN)/avgstVEN
> rranua2VEN<-(stVEN-anua2VEN)/stVEN; rranua2VEN
[1] 0.9974097      NA 0.9964489      NA 0.9971634      NA 0.9967468
[8]      NA 0.9862739      NA      NA      NA      NA      NA
> ezVEN<-dat$ezVEN
> rrezVEN<-(avgstVEN-ezVEN)/avgstVEN
> rrezVEN<-(stVEN-ezVEN)/stVEN; rrezVEN
[1]      NA      NA      NA      NA      NA      NA -0.1749118
[8]      NA -3.2148947      NA -1.4652170 -2.8469599 -0.6007124 -0.9776242
> w4VEN<-dat$w4VEN
> rrw4VEN<-(avgstVEN-w4VEN)/avgstVEN
> rrpw4VEN<-(stVEN-w4VEN)/stVEN; rrpw4VEN
[1] 0.9944405      NA 0.9984319      NA 0.9971634      NA 0.9967468
[8]      NA 0.9862739      NA 0.9676938 0.9330696 0.9550391 0.9405952
> w6VEN<-dat$w6VEN
> rrw6VEN<-(avgstVEN-w6VEN)/avgstVEN
> rrpw6VEN<-(stVEN-w6VEN)/stVEN; rrpw6VEN
[1] 0.9974097      NA 0.9982068      NA 0.9971634      NA 0.9914444
[8]      NA 0.9862739      NA 0.9676938 0.9330696 0.9550391 0.9405952
> ###lognormally/normally distributed?
> #null hypothesis - normal distribution
> #p>0.05 indicates normality
> shapiro.test(rranuaVEN)
      Shapiro-wilk normality test
data:  rranuaVEN

```

```

w = 0.56793, p-value = 4.021e-05
> shapiro.test(rrpanuaVEN)
      Shapiro-wilk normality test
data:  rrpanuaVEN
w = 0.61867, p-value = 0.0001584
> shapiro.test(rranua2VEN)
      Shapiro-wilk normality test
data:  rranua2VEN
w = 0.55218, p-value = 0.000131
> shapiro.test(rrpanua2VEN)
      Shapiro-wilk normality test
data:  rrpanua2VEN
w = 0.62622, p-value = 0.001368
> shapiro.test(rrezVEN)
      Shapiro-wilk normality test
data:  rrezVEN
w = 0.75884, p-value = 0.02428
> shapiro.test(rrpezVEN)
      Shapiro-wilk normality test
data:  rrpezVEN
w = 0.91118, p-value = 0.4442
> shapiro.test(rrw4VEN)
      Shapiro-wilk normality test
data:  rrw4VEN
w = 0.38984, p-value = 3.217e-07
> shapiro.test(rrpw4VEN)
      Shapiro-wilk normality test
data:  rrpw4VEN
w = 0.84566, p-value = 0.06674
> shapiro.test(rrw6VEN)
      Shapiro-wilk normality test
data:  rrw6VEN
w = 0.43479, p-value = 1.091e-06
> shapiro.test(rrpw6VEN)
      Shapiro-wilk normality test
data:  rrpw6VEN
w = 0.85392, p-value = 0.08228
> ###independent?
> #null hypothesis - no correlation
> #p>0.05 indicates independence
> dwt(lm(rranuaVEN~time))
lag Autocorrelation D-W Statistic p-value
1      -0.5950128      3.001662  0.162
Alternative hypothesis: rho != 0
> dwt(lm(rrpanuaVEN~time))
lag Autocorrelation D-W Statistic p-value
1      -0.5387422      2.82794  0.3
Alternative hypothesis: rho != 0
> dwt(lm(rranua2VEN~time))
lag Autocorrelation D-W Statistic p-value
1      -0.6213509      3.004965  0.416
Alternative hypothesis: rho != 0
> dwt(lm(rrpanua2VEN~time))
lag Autocorrelation D-W Statistic p-value
1      -0.2883052      2.138111  0.502
Alternative hypothesis: rho != 0

```

```

> dwt(lm(rrezVEN~time))
lag Autocorrelation D-w Statistic p-value
1 0.1645413 1.361692 0.092
Alternative hypothesis: rho != 0
> dwt(lm(rrpezVEN~time))
lag Autocorrelation D-w Statistic p-value
1 -0.529261 2.678481 0.738
Alternative hypothesis: rho != 0
> dwt(lm(rrw4VEN~time))
lag Autocorrelation D-w Statistic p-value
1 -0.05639898 1.371858 0.106
Alternative hypothesis: rho != 0
> dwt(lm(rrpw4VEN~time))
lag Autocorrelation D-w Statistic p-value
1 0.07070128 1.782733 0.378
Alternative hypothesis: rho != 0
> dwt(lm(rrw6VEN~time))
lag Autocorrelation D-w Statistic p-value
1 -0.2290776 2.414729 0.886
Alternative hypothesis: rho != 0
> dwt(lm(rrpw6VEN~time))
lag Autocorrelation D-w Statistic p-value
1 0.01693531 1.908844 0.586
Alternative hypothesis: rho != 0
> ###equal variance?
> #null hypothesis - equal variance
> #p>0.05 indicates equal variance
> rrVEN<-cbind(rranuaVEN,rrezVEN,rrw4VEN,rrw6VEN)
> rrVENstack<-stack(data.frame(rrVEN))
> rrVEN2<-cbind(rranua2VEN,rrezVEN,rrw4VEN,rrw6VEN)
> rrVENstack2<-stack(data.frame(rrVEN2))
> rrpVEN<-cbind(rrpanuaVEN,rrpezVEN,rrpw4VEN,rrpw6VEN)
> rrpVENstack<-stack(data.frame(rrpVEN))
> rrpVEN2<-cbind(rrpanua2VEN,rrpezVEN,rrpw4VEN,rrpw6VEN)
> rrpVENstack2<-stack(data.frame(rrpVEN2))
> #use with nonparametric cases
> leveneTest(rrVENstack$values,rrVENstack$ind)
Levene's Test for Homogeneity of Variance (center = median)
      Df F value  Pr(>F)
group 3  6.0199 0.002562 **
      29
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> leveneTest(rrVENstack2$values,rrVENstack2$ind)
Levene's Test for Homogeneity of Variance (center = median)
      Df F value  Pr(>F)
group 3  5.0609 0.007087 **
      25
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> leveneTest(rrpVENstack$values,rrpVENstack$ind)
Levene's Test for Homogeneity of Variance (center = median)
      Df F value  Pr(>F)
group 3 14.842 4.846e-06 ***
      29
---

```

```

Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> leveneTest(rrpVENstack2$values,rrpVENstack2$ind)
Levene's Test for Homogeneity of Variance (center = median)
      Df F value    Pr(>F)
group  3 13.302 2.179e-05 ***
      25
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> ###are column means the same?
> #null hypothesis - column means are the same
> #p<0.05 indicates significantly different means
> #use with nonparametric cases
> kruskal.test(rrVENstack$values~rrVENstack$ind)
      Kruskal-wallis rank sum test
data:  rrVENstack$values by rrVENstack$ind
Kruskal-wallis chi-squared = 20.019, df = 3, p-value = 0.0001682
> kruskal.test(rrVENstack2$values~rrVENstack2$ind)
      Kruskal-wallis rank sum test
data:  rrVENstack2$values by rrVENstack2$ind
Kruskal-wallis chi-squared = 19.361, df = 3, p-value = 0.0002302
> kruskal.test(rrpVENstack$values~rrpVENstack$ind)
      Kruskal-wallis rank sum test
data:  rrpVENstack$values by rrpVENstack$ind
Kruskal-wallis chi-squared = 14.698, df = 3, p-value = 0.002094
> kruskal.test(rrpVENstack2$values~rrpVENstack2$ind)
      Kruskal-wallis rank sum test
data:  rrpVENstack2$values by rrpVENstack2$ind
Kruskal-wallis chi-squared = 15.001, df = 3, p-value = 0.001816
> ###if column means are different, which columns?
> #null hypothesis - column means are the same
> #p<0.05 indicates significantly different means
> #use with nonparametric case
> kruskalmc(rrVENstack$values~rrVENstack$ind)
Multiple comparison test after Kruskal-wallis
p.value: 0.05
Comparisons
              obs.dif critical.dif difference
rranuaVEN-rrrezVEN 14.722222    13.44534      TRUE
rranuaVEN-rrw4VEN  3.444444    12.02588     FALSE
rranuaVEN-rrw6VEN  1.888889    12.02588     FALSE
rrrezVEN-rrw4VEN  18.166667    13.44534      TRUE
rrrezVEN-rrw6VEN  16.611111    13.44534      TRUE
rrw4VEN-rrw6VEN   1.555556    12.02588     FALSE
> kruskalmc(rrVENstack2$values~rrVENstack2$ind)
Multiple comparison test after Kruskal-wallis
p.value: 0.05
Comparisons
              obs.dif critical.dif difference
rranua2VEN-rrrezVEN 14.100000    13.60260      TRUE
rranua2VEN-rrw4VEN  1.177778    12.52979     FALSE
rranua2VEN-rrw6VEN  0.155556    12.52979     FALSE
rrrezVEN-rrw4VEN  15.277778    11.83954      TRUE
rrrezVEN-rrw6VEN  13.944444    11.83954      TRUE
rrw4VEN-rrw6VEN   1.333333    10.58961     FALSE
> kruskalmc(rrpVENstack$values~rrpVENstack$ind)
Multiple comparison test after Kruskal-wallis

```

p.value: 0.05

Comparisons

	obs.dif	critical.dif	difference
rrpanuaVEN-rrpezVEN	14.9444444	13.44534	TRUE
rrpanuaVEN-rrpw4VEN	2.1666667	12.02588	FALSE
rrpanuaVEN-rrpw6VEN	2.5000000	12.02588	FALSE
rrpezVEN-rrpw4VEN	17.1111111	13.44534	TRUE
rrpezVEN-rrpw6VEN	17.4444444	13.44534	TRUE
rrpw4VEN-rrpw6VEN	0.3333333	12.02588	FALSE

```
> kruskalmc(rrpVENstack2$values~rrpVENstack2$ind)
```

Multiple comparison test after Kruskal-wallis

p.value: 0.05

Comparisons

	obs.dif	critical.dif	difference
rrpanua2VEN-rrpezVEN	18.1000000	13.60260	TRUE
rrpanua2VEN-rrpw4VEN	4.7666667	12.52979	FALSE
rrpanua2VEN-rrpw6VEN	4.4333333	12.52979	FALSE
rrpezVEN-rrpw4VEN	13.3333333	11.83954	TRUE
rrpezVEN-rrpw6VEN	13.6666667	11.83954	TRUE
rrpw4VEN-rrpw6VEN	0.3333333	10.58961	FALSE

```
> #compare removal rate CIs for systems in lowest group(s) to zero
```

```
> n<-100000
```

```
> rranuaVENbs<-rranuaVEN[!is.na(rranuaVEN)];rranuaVENbs
```

```
[1] 0.9951127 0.9889324 0.9951127 0.9951127 0.9951127 0.9871687 0.9951127
```

```
[8] 0.9612790 0.9951127
```

```
> bootrranuaVEN<-numeric(n)
```

```
> for (i in 1:n) {
```

```
+ bootrranuaVEN[i]<-mean(sample(rranuaVENbs,replace=T),na.rm=TRUE)
```

```
+ }
```

```
> hist(bootrranuaVEN)
```

```
> quantile(bootrranuaVEN,probs=c(0.025,0.975))
```

```
2.5% 97.5%
```

```
0.9820695 0.9951127
```

```
> mean(bootrranuaVEN)
```

```
[1] 0.9897617
```

```
> rranua2VENbs<-rranua2VEN[!is.na(rranua2VEN)];rranua2VENbs
```

```
[1] 0.9951127 0.9889324 0.9951127 0.9951127 0.9951127
```

```
> bootrranua2VEN<-numeric(n)
```

```
> for (i in 1:n) {
```

```
+ bootrranua2VEN[i]<-mean(sample(rranua2VENbs,replace=T),na.rm=TRUE)
```

```
+ }
```

```
> hist(bootrranua2VEN)
```

```
> quantile(bootrranua2VEN,probs=c(0.025,0.975))
```

```
2.5% 97.5%
```

```
0.9914045 0.9951127
```

```
> mean(bootrranua2VEN)
```

```
[1] 0.9938781
```

```
> rrezVENbs<-rrezVEN[!is.na(rrezVEN)];rrezVENbs
```

```
[1] -0.7650549 -0.5007477 0.6270611 0.7190927 0.8260008 0.8372986
```

```
> bootrrezVEN<-numeric(n)
```

```
> for (i in 1:n) {
```

```
+ bootrrezVEN[i]<-mean(sample(rrezVENbs,replace=T),na.rm=TRUE)
```

```
+ }
```

```
> hist(bootrrezVEN)
```

```
> quantile(bootrrezVEN,probs=c(0.025,0.975))
```

```
2.5% 97.5%
```

```

-0.2347030 0.7763127
> mean(bootrrrezVEN)
[1] 0.2900181
> rrw4VENbs<-rrw4VEN[!is.na(rrw4VEN)]; rrw4VENbs
[1] 0.9895107 0.9951127 0.9951127 0.9951127 0.9951127 0.9951127 0.9951127
[8] 0.9951127 0.9951127
> bootrrw4VEN<-numeric(n)
> for (i in 1:n) {
+ bootrrw4VEN[i]<-mean(sample(rrw4VENbs, replace=T), na.rm=TRUE)
+ }
> hist(bootrrw4VEN)
> quantile(bootrrw4VEN, probs=c(0.025, 0.975))
      2.5%      97.5%
0.9932454 0.9951127
> mean(bootrrw4VEN)
[1] 0.9944941
> rrw6VENbs<-rrw6VEN[!is.na(rrw6VEN)]; rrw6VENbs
[1] 0.9951127 0.9944112 0.9951127 0.9871470 0.9951127 0.9951127 0.9951127
[8] 0.9951127 0.9951127
> bootrrw6VEN<-numeric(n)
> for (i in 1:n) {
+ bootrrw6VEN[i]<-mean(sample(rrw6VENbs, replace=T), na.rm=TRUE)
+ }
> hist(bootrrw6VEN)
> quantile(bootrrw6VEN, probs=c(0.025, 0.975))
      2.5%      97.5%
0.9923795 0.9951127
> mean(bootrrw6VEN)
[1] 0.9941519
> n<-100000
> rrpanuaVENbs<-rrpanuaVEN[!is.na(rrpanuaVEN)]; rrpanuaVENbs
[1] 0.9974097 0.9964489 0.9971634 0.9967468 0.9862739 0.9151818 0.9330696
[8] 0.6437849 0.9405952
> bootrrpanuaVEN<-numeric(n)
> for (i in 1:n) {
+ bootrrpanuaVEN[i]<-mean(sample(rrpanuaVENbs, replace=T), na.rm=TRUE)
+ }
> hist(bootrrpanuaVEN)
> quantile(bootrrpanuaVEN, probs=c(0.025, 0.975))
      2.5%      97.5%
0.8548944 0.9866885
> mean(bootrrpanuaVEN)
[1] 0.9340241
> rrpanua2VENbs<-rrpanua2VEN[!is.na(rrpanua2VEN)]; rrpanua2VENbs
[1] 0.9974097 0.9964489 0.9971634 0.9967468 0.9862739
> bootrrpanua2VEN<-numeric(n)
> for (i in 1:n) {
+ bootrrpanua2VEN[i]<-mean(sample(rrpanua2VENbs, replace=T), na.rm=TRUE)
+ }
> hist(bootrrpanua2VEN)
> quantile(bootrrpanua2VEN, probs=c(0.025, 0.975))
      2.5%      97.5%
0.9904868 0.9971786
> mean(bootrrpanua2VEN)
[1] 0.9948034
> mu<-mean(rrpezVEN, na.rm=TRUE); mu

```

```

[1] -1.54672
> s<-sd(rrpezVEN,na.rm=TRUE); s
[1] 1.231319
> n<-length(which(rrpezVEN!="NA"));n
[1] 6
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] -2.83891 -1.54672 -0.25453
> mu<-mean(rrpw4VEN,na.rm=TRUE); mu
[1] 0.9743838
> s<-sd(rrpw4VEN,na.rm=TRUE); s
[1] 0.02600114
> n<-length(which(rrpw4VEN!="NA"));n
[1] 9
> a<-0.05
> t<-qt(1-a/2,n-1)
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.9543975 0.9743838 0.9943700
> mu<-mean(rrpw6VEN,na.rm=TRUE); mu
[1] 0.9740995
> s<-sd(rrpw6VEN,na.rm=TRUE); s
[1] 0.02577754
> n<-length(which(rrpw6VEN!="NA"));n
[1] 9
> t<-qt(1-a/2,n-1)
> a<-0.05
> se<-s/sqrt(n)
> ci.mu<-c(mu-t*se,mu,mu+t*se);ci.mu
[1] 0.9542852 0.9740995 0.9939139

```

## CBZ and LAM Correlations

```

> dat<-read.csv("anovaPPCP.csv")
> time<-dat$time
> w6pH<-dat$w6pH
> w4pH<-dat$w4pH
> anuapH<-dat$anuapH
> AW1pH<-dat$AW1pH
> w4CBZ<-dat$w4CBZ
> w4LAM<-dat$w4LAM
> w6CBZ<-dat$w6CBZ
> w6LAM<-dat$w6LAM
> AW1CBZ<-dat$AW1CBZ
> AW1LAM<-dat$AW1LAM
> anuaCBZ<-dat$anuaCBZ
> anuaLAM<-dat$anuaLAM
> cwde1tapH<-w4pH-w6pH

```

```

> anuade1tapH<-AW1pH-anuapH
> rrcwCBZ<-(w4CBZ-w6CBZ)/w4CBZ
> rrcwLAM<-(w4LAM-w6LAM)/w4LAM
> rranuaCBZ<-(AW1CBZ-anuaCBZ)/AW1CBZ
> rranuaLAM<-(AW1LAM-anuaLAM)/AW1LAM
> 1mrrcwCBZpH<-1m(rrcwCBZ~cwde1tapH)
> 1mw4pHCBZ<-1m(w4CBZ~w4pH)

```

### *Correlations between time and CBZ and LAM for CW4 and Anua Effluent*

```

> 1mw6CBZtime<-1m(w6CBZ~time)
> summary(1mw6CBZtime)

```

Call:

```
1m(formula = w6CBZ ~ time)
```

Residuals:

Min	1Q	Median	3Q	Max
-7.3572	-0.2103	0.7721	1.8489	2.5268

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	9.24180	1.71203	5.398	0.00101 **
time	0.05332	0.02328	2.291	0.05576 .

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 3.197 on 7 degrees of freedom  
(3 observations deleted due to missingness)

Multiple R-squared: 0.4284, Adjusted R-squared: 0.3468

F-statistic: 5.247 on 1 and 7 DF, p-value: 0.05576

>

```

> 1mw6LAMtime<-1m(w6LAM~time)
> summary(1mw6LAMtime)

```

Call:

```
1m(formula = w6LAM ~ time)
```

Residuals:

Min	1Q	Median	3Q	Max
-6.1322	-0.1768	0.6708	1.6833	4.2712

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	3.40609	1.78822	1.905	0.0985 .
time	0.04873	0.02431	2.004	0.0851 .

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 3.34 on 7 degrees of freedom  
(3 observations deleted due to missingness)

Multiple R-squared: 0.3646, Adjusted R-squared: 0.2738

F-statistic: 4.017 on 1 and 7 DF, p-value: 0.08509

```

>
> lmanuaCBZtime<-lm(anuaCBZ~time)
> summary(lmanuaCBZtime)

Call:
lm(formula = anuaCBZ ~ time)

Residuals:
    Min       1Q   Median       3Q      Max
-5.9809 -3.0882 -2.2879 -0.9983 17.1334

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  2.48867    3.87428   0.642  0.5411
time         0.12944    0.05268   2.457  0.0436 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 7.236 on 7 degrees of freedom
(3 observations deleted due to missingness)
Multiple R-squared:  0.4631, Adjusted R-squared:  0.3864
F-statistic: 6.038 on 1 and 7 DF, p-value: 0.04364

```

```

>
> lmanuaLAMtime<-lm(anuaLAM~time)
> summary(lmanuaLAMtime)

Call:
lm(formula = anuaLAM ~ time)

Residuals:
    Min       1Q   Median       3Q      Max
-1.1922 -0.8849 -0.7780  0.0619  4.9016

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.793848    1.090661   0.728  0.49
time         0.007253    0.014830   0.489  0.64

Residual standard error: 2.037 on 7 degrees of freedom
(3 observations deleted due to missingness)
Multiple R-squared:  0.03304, Adjusted R-squared: -0.1051
F-statistic: 0.2392 on 1 and 7 DF, p-value: 0.6397

```

### ***Correlations between pH and CBZ and LAM for CW4 and Anua Effluent***

```

> lmw6pHCBZ<-lm(w6CBZ~w6pH)
> summary(lmw6pHCBZ)

Call:
lm(formula = w6CBZ ~ w6pH)

Residuals:
    Min       1Q   Median       3Q      Max

```

-3.7117 -1.6526 0.4432 1.5143 2.7394

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-89.987	27.196	-3.309	0.01296 *
w6pH	16.261	4.321	3.763	0.00705 **

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 2.432 on 7 degrees of freedom

(3 observations deleted due to missingness)

Multiple R-squared: 0.6692, Adjusted R-squared: 0.622

F-statistic: 14.16 on 1 and 7 DF, p-value: 0.007046

> `lmw6pHLAM<-lm(w6LAM~w6pH)`

> `summary(lmw6pHLAM)`

Call:

`lm(formula = w6LAM ~ w6pH)`

Residuals:

Min	1Q	Median	3Q	Max
-2.7672	-1.0903	-0.4575	1.6329	3.1102

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-98.489	25.040	-3.933	0.00565 **
w6pH	16.642	3.978	4.183	0.00412 **

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 2.24 on 7 degrees of freedom

(3 observations deleted due to missingness)

Multiple R-squared: 0.7143, Adjusted R-squared: 0.6735

F-statistic: 17.5 on 1 and 7 DF, p-value: 0.004121

> `lmanuapHCBZ<-lm(anuaCBZ~anuapH)`

> `summary(lmanuapHCBZ)`

Call:

`lm(formula = anuaCBZ ~ anuapH)`

Residuals:

Min	1Q	Median	3Q	Max
-6.042	-4.473	-2.937	-1.072	17.147

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-64.884	45.520	-1.425	0.197
anuapH	11.795	7.163	1.647	0.144

Residual standard error: 8.384 on 7 degrees of freedom

(3 observations deleted due to missingness)

Multiple R-squared: 0.2792, Adjusted R-squared: 0.1763

F-statistic: 2.712 on 1 and 7 DF, p-value: 0.1436

```
> lmanuapHLAM<-lm(anuaLAM~anuapH)
> summary(lmanuapHLAM)
```

Call:

```
lm(formula = anuaLAM ~ anuapH)
```

Residuals:

Min	1Q	Median	3Q	Max
-1.0905	-0.8922	-0.7183	-0.1393	4.8910

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-4.9443	11.0032	-0.449	0.667
anuapH	0.9704	1.7313	0.560	0.593

Residual standard error: 2.026 on 7 degrees of freedom

(3 observations deleted due to missingness)

Multiple R-squared: 0.04295, Adjusted R-squared: -0.09377

F-statistic: 0.3142 on 1 and 7 DF, p-value: 0.5926

### ***Change between CW4 and Tank 6 and Anua Well 1 and Effluent***

```
> summary(lmrrcwbZPH)
```

Call:

```
lm(formula = rrcwCBZ ~ cwde1tapH)
```

Residuals:

2	4	5	7	9	10	11
0.112165	0.007257	-0.038732	-0.069783	-0.018317	0.043323	-0.035913

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	0.3025	0.1774	1.706	0.1487
cwde1tapH	0.5553	0.2372	2.341	0.0663 .

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.06709 on 5 degrees of freedom

(5 observations deleted due to missingness)

Multiple R-squared: 0.5229, Adjusted R-squared: 0.4275

F-statistic: 5.481 on 1 and 5 DF, p-value: 0.06629

```
> lmrrcWLAMPH<-lm(rrcWLAM~cwde1tapH)
```

```
> summary(lmrrcWLAMPH)
```

Call:

```
lm(formula = rrcWLAM ~ cwde1tapH)
```

Residuals:

2	4	5	7	9	10	11
0.039922	-0.006119	-0.004635	-0.055609	0.019711	0.034108	-0.027378

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
--	----------	------------	---------	----------

```
(Intercept) 0.69790 0.09924 7.033 0.000897 ***
cwdeltapH 0.29844 0.13273 2.249 0.074410 .
---
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Residual standard error: 0.03754 on 5 degrees of freedom
(5 observations deleted due to missingness)
Multiple R-squared: 0.5028, Adjusted R-squared: 0.4033
F-statistic: 5.056 on 1 and 5 DF, p-value: 0.07441
```

```
> lmrranuacBzPH<-lm(rranuacBZ~anuadeltapH)
> summary(lmrranuacBzPH)
```

```
Call:
lm(formula = rranuacBZ ~ anuadeltapH)
```

```
Residuals:
    5     7     9    10    11    12
0.16201 0.15951 -0.19605 0.07765 -0.17456 -0.02857
```

```
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) 0.5029     0.1033   4.87 0.00822 **
anuadeltapH 0.1968     0.1137   1.73 0.15862
---
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Residual standard error: 0.1785 on 4 degrees of freedom
(6 observations deleted due to missingness)
Multiple R-squared: 0.4281, Adjusted R-squared: 0.2851
F-statistic: 2.994 on 1 and 4 DF, p-value: 0.1586
```

```
> lmrranuaLAmPH<-lm(rranuaLAM~anuadeltapH)
> summary(lmrranuaLAmPH)
```

```
Call:
lm(formula = rranuaLAM ~ anuadeltapH)
```

```
Residuals:
    5     7     9    10    11    12
0.012974 0.013713 -0.034415 0.004865 -0.003033 0.005895
```

```
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) 0.989665 0.011600 85.315 1.13e-07 ***
anuadeltapH -0.003976 0.012775 -0.311 0.771
---
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Residual standard error: 0.02005 on 4 degrees of freedom
(6 observations deleted due to missingness)
Multiple R-squared: 0.02365, Adjusted R-squared: -0.2204
F-statistic: 0.09689 on 1 and 4 DF, p-value: 0.7711
```

Table C.6.2: anova.csv

Sample	Date	time	w4adj	w6adj	anuaAlk	anua2Alk	ezAlk	w4Alk	w4AlkN	w6Alk	w6AlkN	w6Alk_use
	1 6/19/2016		0 1.171429	1.48								
theoretical	6/24/2016		5									
theoretical	6/28/2016	9.333333										
	2 6/29/2016		10 0.782857	0.96	16	16		267.5	209.4143	328.6	315.456	
	3 7/7/2016		18 0.782857	0.497143								
theoretical	7/12/2016		23									300.6763
	4 7/17/2016		28 0.422857	0.457143								
theoretical	8/1/2016		43									
	5 8/7/2016		49	1 1.182857	63.3	63.3	33.8	240.3	240.3	241.7	285.8966	
quarterly	8/12/2016		54				39.4					
	6 8/16/2016		58 0.571429	0.662857								
	7 8/29/2016		71	0.48 0.48								
theoretical	9/12/2016		85									
	8 9/26/2016		99 0.794286	0.862857	35.5		10	293.3	232.964	397.7	343.1583	343.1583
	9 10/22/2016		125 0.811429	0.817143	74.5							
	10 11/16/2016		150 0.937143	0.96				24.1	248.2	232.5989	316.4	303.744 303.744

stAlk	anuaBOD	anua2BOD	ezBOD	w4BOD	w4BODN	w6BOD	w6BODN	stBOD	anuaCOD	anua2COD	ezCOD	w4COD
									91	91		
212.1									79	79		34
	3.5	3.5		3.1	2.426857	25.6	12.72686	112	82	82		38
									105	105		42
284.9				4.8								33
173.2	3.3	3.3	3.4	1.7	0.971429	19.7	13.05829	72.2	67	67	27	34
232.9	1.6		4.6	0.58	0.2784	23.6	11.328	75.1	85		20	26
208.1	1		3	1.8	1.429714	21.4	18.46514	96.9	64		40	45
238.2	1.4		2.2	1.3	1.054857	18	14.70857	86.7	78		26	30
	1.7		2.8	6.6	6.185143	17.2	16.512	61.4	58		16	17

w4CODN	w6COD	w6CODN	w6COD_u:stCOD	anuaTSS	anua2TSS	ezTSS	w4TSS	w4TSSN	w6TSS	w6TSSN	stTSS
	204	301.92		316							
			194.5886								
26.61714	204	195.84		291							
29.74857	173	86.00571		319	3	3	2	1.565714	8	3.977143	68
17.76	190	86.85714		335							
			95.13143								
							2				
19.42857	156	103.4057		189	1	1	1	0.571429	5	3.314286	14
12.48	167	80.16		195							
			106.9514								
35.74286	155	133.7429		231							
24.34286				155	4		4	0.811429	2	1.634286	23
15.93143	91	87.36	87.36	226	8		3	0.937143	11	10.56	43

anuaAMM	anua2AMM	ezAMM	w4AMM	w4AMMN	w6AMM	w6AMMN	stAMM	anuaTIN	anua2TIN	ezTIN	w4TIN	w4TINN
0.1	0.1		0.1	0.078286	0.004	0.00384	31.87	10.8	10.8		0.2	0.156571
0.004	0.004		0.004	0.003131	0.004	0.001989	41.02	7.5	7.5		0.65	0.508857
0.03	0.03		0.04	0.016914	0.08	0.036571	33.71	5.76	5.76		0.04	0.016914
0.2	0.2	0.74	0.02	0.02	0.03	0.035486	29.56	10.5	10.5	24.74	0.02	0.02
		0.94								22.25		
0.04	0.04	1.3	0.1	0.057143	0.004	0.002651	22	7.03	7.03	22.1	0.4	0.228571
0.1		2	0.004	0.00192	0.004	0.00192	32.45	19		25.3	0.31	0.1488
0.08		4.12	0.06	0.047657	0.004	0.003451	30.35	25.53		29.05	0.44	0.349486
0.05		2	0.004	0.003246	0.004	0.003269	32.73	35.85		25.1	0.71	0.576114
0.06		2.1	3.2	2.998857	0.004	0.00384	29.68	15.88		30.7	7.2	6.747429

w6TIN	w6TINN	stTIN	anuaTN	anua2TN	ezTN	w4TN	w4TNN	w6TN	w6TNN	stTN
0.37	0.3552	32.39								
0.65	0.323143	41.75	8	8		0.8	0.626286	1.6	0.795429	50
0.31	0.141714	34.14								
0.03	0.035486	29.67								
					23					
0.31	0.205486	22.1	7	7	22	0.5	0.285714	1	0.662857	24
0.33	0.1584	32.55								
0.73	0.629886	30.44								
0.35	0.286	32.75	42		32	2	1.622857	3	2.451429	43
0.71	0.6816	30.41	15		31	7	6.56	2	1.92	30

Table C.6.3: *anovaPPCP.csv*

Sample	Date	time	w4adj	w6adj	w6pH	anuapH	anuaACE	anua2ACE	ezACE	w4ACE	w4ACEN
	2	6/29/2016	0	0.782857	0.96	6.39	6.03	0.015	0.015	0.044697	0.034991
	3	7/7/2016	8	0.782857	0.497143	6.24	5.93	1.107682	1.107682	0.1857	0.145377
theoretical		7/15/2016	16.25								
	4	7/17/2016	18	0.422857	0.457143	5.98	5.89	0.015	0.015	0.015	0.015
	5	8/7/2016	39	1	1.182857	6.41	6.4	0.054527	0.054527	0.037887	0.015
theoretical		8/11/2016	43.5								
	6	8/16/2016	48	0.571429	0.662857	6.28	6.4	0.015	0.015	0.949607	0.015
theoretical		8/22/2016	54.5								
	7	8/29/2016	61	0.48	0.48	6.15	6.38	0.015	1.30656	0.015	0.015
	8	9/26/2016	89	0.794286	0.862857	6.08	6.09	0.015	0.714522	0.015	0.015
	9	10/22/2016	115	0.811429	0.817143	6.55	6.98	0.915365	0.509467	0.015	0.015
	10	11/16/2016	140	0.937143	0.96	6.54	6.99	0.060312	3.628867	0.095373	0.089378

w6ACE	w6ACEN	stACE	anuaCBZ	anua2CBZ	ezCBZ	w4CBZ	w4CBZN	w6CBZ	w6CBZN	stCBZ	anuaLAM	anua2LAM
0.101377	0.097322	181.3176	0.20078	0.20078		56.12616	43.93877	10.20708	9.798798	71.08298	0.015	0.015
0.744293	0.37002	182.5486	1.176868	1.176868		47.31657	37.04211	9.827063	4.885454	74.99487	1.217517	1.217517
0.04611	0.021079	154.1928	1.717033	1.717033		52.4678	22.18638	9.991273	4.567439	61.3076	0.195507	0.195507
0.176725	0.20904	56.08688	6.13446	6.13446	52.60027	36.16847	36.16847	13.17032	15.5786	35.46163	0.191823	0.191823
0.015	0.015	11.89387	5.613777	5.613777	60.57946	40.31311	23.03606	12.57333	8.334322	43.41624	0.17576	0.17576
0.015	0.015	32.18582	27.51808		54.27805	40.00983	19.20472	11.53051	5.534646	40.61334	6.13784	
0.015	0.015	85.87832	16.08246		61.17824	30.77476	24.44395	6.630292	5.720995	53.84547	0.24719	
0.015	0.015	9.84899	16.37642		42.11302	41.82816	33.94056	17.90064	14.62738	31.26747	1.689828	
0.015	0.015	63.65948	14.62991		33.5029	30.23499	28.33451	18.96661	18.20795	21.57307	1.031287	

ezLAM	w4LAM	w4LAMN	w4LAMus	w6LAM	w6LAMN	stLAM	anuaLEV	anua2LEV	anua2LEV <sub>i</sub>	ezLEV	w4LEV	w4LEVN
	93.38736	73.10896		5.08944	4.885862	125.7606	0.341992	0.341992			7.291122	5.707907
	92.31777	72.27162		5.468583	2.718667	147.0336	0.481463	0.481463			1.793705	1.404215
			61.39582									
	104.5796	44.22222		4.143422	1.894136	151.3902	0.14705	0.14705	0.14705		0.273612	0.115699
124.6184	55.98047	55.98047		5.97746	7.070481	104.8457	0.271043	0.271043	0.271043	10.77514	7.166453	7.166453
134.3962	49.2844	28.16252		5.568403	3.691056	98.41451	0.056118	0.056118	0.056118	29.1671	0.50787	0.290211
			26.7325									
149.9628	52.71349	25.30247		2.772073	1.330595	134.2766	0.13676			17.11475	0.07783	0.037358
154.2066	49.99651	39.71151	39.71151	1.61099	1.390054	122.6814	0.479157			20.90532	0.31147	0.247396
124.2414	80.33059	65.18254	65.18254	13.28141	10.85281	108.5444	4.440898			17.2136	3.863272	3.134769
147.0126	88.78219	83.20159	83.20159	11.98592	11.50649	119.4543	0.399777			19.2269	1.290945	1.2098

w6LEV	w6LEVN	stLEV	anuaMET	anua2MET	ezMET	ezMETuse	w4MET	w4METN	w6MET	w6METN	stMET	anuaTOP
0.483702	0.464354	131.9878	0.015	0.015			0.036933	0.028914	0.015	0.015	0.074208	0.015
0.37225	0.185061	43.62001	0.015	0.015			0.015	0.015	0.015	0.015	0.055753	0.015
0.081062	0.037057	142.7043	0.015	0.015			0.015	0.015	0.015	0.015	0.06273	0.015
1.108773	1.31152	114.2346	0.015	0.015	0.685813		0.015	0.015	0.050763	0.060046	0.268473	0.015
						0.512618						
0.057123	0.037865	118.7299	0.015	0.015	0.339423		0.015	0.015	0.015	0.015	0.125092	0.015
0.056313	0.02703	129.8446	0.015		0.04897	0.04897	0.015	0.015	0.015	0.015	0.051802	0.11279
0.570428	0.492198	124.888	0.015		0.015	0.015	0.015	0.015	0.015	0.015	0.147975	0.015
0.055803	0.045599	131.0108	1.120725		0.015	0.015	0.015	0.015	0.015	0.015	0.096393	0.154063
0.20121	0.193162	117.3033	0.106395		0.015	0.015	0.015	0.015	0.015	0.015	0.132498	0.12872

anuaTOPu	anua2TOP	ezTOP	w4TOP	w4TOPN	w6TOP	w6TOPN	stTOP	anuaTCS	anua2TCS	ezTCS	w4TCS	w4TCSN
0.015	0.015		0.015	0.015	0.015	0.015	0.015	2.477368	2.477368		3.70811	2.90292
0.015	0.015		0.015	0.015	0.015	0.015	0.015	1.722243	1.722243		0.5	0.5
0.015	0.015		0.015	0.015	0.015	0.015	0.015	1.00387	1.00387		0.5	0.5
0.015	0.015	0.015	0.015	0.015	0.067093	0.079362	0.015	0.5	0.5	1.74365	0.5	0.5
	0.015	0.015	0.015	0.015	0.015	0.015	0.015	1.460173	1.460173	1.245927	0.5	0.5
0.063895		0.068632	0.015	0.015	0.015	0.015	0.08071	0.5		0.5	0.5	0.5
0.015		0.015	0.069703	0.055364	0.015	0.015	0.015	0.5		0.5	0.5	0.5
0.154063		0.015	0.015	0.015	0.015	0.015	0.015	0.5		0.5	0.5	0.5
0.12872		0.015	0.015	0.015	0.015	0.015	0.015	0.5		0.5	0.5	0.5

w6TCS	w6TCSN	stTCS	anuaVEN		anua2VEN	ezVEN	w4VEN	w4VENN	w6VEN	w6VENN	stVEN
2.185618	2.098194	9.167433	0.015	0.995113	0.015		0.041123	0.032194	0.015	0.015	5.790798
0.5	0.5	4.186408	0.033968	0.988932	0.033968		0.015	0.015	0.034503	0.017153	9.565627
0.5	0.5	53.27594	0.015	0.995113	0.015		0.015	0.015	0.015	0.015	5.288052
1.072527	1.268646	27.75055	0.015	0.995113	0.015	5.417272	0.015	0.015	0.03335	0.039448	4.61079
0.5	0.5	14.63649	0.015	0.995113	0.015	4.606065	0.015	0.015	0.015	0.015	1.092807
0.5	0.5	18.1838	0.039382	0.987169		1.144617	0.015	0.015	0.015	0.015	0.464307
2.468422	2.129895	8.265385	0.015	0.995113		0.862155	0.015	0.015	0.015	0.015	0.224113
0.5	0.5	1.999138	0.118842	0.961279		0.534035	0.015	0.015	0.015	0.015	0.333623
0.5	0.5	1.07028	0.015	0.995113		0.49936	0.015	0.015	0.015	0.015	0.252505

## C.7 Pilot Study Photos

March



CW Tank 1 two weeks after planting



CW Tank 1 two weeks after planting



**CW Tank 2 two weeks after planting**



**CW Tank 3 two weeks after planting**



CW Tank 4 two weeks after planting



*Trametes versicolor* and *Pleurotus ostreatus* spawn for mycoremediation filters



*Trametes versicolor* spawn blended with wood chips, hardwood mulch, and a small amount of compost



*Trametes versicolor* spawn blended with wood chips, hardwood mulch, and a small amount of compost

April



Peristaltic pumps for CW system



CW Tank 1 effluent piping, including level control, air gap, and sampling port



CW Tank 1 effluent piping and sampling port



CW Tank 2 three weeks after planting



CW Tank 2 influent piping and aeration waterfall



CW Tank 3 three weeks after planting



CW Tank 3 three weeks after planting



**CW Tank 5 with shade cloth; distribution piping replaced prior to sampling period**

**May**



**CW Tank 4 eight weeks after planting**



*Pleurotus ostreatus* blooms in CW Tank 6; distribution piping replaced prior to sampling period



*Iris versicolor* blooms in CW Tank 2



*Trametes versicolor* blooms in CW Tank 5



Fungal blooms in CW Tank 6

**June**



**CW Tank 1 12 weeks after planting with distribution piping used throughout sampling period**



**CW Tank 2 12 weeks after planting**



**CW Tank 3 12 weeks after planting**



**CW Tank 4 12 weeks after planting with distribution piping used throughout sampling period**



CW Tank 5 with distribution piping used throughout sampling period



CW Tank 5 with distribution piping installed throughout sampling period



**CW Tank 6 with distribution piping installed throughout sampling period**



**CW Tank 6 effluent piping, including level control, air gap, sampling port, and backflow prevention device (bucket)**



**E-Z Treat biofilter and holding tank wrapped in reflective material for temperature control**



**E-Z Treat holding tank and backflow prevention device (barrel)**



**Pilot system**



**Pilot system**



Pilot system



*Coprinus comatus* blooms in CW Tank 6



Unknown fungal blooms in CW Tank 5



*Pontedaria cordata* blooms in CW Tank 2

**July**



**CW Tank 1 18 weeks after planting**



**CW Tank 2 18 weeks after planting**



*Pontedaria cordata* leaves chewed by Japanese beetles in CW Tank 2



CW Tank 3 18 weeks after planting



CW Tank 4 18 weeks after planting

September



CW Tank 1 27 weeks after planting



CW Tank 2 27 weeks after planting



**CW Tank 3 27 weeks after planting**



**CW Tank 4 27 weeks after planting**



**CW Tank 5**



CW Tank 6