FATE OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS IN DUCKWEED AND WASTE ACTIVATED SLUDGE UNDER AEROBIC AND ANAEROBIC SOLIDS STABILIZATION CONDITIONS

by

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ABSTRACT

Fate of Pharmaceuticals and Personal Care Products in Duckweed and Waste Activated Sludge Under Aerobic and Anaerobic Conditions

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Biosolids used for compost or as feed for anaerobic digesters are common and sustainable biosolids management applications. However, the use of these stabilized biosolids as soil amendment could be a potential means of introducing pharmaceuticals and personal care products (PPCPs) into the environment, increasing human exposure and risk. This study investigated the removal of PPCPs from biosolids after going through aerobic composting and anaerobic digestion. In a lab experiment PPCPs (caffeine, carbamazepine, DEET, estrone, gemfibrozil, triclosan, TCEP in duckweed; plus sulfamethoxazole and fluoxetine in waste activated sludge) were monitored during aerobic composting and anaerobic digestion.

With microorganism sourced from an active food waste compost system and wood chips as a bulking agent, waste activated sludge (WAS) from the Hyrum WWTP and harvested duckweed from Wellsville sewage lagoons spiked with PPCPs were composted over a 21-day period. Temperature and oxygen were monitored daily, while samples of the compost were collected every 3 days and analyzed for PPCPs.

In a second experiment, powdered duckweed spiked with PPCPs was pulse-fed along with bromide into a lab-scale anaerobic digester. Digestate samples were collected as the digester was fed with fresh duckweed (with no PPCPs or bromide spikes) every 3 days until the bromide spike was completely removed from the digester. Digestate samples were monitored for bromide concentration in the liquid, and PPCPs in both liquids and solids.

Solid samples from the compost and digester were extracted using pressurized fluid extraction. Liquid samples from the digester were extracted with acetonitrile. PPCPs were analyzed using LC/QQQ/MS.

It was determined that composting was a more effective process for the removal of PPCPs from biosolids than anaerobic digestion, suggesting that composting of biosolids can be effectively used to reduce human and environmental exposure to many PPCPs found in municipal wastewater. It was also determined that hydrophilic PPCPs like DEET and sulfamethoxazole had shorter half-lives in compost than hydrophobic PPCPs like estrone and triclosan. Carbamazepine and tris-2-chloroethyl phosphate were recalcitrant chemicals that persisted in WWTP and after solids stabilization, whereas estrone, β-estradiol, acetaminophen, caffeine and DEET were effectively removed by WWTPs through transformation into daughter products.

(166 pages)

PUBLIC ABSTRACT

Fate of Pharmaceuticals and Personal Care Products in Duckweed and Waste Activated Sludge Under Aerobic and Anaerobic Conditions

Kwame T. Duodu

Twelve commonly used pharmaceuticals and personal care products (PPCPs) (acetaminophen, β-Estradiol, caffeine, carbamazepine, DEET, estrone, gemfibrozil, triclosan, TCEP, sulfamethoxazole, progesterone, fluoxetine) were investigated to determine their fate in biosolids. These compounds were selected due to their wide usage, and varying properties, that makes them representative of many other PPCPs.

Commonly used PPCPs (caffeine commonly found in tea, coffee, chocolate and soda, the pain reliever acetaminophen, and the pesticide DEET) were detected in high amounts in the influent of the Hyrum WWTP and Wellsville sewage lagoons. Although conventional WWTPs are not designed to remove PPCPs from wastewater, some PPCPs, such as acetaminophen, caffeine and DEET, were removed from the wastewater while other PPCPs persisted in the effluent and biosolids.

The application of biosolids to land as fertilizer could introduce PPCPs into the soil, which may be bioavailable to soil flora and fauna. In this study, two solid stabilization processes were investigated to determine their effect on PPCPs removal from biosolids. Composting proved to be an effective option for removing PPCPs from biosolids.

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"A journey of a thousand mile begins with a single step." Lao Tzu

In 2017 when I first set foot on USU campus I did not know it was a whole new adventure that was about to unfold in the next phase of my young adult life. It has been a mixture of experiences. I must say I owe a depth of gratitude to countless number of people who made my dream for pursuing masters in environmental engineering a reality.

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CHAPTER 1

INTRODUCTION

The use of pharmaceutical compounds worldwide has increased due to global population rise and the manufacturing of new drugs to cure old and new diseases (Carter et al. 2014). After pharmaceuticals have been used by patients, active pharmaceutical ingredients (APIs) and their metabolites are excreted and end up in the sewage system (Fig. 1). They may then be transported to a wastewater treatment plant (WWTP). At the WWTP these pharmaceutical compounds may be transformed, adsorbed to sludge, or released into the environment in the effluent based on their physico-chemical properties (Carter et al., 2014). This represents a risk to humans and the environment of exposure to pharmaceutical compounds when sludge is applied to land as fertilizer or when effluent from WWTPs is used for irrigation.

Another group of contaminants of concern are personal care products (PCPs). PCPs, such as deodorants, shampoos, bar soaps, etc., used mainly to enhance the quality of life (Ebele et al. 2016), also contain chemicals that are deleterious to the environment.

Pharmaceuticals and personal care products (PPCPs) detected in treated wastewater have been described as Contaminants of Emerging Concern (CEC) by the United States Environmental Protection Agency (U.S. EPA 2013) due to their recent detection in low concentrations in the environment and their deleterious effects on ecological systems. Although, there is still much research going on to ascertain the direct risk posed to humans from exposure to these PPCPs there is evidence that some of these pharmaceuticals act as Endocrine Disrupting Chemicals and affect reproduction in aquatic organisms (Gibbs et al. 1991). PPCPs have also been detected in drinking water supplies.

In a study by Benotti et al. (2009), 19 municipal drinking water systems were screened for 51 pharmaceuticals. Sulfamethoxazole, gemfibrozil, carbamazepine and estrone were some of the chemicals commonly detected.

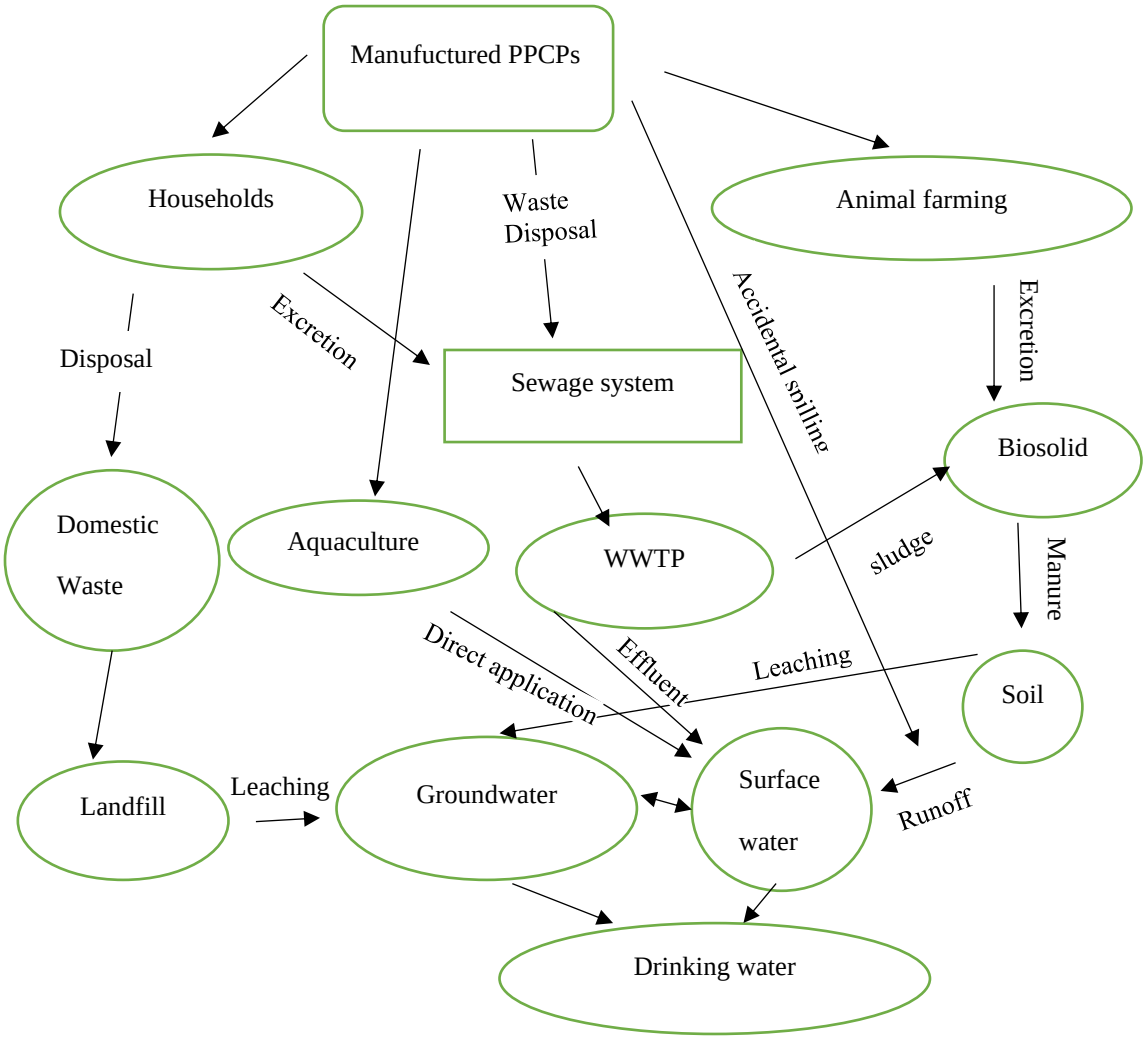


Fig. 1. Routes for transport of PPCPs in the environment (after Magureanu et al. 2015).

Preliminary research conducted by Dupont et al. (2019), on the risk of exposure to PPCPs with the use of reclaimed water for irrigation, detected PPCPs in effluents from the Hyrum UT WWTP. PPCPs were also detected in trace concentrations in vegetable

samples that had been irrigated with this treated wastewater. Other research has also detected PPCPs in WWTP effluents and has shown the uptake and translocation of PPCPs in vegetables (Wu et al. 2013; Wu et al. 2014; Wu et al. 2015; Riemenschneider et al. 2016; Paltiel et al. 2016; Christou et al. 2017; Mordechav et al. 2018).

Activated sludge uses biological processes to remove biodegradable organic contaminants from wastewater. The biosolids may also serve as a sink for some PPCPs that do not biodegrade although the WWTPs are not specifically designed to remove them (Roth, 2012). Roth (2012) discovered that PPCPs with low biodegradability and high partition coefficients (Log K_{ow}) associated with biosolids from the Hyrum WWTP. In addition to sludge removing PPCPs from WWTPs, duckweed (*Lemma minor*) has also been shown to be effective in removing PPCPs from wastewater lagoons (Farrell, 2012).

In Farrell's studies, duckweed, a fast-growing plant that floats on the surface of stagnant water bodies, removed five PPCPs and phosphorus from the wastewater lagoons in Wellsville, Utah. Further studies by Kesaano (2011) showed that duckweed grown for nutrient control in municipal wastewaters could be harvested and processed for other purposes. This study showed that harvested duckweed from the Wellsville lagoons could serve as animal feed, be used to produce energy through anaerobic digestion, or could be fermented for alcohol production (Kesaano 2011), but use may be limited if PPCPs are present.

In this study, two biosolid types (waste activated sludge and duckweed) were investigated to determine the fate of the various PPCPs in the biosolids under aerobic and anaerobic treatment environments. These biosolids are rich in slow releasing nutrients (phosphorus, nitrogen) so they can be applied as fertilizers and for soil amendment. Since

the use of these biosolids for land application is a pathway for exposure to PPCPs, it is important to investigate the fate of the PPCPs in these biosolids as the biosolids are processed for other uses or disposed in a landfill.

In the available literature, PPCPs in wastewater have been shown to be more effectively removed from sludge in aerobic environments than in anaerobic environments. However, there is no published research that specifically compares removal of PPCPs from duckweed using aerobic composting and anaerobic digestion in one study. In this study, the removal of PPCPs in WAS and duckweed through aerobic composting and anaerobic digestion are investigated.

Knowledge was gained on the PPCPs that persist in the biosolids and those that are removed when the biosolids are subjected to aerobic and anaerobic stabilization methods. Information was also gained on the solid stabilization processes (aerobic or anaerobic) which are most effective in removing PPCPs from the two biosolids.

Twelve PPCPs were selected for this study, namely, β-estradiol, carbamazepine, estrone, progesterone, triclosan, acetaminophen, caffeine, DEET, tris-2-chloroethyl phosphate (TCEP), gemfibrozil, sulfamethoxazole and fluoxetine. These were selected based on variations in the chemical and physical properties that makes them representative of the many PPCPs available.

CHAPTER 2

HYPOTHESIS AND OBJECTIVES

In WWTPs, activated sludge can act as a sink for the removal of PPCPs in wastewater. The treatment processes at the various stages in the WWTP provide enabling environments for the transformation of PPCPs in sludge through a range of aerobic and anaerobic transformation pathways. For duckweed, which takes up PPCPs, typical solids processing steps such as composting and anaerobic digestion may lead to PPCP transformation. Anaerobic environments are low in free oxygen and will favor the growth of microbes that will biodegrade PPCPs if PPCPs are anaerobically biodegradable. Composting combines mainly oxidation (oxygen) and biodegradation processes with elevated temperatures to aerobically breakdown PPCPs associated with biosolids. In aerobic and anaerobic environments PPCPs are expected to transform at different rates and to different extents depending on their physico-chemical properties. This leads to the following hypotheses:

- PPCPs that exist mostly as uncharged compounds and have low solubility and high Log K_{ow} (e.g., β-Estradiol, triclosan, estrone, carbamazepine and progesterone), and cationic PPCPs (e.g., fluoxetine) will associate more with biosolids (waste activated sludge or duckweed) compared with the liquid phase. PPCPs that exist mostly as uncharged compounds with high solubility and low Log K_{ow} (e.g., acetaminophen, DEET, TCEP, and caffeine), and anionic PPCPs (e.g., gemfibrozil and sulfamethoxazole) will associate more with the liquid or effluent from the WWTPs. This was tested by analysis of water, duckweed, and sediment from the Wellsville lagoons and effluent and biosolids from Hyrum

WWTP; and the digestate sampled from the anaerobic digester which was separated into solids and liquids.

- The rate of transformation of PPCPs in aerobic environments is faster than the rate of transformation of PPCPs in anaerobic environments. This was tested by a comparison between composting and anaerobic digestion of the duckweed using first order kinetics.
- PPCPs that associate more with the solids (hydrophobic) will have a lower rate of transformation than PPCPs that associate more with the liquids (hydrophilic) due to differences in bioavailabilites. Transformation rates of hydrophilic and hyrophobic PPCPs in compost and anaerobic digestion were compared.

The following objectives were met to test the above hypothesis:

- Measure the PPCP concentrations in effluent of Hyrum WWTP, Wellsville sewage lagoons and digestate liquid from an anaerobic digester to determine which PPCPs are associated with the liquid phase; measure PPCP concentrations in the duckweed, sediments of Wellsvile sewage lagoons, waste activated sludge (WAS) from the Hyrum WWTP and digestate solid from an anaerobic digester to determine which PPCPs associated with biosolids. Use mass balance calculations to determine transformation and distribution of PPCPs between solid and liquid phase.
- Determine the rate of transformation/half-lives of PPCPs in duckweed and WAS composts and compare the tranformation rates/half-lives of PPCPs in duckweed compost to transformation rates of PPCPs in anaerobically digested duckweed.
- Compare rates of transformation/half-lives of hydrophillic and hyrdrophobic PPCPs in compost and anerobic digestion units.

CHAPTER 3

LITERATURE REVIEW

3.1 Sources of Pharmaceutical and Personal Care Products in the Environment

The use of PPCPs have become a part of daily life. Drugs are mainly taken to cure the body of ailments but may find their way into the environment through several routes as shown in Fig. 1. PPCPs may enter water systems from various sources such as human excretion, inappropriate disposal of unused medicines, leaching from landfills, or in run-off. It is still not clear whether the levels of the PPCPs present in the environment can directly cause harmful physiological effects in wildlife and humans (Archer et al. 2017).

Excretion is one of the many ways through which water resources become contaminated with PPCPs. Excreted human pharmaceuticals pass through the sewage collection system and reach wastewater treatment plants (WWTPs). PPCPs that are not removed by WWTPs may end up in surface water through WWTP effluent or may end up in the soils through land application of biosolids as fertilizer. Waste activated sludge (WAS) from WWTPs may contain undegraded pharmaceuticals, and when used as fertilizer would pose the risk of soil contamination (Magureanu et al. 2015). Table 1 shows the removal efficiencies of some PPCPs by WWTPs, and indicates that WWTPs have demonstrated high percent removal efficiency for caffeine and ibuprophen, while carbamazepine, triclosan and gemfibrozil are not very well removed via conventional WWTPs.

3.2 Properties of PPCPs.

Pharmaceutical compounds can be grouped into antibiotics, hormones, anticonvulsants, non-steroidal anti-inflammatory drugs (NSAIDS), lipid regulators,

antihypertensive and antidepressants (Magureanu et al. 2015). Out of the 12 PPCPs being investigated in this study, progesterone, β-Estradiol and estrone are hormones.

Table 1. Concentrations of some PPCPs in Wastewater Treatment Plant Influent and Percent Removal in Effluents as Summarized by Xia et al. (2005**).**

PPCPs	Influent Concentrations (μg/L)	Decrease in Effluent (%)	Reference
Caffeine	230	99.9	Heberer et al. (2002)
Carbamezipine	1.78 to 2.1	7 to 8	Ternes (1998), Heberer et al. (2002)
Gemfibrozil	0.35 to 0.9	16 to 69	Ternes (1998), Stumpf et al. (1999)
Ibuprofen	0.3 to 4.1	90	Ternes (1998), Stumpf et al. (1999)
Triclosan	0.5 to 1.3	34 to 92	Lindstrom et al. (2002)

Gemifibrozil acts as a lipid regulator, fluoxetine is an antidepressant and carbamezipine is an anticonvulsant used in the treatment of epilepsy and other psychotherapy applications. Acetaminophen, which is an analgesic, is classified as an NSAIDS, whereas sulfamethoxazole serves as an antibiotic. Triclosan is an antimicrobial agent with germ-fighting capabilities and is found in personal care products like hand sanitizers, soaps and toothpaste (Cooney 2010).

DEET is a chemical (N,N-diethyl-meta-toluamide) used as the active ingredient in many insect repellent products. Caffeine is a drug that stimulates the central nervous system to increase alertness and can be found in beverages such as coffee, tea and colas. Tris-2-chloroethyl phosphate (TCEP) is a flame retardant used in the textile, plastic and furniture industry.

PPCPs exhibit various physical, chemical and biological properties which determine their behavior in the environment. For example (Table 1) carbamazepine

removal in WWTPs is very poor, mostly below 10%, since it is resistant to biodegradation at low concentrations (Magureanu et al. 2015). One chemical known to persist in the environment is TCEP. TCEP is considered nonbiodegradable and is not expected to hydrolyse under environmental conditions. This chemical also does not photolyse directly in light is predicted to have 1.4 percent removal from wastewater in a conventional WWTP (European Union Risk Assessment 2009). Tables 2, 3, and 4 list some physico-chemical properties that characterise the 12 PPCPs being investigated in this study and determine their fate in the environment.

3.3 Partitioning of PPCPs Between Solid and Liquid Phases

Once PPCPs are released into the environment, they can migrate in the environment, persist, or degrade based on their physicochemical properties and that of the receiving environment. Many PPCPs have low volatility and are highly polar and hydrophilic in nature. Thus their distribution through the environment occurs mainly through aqueous transport and food chain dispersal (Ebele et al. 2016). Transport of PPCPs across various environmental media depends on their sorption behaviour in wastewater treatment plants, soil, water and sediment.

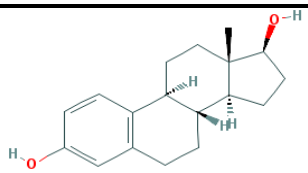
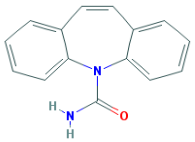
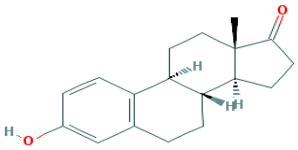
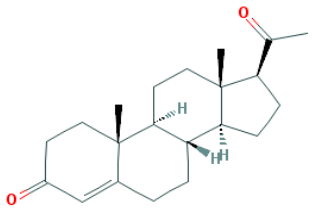
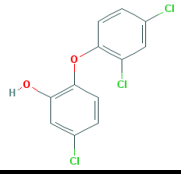
The fate of the neutral compounds in solids or effluent is determined by their octanol-water partition coefficient (Log K_{ow}). This determines the hydrophobicity or hydrophilicity of a compound.

Neutral hydrophobic PPCPs (Table 2) such as triclosan, progesterone, estrone, and carbamazepine and cation (fluoxetine) have the potential to sorb onto biosolids whereas neutral hydrophilic PPCPs (Table 2) such as acetaminophen, DEET, caffeine,

TCEP and negatively charged PPCPs gemfibrozil and sulfamethoxazole will associate with effluent from WWTPs (Wu et al. 2013).

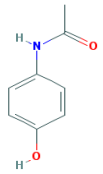
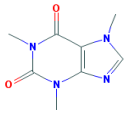
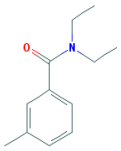
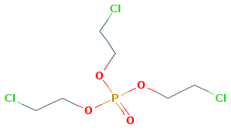
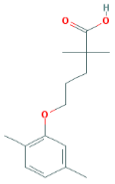
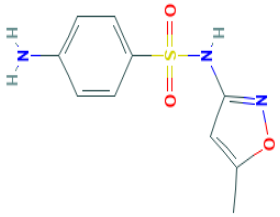
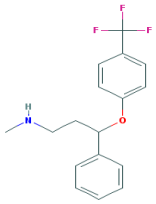
In Table 2 PPCPs with Log K_{ow} values less than 2 are considered hydrophilic, while PPCPs with Log K_{ow} greater than 2 are considered hydrophobic. An exception to this rule is gemfibrozil which has a high Log K_{ow} but exists in mostly the negatively ionized form, and thus behaves hydrophilically at environmentally relevant pHs.

Table 2. Molecular Structure, Log K_{ow}, Molecular Weight, Density and Water Solubility of Target PPCPs.

Compound (Molecular Formula) (CAS Number)	Molecular Structure	Molecular Weight (g/mol)	Log K_{ow}	Density g/cm^3	Water Solubility (mg/L)
β-Estradiol ($C_{18}H_{24}O_2$) 000050-28-2		272.8	4.01		3.6 at 27°C
Carbamazepine ($C_{15}H_{12}N_2O$) 000298-46-4		236.27	2.45		18 at 25°C
Estrone ($C_{18}H_{22}O_2$) 000053-16-7		270.37	3.13	1.236 at 25°C	30 at 25°C
Progesterone ($C_{21}H_{30}O_2$) 000057-83-0		314.47	3.87	1.66 at 23°C	8.81 at 25°C
Triclosan ($C_{12}H_7Cl_3O_2$) 003380-34-5		289.54	4.76	1.49[2]	10 at 20°C

[1]LookChem (2018) ; All other data were obtained from PubChem (2018)

Table 2. (continued). Molecular Structure, Log K_{ow}, Molecular Weight, Density and Water Solubility of Target PPCPs.

Compound (Molecular Formula) (CAS Number)	Molecular Structure	Molecular Weight (g/mol)	Log K_{ow}	Density g/cm^3	Water solubility (mg/L)
Acetaminophen ($C_8H_9NO_2$) 000103-90-2		151.16	0.46	1.293 at 21°C	14,000 at 25°C
Caffeine ($C_8H_{10}N_4O_2$) 000058-08-2		194.19	-0.07	1.23 at 18°C	21,600 at 25°C
DEET ($C_4H_{13}N_3$) 000134-62-3		191.27	2.02	0.996 at 20°C	912 at 25°C
TCEP ($C_6H_{12}Cl_3O_4P$) 000115-96-8		283.94	1.43	1.39 at 25°C	7,820 at 20°C
Gemfibrozil ($C_{15}H_{22}O_3$) 025812-30-0		250.34	4.7		11 at at 25°C
Sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) 000723-46-6		253.28	0.89	1.462[1]	610 at 37°C
Fluoxetine ($C_{17}H_{18}F_3NO$) 000002-84-9		309.23	4.05		1.7 at 25°C

[2] Wikipedia (2019); All other data was obtained from PubChem (2018).

In Table 3 additional properties of PPCPs that determine their fate in the environment are listed. Out of the properties listed, the most important pertaining to this research is the pKa which determines what fraction of PPCPs will exist as ionized or neutral compounds in the environment. Based on the pKa values and the pH (6.5-7.5) for water samples collected from WWTPs in which the PPCPs were found, sulfamethoxazole, gemfibrozil and fluoxetine are the only compounds that existed mainly as ionized compounds. Assuming the lowest pKa for caffeine, it will exist mainly as a neutral compound, while assuming a high pKa 10 or above makes it a cation. In this study caffeine is grouped with neutral compounds, assuming it exists in the form with the lowest pka (Table 3).

Gemfibrozil existed as mostly a negatively charged compound in the water samples from the WWTPs that had pH measurements of approximately 7 (Appendix D, Table D.4). Fluoxetine was the only compound that existed as a mostly positively charged compound. Sulfamethoxazole existed mostly as negatively ionized with a small percentage as neutral. All other PPCPs investigated in this study existed as mostly neutral compounds.

The partitioning of the PPCPs between the biosolids (WAS and duckweed) and the effluent is first determined by the charge. Negatively charged compounds will not sorb to negatively charged biosolid surfaces due to elecrostatic repulsion. However, positively charged PPCPs will sorb to negatively charged surfaces due to electrostatic attraction. The fate of the neutral compounds in the biosolids or effluent is determined by their octanol-water partition coefficient (Log K_{ow}).

Table 3. pKa, Ionic Charge at pH 7, Henry's Constant (K_h) and Common Use of Target PPCPs Analyzed in This Study.

Compound	pKa	Ionic Charge at pH 7	Acid/Base	f_n	Henry's Constant K_h (atm-m^3/mol)	Common Use
β-Estradiol	NA	Neutral	Neutral[b]	1	$3.64x10^{-11}$	Female Hormone
Carbamazepine	13.9[a], 2.3[b]	Neutral[c]	Very Weak Base[b]	1	$1.08x10^{-10}$ [d]	Mood Stabilizer
Estrone	10.49[d]	Neutral	Neutral[b]	1	$3.8x10^{-10}$ [d]	Female Hormone
Progesterone	NA	Neutral[c]	Neutral[b]	1	$6.49x10^{-8}$ [d]	Female Hormone
Triclosan	7.9[a,b]	Mostly neutral	Weak Acid[b]	0.89	$4.99x10^{-9}$	Antimicrobial agent
Acetaminophen	9.38[a]	Mostly Neutral. Small Percent Negative[c]	Acid[b]	1	$6.42x10^{-13}$ [c,d]	Pain reliever
Caffeine	10.4[b], 14[a], 0.61[d], 3.6[d],0.5[c]	Neutral[c]	Base[b]	1	$3.58x10^{-11}$ [d]	Stimulant
DEET	0.67[b]	Mostly Neutral[g]	Very Weak Base[b]	1	$5.1 x10^{-8}$	Pesticide
TCEP	NA	Neutral	Neutral[b]	1	$1.1x10^{-6}$ [d]	Flame retardant
Gemfibrozil	4.5[a], 4.48[d]	Negative	Weak Acid[b]	$3.2x10^{-3}$	$1.19x10^{-8}$ [d]	Lipid Regulator
Sulfamethoxazole	1.6[a], 5.7[a], 1.85[b]	Mostly Negatively Charged[f,a]	Acid[e]	$4.7x10^{-6}$	$9.56x10^{-13}$ [d]	Antibiotic
Fluoxetine	10.09[b]	Mostly Positive, Small Percent Neutral[c].	Base[b]	$9.9x10^{-4}$	$8.9x10^{-8}$	Antidepressant

[a]Pubchem Database (2018)**;** [b]Wu et al. (2013) (Syracuse Research Corporation Database).
[c]Beardall (2015), [d]Roth (2012, [e]Farrell (2012), All other data were obtained from EPI Suite (2000 – 2012**)**
Henry's constant (Kh): ratio of the concentration of a substance in air and its concentration in water at equilibrium.
Solubility in water: maximum possible concentration of a chemical compound that can be dissolved in water at a particular temperature.
pKa: the measure of the strength of an acid or base. It is used to determine the charge on a molecule at a given pH.

Generally, compounds with low Log K_{ow} values have high water solubilities. Thus solubilities of the PPCPs can also be used to predict the distribution of the compounds between biosolids or effluents. In Table 3 the Henry's law constants are given which also predicts whether the PPCPs is volatile. Based on the very low Henry's

law constants seen for the all the PPCPs being investigated, the compounds are not expected to volatilize at the prevailing environmental pH values measured during the study.

Runoff from biosolids containing PPCPs either from landfills or applied on agricultural land may be transported into the surrounding surface water or leach into the groundwater, thereby posing a risk to aquatic life and public health. Sorption on sediment is another mechanism through which PPCPs are transported to the aquatic environment. Several studies have shown some PPCPs (e.g., sulfamethoxazole, carbamazepine, triclosan and ciprofloxacin) to be more persistent in sediment than water (Halden et al. 2005; Ebele et al. 2016).

Conkle et al. (2012) determined that gemfibrozil degraded faster under aerobic conditions than under anaerobic conditions. The half-lives for gemfibrozil degradation under aerobic and anaerbic conditions were approximately 22 days and 7 months, respectively.

Ionization enhances the solubility of a compound in water and reduces its ability to partition onto solid surfaces. For this reason, the knowledge of a compound's pKa is an important factor to consider in determining the potential sorption of a compound. The pKa helps to determine the fraction of the chemical that exist as neutral compounds. This depends on the prevailing pH of the surrounding media and the pKa of the compound which ultimately determines the fate of the compound (Jjemba 2008).Weak acids or bases undergo partial dissociation under environmental pH conditions and are present in either the neutral molecule, ionized species or both. Using Equation 1 the fraction of neutral (f_n) and charged PPCPs were calculated using an environmental pH of 7 and the results were

similar to that found in the available literature. The fraction of neutral molecule, f_n, was calculated as (Trapp 2009; Wu et al. 2013):

$$f_n = \frac{1}{1+10^{i(pH-pKa)}} \quad (1)$$

where i = 1 for acids and -1 for bases (Wu et al. 2013).

Lipophilicity is an important physicochemical descriptor used to relate chemical structure to biological activity. It is represented by the octanol-water partitioning (K_{ow}) as the ratio of the concentration of the compound in octanol to the concentration in water (Equation 2), where octanol is used to represent the lipid surface (Jjemba 2008).

$$K_{ow} = \frac{Concentration\ in\ octanol}{Concentration\ in\ water} \quad (2)$$

K_{ow} represents the neutral fraction of the compounds that predominantly partitions into the organic or lipid surface. It is usually represented as Log K_{ow} by taking the logarithm of the K_{ow} value.

For the charged chemicals in Table 3 where Log K_{ow} values may not necessarily predict their hydrophobicity due to the small proportion of neutral a pH adjusted octanol-water partiton coeffecient Log D_{ow} could be used to determine its hydrophobicity. This parameter could be found using Equation 3.

$$LogD_{ow} = LogK_{ow} + Logf_n \quad (3)$$

According to Equation 3 fluoxetine, gemfibrozil and sulfamethoxazole have $LogD_{ow}$ values of 1.05, 2.2 and -4.4 respectively, which better determines their hydrophilicity than using $LogK_{ow}$ since they are charged compounds.

3.4 Process that Influence the Transformation of PPCPs

Transformation or persistence of PPCPs and their metabolites in the environment are influenced by several factors namely, hydrolysis, adsorption, photodegradation, atmospheric oxidation and biodegradation (Jjemba 2008). Other processes include complexation, mineralization, thermolysis, volatilization, and redox reactions (Jjemba 2008). Table 4 summarizes how some physico-chemical properties influence the fate of PPCPs in the environment. In this table, emphasis is placed on octanol-water partition coefficient, Henry's constant, solubility and pKa which are the important factors determining the fate of PPCPs in this study.

3.5 Environmental Effects on Transformation of PPCPs

Moisture and Oxygen

Low moisture content is known to limit biochemical processes, thus transformation of PPCPs due to biodegradation is reduced under low moisture content conditions (Jjemba, 2004). Biodegradation of chemicals in surface water is generally enhanced when there is free dissolved oxygen (Jjemba 2008). Halden and Paul (2005) estimated the half-life of Triclosan to be 1 day and 540 days in air and sediments, respectively. In a degradation experiment by Huang (2014), the half-life of triclosan in water was found to range between 89 and 161 days, similar to that determined by Halden and Paul (2005).

Table 4. Fate of Organic Compounds Based on Their Octanol-water Partitioning (Log K_{ow}), Henry's Constant (K_h), Solubility in Water and Acid or Base Properties (Miren Lopez 2007).

Log K_{ow}	K_h	Solubility	Acid-Base ionization (pKa)
log K_{OW} > 4 to 5 are non-polar compounds	K_h < 9.9E-11 atm m^3 mol^{-1} low volatile compounds	< 0.5 to 1 mg/L is very insoluble	Substances with a pKa < 3 to 4 tend to move to the aquatic medium
log K_{ow} of 1.5 to 4 are moderately polar compounds	High values mean high volatilization	Highly water soluble substances are less likely to volatilize from water and likely to enter the aquatic environmental through run-off	Substances with a pKa > 10 tend to be retained in soil/on solids
log K_{ow} < 1 to 1.5 are polar compounds.		The higher the water solubility, the greater the tendency to remain dissolved.	Ionization of a compound increases its solubility in water and decreases its lipophilicity. The pH of the water/soil media is 5 to 8
The higher the Log K_{ow} the greater the tendency of the compound to absorb to solid phases and bioaccumulate in organisms		Low water soluble substances volatilize more readily in water, tend to precipitate, to partition to soil, and to bioconcentrate in aquatic organisms	

Temperature

The rate at which PPCPs degrade depends on the prevailing temperature. Studies have shown that higher temperatures favor the transformation of PPCPs while low temperatures favor persistence of PPCPs (Jjemba 2008). For example, the half-life for Ivermectin in the environment was six times greater in winter than in summer (Halley et al. 1993).

Light

Light has the capacity to transform PPCPs through photolysis. Photolysis is the direct absorption of light by a compound followed by a reaction that transforms the parent

compound (Jjemba 2008). High-energy UV radiation can damage organic compounds and interfere with their function. Generally, photosensitive drugs have substituents of chlorine atoms that are substituted or reduced during photodegradation (Glass et al. 2001; Konstantinou et al. 2001). Two chemicals easily susceptible to photodegradation in this study are triclosan and fluoxetine (Aranami et al. 2007; Tisler et al. 2019), however certain compounds could catalyze photolysis of many other pharmaceutical compounds. In this lab study, limitations to light exposure established conditions that limited the photodegradation of any of the chemicals studied.

3.6 Detection and Effects of PPCPs in the Environment

There is vast literature on studies that have detected PPCPs in the environment and how these PPCPs could act as endocrine disruptors. Ebele et al. (2017) reviewed the literature available on PPCPs detected in water, sediment and biota for studies conducted on the six inhabited continents. PPCPs were in surface and groundwater across the US. Krogh et al.(2017) have also detected several PPCPs in untreated sewage, WWTP effluents, receiving marine water and sediments in samples collected between 2009 and 2016 in Victoria, Canada. These findings show the prevalence of PPCPs in the environment and why they should be a concern mainly because they could act as endocrine disruptors. These chemicals bind to receptors in the body and can increase or decrease hormone levels (nih.gov 2020). Table 5 lists examples of endocrine disruptors.

Endocrine disrupting pharmaceuticals include sex hormones, glucocorticoids, veterinary growth hormones and a few non-steroidal pharmaceutical (Ebele et al. 2012). Some hormones released into the environment have the capacity to feminize or

masculinize fish (Ternes 2004). There has been an incident where a protein (vitellogenen) that is used for egg production, and thus only expected to be produced in female fishes, was observed in male fishes in an environment that had been exposed to hormones such as ethylyn oestradiol. In the study by the UK government's Environment Agency, 86 % of male fish sampled from 51 sites across the country were intersex. (Gilbert 2012). Table 6 lists some pharmaceutical compounds that also act as EDs in living organisms.

Additionally, Brooks et al. (2005) reported detections of PPCPs in the liver and brains of fish samples from an effluent dominated stream in the US. Finally, another drug, the anti-inflammatory diclofenac, has been shown to have damaged the gills and lungs of fish (Gilbert 2012).

Antibiotics can also negatively affect microbial communities in sewage systems by influencing the degradation processes. For mixtures of PPCPs, biodegradation of other, non-antimicrobial PPCPs can also be reduced by antibiotics that deactivate the microbial community (Jjemba 2008). A study in Australia that detected antibiotics (ciprofloxacin, tetracycline, ampicillin, trimethoprim, erythromycin and trimethoprim/sulfamethoxazole) in the effluent of a WWTP showed that there was an increase in antibiotic resistance of two natural bacterial strains in the receiving water of the WWTP effluent (Ebele et al. 2016).

Table 5. Endocrine Disrupting PPCPs (Ebele et al. 2017).

Steroids	Personal care products	Non-steroidal pharmaceuticals
Estrone	Galaxolide	Fluoxetine
Progesterone	Tonalide	Diclofenac
Testosterone	Homosalate	Naproxen
β-estradiol	Celestolide	Ibuprofen
		Acetaminophen

3.7 Fate of PPCPs in WWTPs

PPCPs have been detected in the effluent of WWTPs worldwide (Daughton 2019) due to their increased usage as well as not being targeted for removal during wastewater treatment (Deziel 2014). During wastewater treatment, PPCPs, their conjugates, or metabolites may completely transform to carbon dioxide, partially transform producing metabolites, or may remain unchanged (Xia et al. 2005). The various stages of a WWTP influence the transformation or degradation of PPCPs (Xia et al. 2005).

Activated sludge is a common method for wastewater treatment. It is a suspended growth biological treatment method which uses microorganisms to degrade the organic compounds in the wastewater (Ternes 2004; Deziel 2014). Although WWTPs are not designed to remove PPCPs, some PPCPs are biologically degraded, or can be sorbed onto activated sludge solids and be removed from the system when the solids are wasted.

Table 6 predicts, using EPI Suite (USEPA 2012), the potential for degradation of the 12 PPCPs evaluated in this study through atmospheric oxidation, hydrolysis, and biodegradation in aerobic and anaerobic environments. EPI Suite uses QSAR (quantitative structure activity relationship) to develop models based on the physical properties of chemicals in order to make predictions. This model predicts the activity of a chemical based on the fragments of a chemical by assigning values to bonds, functional groups, number of carbon etc.

A linear model which is a function of the chemical structure is obtained which is used to predict the properties of the chemical. For example in predicting biodegradability of acetaminophen (Table 6), BIOWIN 1 uses the one aromatic alcohol functional group and the one amide functional group to develop the model. Since functional groups are

Table 6. Prediction of Transformation of PPCPs by Aerobic and Anaerobic Biodegradation, Atmospheric Oxidation and Hydrolysis Using EPI Suite (USEPA 2012, v 4.1).

PPCPs	Aerobic[a] Biodegradation Potential	Anaerobic[b] Biodegradation Potential	Atmospheric[c] Oxidation Potential (Half-life, days)	Potential[d] for Hydrolysis
Acetaminophen	Biodegrades fast	Does not biodegrade quickly	7.26	In years [e] or not susceptible to hydrolysis
TCEP	Biodegrades fast	Biodegrades quickly	5.84	19.88 days
DEET	Biodegrades fast	Does not biodegrade quickly	5.07	Not susceptible to hydrolysis
Estrone	Biodegrades fast	Does not biodegrade quickly	1.02	Not susceptible to hydrolysis
Progesterone	Does not biodegrade fast	Does not biodegrade quickly	1.23	Not susceptible to hydrolysis
Sulfamethoxazole	Does not biodegrade fast	Does not biodegrade quickly	0.64	Not susceptible to hydrolysis
Fluoxetine	Does not biodegrade fast	Biodegrades quickly	3.48	Not susceptible to hydrolysis
Carbamazepine	Biodegrades fast	Does not biodegrade quickly	1.59	Half life greater than 1 year or not susceptible to hydrolysis
Caffeine	Biodegrades fast	Biodegrades quickly	6.61	Not susceptible to hydrolysis
Gemfibrozil	Biodegrades fast	Does not biodegrade quickly	1.56	Not susceptible to hydrolysis
β-Estradiol	Biodegrades fast	Does not biodegrade quickly	1.05	Not susceptible to hydrolysis
Triclosan	Does not biodegrade fast	Does not biodegrade quickly	7.96	Not susceptible to hydrolysis

[a]BIOWIN 1; [b]BIOWIN 7; [c]AOPWIN(based on $OH^{\bullet}$ rate constant); [d]HYDROWIN([e]Mabey and Mill (1978).

expected to influence chemical property in the same way, BIOWIN gives the same values to same functional groups found in different chemicals when making its predictions.

Predictions for aerobic biodegradation using BIOWIN 1 model is intended to represent general aerobic environment and not for any specific medium. BIOWIN 7 estimates however, are assumed to be predicitive of the conditions in an anaerobic digester. The AOPWIN model estimates the half-lives of chemicals based on the rate constants of photochemically produced hydroxyl radicals. Predictions by HYDROWIN are based on hydrolysis rate constant of chemicals which are used to calculate half-lives.

The prediction of the potential for aerobic degradation is low for progesterone, sulfamethoxazole, fluoxetine and triclosan. Acetaminophen, caffeine, DEET, β-Estradiol, estrone, carbamazepine and tris-2 chloro-ethyl phosphate are predicted to biodegrade rapidly under aerobic conditions. Caffeine, TCEP and fluoxetine were the only compounds that were predicted by EPI Suite to biodegrade under anaerobic conditions. All the compounds being studied had low potential for hydrolysis. Atmospheric oxidation, as predicted by AOPWIN using OH* rate constants, was indicated to be high for many of the PPCPs in the presence of UV light.

3.8 Components of WWTP Sludge

Activated sludge is a type of biological wastewater treatment process whose primary role is to remove dissolved and colloidal biodegradable organic material in the waste stream. It uses a mixed culture of microbes that made up of five major groups. They are: bacteria, protozoa, metazoa (primarily rotifers), filamentous bacteria, and fungi; with bacteria making up the largest population in the activated sludge community (Seman

2019). Amongst many other components, biosolids from WWTPs are also known to be rich in nutrients (MacFarland 2000).

Biosolids from WWTPs also contain metals sourced from domestic and industrial waste (MetCalf and Eddy 2003). These metals play an important role in transferring electrons during oxidation-reduction reactions which could also influence transformation of PPCPs (Jjemba 2008; Crittenden et al. 2012).

3.9 Sludge Processing

WAS is taken through various treatment steps to reduce pathogens, remove odor, reduce putrefaction, and reduce moisture to make it easy for disposal (MacFarland 2000). Fig. 2 shows the various stages of sewage sludge generation, treatment, use and disposal. Some of the sludge treatment processes include, thickening, dewatering and conditioning. Examples of sludge stabilization processes include aerobic composting and anaerobic digestion (MacFarland 2000; Metcalf and Eddy 2003) which were considered in this study. Each stage of sewage sludge treatment may subject PPCPs in sludge to a myriad of transformation mechanisms through the addition of chemicals and removal of moisture. However, some PPCPs do persist in the sludge and may pose risk of exposure to humans and animals. Table 7 shows the various pathways through which one can be exposed to contaminated biosolids when applied to land.

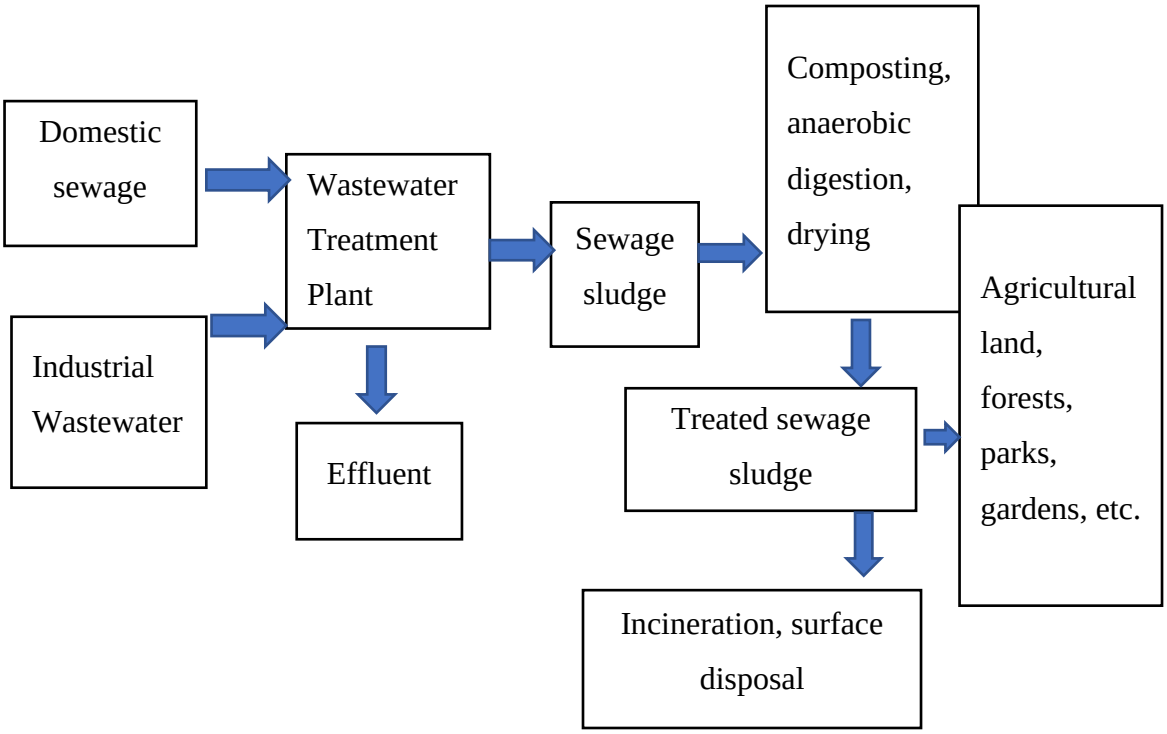


Fig. 2. Schematic of generation, treatment, use and disposal of sewage sludge (McFarland 2000).

Humans and animals could be exposed to the contaminants on biosolids directly through ingestion of biosolids or indirectly through consumption of plants that have taken up contaminants from biosolids after land application.

3.9.1 Thickening

Thickening is described as the removal of water from sludge to achieve an overall volume reduction (MacFarland 2000). Thickened biosolids is still fluid and pumpable and the solid content is about 2 percent (MacFarland 2000). Thus, the thickened biosolids may be conveyed within the WWTP or to a land-application site through pipelines (McFarland 2000). Sludge thickening may be achieved in the primary clarifier, in the digestion facility or in a specially designed separate unit (Metcalf and Eddy 2003).

Table 7. Exposure Pathways for Conducting a Risk Assessment of the Land Application of Biosolids (McFarland 2000**).**

Pathway	Description
Biosolids → Soil → Plant → Human	Human (except home gardener) lifetime ingestion of plants grown in biosolids-amended soil
Biosolids → Human	Human (child) ingesting biosolids
Biosolids → Soil → Plant → Animal → Human	Human lifetime ingestion of animal products
Biosolids → Soil → Animal → Human	Human feeding on animals that have ingested biosolids directly
Biosolids → Soil → Plant → Animal	Animal ingests plants grown on biosolids-amended soil
Biosolids → Soil → Animal	Animal ingests biosolids directly
Biosolids → Soil → Plant	Plants become toxic by taking up biosolids pollutants from soils amended with biosolids.
Biosolid→ Soil → Airborne Dust → Human	Inhalation of particles from biosolids. Eg. When tractor driver is tilling the land
Biosolids → Soil → Air → Human	Human inhalation of biosolids components that is volatile
Biosolids → Soil → Surface Water → Human	Humans drinking surface water that receives run off from a land applied with biosolids.
Biosolids → Soil → Ground Water → Human	Humans drinking well water that has pollutants leaching from biosolids applied to land

3.9.2 Conditioning

Conditioning is done prior to dewatering to facilitate water removal. It involves the addition of chemical or physical treatment of sludge to enhance water removal and to improve solids capture. Most sludge conditioning systems employ inorganic chemicals, organic polymers, or heat. Solids are suspended in sludge due to negative surface charges that repel one another. Conditioning chemicals (e.g., lime, ferric chloride, polymers, etc.) are used to introduce cations into the sludge to overcome the repulsive effects of the negative surface charges and cause particles to flocculate (McFarland, 2000).

3.9.3 Dewatering

Dewatering is the step in the sludge treatment process in which stabilized biosolids are concentrated to the point that they can be handled as a dry solid material rather than a viscous liquid. Dewatering processes are designed to increase the solids

content to more than 18% solids using various mechanical methods including: vacuum filtration, belt filter presses, centrifugation, or plate filters. These mechanical devices require polymer addition to enhance particle flocculation and water removal from the digested biosolids to produce dry solids cakes. Small WWTPs in arid areas may also use sand drying beds for their biosolids dewatering that relies on evaporation and filtration to produce a dry solids cake. Sand drying beds do not require polymer addition but do require high evaporation rates and large land areas for drying to be effective.

Dewatered biosolids are not pumpable and therefore must be conveyed within and outside the wastewater treatment facility by means other than a pipeline (e.g., front-end loader, belt conveyor, truck, rail, barge, etc., McFarland (2000)). Dewatered sludge reduces the cost of transportation and is easier to handle (Metcalf and Eddy 2003) than wet sludges for most reuse and disposal applications.

3.9.4 Stabilization

Stabilization is done to remove pathogens, eliminate offensive odors and reduce putrefaction (Metcalf and Eddy 2003). Chemical or biological processes can be used to achieve this result. Alkaline stabilization, aerobic digestion, anaerobic digestion, and composting are some of the processes used to stabilize biosolids (Metcalf and Eddy 2003).

3.9.4.1 Composting of WWTP sludge

Composting is a commonly used solid stabilization peocedure which involves a series of biological reactions that break down organic matter and produce humic materials. There are three separate stages involved in composting: the mesophilic stage, the

thermophilic stage, and the cooling stage (Metcalf and Eddy, 2003). In the mesophilic stage the temperature of the compost pile increases to 40^{o}C with the appearance of fungi and acid producing bacteria. The thermophilic stage is where temperature increases from 40 to up to 70^{o}C leading to water evaporation and the appearance of thermiphilic bacteria, actinomycetes and thermophilic fungi. This is the stage where maximum degradation of organic matter is likely to occur. In the cooling stage, pH is stabilized and humic acids and other humic materials are produced (Metcalf and Eddy 2003). Composting has been used as an effective means of degrading organic contaminants in biosolids such as pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated phenyls (PCBs), trinitrotoluene (TNT), and perchlorate (Xia et al. 2005).

3.9.4.2 Anaerobic Digestion of Sludge.

Anaerobic digestion is the biological degradation of organic compounds under low or no oxygen conditions to produce methane, carbon dioxide, new bacterial cells and stabilized sludge (Metcalf and Eddy 2003). In the first stage of anaerobic digestion, the solids are hydrolyzed leading to the production of volatile fatty acids and alcohols (Gerardi 2003). The second stage is acetogenesis, which involves the conversion of the volatile fatty acids and alcohols to substrates such as acetic acid or acetate and hydrogen gas that can be used by methane-forming bacteria in the final stage of the stabilization process (Gerardi 2003).

The optimum pH range for proper anaerobic digester operation is generally reported to be between 6.4 to 7.5 (MacFarland 2000). However, studies of a lab-scale

reactor used for anaerobic digestion at the UWRL has shown a greater pH range of between 6 to 8 for suceesful anaerobic digestion of various organic materials.

In the available literature, anerobic digestion is not effective in removing many PPCPs from sludge. Ahmad and Eskicioglu (2019) determined that, between anaerobic digestion and a sequential anaerobic/aerobic/anoxic sludge treatment, ibuprofen and diclofenac, had increased removal from the solids from the latter solids stabilization method.

In other studies sulfamethoxazole was degraded in an anaerobic digester whereas acetaminophen, triclosan and carbamazepine were not degraded (Musson et al. 2010; Narumiya et al. 2013). In assessing whether the distribution of chemicals in liquid/solid phase in anaerobic digestion, Gonzalez-Gil et al. (2018) determined that biotransformation of a chemical was not dependent on where the chemical was distributed.

3.10 Removal of Pharmaceutical Compounds by Duckweed

The use of duckweed for phytoremediation of contaminated water is promising due to duckweed's ability to grow under a wide range of temperature, pH, and nutrient conditions. Duckweed are aquatic macrophytes that grows very fast and float on the surface of stagnant or slow moving water bodies (Skillicorn et al. 1993). They are classified under the *Lemnaceae* family which consists of about 40 species in five genera namely; *Spirodela, Lemna, Landolita, Wolffiella* and *Wolffia* (Skillicorn et al. 1993; Lyerly 2004)

The species found on the Wellsville Sewage Lagoons are *Lemna minor* and *Wolffia* spp. These two species coexist on the lagoons although *Lemna minor* appears to

be the dominant species. The species are easily differentiated by size, i.e., the fronds of *Lemna* species typically average between 6 to 8 mm while those of the *Wolffia* species are about 2 mm or less in diameter (Skillicorn et al. 1993; Cheng and Stomp 2009).

In the winter months, the duckweed survive the low temperatures by forming a starchy survival frond known as a turion, which sinks to the bottom of the pond and remains dormant until spring (Zirschky et al. 1988, Skillicorn et al. 1993). The increase in temperatures in spring triggers their return to normal growth (Kesaano 2011).

3.10.1 Fate of Pharmaceutical Compounds in Harvested Duckweed

Duckweed needs to be harvested in order to completely remove contaminants from wastewater (Keesano 2011). Harvested duckweed can be used as feed for animals, for energy production through anaerobic digestion, or fermented to produce ethanol (Kessano 2011). The ability of duckweed to remediate organic chemicals, including pharmaceuticals, has been investigated by Farrell (2012) and Allam et al. (2015).

Laboratory experiments by Farrell (2012) showed that acetaminophen was taken up by duckweed, while progesterone, fluoxetine, and sulfamethoxazole sorbed onto duckweed surfaces and could be desorbed following water rinsing. Allam et al. (2015) found that acetaminophen, diclofenac and progesterone were removed by duckweed through passive uptake and sorption.

3.11 Focus of this Study

There have been prior experiments by other researchers that lead to this study. Roth (2012) evaluated the effectiveness of three WWTPs in Utah in removing PPCPs and found that biodgradability and partitioning coeffecients influence the partitioning of

PPCPs between water and activated sludge. Chenxi et al. (2008) did lab studies on PPCPs under aerobic, anaerobic and light treatments to determine the persistence of the PPCPs under these various treatments and found that aerobic conditions favor degradation of most PPCPs over anaerobic conditions. Farrell (2012) found that duckweed was capable of removing PPCPs from the Wellsville Lagoons in Utah. Similarly, Allam et al. (2015) found that acetaminophen, diclofenac and progesterone were removed by duckweed through passive uptake and sorption.

Ahmadi and Dupont (2018) demonstrated the successful use of duckweed as a feed source for biofuel production through anaerobic digestion, and Kesaano (2012) showed that harvested duckweed could be used for ethanol as well as biogas production. A review by Xia (2005) showed that some phamaceuticals (eg., nonylphenol) are effectively removed during composting.

Based on findings in the literature there remains a concern that biosolids containing PPCPs may potentially become a source of contamination when land applied. Therefore, in order to determine the extent to which PPCPs in biosolids are treated by solid stabilization processes, this study seeks to determine the fate of PPCPs in duckweed and WAS after undergoing aerobic composting and anaerobic digestion.

CHAPTER 4

MATERIALS AND METHOD

4.1 Sampling Locations

Samples were obtained from two locations; the Wellsville wastewater treatment lagoons and the Hyrum WWTP. Duckweed and sediment samples were obtained from the Wellsville lagoons whereas dewatered waste activated sludge was obtained from the drying beds at the Hyrum WWTP. Water samples were also collected at the two locations.

4.1.1 Wellsville Lagoons

Wellsville is located in Cache County, Utah. The city has a population of approximately 3,432 (US Census Bureau, 2010). The city uses a four cell lagoon system (Fig. 3) to treat its wastewater through biological activity of microbes and the uptake of contaminants by duckweed that grows on the surface of the lagoons. The lagoons treat approximately 230,000 gallons of sewage per day. UV disinfection is carried out on the effluent when the plant discharges to the Little Bear River. The plant discharges effluent into river from October to April. For the remaining months the effluent is retained in Cell 4 prior to being used during summer months to irrigate adjacent feed crop land.

These lagoons are located in a valley sheltered from the wind by the hills and trees found along the portion of the Little Bear River that flows besides the lagoons (Fig. 4). This site provides ideal growing conditions for the duckweed due to the abundance of nutrients from the sewage discharge and shelter from the wind. The growth period of duckweed in the lagoons spans from late spring to early winter months (early May to

early November) (Kessano 2011). Influent and effluent samples, as well as duckweed, sediments and lagoon water were collected from all four cells.

Fig. 3. Wellsville municipal sewage lagoons, Wellsville Utah. (a) Aerial view from Google maps (b) Cell 4 of Wellsvile sewage lagoons viewed closely from the north.

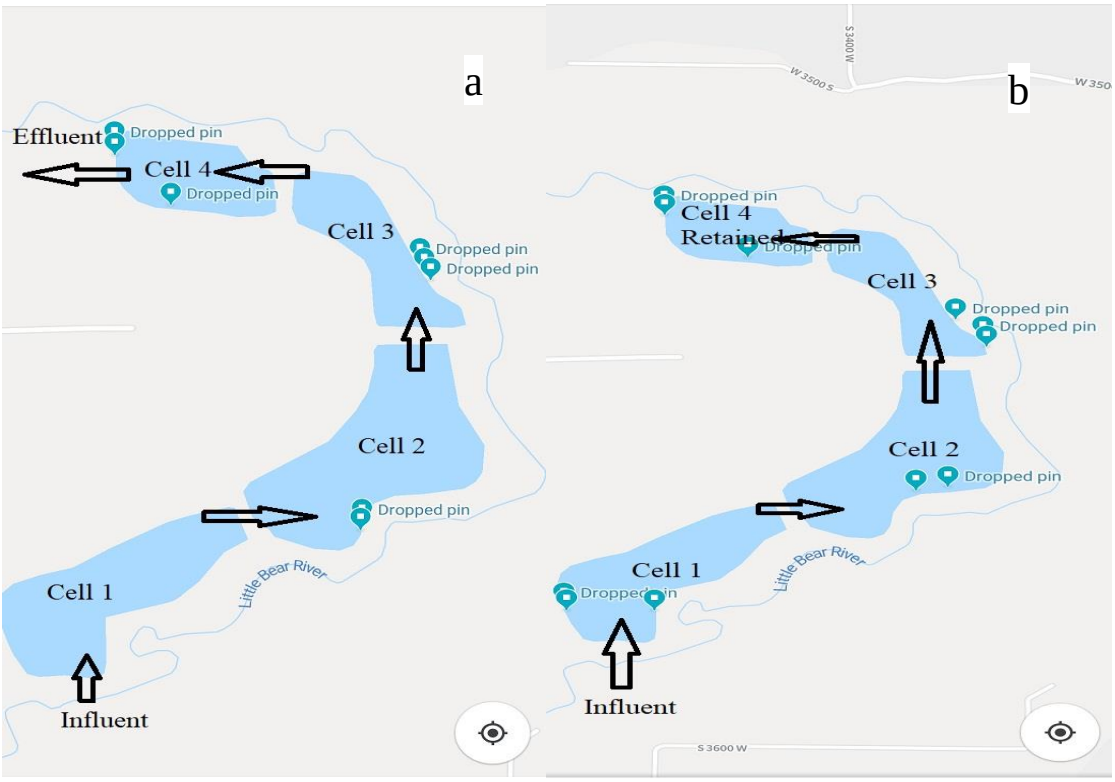


Fig. 4. Sampling locations at Wellsville sewage lagoons : (a) Sampling location for samples collected on 6/5/2019 when effluent from the ponds was being used for irrigation. (b) Sampling locations for samples colected on 8/15/2019 when water was retained in Cell 4.
Sample locations indicated by the pins. Water, duckweeed and sediments were co-located.

The first round of samples (influent, effluent, sediment and duckweed from each cell; no pond water was collected at this time) were collected on 6/5/2019 when the eflluent of the lagoons was being used to irrigate surrounding farmlands (Fig. 4(a)). The second round of samples were collected on 8/15/19 when water was retained in Cell 4 and not being discharged (Fig. 34(b)).

The samples collected during the second sampling event were influent and effluent, pond water, sediments and duckweed from all four cells. The Figs. 4(a) and 4(b) show the locations in the lagoons where samples were collected. Sample locations from Cell 1 for the 6/5/19 sampling event could not be taken because the markers that were being used to identify sampling locations were blown away in the wind. Influent, sediments and duckweed were collected at the same locations, with pond water in the cells also collected on the second samplng event. For the two sampling events, samples were collected about 8 ft into the lagoons using a 1 L plastic bottle attached to a pole. The sediment was measured to be approximately 5 inches thick at locations where samples were collected. Sediments and duckweed were collected using the same plastic bottle attched to a pole and the water drained with a sieve.

Water samples were collected into 1 L glass amber bottles and capped tightly. Sediment from the lagoons was collected from the bottom of the lagoons at the same location that duckweed and water were collected. During the second sampling event on the 8/15/2019 there was more duckweed on the lagoons than there was during the 6/5/2019 sampling event.

Duckweed and sediment samples were kept in ziploc bags. Samples were kept in the cooler at 4^{o}C using ice blocks and transported to the Utah Water Research Lab.

Sediments and duckweed were processed in a 25^{o}C constant temperature room where the ziploc bags were opened and the solids were spread out and allowed to dry in the bags. Samples were collected from three locations from each cell were mixed together before being split into triplicate for processing and analysis. Sampling locations were next to each other within 20 ft and are expected to be representative of the cells.

4.1.2 Hyrum City Wastewater Treatment Facility

Hyrum City is located in Cache County, UT, with a total population of approximately 7,609 (U.S. Census Bureau 2010). The average inflow to the plant is approximately 1.0 MGD. Wastewater is treated using two anoxic basins and two aeration tanks by aerobic activated sludge in a membrane bioreactor (Fig. 5). The plant produces approximately 18 tons of waste activated sludge a year.

Fig. 5. The anoxic basins in the Hyrum WWTP.

Phosphorus removal is accomplished by the addition of alum. The Class B biosolids (18% solid) produced are annually applied to 160 acres of city-owned land. After UV disinfection the secondary effluent is discharged to an irrigation ditch that flows into Spring Creek. The Hyrum WWTP uses the treated effluent for secondary irrigation for both feed crops and home gardens during irrigation season from April through October.

The first round of samples from the Hyrum WWTP were collected on 4/1/2019 when the plant was removing phosphorus from the wastewater using alum. The second round of samples were collected on 8/15/2019 when they were using alum and an organic polymer, T-floc (B-135 and B-1419), to enhance coagulation of the activated sludge to prevent premature membrane fouling. Influent, effluent and WAS were collected. Influent and effluent samples were collected into 1 litre amber glass bottles and closed tightly. A stainless steel spoon was used to collect WAS from the drying beds into clear wide mouth glass bottles and covered tightly. Samples were kept in the cooler at 4^{o}C using ice blocks and transported to the Utah Water Research Lab where they were kept in a walk-in refridgerater ($\leq$ 4°C) before processing and analysis.

4.2 Analytical Methods

4.2.1 Chemicals

A 200 mL stock solution of the 12 PPCPs (Table 8) was prepared from pure standards of these compounds in LC-MS grade methanol at a concentration of 5,000 µg/L. The stock solution was kept in the refrigerator at 4^{o}C. A 10 point calibration curve was made from the stock with concentrations from 0.02 µg/L to 100 µg/L.

Table 8. Chemical Manufacturers and Purities of PPCPs and Other Chemicals Used in Study

PPCP	Manufacturer	CAS Number	Purity
Acetaminophen	Aldrich	103-90-2	0.98
Caffeine	Sigma - Aldrich	58-08-2	1
Sulfamethoxazole	Bioworld	723-46-6	0.99
Carbamazepine	Sigma - Aldrich	298-46-4	1
TCEP	Aldrich	115-96-8	0.97
Fluoxetine Hydrochloride	Spectrum	2-84-9	0.89
DEET	Aldrich	134-62-3	0.97
β-Estradiol	Sigma	50-28-2	1
Estrone	Acros	53-16-7	0.99
Progesterone	Aldrich	57-83-0	0.98
Gemfibrozil	Spectrum	25812-30-0	1
Triclosan	Aldrich	3380-34-5	0.97
Potassium Bromide	Fisher	7758-02-3	0.99
Acetic acid	Fisher	64-19-7	0.99
Formic acid	Fisher	64-18-6	0.99
Sodium Nitrate	Fisher	7631-99-4	0.99

For anaerobic digestion, a tracer study was conducted in a lab-scale anaerobic digester. A six point calibration curve for bromide standards was made ranging from 1 µM to 0.1 M. Two mL of 5 M sodium nitrate solution were added to 100 mL of liquid samples collected from the digester to adjust for ionic strength before measuring for bromide.

4.2.2 Analysis of liquid samples from the field

Water samples from the field (influent and effluent) were analyzed using EPA 1694 with two dilution factors. In Method 1, 0.9 mL of sample were measured into 2 mL centrifuge tubes with 0.1 mL acetonitrile added and centrifuged at 14,000 rpm for 10 minutes. A total of 0.5 mL of the supernatant were measured into autosampler vials and analyzed for PPCPs using an Agilent 1290 Infinity LC system with an Agilent 6490 QQQ

MS and an Agilent Eclipse Plus C18 column (2.1 x 50 mm, 1.8 µm I.D, 0.45 mL/min flow rate, 15 minutes run time, 5 µL injections). The LC-QQQ-MS system utilizes a binary pump with mobile phase A made of 0.1% formic acid and methanol (by volume) in DI water and mobile phase B made of 0.1% formic acid (by volume) in LC-MS grade acetonitrile. In Method 2 PCPPs were extracted from water samples using an Oasis™ HLB (500 mg, 6 cc; Waters Corporation, Milford, MA) solid phase extraction (SPE) cartridge. About 500 mL of the approximately 1,000 mL of water in the amber bottles were run through the cartridges. The analytes were eluted from the cartridges with 10 mL LC-MS grade methanol, and 1 mL of eluate was measured into autosampler vials for analysis using the same LC-QQQ-MS method.

4.2.3 Analysis of solid samples from the field

Prior to selecting an appropriate method for solids processing and analysis, different drying methods were used to determine which method gave the best recoveries from duckweed spiked at 200 ng/g of the PPCP stock solution. Separate batches of duckweed were air-dried in the presence of light and in the dark at a constant room temperature of 25°C. Duckweed was also dried in a 40°C oven, while another batch was freeze-dried at -40°C. Upon drying, samples were ground into powder and analyzed for PPCPs using Method 2 described below. Air-drying at a room temperature of 25 °C was selected as the drying method for the solids because it was fast, had good recoveries by comparison with the other drying methods, and made solids easy to handle (Appendix A, Table A.1).

Two methods were used for anlyzing PPCPs in the the biosolids from the field after extraction. Biosolids samples were first air-dried at constant room temperature of 25^{o}C. The dry solids were ground into powder using a mortar and pestle. 1 g of dry powdered sample was weighed into Q cups made up of ultra thin aluminium having an M2 filter on top of a C9 filter and placed in an EDGE instrument (CEM Corporation, NC). The EDGE combines pressurized fluid extraction and dispersive solid phase extraction in one instrument to produce fast, efficient extraction, filtering, washing and cooling. The PPCPs were extracted at a pressure of 40 psi and a temperature of 100^{o}C with 20 mL of methanol:water (80:20, vol/vol) and 0.1 % volume acetic acid. In Method 1 of analyzing PPCPs in solids, 1 mL of the 20 mL extract from the EDGE instrument was diluted with 9 mL Mili Q water and centrifuged at 10,000 rpm for 15 minutes in 50 mL Teflon centrifuge bottles. One mL of the supernatant was measured into autosampler vials and analyzed using same LC-QQQ-MS method used for the liquids. In Method 2, the remaining extract from the EDGE instrument was diluted to 1,000 mL with DI water to change the mobile phase from methanol to water. The diluted sample was concentrated using an Oasis^{TM} HLB (500 mg, 6 cc; Waters Corporation, Milford, MA) solid phase extraction. Approximately 500 mL were run through the cartridges. Methanol was used to elute the PPCPs from the cartridges and 1 mL was anlyzed using same LC-QQQ-MS method.

4.2.4 Analysis of Samples from Lab-Scale Anaerobic Digester

One of two 10 L anaerobic digesters made of glass was fed with powdered duckweed from the Wellsville lagoons to evaluate the energy production potential

(Keesano, 2011) of the harvested duckweed (Fig 6). Tygon tubes connected each bioreactor to a gas collection system made of two calibrated plastic containers which uses fluid displacement to measure gas production. The fluid in the gas collection system contains methyl red indicator and a 5 % solution of sulfuric acid with 4 g/L NaCl. The salt and the acid are added to prevent microbial growth and also reduce carbon dioxide absorption in the fluid.

The reactors were placed in a a constant temperature room at 25°C. The temperature inside the reactor was maintained at an average of 28˚C using an external heating jacket. A circulation pump (Master flex pump) was used to constantly circulate and mix the contents of the digesters at a flow rate of 300 mL/min. Microbes in the reactor feed on the duckweed biomass and produce biogas (Ahmadi and Dupont 2018).

At the start of the experiment the volume of reactor based on the level of fluid observed in the glass was estimated to be 2.5 L. Tracer analysis preformed on the reactor over a 30-day period in September 2019, showed an average retention time of 10 days and an active volume of 2.4 L. The digester was regularly run by collecting 600 mL of digestate from the reactor and feeding with 5.8 g duckweed in 600 mL DI water daily to ensure constant gas production. The pH and temperature in the reactor were monitored daily in the samples collected from the reactor using a pH probe and a waterproof Hanna temperature probe, respectively.

In a 30-day experiment, the reactor was given a pulse feed of powdered duckweed spiked with PPCPs at approximately 60 µg/g alongside a bromide tracer at 830 mg/L in the 600 mL DI water (Appendix C,Table C.1) after collecting 600 mL digestate from the digester. The reactor was allowed to run for 24 hours with the pump circulating the fluid,

after which the first sample of digestate was collected, and the digester was fed with 5.8 g powdered duckweed (Appendice D,Table D.5) in 600 mL DI water, with no PPCP or bromide spike.

The digester was returned to regular operation with samples collected and fed every 3 days. Each time digestate samples were collected total suspended solids (TSS) and volatile suspended solids (VSS), Br and PPCPs in the digestate were measured (Appendix C, Table C.1).

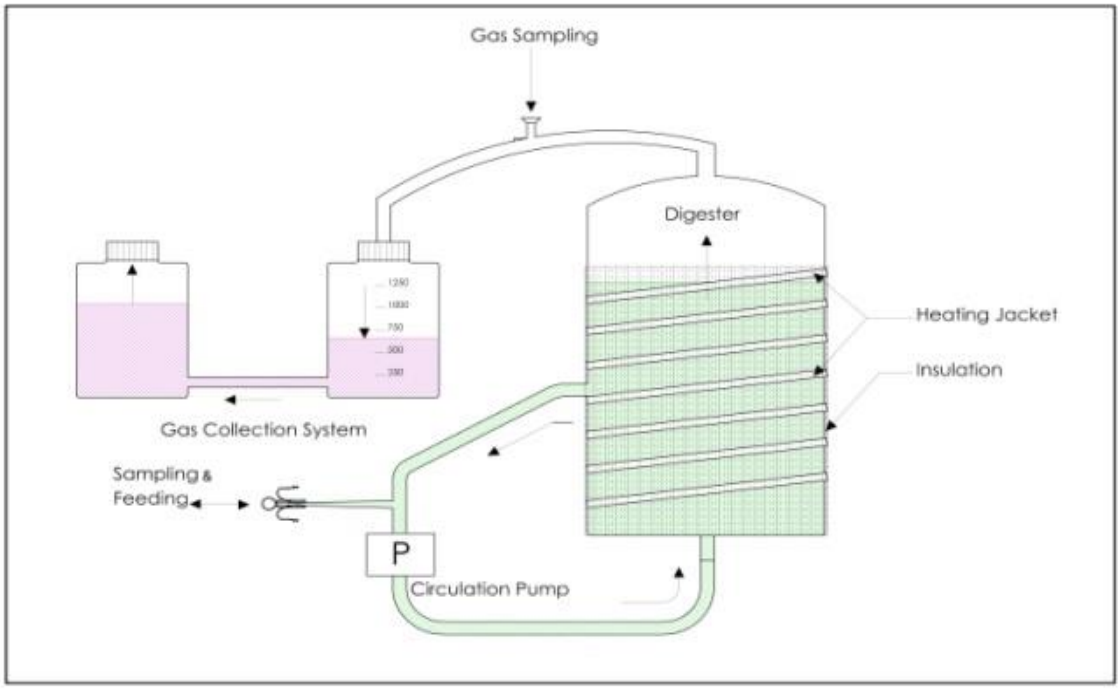


Fig. 6. Lab-scale anaerobic digester configuration (from Ahmadi and Dupont 2018).

A 100 mL subsample of digestate was centrifuged at 5,000 rpm for 10 minutes and the supernantant (approximately 100 mL) analysed for Br concentration. The Br concentration in the 100 mL sample was measured using an Orion Bromide ISE and meter from Thermo Scientific.

The 500 mL of remaining sample were centrifuged in two 250 mL bottles at 5,000 rpm for 10 minutes and filtered through 1.5-µm pores size, 42.5-mm diameter Whatman microfiber filter paper to separate the solids from the liquids for PPCP analysis. PPCPs in digestate liquid was analyzed using Method 1 used for PPCPs analysis in liquids in field samples.

Solid samples obtained after centrifuging 500 mL of digestate were air-dried in the open for 2 weeks at constant room temperature of 25 ^{o}C, processed and analyzed for PPCPs using Method 1 as shown for PPCPs analysis for field samples.

Caffeine, carbamazepine, DEET, estrone, gemfobrozil, triclosan and TCEP were monitored in the anaerobic digester based on their recoveries in Tables A.2 and A.3 (Appendix A).

4.2.4.1 Gas Composition Analysis

Gas samples were collected from the sampling nozzle as shown in Fig. 6 using a gas tight syringe. Gas samples were collected on the days as the digestate samples. A sample volume of 5 mL was injected into a GOW-MAC series 400-GP gas chromatograph that uses a thermoconductivity detector, helium as the carrier and dual columns (20% Carbowax 20M column and 20% DC 200 column); with temperature set for column, detector and injector at 72, 110 and 100 ^{o}C respectively and detector current set at 100 mA. A one point standard was made for each of the gases using 100% for carbon dioxide, 100% for methane, and a 20.5 % for oxygen, 78 % for nitrogen from a mixture of lab air. The percent composition of carbon dioxide, nitrogen, oxygen and methane in the biogas samples were found by matching their retention times to the retention times of the pure

standards and comparing the area under the sample chromatogram to that for each peak determined from the standard.

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4.2.4.2 TSS and VSS Measurement

Digestate samples from the reactor were stirred to make sure the mixture was completely mixed. A 1.5-µm pore size, 42.5-mm diameter, Whatman glass microfiber filter paper was weighed on a mass balance. Five mL of well mixed digestate were filtered through the filter paper. The filter paper with the deposited solids (digested duckweed) was dried in a 103^{o}C oven overnight. The weight of the filter paper with the solids deposit was measured to determine the TSS of each sample that was collected during the experiment. Following the TSS measurement, the dried sample was kept in the 550^{o}C oven for 15 minutes and then reweighed after cooling. The VSS concentration was calculated based on the loss in mass of the solids after drying in the 550^{o}C oven for 15 minutes.

4.2.4.3 Mass Balance in Anaerobic Digester

Concentrations of PPCPs in duckweed at all 11 sampling times collected from the lab-scale anaerobic reactor were included in the following mass balance calculations to determine percent recovery (Table 25). Using Equations 4 and 5 and the average PPCP concentrations as shown in Appendix C (Table C.2 and C.3) the mass of PPCPs collected from the digester at each time interval were calculated as follows:

M_T = Mass of PPCP in Digestate Solids + Mass of PPCP in Digestate Liquid (4)

$M_T = Cs*TSS*V + Cw*V$ (5)

where M_T = Total mass of a PPCP sampled from digester at a time T, µg; Cs = PPCP concentration on digestate solids, µg/g; TSS = Total suspended solids in digestate sample, g/L; V= Volume of digestate, L; and Cw = Concentration of PPCP in digestate liquid, µg/L.

4.2.5 Analysis of Compost Samples

WAS samples and duckweed samples were composted concurrently in two separate chambers in a lab composting unit (Fig. 7). Table 9 shows the concentrations of PPCPs in the components that were mixed together to make the initial compost mixture.

Table 9. Concentrations of PPCPs (mean ± 95% CI) in Materials Used for Composting.

PPCP Concentrations in Compost Constituents				
PPCPs	USU compost (ng/g)	Wood dust (ng/g)	WAS Spiked with PPCPs (ng/g)	Duckweed Spiked with PPCPs (ng/g)
Acetaminophen	<3.54	<3.54	8,940±1,890	78,100±8,790
B-Estradiol	<47.0	<47.0	4,030±3,980	37,400±8,720
Caffeine	6.41±5.55	<2.00	35,800±8,800	402,000±33,700
Carbamazepine	<2.00	6.60±6.00	40,500±6,700	536,000±84,900
DEET	<11.0	<11.0	47,500±8,920	673,000±88,800
Estrone	<136	<136	74,200±5,220	659,000±82,000
Fluoxetine	<1.20	8.50±4.90	6,410±5,270	22,500±7,270
Gemfibrozil	<51.9	<51.9	27,600±10,500	505,000±55,600
Progesterone	4.42±0.57	25.9±12.4	17,300±3,360	163,000±25,200
Sulfamethoxazole	<3.32	<3.32	22,600±4,500	219,000±17,800
Triclosan	<61.2	<61.2	42,100±6,260	553,000±50,700
TCEP	<39.05	<39.05	55,900±9,460	716,000±77,100

The background concentrations of PPCPs in the WAS and the duckweed were low so they were spiked to a higher concentration with PPCPs so that any reduction in concentration in the compost could be detected. Background concentrations of PPCPs in

the other components of the compost (USU compost (Fig. 8), and wood dust/cutiings) were also measured as shown in Table 9.

4.2.5.1 Duckweed Compost

Duckweed for composting was spiked with 12 PPCPs at a starting concentration of approximately 60 µg/g dry weight. Compost was made at the lab from duckweed collected from Wellsville sewage lagoons seeded with compost from a USU industrial food waste composting unit operated by the USU Food Services Department and wood cuttings as a bulking agent . The C/N ratios of the compost mixes was found by entering the values of Tables 10 and 11 into Equation 6:

$$C/N = \frac{W1*(1-\%moisture)*\%C+W2*(1-\%moisture)*\%C+W3*(1-\%moisture)*\%C}{W1*(1-\%moisture)*\%N+W2*(1-\%moisture)*\%N+W3*(1-\%moisture)*\%N} \quad (6)$$

where W1= weight of duckweed/WAS, g; W2 = weight of USU food waste compost, g; and W3 = weight of wood cuttings, g.

Composting was done in a black, eight sided tumbling composter (Fig. 7). The tumbling composter has two chambers with each having a volume of 18.5 gallons. The composter is constructed from UV inhibited, recycled propylene.

The composter was turned five to six times every 3 days for proper mixing and aeration of the prepared compost mixture. Compost samples were collected immediately after mixing the constitituents and every 3 days thereafter, and analyzed for PPCPs of interest. One g of compost sample was measured and extracted with the EDGE automated

extraction system using the same method used for the digestate solids as described above in Method 1 for PPCPs analysis in solids.

Fig. 7. FCMP Outdoor IM4000 Dual-Chamber Tumbling Composter

Fig. 8. USU Industrial Food Waste Composting Unit

Table 10 shows the proportions, moisture content and C:N ratios of duckweed, USU Food Waste Compost and wood cuttings combined to make the duckweed compost. Caffeine, carbamazepine, DEET, estrone, gemfibrozil, triclosan and TCEP were the compounds that were monitored in the duckweed compost study.

Table 10. Components of Compost Made from Duckweed from the Wellsville Sewage Lagoons.

	% Moisture	% C	% N	Mass Composted (Wet), g
USU Compost	50	10.2[2]	0.48[2]	2,800
Wood Dust/Cuttings (Honey Locust)	10	50[1]	0.1[1]	1,400
Duckweed	90	39[2]	4.47[2]	6,183
Final Compost Mixture	68	30.9	1.08	10,383

[1] http://www.carryoncomposting.com/416920203; [2] USU Analytical Lab

The duckweed was mixed with wood dust and an active compost from USU Food Waste Compost pile at a ratio of 1:2:2, respectively. The compost mix had a starting moisture content of approximartely 70% and a starting temperature of 23ºC

4.2.5.2 WAS Compost

WAS for composting was spiked with the 12 PPCPs at a starting concentration of approximately 15 µg/g dry weight. Composting was done in a black, eight sided tumbling composter (Fig. 7) and was operated as indicated above for the duckweed composting experiment. Table 11 shows the proportions, moisture content, and C:N ratios of WAS, USU Food Waste Compost and wood dust/cuttings combined to make the WAS compost.

WAS compost was made by mixing WAS, wood dust and USU Food Waste compost in a ratio of 1:1:1. The compost mix had a starting temperature of 25ºC and a moisture content of approximately 40 %. A small amount of water was added to the

compost to increase the moisture content to approximately 50%. Samples were collected from the compost mix right after mixing and every 3 days after that for the duration of the 21-day experiment. Temperature and oxygen in the compost mix were monitored every day throughout the composting period.

Table 11. Components of Compost Made from WAS from Hyrum WWTP.

	% Moisture	% C	% N	Mass Composted (Wet), g
USU Compost	50	10.2[2]	0.48[2]	2,800
Wood Dust/Cuttings(Honey Locust)	10	50[1]	0.1[1]	1,400
WAS	40	7.72[2]	1.26[2]	2,332
Final Compost Mixture	37	21.7	0.63	6,532

[1] http://www.carryoncomposting.com/416920203; [2] USU Analytical Lab

WAS compost samples were air-dried at a constant room temperature and ground into powder using a mortar and pestle. One g samples was measured and extracted with the EDGE automated extraction system using the same method used for the duckweed biosolids from the digester as described above in Method 1 for solids analysis. Caffeine, carbamazepine, DEET, estrone, gemfobrozil, triclosan, TCEP, sulfamethoxazole and fluoxetine were the compounds that were monitored in the WAS compost.

Temperature and oxygen levels in the two compost were monitored throughout the composting process which took 21 days. Temperature was monitored with a Reotemp compost thermometer (Reotemp Instruments, San Diego, California) and oxygen was monitored with Hti HT-1805 multigas detector (High Tech Instrument Co. Ltd., China) for the two compost types.

4.3 Quality Control

Individual standards were prepared by dissolving known masses of pure compounds in LC-MS grade methanol. These standard solutions were used to prepare all spike solutions and calibration curve standards. Lab Control Samples (LCS) samples were made to determine percent recovery of the PPCPs with the procedure. Triplicate samples were collected and used for PPCP analysis. Matrix spikes were done at the begining of the study to determine the PPCPs with better recoveries that would be monitored in the lab studies.

All glassware used for this experiment was washed with DI water one time and washed three times with methanol to eliminate all forms of contamination. DI water was obtained directly from the Milli Q DI dispenser. Interferences were checked by spiking directly into samples before running them in the EDGE instrument. Instrument drift of the LC-QQQ-MS was checked by running CCVs after every 10 samples. Blank samples were run between approximately every 10 samples to check for contamination or carryover during analysis.

CHAPTER 5

RESULTS AND DISCUSSIONS

5.1 QC Results

Drying Methods and Spike Recoveries.

Twelve PPCPs were originally investigated in this study (Table 4); however, only seven compounds, namely caffeine, carbamazepine, DEET, estrone, gemfibrozil, triclosan and TCEP, were monitored in the composted and anaerobically digested duckweed due to issues with reliable recovery and quantition of the other five compounds. In the WAS from the Hyrum WWTP, the aforementioned seven compounds in addition to fluoxetine and sulfamethoxazole, were spiked and monitored in the composted WAS, again due to recovery and quantitation issues for the remaining three compounds. The details of the compound recovery stuides are discussed below.

Solids processing was an important step in quantifying PPCPs in field samples and lab experiments. It was important to determine the optimum way of handling the solids from the compost or anerobic digester so that any loss in PPCPs due to solids handling would not be mistakenly attributed to contaminant degradation. WAS and duckweed from the field, as well as digestate and compost from the lab, had to be dried and powdered before extracting PPCPs for analysis. Using duckweed to represent all solids, and Method 1 for solids analysis as described in the Materials and Method section, duckweed was spiked at 200 ng/g and dried using four different methods to determine which method yielded the best PPCPs recovery efficiency. Samples of spiked duckweed were freeze-dried at -40^{o}C. Samples were also dried in the oven at 40^{o}C, while other samples were dried at a room temperature of 25 ^{o}C with a cover to isolate them from

room lighting, and a final set was air dried without a cover. As shown in Fig. 9, the PPCP recoveries from the spiked duckweed using the various drying methods were different.

There was no recovery of gemfibrozil from duckweed after oven drying at 40 ºC. β-Estradiol was not recovered in any of the drying methods even though it was spiked at the same level of 200 ng/g on the duckweed. The same amount of estrone was recovered from duckweed in all the different drying methods. For all compounds there was no statistical difference between PPCPs recoveries when duckweed was air-dried with light or without light.

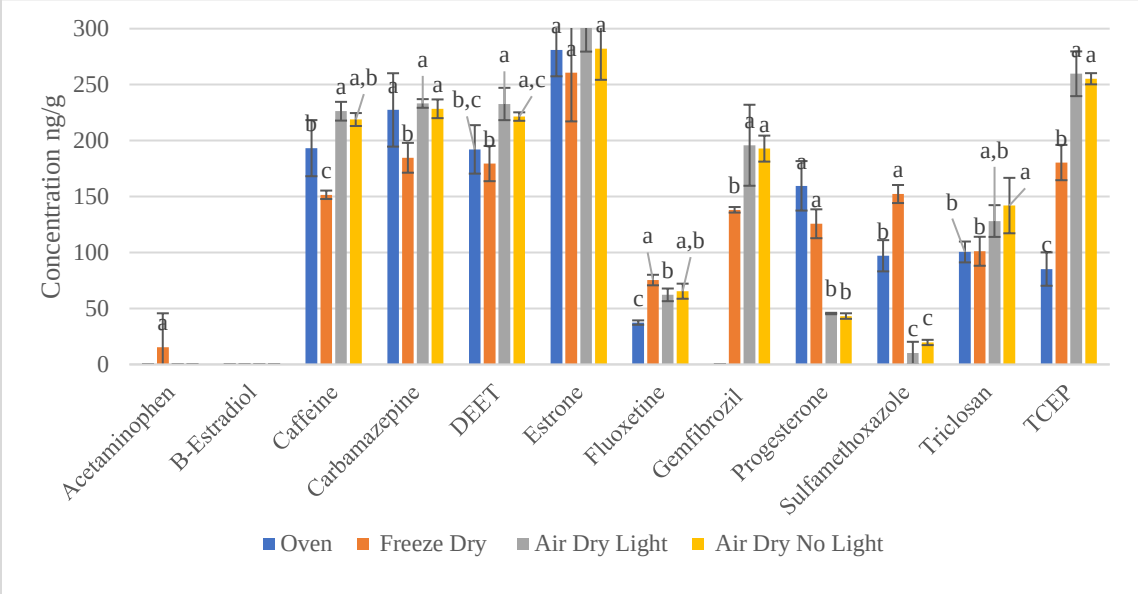

Fig. 9. PPCPs recovery on duckweed initially spiked to 200 ng/g using different drying methods (Error bars reperesent 95% confidence interval). Different letters for chemicals indicate statistical difference in drying methods using ANOVA and post hoc testing with Tukey's HSD.

Apart from progesterone and sulfamethoxazole, air-drying with and without light achieved higher PPCPs recoveries from duckweed as compared to oven drying and freeze-drying. Thus, air-drying with light was selected for drying solids samples from the

field and the lab due to the ease of drying and the higher recoveries of PPCPs. Air-drying with or without light took 2 weeks to complete, and the moisture content was found to be approximately 10% after drying.

The moisture content of air-dried duckweed in the dark using a box cover was slightly higher than the moisture content in duckweed air-dried with no cover. It took about a week for duckweed to be completely dry in the oven whereas it took about 3 weeks for duckweed to completely freezedry. The moisture content in oven dried duckweed and freezedried duckweed were about the same at approximately 5%.

Table 12 shows the method detection limits of the methods used for analysis of the PPCPs in this study. Method 2 was used to detect PPCPs found at much lower concentrations in the field and laboratory samples. WAS and duckweed samples from the field had low levels of PPCPs. For this reason Methods 1 and 2 were run on both solids and liquids in order to achieve high recoveries and lower detection limits.

In Method 2 for both solids and liquids, samples were concentrated before analysis in the LC-QQQ-MS. However, Method 1 for both solids and liquids had higher recoveries for PPCP spikes as shown in Tables 13, 14, 15 and 16. In reporting results for field samples, detectable concentrations of PPCPs were first selected from Method 1 due to their high recoveries in matrix spikes. In cases where the PPCP concentrations were below the method detection limits of Method 1, detectable concetrations from Method 2 were selected. This was not the case for lab samples. In lab samples only Method 1 was used for both solids and liquids since samples had been initially spiked at high concentrations and could be detected easily throughout the studies using Method 1.

Table 12. Method Detection Limits for the Methods used to Process and Analyze PPCPs in Solids and Liquids.

PPCP	Solids		Liquids	
	Method 1 (ng/g)	Method 2 (ng/g)	Method 1 (ng/L)	Method 2 (ng/L)
Acetaminophen	3.54	0.31	17.5	0.35
β-Estradiol	47.0	4.11	232	4.6
Caffeine	2.00	0.17	9.9	0.20
Carbamazepine	2.00	0.17	9.9	0.20
DEET	11.0	0.96	54.1	1.1
Estrone	136	11.9	672	13.4
Fluoxetine	1.20	0.10	5.9	0.12
Gemfibrozil	51.9	4.54	256	5.1
Progesterone	1.96	0.17	9.7	0.20
Sulfamethoxazole	3.32	0.29	16.4	0.33
Triclosan	61.2	5.36	302	6.0
Tris 2 chloroethyl phosphate	39.0	3.42	193	3.9

Tables 13 and 14 show the matrix spike recoveries of PPCPs for effluents from the Wellsvilles sewage lagoons and the Hyrum WWTP spiked at 1.5 µg/L, respectively. As seen above, β-Estradiol was not detected by Method 1 for liquids at the level at which it was spiked. This may be due to an interference or a possible transformation of β-Estradiol into other compounds. Estrone was not recovered in the the matrix spikes in Hyrum WWTP effluent but it was detected in the Wellsville sewage lagoons effluent matrix spikes. The concentration method for liquids (Method 2) enabled the detection of the spikes in the matrices of the Wellsville sewage lagoons and Hyrum WWTP effluent for all 12 compounds. However, the recoveries of PPCPs in the the matrices of the two effluents were generally higher in Method 1 than in Method 2.

Table 13. Percent Matrix Spike Recovery (mean ± 95% CI) from Wellsville Sewage Lagoon Effluent (n=3) Spiked at 1.5 ug/L.

	Method 1, (%)	Method 2, (%)
Acetaminophen	94.4±4.13	52.0±2.84
β-Estradiol	ND	59.2±3.42
Caffeine	88.0±5.26	66.0±2.73
Carbamazepine	91.8±7.9	74.9±2.50
DEET	86.3±1.53	65.4±3.62
Estrone	113±46.1	50.9±9.78
Fluoxetine	71.8±4.11	50.9±2.19
Gemfibrozil	81.7±24.5	86.4±8.49
Progesterone	86.6±5.8	53.2±2.40
Sulfamethoxazole	109±5.80	72.2±3.33
Triclosan	60.7±7.24	42.8±4.92
TCEP	94.1±6.58	72.3±3.61

Table 14. Percent Matrix Spike Recovery (mean ± 95% CI) from Hyrum WWTP Effluent (n=3) Spiked at 1.5 ug/L.

	Method 1, (%)	Method 2, (%)
Acetaminophen	111±4.13	45.4±0.77
β -Estradiol	ND	62.2±1.72
Caffeine	92.4±2.20	43.0±0.06
Carbamazepine	90.7±0.68	61.2±1.99
DEET	144±3.90	86.8±2.31
Estrone	ND	49.2±4.73
Fluoxetine	70.7±1.43	42.9±4.49
Gemfibrozil	98.6±18.3	82.1±0.79
Progesterone	91.1±1.62	66.9±2.34
Sulfamethoxazole	264.7±11.7	180±2.48
Triclosan	76.4±9.34	70.6±1.53
TCEP	95.8±1.82	61.1±4.31

WAS and duckweed from the field were spiked at 2,000 ng/g and air dried in the room uncovered. As shown in Table 15, Method 1 recoveries of PPCPs from the duckweed matrix were higher than recoveries by Method 2.

Table 15. Percent Matrix Spike (mean ± 95% CI) Recovery from Duckweed (n=3) Spiked at 2 ug/g.

	Method 1, (%)	Method 2, (%)
Acetaminophen	0.26±0.03	0.03±0.01
β-Estradiol	ND	ND
Caffeine	91.5±1.69	55.8±2.25
Carbamazepine	103±2.16	66.6±4.66
DEET	83.7±5.15	51.0±3.30
Estrone	99.2±3.36	55.0±4.38
Fluoxetine	22.6±1.73	13.7±3.13
Gemfibrozil	91.2±10.3	50.8±1.15
Progesterone	5.20±0.30	2.42±0.07
Sulfamethoxazole	6.56±0.30	2.70±0.03
Triclosan	62.7±3.43	28.0±4.28
TCEP	92.4±3.44	49.5±5.35

The chemicals that had better matrix spike recoveries in the duckweed when spiked at 2 ug/g with PPCPs were the same chemicals that had better recoveries from duckweed spiked with PPCPs at 0.2 ug/g (Fig. 9; Table 15). β-Estradiol was not detected in Method 1. Table 16 shows similar results for WAS solids as β -Estradiol was not detected on the WAS matrix using Method 1 or 2 for solids. Moreover, acetaminophen was not detected in WAS using Method 1, and Method 1 recoveries for both matrices were generally higher than Method 2 recoveries (Table 15 and 16). Caffeine, carbamazepine, DEET, estrone, gemfibrozil, triclosan and TCEP were monitored in duckweed compost and anaerobic digester experiments due to their consistent higher recoveries in both solid and liquid matrices (Table 13 and 1). Sulfamethoxazole and

fluoxetine, in addition to the PPCPs monitored in duckweed compost, were selected for monitioring in WAS compost due to their high recoveries in the WAS matrix (Table 16).

Table 16. Percent Matrix Spike (mean ± 95% CI) Recovery from WAS (n=3) Spiked at 2 ug/g.

	Method 1, (%)	Method 2, (%)
Acetaminophen	ND	0.04±0.08
β -Estradiol	ND	ND
Caffeine	74.2±4.09	61.7±4.63
Carbamazepine	80.3±7.32	63.6±6.13
DEET	84.9±3.57	57.4±2.79
Estrone	141±18.5	113±14.1
Fluoxetine	87.4±15.8	60.1±6.92
Gemfibrozil	113±10.3	88.3±6.67
Progesterone	21.8±1.83	11.8±1.08
Sulfamethoxazole	62.1±3.56	18.9±1.35
Triclosan	141±20.5	88.1±8.31
TCEP	76.5±5.86	57.9±2.76

5.2 Field Sampling Results

5.2.1 Wellsville Sewage Lagoons

Wellsville sewage lagoons discharge effluent from the treatment facility to the Little Bear River from October to April. For 3 months (June, July and August) the effluent is applied to the surrounding farmlands. When the effluent is not let into the Little Bear River or applied to land, the lagoon system retains the treated sewage in Cell 4.

As the influent moves through the cells of the lagoons, biological activity and uptake or sorption to duckweed are the main mechanisms that remove contaminants from the wastewater (Farrell 2012). The surface of the lagoons is also exposed to the atmosphere and direct sunlight so there is also a potential for photodegradation. The

duckweed that grows in each cell does not transfer across the cells but the liquid does. In Fig. 10 and 11 the concentrations of PPCPs in influent, effluent and pond water reatined retained in Cell 4 grab samples are shown. Apart from β-estradiol, estrone and gemfibrozil, all other chemicals considered in this study were detected in duckweed and sediments (Fig. 12).

PPCPs that were being removed from the liquid were accummulating on the solids or transforming in the liquid. PPCPs that are removed from the liquid and are not detected on either duckweed or sediments indicate transformation.

Neutral hydrophobic compounds (β-estradiol, carabamazepine, estrone, progesterone, triclosan)

β-Estradiol, estrone, progesterone and triclosan were completely removed from the effluent to concentration below the detection limit (Table 17; Fig. 10) in 6/5/2019.

Table 17. Percent removal (mean ± 95% CI) of PPCPs from the Liquid Phase in the Wellsville Sewage Lagoons.

Percent Removal of PPCPs		
	6/5/2019	8/15/2019
β-Estradiol	100±0.91	ND
Carbamazepine	NSD	15.1±6.58
Estrone	100±19.4	ND
Progesterone	100±56.2	ND
Triclosan	100±0.58	100±1.36
Acetaminophen	100±3.97	100[b]±8.96
Caffeine	100±4.10	99.6±0.09
DEET	97.1±0.56	98.5±18.8
TCEP	NSD	NSD
Gemfibrozil	NSD	NSD
Sulfamethoxazole	32.3±1.81	96.1±2.17
Fluoxetine	98.7±0.6	76.8±2.67

ND: Not detected in influent and effluent; NSD: No significant removal.
[b] Based on imputed values for cell 4 retained liquid

β-Estradiol, estrone, and progesterone were not detected in the influent for the 8/15/209 sampling event (Table 17, Fig. 11).Duckweed -water partition coeffecients could not be determined for β-estradiol and estrone due to their non-detection in effluent and duckweed (Table 19 and 20).

Estrone and β-Estradiol are examples of PPCPs that showed likelihood of transformation due to their lack of detection in any compartment in the cells of the lagoons.

Carbamazepine was poorly removed from liquid and did not have significant duckweed-water partition coeffecient, but had significant sediment-water partition coeffecient in cells 1, 2 and 3 (Tables 18 and 19). Progesterone was detected in duckweed and sediment (Fig. 12) but not in pond water whereas triclosan had significant duckweed-water partition coeffecient in Cells 2, 3, and 4 and significant sediment-water partition in Cell 4.

Neutral hydrophilic compounds (acetaminophen, caffeine, DEET, TCEP)

The commonly used painkiller, acetaminophen, had the highest concentration in the influent. It was followed by caffeine commonly found in coffee, soda, tea, chocolate, etc. DEET an insect repellent commonly used during the summer, had the next highest concentration in the influent wastewater (Fig. 10 and 11).

Acetaminophen, caffeine, and DEET, although seen in high concentrations in the influent, were significantly reduced in the effluent liquid, thus had high percent removals (Table 17). As seen in Fig. 10, TCEP had poor removal from the liquid and significant duckweed-water and sediment-water partition coeffecient in all cells. Fig. 11 shows the

levels of PPCPs in the liquids as wastewater moves across the cells. Acetaminophen did not have a significant duckweed-water partition coeffecient but had significant sediment-water partitioning in only Cell 4 (Table 17). Caffeine did not have significant duckweed-water partition coeffecient but had a significant sediment-water partition coeffecient in Cell 3. DEET had a significant duckweed water-coeffecient in Cell 4 and a significant sediment water partiton in Cells 1, 3 and 4.

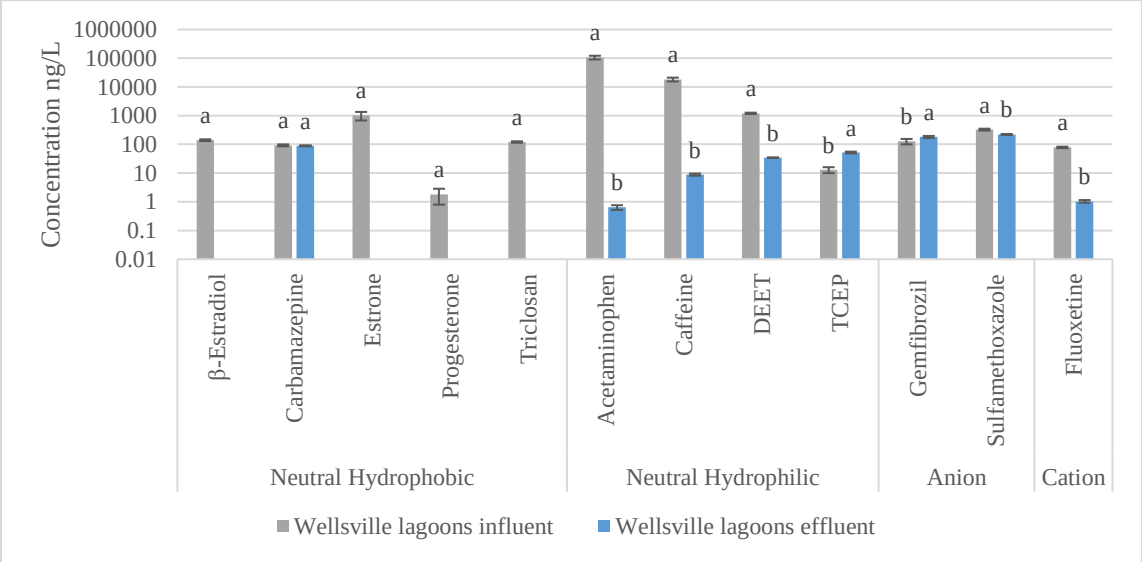

Fig. 10. PPCP concentration in influent and effluent collected from Wellsville sewage lagoons on 6/5/2019. Error bars represent 95% confidence intervals of replicate measurements. Water was not collected from the cells. Different letters for chemicals indicate statistical difference in concentrations using ANOVA and post hoc testing with Tukey's HSD.

Anionic compounds (gemfibrozil, sulfamethoxazole)

Gemfibrozil and sulfamethoxazole persisted in the liquid (Fig. 10; Table 17). Gemfribrozil was only detected in pond water and was never detected in duckweed or sediments (Fig.11 and Fig 12). Sulfamethoxazole although detected in duckweed and sediments at low concentrations, did not have significant duckweed-water or sediment-

water partition coeffecients (Tables 18 and 19). Electrostatic repulsion could be the reason for nondetection of gemfibrozil in duckweed and sediments.

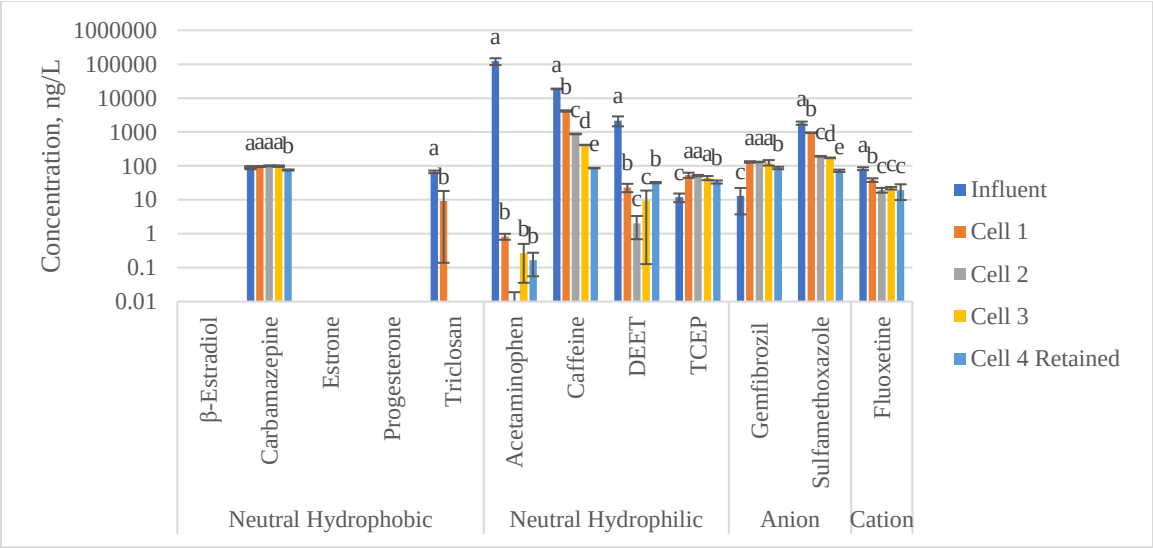

Fig. 11. PPCP concentration in influent, effluent and water from cells in Wellsville sewage lagoons on 8/15/2019. Error bars represent 95% confidence intervals of replicate measurements. Different letters for chemicals indicate statistical difference in concentration using ANOVA and post hoc testing with Tukey's HSD.

Table 18. Duckweed-water Partition Coefficient (mean ± 95% CI) of PPCPs in Wellsville Sewage Lagoons (n=3).

Duckweed-water partition coefficient (L/g)				
	Cell 1	Cell 2	Cell 3	Cell 4
β-Estradiol	NA	NA	NA	NA
Carbamazepine	*NP*			*NP*
Estrone	NA			
Progesterone	D			
Triclosan	*NP*	2.07±0.80	0.332±0.031	
Acetaminophen	*NP*	*NP*	*NP*	*NP*
Caffeine	*NP*	*NP*	*NP*	*NP*
DEET	*NP*	*NP*	*NP*	0.044±0.029
TCEP	0.357± 0.041		0.267±0.055	0.396±0.030
Gemfibrozil	W			
Sulfamethoxazole	*NP*	*NP*	*NP*	*NP*
Fluoxetine	0.517±0.201	0.356±0.052	*NP*	

NA: Not detected in either duckweed or water; W: Not detected in duckweed but detected in water; D: Not detected in water but detected in duckweed.
NP: partition coefficient is not significant

Cationic compound (fluoxetine)

Fluoxetine had high removal efficiency from the liquid in the two sampling events (Fig. 10). Fluoxetine had a significant duckweed-water partition coeffecient in Cells 1 and 2 and a significant sediment-water partition coefficient in Cells 1, 2, and 3 (Tables 18 and 19). This may be due to cation exchange between the negatively charged solid surfaces and the positively charged fluoxetine.

Table 19. Sediment-water Partition Coefficient (mean± 95% CI) of PPCPs in Wellsville Sewage Lagoons (n=3).

Sediment-water partition coefficient (L/g)				
	Cell 1	Cell 2	Cell 3	Cell 4
β-Estradiol	NA			
Carbamazepine	0.13±0.01	0.07±0.01		*NP*
Estrone	NA			
Progesterone	S			
Triclosan	*NP*	*NP*		3.64±1.60
Acetaminophen	*NP*	*NP*	*NP*	1.57±0.43
Caffeine	*NP*	*NP*	0.02±0.01	*NP*
DEET	0.36±0.13	*NP*	1.47±0.92	0.12±0.01
TCEP	0.27±0.04			0.40±0.03
Gemfibrozil	*W*			
Sulfamethoxazole	*NP*	*NP*	*NP*	*NP*
Fluoxetine	1.91±0.28	0.58±0.05		*NP*

NA: Not detected in either duckweed or water. W: Not detected in sediments but detected in water; S: Not detected in water but detected in sediments.
NP: partition coefficient is not significantly different from zero.

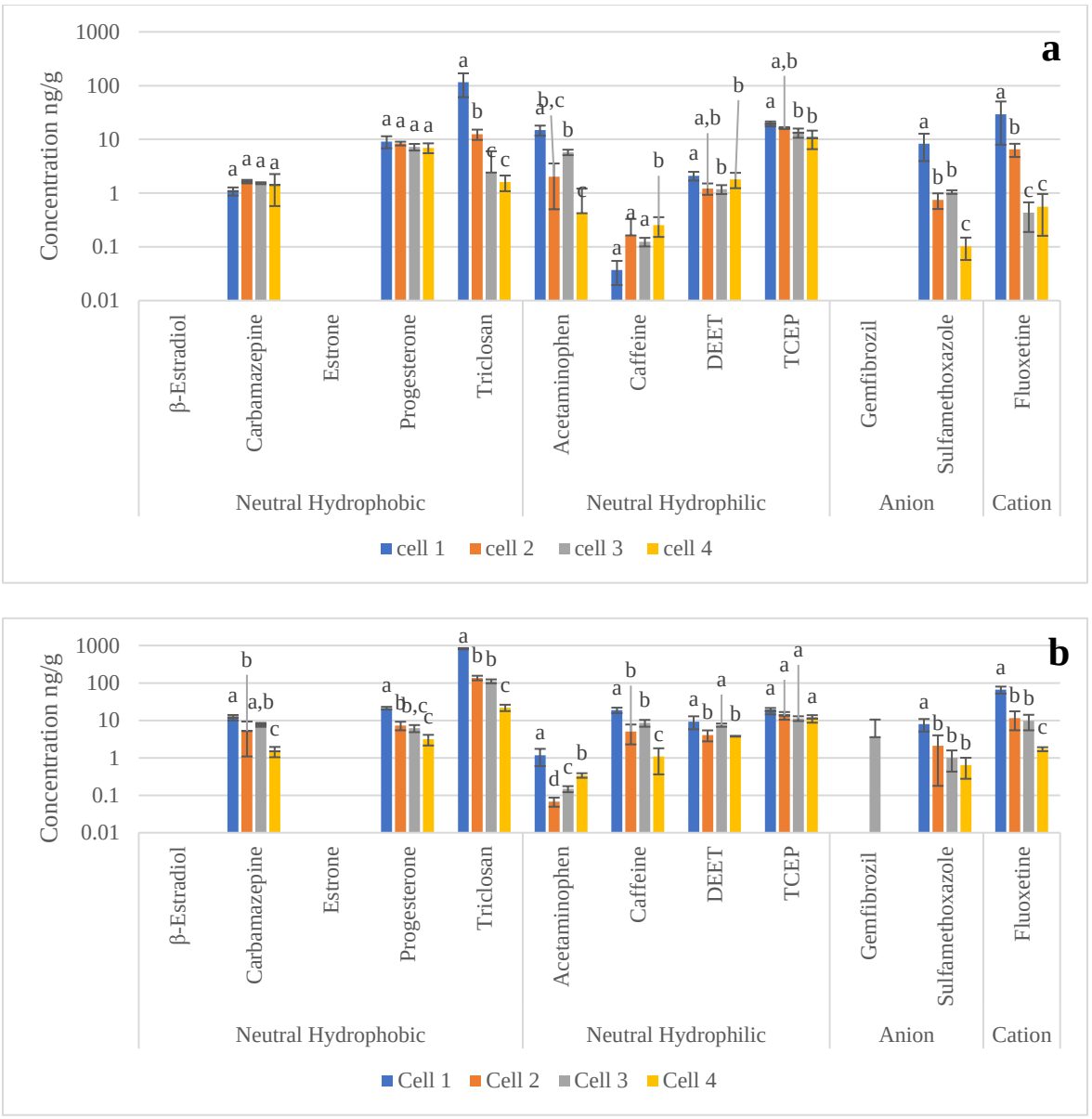

Fig. 12. PPCP Concentrations in: (a) Duckweed (b) Sediments From Wellsville Sewage Lagoons (8/15/19). Error bars represent 95% confidence interval and different letters for chemicals represent statistical difference in concentration using ANOVA and post hoc testing with Tukey's HSD.

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5.2.2 Hyrum WWTP

In the two sampling events at the Hyrum WWTP, the difference in treatment was associated with different chemical addition processes. During the 4/1/2019 sampling event phosphorus removal and coagulation was being done with alum, while for the

6/5/2019 sampling event, coagulation was being done with T-floc and alum. Table 20 shows the removal efficiencies of PPCPs from the Hyrum WWTP.

Table 20. Removal of PPCPs (mean ± 95% CI) from the Effluent of the Hyrum WWTP Based on Influent and Effluent Concentration Measurements.

	% Removal of PPCPs	
	4/1/2019	6/5/2019
β-Estradiol	NA	NA
Carbamazepine	13.7±2.0	NSD
Estrone	100±2.5	ND
Progesterone	100±15.6	99.5±0.2
Triclosan	71.1±1.04	100±0.97
Acetaminophen	99.6±0.97	99.9±0.02
Caffeine	92.2±0.23	99.9±0.19
DEET	93.1±55.8	78.9±0.11
Tris-(2-chloroethyl) Phosphate	NSD	NSD
Gemfibrozil	NSD	ND
Sulfamethoxazole	NSD	30.7±2.00
Fluoxetine	76.5±7.28	81.9±1.77

NA: Influent and Effluent<MDL;ND: Influent and Effluent statistically the same; NSD: No significant removal.

Fig. 13, 14 and 15 shows the concetrantions of PPCPs detected in influent, effluent and WAS, respectively. Acetaminophen, caffeine and DEET were detected at high levels in the influent since they are found in commonly used drugs or personal care products (Fig. 13).

The use of DEET, gemfibrozil and caffeine increased during the second sampling event when the weather was warmer. The increase in DEET in the influent could be explained by the increase in insect repellent usage by people in Hyrum to fight insects during the summer. Cationionic (fluoxetine), anionic (sulfamethoxazole and gemfibrozil) and neutral hydrophilic PPCPs (acetaminopen, caffeine, DEET, TCEP) were consistently detected in the effluent whereas neutral hydrophobic PPCPs were inconsistently detected

(Fig. 14). In Fig. 15 all studied PPCPs apart from β-estradiol, estrone and gemfibrozil were detected in the WAS from Hyrum WWTP.

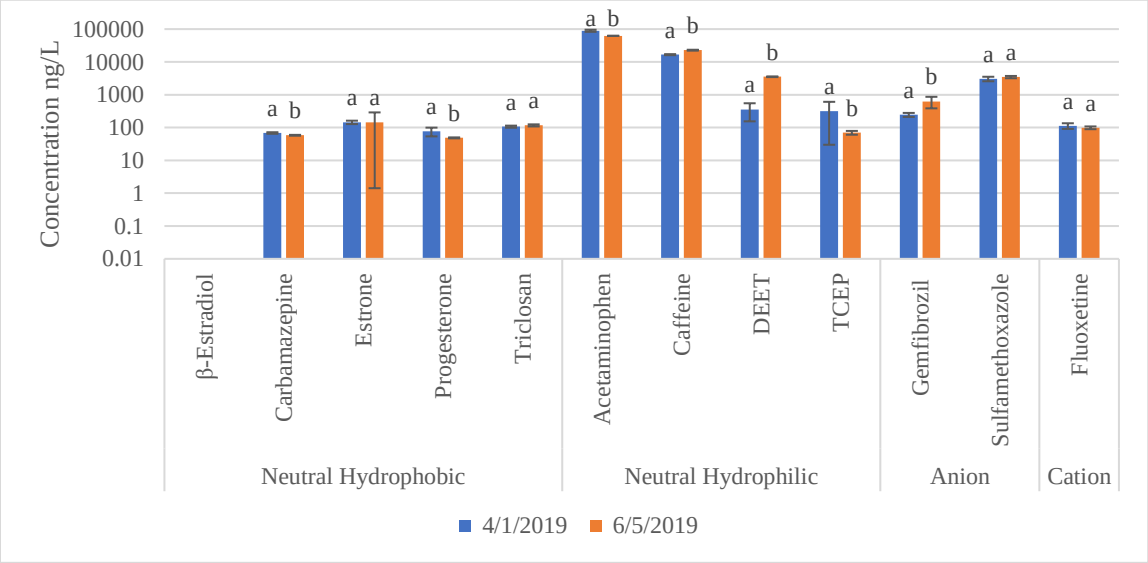

Fig. 13. PPCP cocentrations in the influent of the Hyrum wastewater treatment plant. Error bars represent 95% confidence interval and different letters for chemicals represent statistical difference in concentration using ANOVA and post hoc testing with Tukey's HSD.

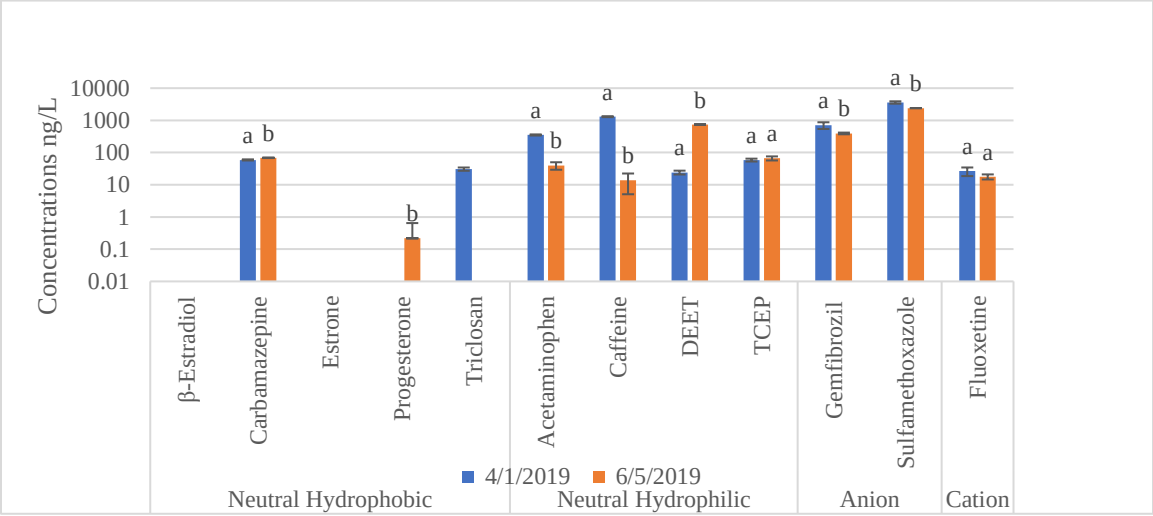

Fig. 14. PPCP concentration in the effluent of Hyrum wastewater treatment plant. Error bars represent 95% confidence interval and different letters for chemicals represent statistical difference in concentration using ANOVA and post hoc testing with Tukey's HSD.

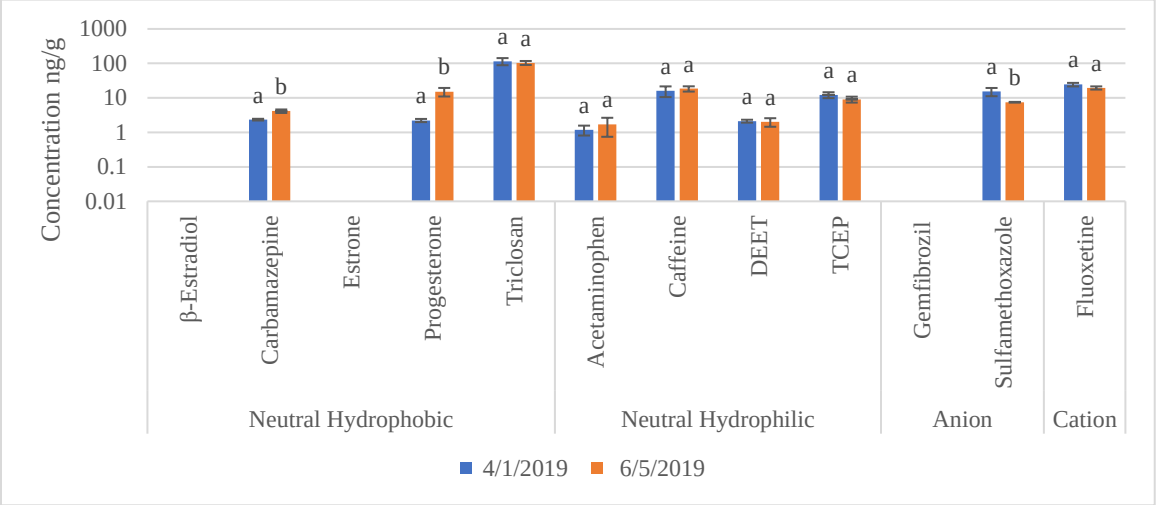

Fig. 15. PPCP concentration in WAS from the Hyrum wastewater treatment plant. Error bars represent 95% confidence interval and different letters for chemicals represent statistical difference in concentration using ANOVA and post hoc testing with Tukey's HSD.

EPI Suite model STPWIN (USEPA 2012) was also used to predict the removal of chemicals from the effluent of a conventional sewage treatment plant and the partitioning of chemicals between WAS and the effluent liquid (Fig. 16 and 17).

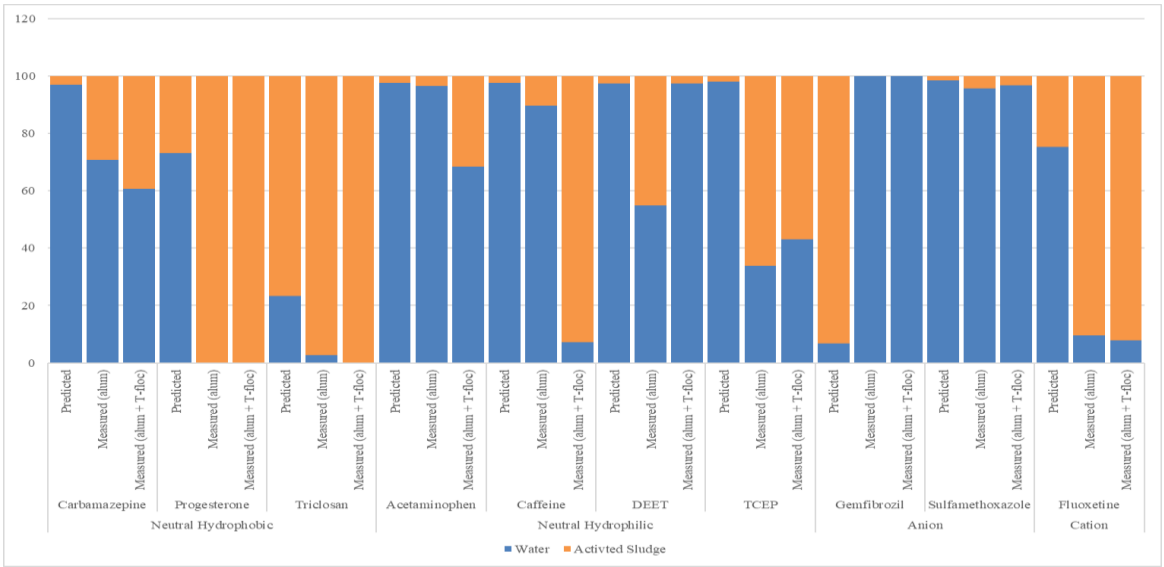

Fig. 16. Predicted and measured distributions of PPCPs between solid and liquid phase of the Hyrum WWTP.

This model uses a fugacity approach to predict the fate of chemicals based on standard operating parameters for a conventional secondary wastewater treatment plant (SWTP). The flowrate, MLSS concentration, tank volume and configuration of the SWTP used by EPI Suite are different from that of Hyrum's WWTP and the program does not allow parameters to be changed to model a specific SWTP.

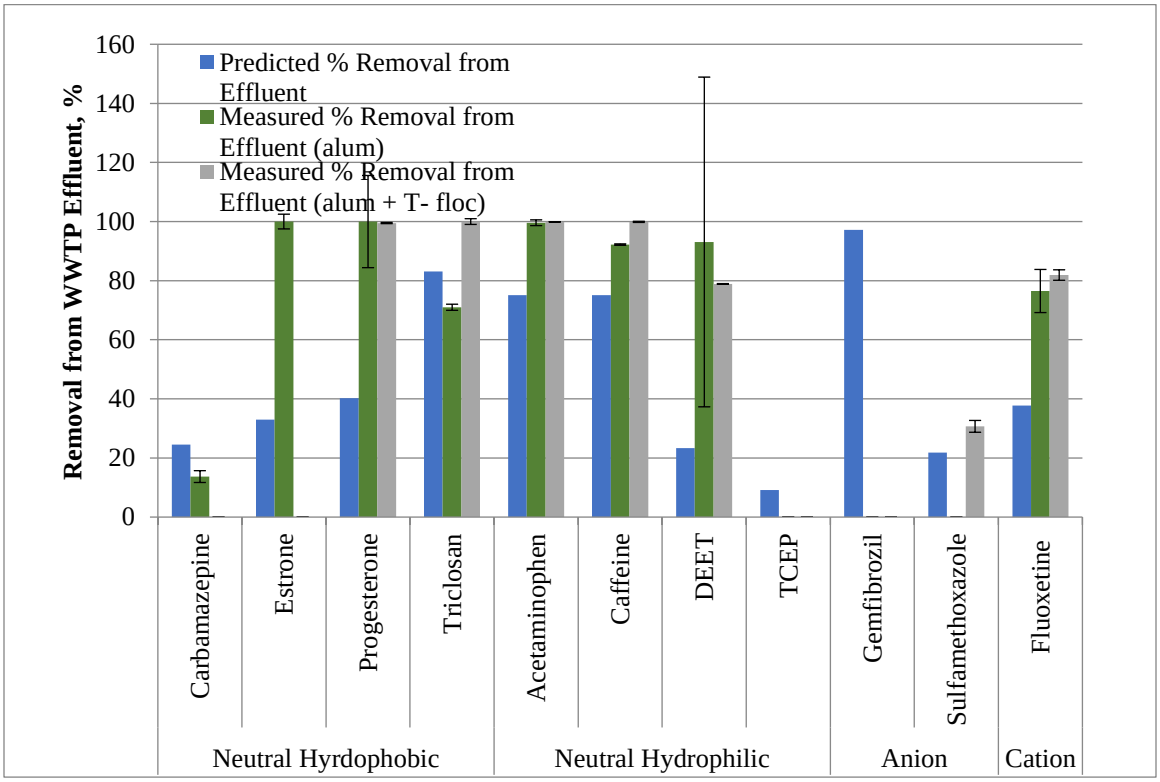

Fig. 17. PPCP removal efficiencies from the wastewater based on prediction by STPWIN versus measured values from the Hyrum WWTP. Error bars represent 95% confidence interval.

The main difference between the model and the Hyrum WWTP is chemical addition. The addition of the chemicals to enhance coagulation and flocculation for P removal and reduction in membrane fouling may have improved sorption of some chemicals since the percent distribution of chemicals on the solids measured were

generally higher than what was predicted by the model (Fig. 16). There was also significantly increased solids concentrations (9,000 mg/L) compared to conventional activated sludge systems (2,500 mg/L) for adsorption of chemicals due to increased sludge formation. Table 21 shows the partitioning of PPCPs between WAS and effluent liquid from the Hyrum WWTP.

Table 21. Partitioning of PPCPs (mean ± 95% CI) between WAS and Effluent Liquid at the Hyrum WWTP.

Hyrum WWTP Sludge/Water Partitioning Coefficient (L/g)		
	4/1/2019	6/5/2019
β-Estradiol	NA	
Carbamazepine	0.04±0.03	0.07±0.003
Estrone	NA	
Progesterone	S	
Triclosan	3.56±1.40	S
Acetaminophen	*NP*	*NP*
Caffeine	*NP*	1.26±0.39
DEET	NA[a]	*NP*
Tris-(2-chloroethyl) Phosphate	0.17±0.07	
Gemfibrozil	W	
Sulfamethoxazole	*NP*	*NP*
Fluoxetine	1.10±0.09	

NA: Not detected in sludge and water;
W : Not detected in sludge but detected in water; S: Not detected in water but detected in sludge

A mass balance was carried out on the PPCPs in the Hyrum WWTP using the following parameters: volume of plant tankage; 0.229 MG, flow rate of influent, 1.38 MGD (4/1/2019), 0.925 MGD (6/5/2019); flowrate of WAS, 0.025 MGD; MLSS, 9,388 mg/L (4/1/2019), 9,731 mg/L (6/5/2019); HRT, 0.229 days. The results of these calculations are shown in Table 22.

Table 22. Percent Degraded and Distribution of PPCPs (mean ± 95% CI) Based on Mass Balance in Hyrum WWTP.

	4/1/2019 (alum only)			6/5/2019 (alum + T-floc)		
PPCPs		% Distribution			% Distribution	
	%Degraded	Solids	Liquid	%Degraded	Solids	Liquid
β-Estradiol	NA	NA	NA	NA	NA	NA
Carbamazepine	*NSD*	29.2±0.06	70.8±0.04	*NSD*	39.4±0.01	60.6±0.004
Estrone	100±1.43	NA	NA	*NSD*	NA	NA
Progesterone	77.7±7.54	100±0.01	NA	*NSD*	99.9±0.04	NA
Triclosan	*NSD*	97.2±0.03	2.8±0.53	*NSD*	100±0.10	NA
Acetaminophen	99.4±0.98	3.4±1.08	96.6±0.07	99.8±0.02	31.5±2.49	68.5±1.68
Caffeine	86.7±0.12	10.3±0.22	89.7±0.05	99.0±0.19	92.9±0.07	7.1±0.78
DEET	85.9±28.5	45.1±0.30	54.9±0.32	48.8±0.14	2.6±0.15	97.4±0.01
Tris-(2-chloroethyl) Phosphate	*NSD*	66.1±0.05	33.9±0.11	*NSD*	57.0±0.17	43.0±0.26
Gemfibrozil	*NSD*	NA	100±2.67	*NSD*	NA	100±0.18
Sulfamethoxazole	*NSD*	4.2±3.83	95.8±0.32	*NSD*	3.3±0.03	96.7±0.002
Fluoxetine	*NSD*	90.5±0.03	9.5±0.24	*NSD*	92.2±0.01	7.8±0.09

NA: Not available due to non-detection of chemical; NSD: Not degraded.

Neutral hydrophobic compounds (β-estradiol, carabamazepine, estrone, progesterone, triclosan)

β-Estradiol was not detected in the influent and effluent so no WAS-water partition coeffecient could be calculated. Progesterone (neutral with high Log K_{ow} 3.87) and triclosan (neutral with high Log K_{ow} 4.7) persisted in the solids. Removal of progesterone and estrone from the effluent were higher than what was predicted by STPWIN. Carbamazepine and triclosan had same removal efficiency from the liquid as predicted by STPWIN. Mass balance results showed that estrone transformed while progesterone and triclosan accummulated in the WAS. Carbamazepine however, persisted and accummulated in the liquid phase.

Progesterone and triclosan all had above 70% removal from the Hyrum WWTP effluent (Table 20) in both sampling events which could be attributed to accummulation in solids. Progesaterone was exclusively detected in WAS whereas triclosan had a significant WAS-water partition coeffecient in 4/1/2019 samples and was exclusively measured in WAS on 6/5/2019. Carbamazepine also had a significant WAS-water partition coeffecient.

Neutral hydrophilic compounds (acetaminophen, caffeine, DEET, TCEP)

Acetaminophen, caffeine, and DEET, had above 70% removal from the Hyrum WWTP influent (Table 20) in both sampling events. The removal of caffeine and acetaminophen from the effluent was the same as was predicted by STPWIN but the model predicted lower removal efficiency for DEET. TCEP was poorly removed from the effluent as predicted by STPWIN and persisted in the WAS. Acetaminophen and DEET did not have significant WAS-water partition coeffecients. TCEP had a significant WAS-water partition coeffecient for the two sampling events and caffeine had a significant WAS-water partition coefficient only when t-floc and alum were used together.

The effluent and WAS from the two sampling events were compared to determine any effect from different chemical additions (Fig. 14 and 15, Appendix E). The use of alum and t-floc chemical together had no effect on TCEP levels in the effluent. Acetaminophen and caffeine decreased in effluent after t-floc/alum combination whilst DEET increased. Mass balance results showed that removal of acetaminophen, caffeine and DEET was due to transformation into daughter products.

Anionic compounds (gemfibrozil, sulfamethoxazole)

Gemfibrozil was not detected in any of the WAS samples, which is most likely due

to its negative charge inducing electrostatic repulsion. Gemfibrozil (negatively ionized, log D_{ow}=2.2) and sulfamethoxazole (mostly negatively ionized, log D_{ow}=-4.4) persisted in higher amounts in the liquid. Gemfibrozil was predicted by the model to be highly removed from the effluent, however, measurements at Hyrum WWTP showed that it was poorly removed from the effluent (Fig.17). Sulfamethoxazole did not have a significant WAS-water partition coeffecient and the removal efficiency from the liquid, when both alum/T-floc were used, was higher than what was predicted by STPWIN.

Sulfamethoxazole levels in the WAS was lower when T-floc/alum was used. (Fig. 15). Gemfibrozil was only detected in water but not in WAS. Mass balance analysis showed that the two chemicals persist in the liquid phase and do not degrade (Table 22).

Cationic compouns (fluoxetine)

Fluoxetine had above 70% removal from the Hyrum WWTP effluent (Table 21) in both sampling events. It had a significant WAS-water partition coeffecient and the removal efficiency from the effluent was higher than what was predicted by STPWIN. The addition of T-floc with alum had no effect on fluoxetine levels in the effluent (Table 22). Mass balance results showed that fluoxetine persisted on the solids, which indicates that removal from the liquid phase was due to accummulation on the solids rather than to biodegradation in the liquid.

5.3 Laboratory Studies

5.3.1 Anaerobic Digestion of Duckweed

The active volume in the reactor was found to be 2.4 L from the concentration of bromide in the reactor after mixing for 24 hr. Dividing 2.4 L by 0.6 L/every 3 days gives a

theoretical hydraulic retention time of 12 days. However, using tracer analysis from the potassium bromide tracer, the retention time was found to be approximately 10 days. Since 99% of the tracer is expected to leave the reactor in three hydraulic retention times, the tracer was monitored in the effluent liquid over this time interval (30 days). There was approximately 110% recovery of bromide from the lab-scale anaerobic digester over the three retention times. Background concentrations of bromide in duckweed were found to be approximaately 1 mg/g of dry duckweed, and were accounted for in the bromide mass balance calculations.

Generally, for the PPCPs that were monitored in the anaerobic digester, their background concentrations were 1,000 times lower than the concentrations spiked on the duckweed that was pulse-fed into the reactor at the beginning of the study. Table D.5 in Appendix D shows these background PPCP concentrations in the duckweed feed.

Fig. 18 shows the washout of bromide from the anaerobic digester. Bromide was used as a non-reactive tracer to compare to the washout of PPCPs from the reactor in order to quantify the degradation of the PPCPs due to anaerobic digestion.

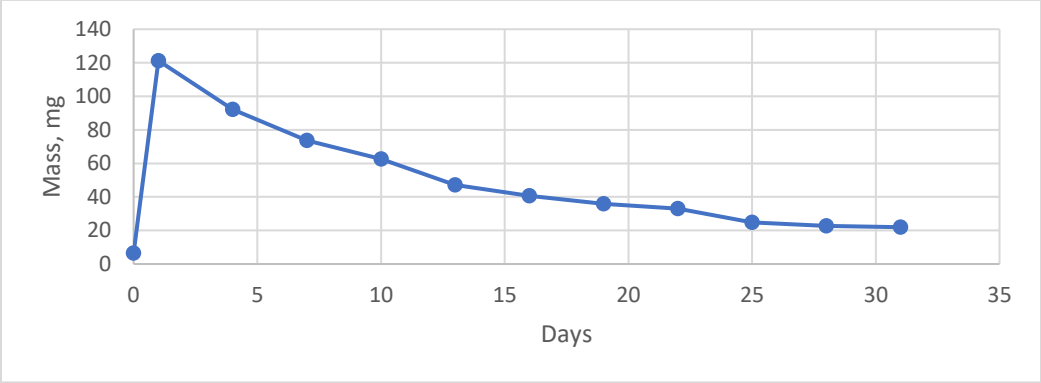

Fig. 18. Washout curve of bromide tracer from anaerobic digester.

Table 23 shows the first order transformation rates and half-lives of PPCPs spiked in duckweed in the lab-scale anaerobic digester. By comparing the washout rates of chemicals to the washout rate of bromide using overlapping confidence intervals, caffeine was the only compound that showed a first order washout rate statisitically different from the washout rate of the bromide tracer (Fig 19). Individual PPCP transformation rates, k*, were calculated by subtracting the Br tracer washout rate from individual PPCP washout rates.

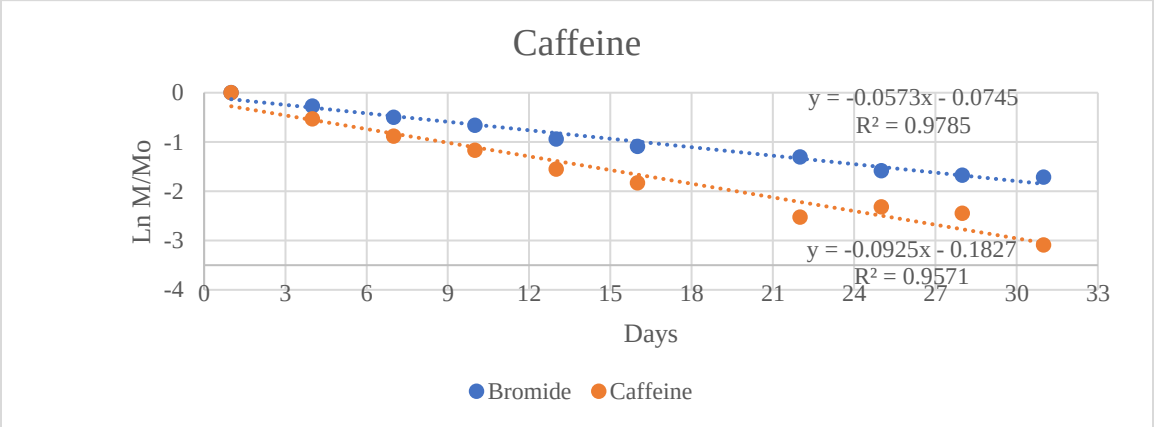

Fig. 19. First order linear regression of of caffeine and bromide washout from lab-scale anaerobic digester.

TCEP and caffeine showed a negative transformation rate although washout rate of the former was not statistically different from washout rate of bromide based on 95 % CI. These result are similar to what was predicted by EPI Suite BIOWIN 7 as shown in Table 6 except that the predicted transformation cannot be confirmed for TCEP based on statistics. All other compounds showed first order washout rates from the anaerobic digester statistically the same as the first order washout rate of bromide based on overlapping washout rate confidence intervals of bromide and the PPCPs. DEET and other neutral hydrophilic, PPCP, did not transform in the anaerobic digester. Anionic gemfibrozil and neutral hydrophobic estrone, carbamazepine and triclosan also did not

transform. Sulafmethoxazole was not monitored monitored due to poor matrix spike recoveries in duckweed (Appendix A, Table A.3).

Table 23. Transformation Rates and Half-lives (mean ± 95% CI) of PPCP Spiked Duckweed under Anaerobic Conditions.

Chemical	k (day^{-1})	r^2	k* (day^{-1})	n	$t_{1/2(upper)}$ (day)	$t_{1/2(mean)}$ (day)	$t_{1/2(lower)}$ (day)	Significant degradation
Br	-0.06±0.01	0.9857	-	10		-		-
Carbamazepine	-0.04±0.01	0.9052	-	10		-		No
Estrone	-0.02±0.03	0.2291	-	10		-		No
Triclosan	-0.03±0.05	0.2338	-	10		-		No
Caffeine	-0.09±0.01	0.9783	-0.03±0.004	10	26.5	23.3	20.7	Yes
DEET	-0.05±0.01	0.959	-	10		-		No
TCEP	-0.06±0.01	0.9719		10	-	-		No
Gemfibrozil	-0.04±0.01	0.9212	-	10		-		No

k: Washout rate from anaerobic digester; k*: Transformation rate of PPCP; $t_{1/2}$: Half-life.

Table 24 shows the mass of PPCPs recovered from the lab-scale anaerobic reactor after the 30-day experiment. Caffeine was the only compound that showed a lower than 100% total mass recovery from the anaerobic digester after 30 days, consistent with its degradation within the reactor over the study period. Fig. 17 shows the first order washout rate of caffeine, a degrading compound, from the anaerobic digester as compared to the first order washout rate of the bromide tracer.

Table 24. Percent of PPCPs (mean ± 95% CI) and Bromide Recovered from Lab-scale Anaerobic Digester after 30 Days.

% Recovery of PPCPs after 30 Days	
Bromide	110
Carbamazepine	113±0.03
Estrone	134±0.95
Triclosan	115±1.26
Caffeine	83.1±0.01
DEET	109±0.28
TCEP	102±0.42
Gemfibrozil	115±0.38

For comparison purposes, Fig. 20 shows the first order washout rate of carbamazepine, a non-degrading compound, from the lab-scale anerobic digester as compared to the bromide washout curve. As seen in Fig. 19 the washout rate of caffeine was statistically different from the washout rate of bromide from the anaerobic digester.

Fig. 20 on the other hand shows no statistical difference between the washout rate of carbamazepine and bromide. This signifies the persistence of carbamazepine, a neutral hydrophobic PPCP in the anaerobic digester.

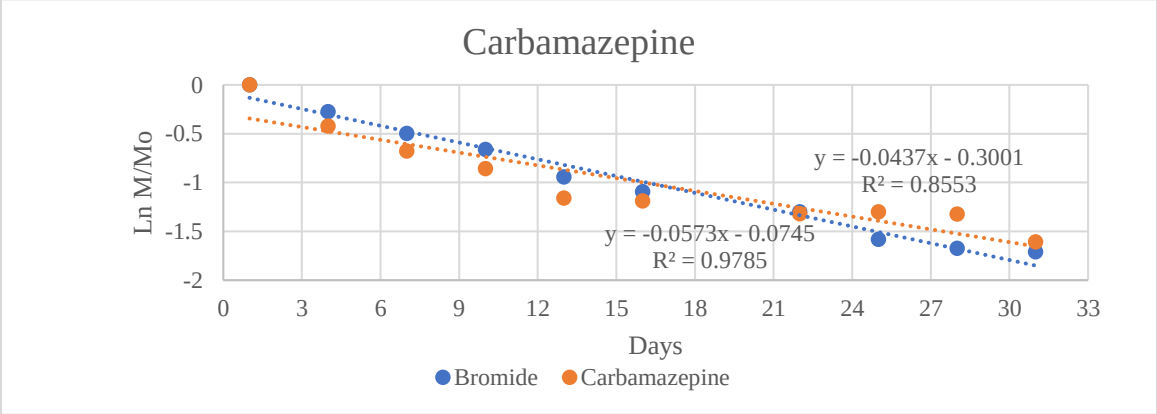

Fig. 20. First order linear regression of of carbamazepine and bromide washout from lab-scale anaerobic digester.

Conkle et al. (2012) found the half-life of carbamazepine in anaerobic sediments incubated for 112 days to range between 382 ± 138.11 to 439 ± 136.73 days. In this study (Table 23) carbamazepine did not show significant degradation in the 30 days that it was monitored in the anaerobic digester. These researchers also found that DEET did not significantly degrade under anaerobic conditions, while gemfibrozil was found to have a half-life ranging between 228 ± 52 and 782 ± 539.70. In the current study, DEET and

gemfibrozil did not significantly degrade in the lab-scale anaerobic digester over the 30 day retention period.

The distribution of PPCP mass collected in the liquid and solids phases over the 30 day experimental period for each of the PPCPs is shown in Figure 21. The blue and orange bars indicate the total initial mass of the PPCPs in the lab-scale anaerobic digester after mixing for 24 hours and the total mass recovered in the effluent digestate over the 30 day period, respectively. The total cumulative mass measured in the solids and liquids are also shown with the gray and yellow bars, respectively.

Based on the total mass of PPCPs recovered in liquids and solids after the 11 sampling events, the distribution of PPCPs between the liquid and solid phases of the digestate were calculated and are shown in Table 25. Neutral hydrophobic PPCPs estrone and triclosan showed higher percentages partitoning to the solid phase than the other PPCPs analyzed, which is expected due to their hydrophobic properties (Tables 2). However, carbamazepine which is also a neutral hydrophobic PPCP dominated in the liquid phase alongside anion gemfibrozil and neutral hydrophilic caffeine, DEET and TCEP.

Table 25. Distribution of PPCPs (mean ± 95% CI) Between Liquid and Solid Phase of Digestate from Lab-scale Anaerobic Digester.

% Distribution of Total Mass of PPCPs Collected from Anaerobic Digester After 30 Days							
Phase	Carbamazepine	Estrone	Triclosan	Caffeine	DEET	TCEP	Gemfibrozil
Solid	12.3±0.06	59.8±0.31	97.9±0.37	8.31±0.04	8.97±0.20	7.52±0.17	14.6±0.25
Liquid	87.7±0.01	40.2±0.35	2.07±9.91	91.7±0.01	91.0±0.03	92.5±0.06	85.4±0.06

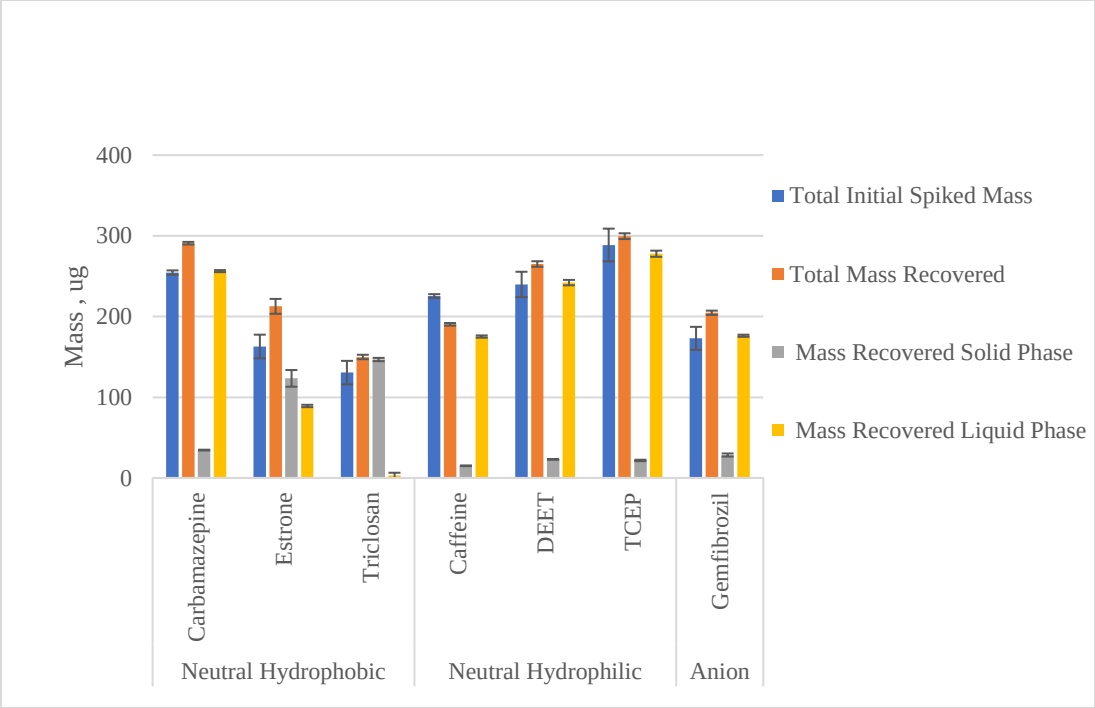

Fig. 21. Total spiked mass and total, liquid, and solid phase PPCP recovery from the lab-scale anaerobic reactor after 30 days (three hydraulic retention times). Error bars represent 95% CI.

Triclosan, which had the highest Log K_{ow} value (4.76) and persists in anaerobic environments, had the highest percentage on the digested solids from the anaerobic digester. The removal of these compounds that stay on the solids after going through anaerobic digestion must be done through further solids processing if their concentrations released to the environment through digested solids disposal are to be controlled. Other compounds, like caffeine and DEET, that dominate in the digestate liquids but are well removed by aerobic wastewater treatment (Tables 20 and 25), would go back into the WWTP through digestate return and be removed through additional liquid treatment.

5.3.2 Compost

5.3.2.1 Composting of Harvested Duckweed

Fig.22 shows the temperature profile of the compost mix over the 21- day study period as an indicator of biological activity. Biological activity increased significantly in

the first 24 hours after mixing as temeperature rose to mesophilic levels (40 ºC).

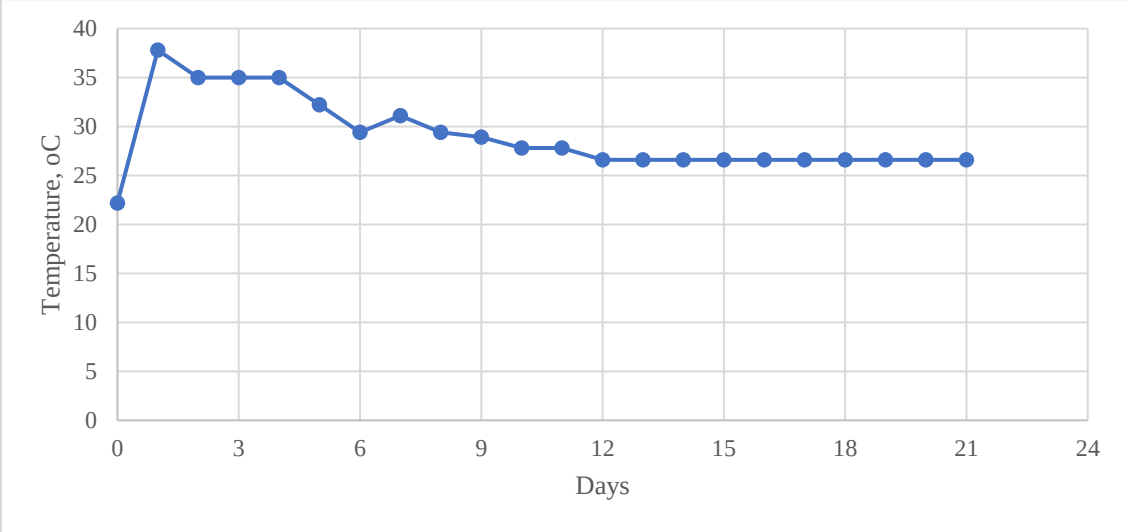

Fig. 22. Temperature profile of duckweed compost over the 21-day compost period.

At this point the microbes begin to grow and decompose the duckweed, and biological activity and corresponding compost pile temperature remained fairly constant and above 30°C during the first week of composting. In the following weeks there was a gradual decline in the biological activity and compost pile temperature as microbes fed on substrate, until there was little remaining substrate for the microbes, and the temperature stabilized slightly above room temperature.

Oxygen was also monitored in the compost mix throughout the composting period and stayed constant at approximately 20.5% from the beginning till the end of the study period. Fig. 23 shows the concentration of PPCPs in the duckweed compost after sampling on the initial day of mixing the compost, and every 3 days through the duration of the study period.

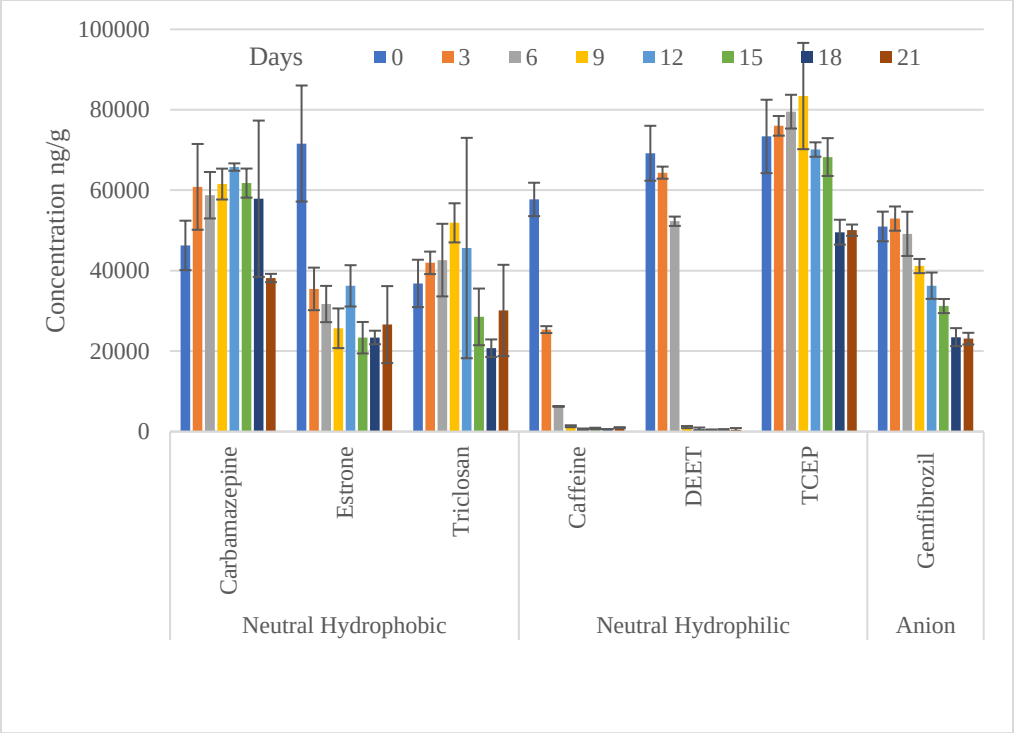

Fig. 23. PPCP concentraion in duckweed over the 21-day study period. Error bars represent 95% confidence interval of replicate measurements.

The mechanisms for transformation of PPCPs in the compost mix are aerobic biodegradation, thermolysis and possible atmospheric oxidation when the compost mix is exposed to UV light (Jjemba, 2008). However, since composting was done in a UV-inhibited container the possibility of atmospheric oxidation can be eliminated. The effect of thermolysis can also be eliminated since the compost only heated up to as high as 40 ^{o}C. As seen in Fig. 9 the effect of drying at 40 ^{o}C on PPCP reduction was insignificant except for gemfibrozil. Thus loss in PPCP concentration during this compost study can mainly be attributed to aerobic biodegradation. Table 26 shows the half-lives of PPCPs in the duckweed compost based on concentration data collected in this lab study.

Table 26. Transformation Rates of PPCPs in Duckweed Composted for 21 Days.

Compound	k(day^{-1})	r^2	n	Significant linear regression	Lag time (day)	$t_{1/2}$ (upper) (day)	$t_{1/2}$ (mean) (day)	$t_{1/2}$ (lower) (day)	% Removal
Carbamazepine	-0.08±0.43	0.8494	3	No	15		-		
Estrone	-0.04±0.03	0.5730	8	Yes	0	132	18.2	9.78	63
Triclosan	-0.06±0.09	0.6355	5	No	9		-		
Caffeine	-0.41±0.08	0.9886	5	Yes	0	2.12	1.71	1.43	98
DEET	-0.39±0.25	0.8195	6	Yes	3	5.12	1.78	1.08	99
TCEP	-0.05±0.03	0.9041	5	Yes	9	37.9	15.2	9.51	31
Gemfibrozil	-0.05±0.01	0.9721	6	Yes	6	17.0	13.0	10.5	54

k; Transformation rates of PPCPS in duckweed compost
$t_{1/2}$: Half-life
n; Number of data points used for first order linear regression

For many of the compounds, the rate of reduction in concentration in the compost decreased when biological activity in the compost mix appeared to slow as indicated by reduced and stable compost temperature (Fig. 19 and Fig. 20) supporting the finding that PPCPs were being aerobically degraded in the compost system as predicted by BIOWIN 1 in Table 6. Neutral hydrophobic PPCPs carbamazepine, estrone and triclosan, and anionic gemfibrozil showed a sustained decline in concentration after compost pile temperatures stabilized.

Neutral hydrophilic PPCPs, caffeine and DEET had high tranformation rates in the compost. These compounds showed sigificant loss in the compost in the first week of composting. Similar to caffeine (Fig. 24), estrone did not exhibit a lag period prior to the initiation of degradation; however, its half-life in the compost was significantly longer as its degradation rate was significantly lower than that of caffeine.

Carbamazepine and triclosan, both neutral hydrophobic compounds, did not have significant first order linear regressions indicating that these compounds did not show significant first order degradation during the 21-day composting study (Appendix D, Figure D.1) as discussed below.

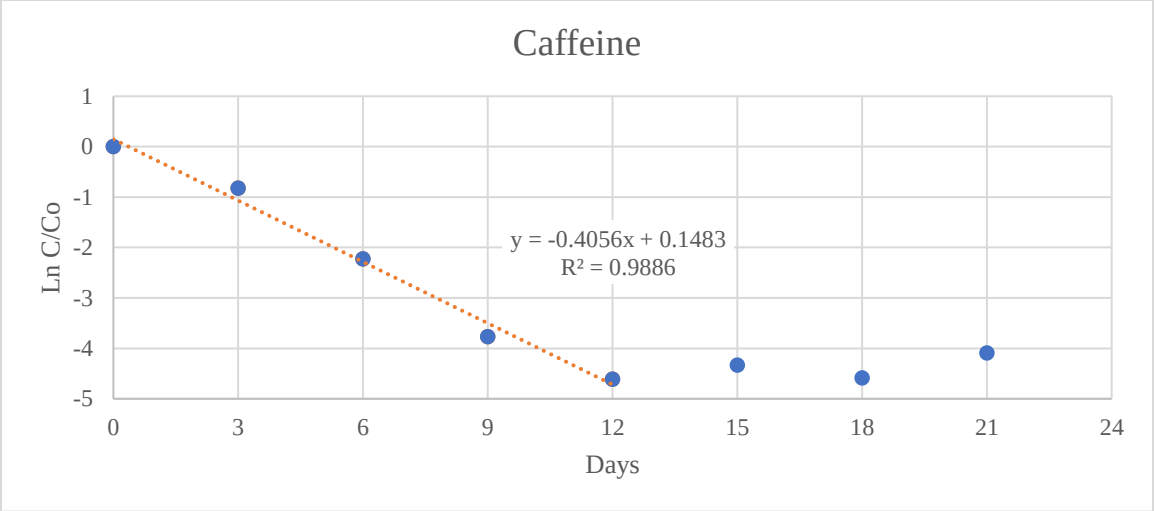

Fig. 24. First order linear regression of caffeine in duckweed composted over the 21-day study period.

Neutral hydrophilic PPCPs (caffeine and DEET) generally had higher percent removals in the compost than did the neutral hydrophobic PPCPs (estrone, triclosan, carbamazepine) (Appendix D, Table D.6). DEET had the highest percent removal of approximately 99%, while estrone removal was found to be only 63% during the 21-day compost period. TCEP had a lag time of 9 days before seeing any reductions in concentrations (Fig. 25).

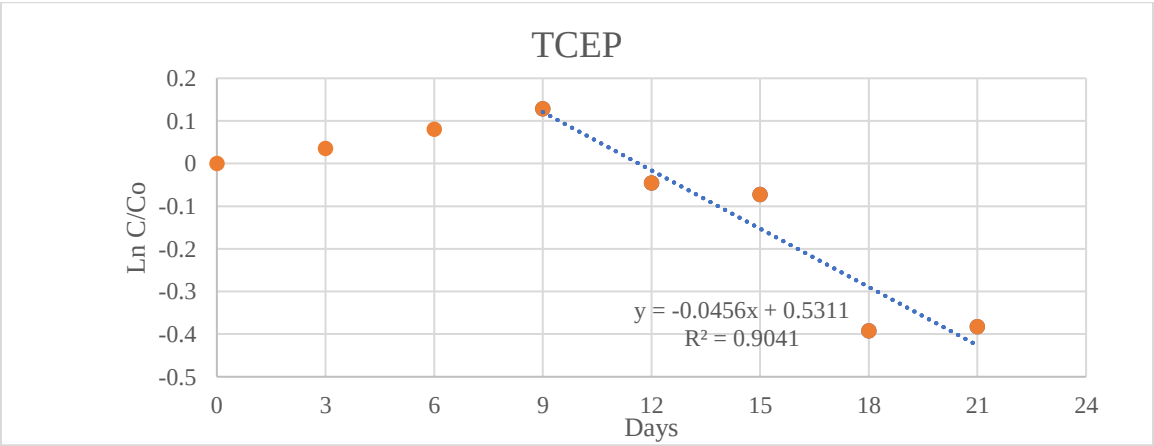

Fig. 25. First order linear regression of TCEP in duckweed composted over the 21-day study period.

A t-test of the starting concentration to the final concentration after composting for triclosan and carbamazepine did not show any statistical difference, with p- values of 0.3785 and 0.1187, respectively.

TCEP, although neutral and hydrophilic, was somewhat persistent, having the lowest percent removal of the hydrophilic PPCPs at 31% and did not transform at a high rate like its fellow neutral hydrophilic PPCPs caffeine and DEET.

The percent removal of gemfibrozil (anionic) in duckweed compost was approximately 54% (Appendix D, Table D.6). In a similar study Fang et al. (2012) determined the loss of gemfibrozil due to aerobic degradation in sandy-loam and silt-loam soils to be 25% and 11.3%, respectively, after 14 days of incubation.

In this laboratory study, aerobic composting was shown to remove PPCPs from biosolids more effectively and at a faster rate than anerobic digestion. Five of seven of the PPCPs quantified in this study showed significant degradation in this compost study compared to only one of seven compounds showing significant degradation via anaerobic digestion (Table 23 and 26). As seen in a comparison of Tables 23 and 26, the half-life of caffeine was 20.7 to 26.6 days in anaerobic digestion, while it was only 2.07 to 6.97 days when the same solids were aerobically composted. DEET, estrone, gemfibrozil and TCEP had significant degradation rates under aerobic composting but no demonstrated degradation in the anerobic digester.

5.3.2.2 Composting of Hyrum WAS

As seen from the temperature profile in Fig. 26, biological activity in the compost mix gradually increased and peaked on Day 3 of the composting study. There was a

gradual decline in biological activity, as indicated by gradually lowering temperatures in the compost unit until compost temperatures reached a steady-state level slightly above room temperature after 2 weeks of composting. Neutral hydrophilic compounds caffeine and DEET, and anionic sulfamethoxazole showed sharp declines in concentration during the composting period indicating that they have short half-lives in the compost as shown in Table 27.

Table 27. Transformation Rates and Half-lives (mean ± 95% CI) of PPCPs in WAS Composted for 21 Days.

Chemical	k (day^{-1})	r^2	n	Significant linear regression	Lag time (day)	$t_{1/2(upper)}$ (day)	$t_{1/2(mean)}$ (day)	$t_{1/2(lower)}$ (day)	% Removal
Carbamazepine	-0.01±0.02	0.4956	6	No	6		-		
Estrone	-0.02±0.03	0.3756	8	No	0		-		
Triclosan	-0.04±0.02	0.8426	8	Yes	0	28.3	16.1	11.2	56
Caffeine	-0.28±0.20	0.7934	6	Yes	6	8.51	2.48	1.45	97
DEET	-0.21±0.06	0.9561	6	yes	6	4.75	3.34	2.57	94
TCEP	-0.04±0.34	0.7099	3	No	15		-		12.3
Gemfibrozil	-0.03±0.03	0.8163	6	Yes	6	59.1	20.2	12.2	46
Sulfamethoxazole	-0.17±0.04	0.9515	8	Yes	0	5.20	4.03	3.28	98
Fluoxetine	-0.03±0.02	0.9022	6	Yes	3	32.5	20.2	14.7	44

k: Transformation rates of PPCPS in WAS compost
$t_{1/2}$: Half-life
n; Number of data points used for first order linear regression

Fig. 27 shows PPCPs concentrations measured in the WAS compost samples over time. A longer composting time would be required to completely remove caffeine since there was a lag time of 6 days before caffeine began to degrade in the WAS compost.

The half-lives of DEET, caffeine, gemfibrozil and TCEP were longer in WAS compost than in duckweed compost. Estrone did not transform in WAS but did in duckweed compost (Tables 26 and 27).This may be attributed to low moisture content and inadequate substrate in the stable, very long solids retention time WAS compost; conditions which do not favor biodegradation. The exception was triclosan which transformed in WAS compost but did not transform in duckweed compost. This behaviour

of triclosan is not surprising since triclosan is known to have a longer half-life in water and a shorter half-life in air (Halden et al. 2005; Huang et al. 2014). The fact that triclosan did not transform under conditions that favor biodegradtion may suggest that biodegradation is not the only transform mechanism for PPCPs in compost.

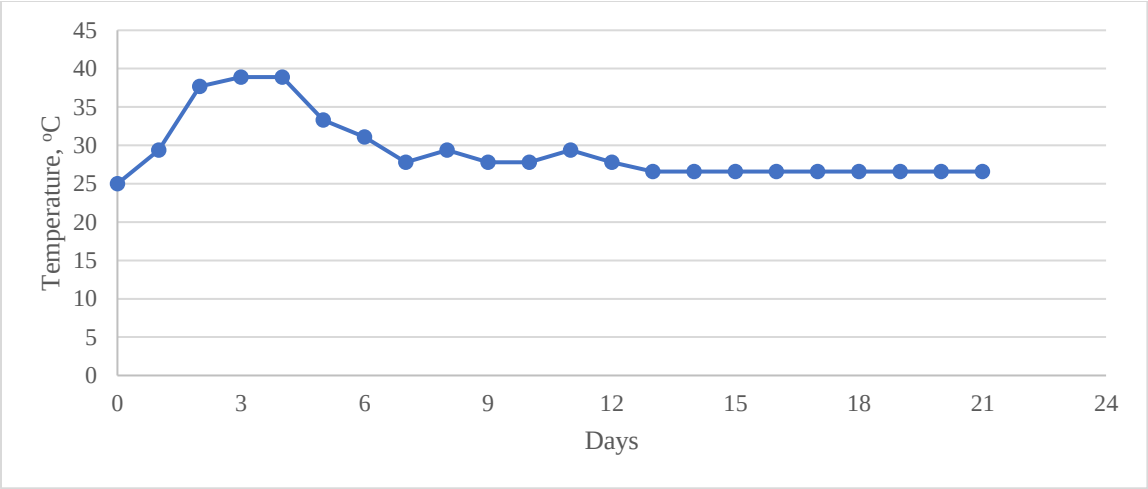

Fig. 26. Temperature profile of WAS compost over the 21-day study period.

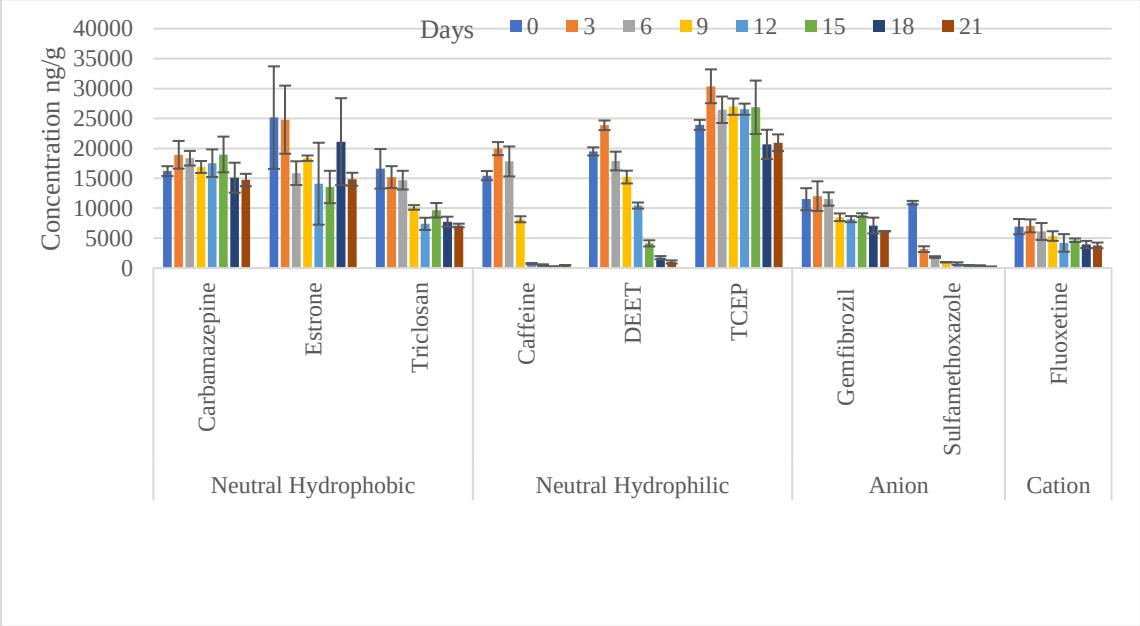

Fig. 27. PPCPs concentration in WAS compost over the 21-day study period. Error bars represent 95% confidence interval of replicate measurements.

Sulfamethoxazole and triclosan did not exhibit a lag period during the composting process, and produced r^2 values of 0.9515 and 0.8426, respectively, for their linear regressions as shown in Table 27. These two compounds, being significantly different in their water solubilities, did show different transformation rates and percent removals after composting. For all the compounds monitored in the WAS compost, the reduction in concentrations was associated with the period of time when the compost was at elevated temperature. Gemfibrozil, DEET, fluoxetine and triclosan continued to decrease in concentration even when the compost temperature dropped to room temperature and biological activity was at its lowest (Appendix D, Fig. D.2 and D.4). In a biodegradation experiment Fang et al. (2012) found that gemfibrozil was reduced by approximately 16% in killed control samples isolated from UV light.

Although it may have suggested abiotic transformation of gemfibrozil, the loss of gemfibrozil was attributed to incomplete extraction. Gemfibrozil exists mainly as a an anion and would not be strongly sorbed onto organic matter in compost. This should make it bioavailable for biodegradation. In this study, the half-life of gemfibrozil in compost was determined to be 12.2-59.9 days averaging at 20.2 days. This result compares to a biodegradation half-life for gemfibrozil in sandy-loamy and silt-loam soils reported by Fang et al. (2012) to be 17.8 and 20.6 days, respectively. Fig. 28 and 29 shows the first order linear regression of sulfamethoxazole (anion) and fluoxetine (cation) in WAS compost. The variations in solubilities of these compounds is reflected in their transformation rates in the compost.

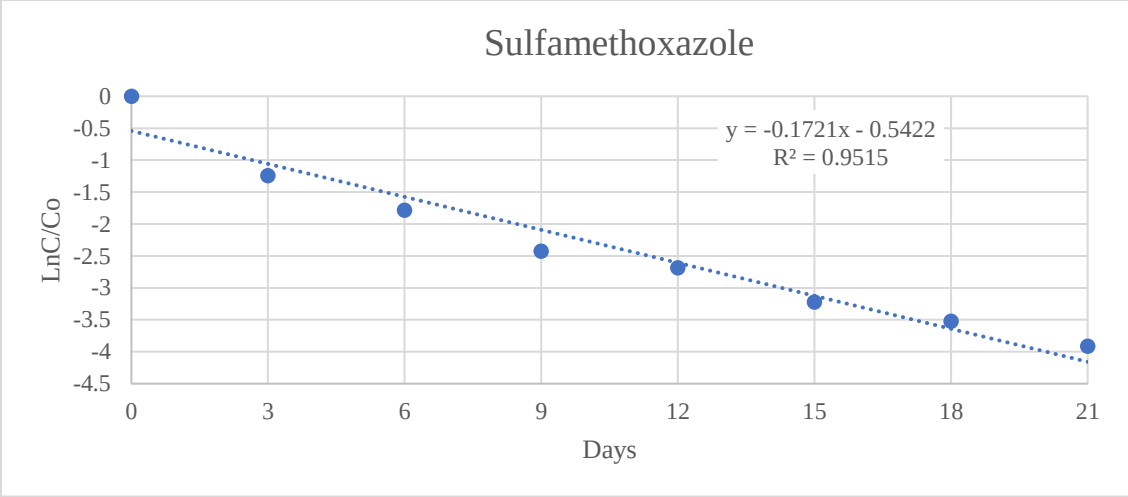

Fig. 28. First order linear regression of sulfamethoxazole in WAS composted over the 21-day study period.

Sulfamethoxazole had no lag time and had an average half-life of 3.28 days with overall removal in the compost of 98%. Fluoxetine, a more lipophilic compound, had a lag time of 3 days in the compost, an average half-life of 14.3 days, and only 44% overall removal from the WAS compost during the 21-day compost period.

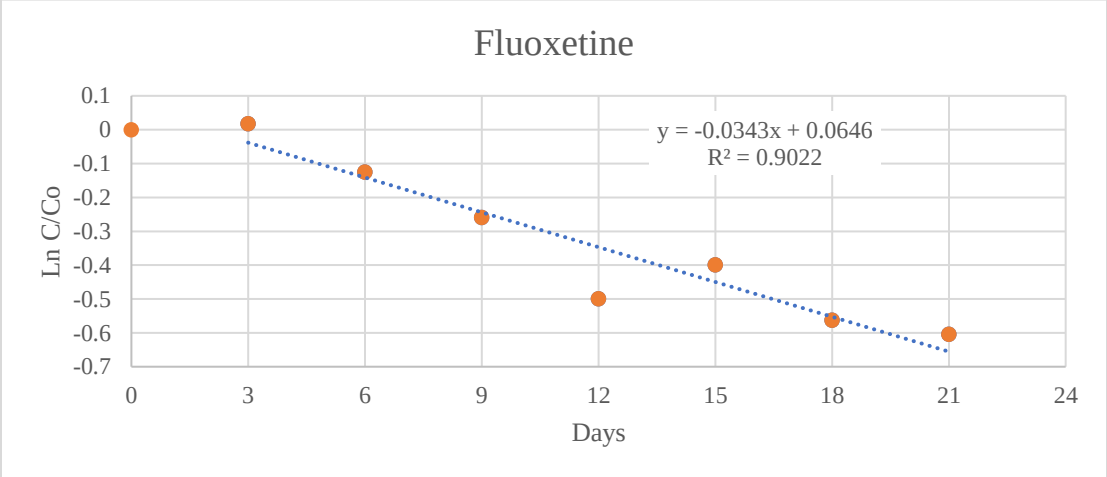

Fig. 29. First order linear regression of fluoxetine in WAS composted over the 21-day study period.

Similar to results obtained for the duckweed compost, hydrophilic PPCPs (caffeine, DEET and sulfamethoxazole) had higher percent removals in the compost than hydrophobic PPCPs (carbamazepine, estrone, fluoxetine, and triclosan).

Gemfibrozil, although a hydrophilic compound, showed a percent removal below 50%. A t-test comparison of the starting concentration to final concentration of carbamazepine and estrone after composting did not show any statistical difference (95% CI with p-values of 0.0944 and 0.1277, respectively), and therefore no degradation due to composting was observed. TCEP did show a statistical difference (95% CI) in concentration after composting with a p-value of 0.0324; its removal was found to be 12.3% over the 21-day study period although degradation did not follow first order kinetics. WAS from Hyrum was only composted and not anaerobically digested because it is considered secondary sludge and thus will not have enough biodegradable material to serve as food for microbes in anaerobic digestion. However, inferring from the results of anaerobic degradation of PPCPs in duckweed, composting achieves higher degradation rates than anaerobic digestion.

These observations are consistent with what has been observed in other literature. Guerra et al. (2015) determined that the concentrations of triclosan, sulfamethoxazole and carbamazepine in WAS were higher in anaerobically treated WAS than in aerobically treated WAS.

5.4. Summary of Fate of PPCPs in Wastewater Treatment.

In summary, Table 28 qualitatively presents the overall removal and corresponding persistence of PPCPs in the wastewater treatment process including both

liquid treatment and solids stabilization steps with data collected from the field study and lab study. Caffeine and DEET are compounds that proved to be very well degraded from the liquid and biosolids by the wastewater treatment process and solid stabilization.

Table 28. Overall Removal of PPCPs by the Wastewater Treatment Processes.

Compound	Removal from Water Phase	Accumulation on Solids - Biomass, Sediment	Degradation via Anaerobic Digestion	Degradation via Composting	Overall Removal, Water + Solids Composting
Carbamazepine	+	+, +	-	-	-
Estrone	+++++	-, -	-	++	+++++
Triclosan	++++	+, +	-	++	++
Caffeine	+++++	+, +	+	+++++	+++++
DEET	++++	+, +	-	+++++	++++
TCEP	+	+, +	-	+	+
Gemfibrozil	+	-, -	-	++	+
Sulfamethoxazole	+	+, +	-	+++++	+
Fluoxetine	+++	+, +	-	++	++

Removal: +++++ > 97%, ++++ >90%, +++>80%, ++ >50%, + >10%, - < 10%
Accumulation: - Not detected, + Detected
Degradation: +++++>95% High rate , ++>50% Moderate Rate,+>10% Low rate,-No degradation

Acetaminophen, however, is effectively degraded in the liquid by the wastewater treatment process but was not monitored in the biosolids during the lab study due to poor recoveries from the spikes. TCEP persisted in the WWTP effluent and also adsorbed onto the biosolids and cannot be removed easily from the biosolids by composting or anaerobic digestion.

Sulfamethoxazole and gemfibrozil (anions), were not effectively degraded by the wastewater treatment process and persisted in the liquid. However, sulfamethoxazole that did accummulate in the solids could be effectively removed through composting. Gemfibrozil did not accummulate on the solids in the WWTP as seen in the field study. If it did, longer composting time could be used to degrade it from the biosolids.

Estrone and and β-estradiol did not accummulate on the solids in the field study and were highly removed from the liquid phase by the WWTP through possible transformation into daughter products. If estrone accummulated in the solids, longer composting time could be used to effectively degrade it from the solids (Table 26). β-estradiol was not monitored in the biosolids during the lab studies due to poor recoveries. Triclosan is effectively removed from the liquid phase through sorption onto solids, and longer composting time could be used to degrade triclosan from the biosolids. Carbamazepine, although neutral hydrophobic in nature, persisted in the wastewater effluent and adsorbed onto the biosolids. The lab study shows that it cannot be removed by composting or anaerobic digestion. Progesterone is removed from wastewater by accummulating in the biosolids but it was not monitored in the biosolids during the lab study due to poor recoveries from the solids.

Fluoxetine is effectively removed from the liquid phase of WWTPs by accumulation on the solids. Longer composting time could be used to degrade fluoxetine that has accummulated in the solids.

Caffeine, DEET and sulfamethoxazole, which are all water soluble, had higher transformation rates in compost than hydrophobic PPCPs like estrone, triclosan and fluoxetine (Tables 26 and 27). Water soluble PPCPs, gemfibrozil and TCEP, had transformation rates in compost similar to those of the hydrophobic PPCPs estrone and fluoxetine. Carbamazepine was found not to have a significant degradation rate in either WAS or duckweed compost, or when associated with duckweed that was anaerobically digested.

The inability of chemicals like sulfamethoxazole, gemfibrozil, tris-2-chloroethyl phsosphate and carbamazepine to tranform due to biodegradation, can be attributed to the molecular structure of these compounds and the lack of particular microbes adapted to produce enzymes to degrade these chemicals (Davis et al. 1996, Kadri et al., 2016). Carbamazepine, which is a tricyclic compound, is expected to be stable (due to many double bonds) in the environment as well as poly-chlorinated chemicals such as TCEP.

Davis et al. (1996) list several factors that affect biodegradation including inorganic nutrients, redox environment, substrate, temperature, water activity and the adaptive response of the microorganisms. The variation in these conditions in the WWTP and compost ennvironment could explain why gemfibrozil and sulfamethoxazole did not transform during wastewater treatment but did during solid stabilization. Chemicals contaning carboxyl, halogen or amide functional groups are resistant to biological treatment (Hai et al. 2018). This explains why gemfibrozil, TCEP, sulfamethoxazole and carbamazepine did not biodegrdade in the WWTP. Gemfibrozil has a carboxyl functional group, TCEP has a a halogen functional group, whereas sulfamethoxazole and carbamazepine have amide functional groups.

Kadri et al. (2016), concluded that the environment, number, type of microrganism and structure of a chemical influences biodegradation. Hai et al. (2018) determined that white-rot fungi could produce enzymes that degraded 99 percent of carbamazepine; a removal efficiency much higher than that reported for conventional activated sludge and membrane bioreactors in the literature.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

Based on the findings of the field and laboratory study, the following conclusions can be made regarding the hypotheses posed at the initiation of this study:

1. The distribution of PPCPs cannot be predicted based solely on their physical/chemical properies. For example, it was determind that neutral hydrophillic PPCPs like caffeine and tris-2 chloroethyl phosphate sorbed significantly onto biosolids, whereas neutral hydrophobic PPCPs, such as carbamazepine (moderately hydrophobic, Log K_{ow} = 2.45), were detected in high concentrations in the liquid phase. Other factors such as concentration of the solute (PPCPs), mass of adsorbent (sludge/duckweed/sediments), retention times and the chemistry of the solvent would also influence the distribution of chemicals between the solid and liquid phase.
2. The rate of transformation of PPCPs in aerobic environments is faster than their transformation in anaerobic environments. Of the compounds monitored in the anaerobic digester, caffeine was the only compound that showed any significant degradation/tranformation. DEET, estrone, triclosan, gemfibrozil and TCEP all showed significant degradation in compost but no significant degradtion through anaerobic digestion.
3. The rate of biodegradation of hydrophillic compounds were in generally faster than for hydrophobic compounds tested. Caffeine, DEET and sulfamethoxazole, which are all hydrophillic PPCPs, had shorter half-lives in compost than estrone, carbamazepine, fluoxetine and triclosan, which are hydrophobic. However there were exceptions. The half-life of gemfibrozil

(hydrophilic) in WAS compost was statistically the same as fluoxetine (hydrophobic).TCEP (hydrophilic) did not have a significant transformation rate but triclosan (hydrophobic) transformed with an average half-life of 12 days.

With the large amounts of biosolids being produced at WWTPs in the US, it is imperative to determine PPCPs that associate with biosolids and monitor their fate during solids stabilization. This research helped to determine the fate of some commonly used PPCPs in biosolids that may eventually end up as soil amendment or in landfills. As a general rule, non-polar PPCPs with high Log K_{ow} values are expected to associate with biosolids. However, some non-polar chemicals (carbamazepine) with high Log K_{ow}s, non-polar chemicals with low Log K_{ow}s(TCEP), and positively charged PPCPs also associated with the biosolids.

For PPCPs that persisted on the biosolids (carbamazepine and TCEP) through both anaerobic and aerobic stabilization steps, it is recommended that other solid stabilization processes be evaluated to determine their effectivness in the removal of these recalcitrant compounds. For the two solid stabilization methods tested, composting was found to be a significantly better process than anaerobic digestion for removing PPCPs from the biosolids generated from both mechanical treatment plants and duckweed populated wastewater treatment lagoons.

The knowledge gained from this study could be used in designing wastewater treatment sytems targeted at the removal of some chemicals such as tris-2-chloroethyl phosphate, fluoxetine and triclosan by the addition of coagulants, which will cause these chemicals to be sorbed unto solids and be removed through aerobic composting.

CHAPTER 7

ENGINEERING SIGNIFICANCE

Knowledge of the effectiveness of composting and anaerobic digestion as an environmental remediation measure for PPCPs removal from biosolids was gained from this study. Composting proved to be a better method for removing PPCPs from biosolids than anaerobic digestion.

The indivividual transformation rates for the target PPCPs in compost can be used to estimate the time required to remove PPCPs from biosolids through composting. The lab compost study should be replicated in field compost piles at thermophilic composting temperatures and variable environmental conditions to determine how these conditions affect the overall degradation of PPCPs in biosolids compared to removal under ideal conditions maintained during the laboratory study. It was also determined that some PPCPs are well degraded in compost when there is high moisture in compost, whereas others degrade better under less moist conditions (i.e., triclosan). This knowledge can be applied to engineered systems to remove targeted PPCPs during composting.

Finally, this study helped to determine chemicals (i.e., sulfamethoxazole) that persisted in the effluent from WWTPs but degraded rapidly in biosolids during composting. This opens the door to further research into how to engineer wastewater treatment systems to accummulate such chemicals on the biosolids and subsequently effeciently degraded them through biosolids composting.

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APPENDICES

Appendix A. Raw Data: Drying Methods and Quality Control

Table A.1 PPCP concentration in duckweed dried with different drying methods.

Drying Method (0.2 ug/g)	Acetaminophen	B-Estradiol	Caffeine	Carbamazepine	DEET	Estrone	Fluoxetine	Gemfibrozil	Progesterone	Sulfamethoxazole	Triclosan	Tris-(2-chloroethyl) Phosphate
	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g
1 Oven (40 °C)	<0.004	<0.05	0.217	0.255	0.212	0.305	0.037	<0.05	0.177	0.109	0.110	0.096
2 Oven (40 °C)	<0.004	<0.05	0.173	0.197	0.173	0.267	0.036	<0.05	0.138	0.084	0.096	0.070
3 Oven (40 °C)	<0.004	<0.05	0.191	0.229	0.191	0.271	0.039	<0.05	0.164	0.098	0.095	0.090
1 Freeze Dry	0.0463	<0.05	0.155	0.198	0.196	0.228	0.079	0.139	0.135	0.160	0.114	0.195
2 Freeze Dry	<0.004	<0.05	0.150	0.180	0.172	0.303	0.076	0.140	0.130	0.150	0.093	0.179
3 Freeze Dry	<0.004	<0.05	0.149	0.176	0.171	0.251	0.071	0.136	0.113	0.146	0.097	0.167
1 Air Dry Light	<0.004	<0.05	0.223	0.229	0.227	0.313	0.068	0.180	0.046	0.016	0.120	0.241
2 Air Dry Light	<0.004	<0.05	0.221	0.234	0.224	0.279	0.058	0.175	0.046	0.015	0.143	0.263
3 Air Dry Light	<0.004	<0.05	0.235	0.236	0.247	0.312	0.061	0.233	0.045	<0.003	0.122	0.276
1 Air Dry No Light	<0.004	<0.05	0.225	0.237	0.223	0.256	0.072	0.189	0.043	0.022	0.150	0.260
2 Air Dry No Light	<0.004	<0.05	0.215	0.223	0.223	0.287	0.060	0.185	0.041	0.020	0.159	0.251
3 Air Dry No Light	<0.004	<0.05	0.216	0.226	0.218	0.304	0.065	0.205	0.046	0.018	0.117	0.255
Method 2 was used for processing and analyzing all samples												

Table A.2. PPCP concentrations in effluent water from Hyrum WWTP and Wellsville sewage lagoons spiked with PPCPs

Matrix spike 1.5 ug/L	Acetaminophen	β- Estradiol	Caffeine	Carbamazepine	DEET	Estrone	Fluoxetine	Gemfibrozil	Progesterc	Sulfometh	Triclosan	Tris-2-chloroethyl phosphate
	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L
wellsville effluent(Method 1)	1.46	<0.232	1.33	1.36	1.32	2.42	1.10	1.03	1.36	1.59	0.83	1.45
wellsville effluent(Method 1)	1.36	<0.232	1.15	1.19	1.20	1.21	0.94	0.97	1.11	1.47	0.83	1.21
wellsville effluent(Method 1)	1.51	<0.232	1.36	1.46	1.24	1.33	1.10	1.56	1.31	1.68	0.98	1.45
wellsville effluent(Method 2)	0.76	0.88	0.97	1.17	0.96	0.75	0.79	1.36	0.83	1.06	0.72	1.07
wellsville effluent(Method 2)	0.71	0.79	0.90	1.01	0.88	0.59	0.71	1.27	0.70	0.98	0.55	0.97
wellsville effluent(Method 2)	0.80	0.92	1.00	1.09	1.01	0.88	0.71	1.14	0.80	1.10	0.60	1.11
Hyrum WWTP Effluent(Method 1)	1.44	<0.232	1.19	1.18	1.87	<0.67	0.94	1.53	1.17	3.60	1.00	1.27
Hyrum WWTP Effluent(Method 1)	1.48	<0.232	1.21	1.20	1.87	<0.67	0.94	1.20	1.23	3.51	0.90	1.25
Hyrum WWTP Effluent(Method 1)	1.52	<0.232	1.30	1.25	2.02	<0.67	0.95	1.20	1.25	3.48	1.16	1.31
Hyrum WWTP Effluent(Method 2)	0.58	0.83	0.56	0.82	1.14	0.69	0.60	1.08	0.87	2.37	0.94	0.75
Hyrum WWTP Effluent(Method 2)	0.60	0.80	0.57	0.82	1.17	0.66	0.51	1.08	0.91	2.40	0.92	0.85
Hyrum WWTP Effluent(Method 2)	0.63	0.86	0.59	0.81	1.16	0.61	0.61	1.12	0.89	2.43	0.96	0.84

Table A.3 PPCP concentrations in WAS from Hyrum WWTP and duckweed from Wellsville sewage lagoons spiked with PPCPs at 2ug/g

Matrix Spike (2 ug/g)	Acetaminophen	β-Estradiol	Caffeine	Carbamazepine	DEET	Estrone	Fluoxetine	Gemfibrozil	Progesterone	Sulfomethoxazole	Triclosan	Tris-2-chloroethyl phosphate
	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g
1 Duckweed (Method 1)	<0.004	<0.05	1.86	2.07	1.77	2.02	0.48	1.93	0.10	0.13	1.32	1.84
2 Duckweed (Method 1)	<0.004	<0.05	1.81	2.08	1.65	2.02	0.45	1.92	0.11	0.13	1.21	1.79
3 Duckweed (Method 1)	0.005	<0.05	1.82	2.01	1.60	1.92	0.42	1.61	0.11	0.14	1.23	1.91
1 Duckweed (Method 2)	<0.0003	0.046	1.16	1.41	1.09	1.01	0.32	1.03	0.05	0.05	0.48	1.10
2 Duckweed (Method 2)	<0.0003	<0.004	1.11	1.33	0.98	1.13	0.28	0.99	0.05	0.05	0.58	0.94
3 Duckweed (Method 2)	<0.0003	0.028	1.08	1.25	0.99	1.16	0.21	1.03	0.05	0.05	0.62	0.94
1 WAS (Method 1)	<0.004	<0.05	1.47	1.72	1.63	3.19	1.56	2.33	0.43	1.25	2.70	1.61
2 WAS (Method 1)	<0.004	<0.05	1.56	1.64	1.76	2.70	2.07	2.40	0.47	1.30	3.24	1.57
3 WAS (Method 1)	<0.004	<0.05	1.42	1.46	1.71	2.58	1.62	2.06	0.41	1.17	2.55	1.41
1 WAS (Method 2)	<0.0003	0	1.15	1.37	1.10	2.20	1.16	1.85	0.22	0.36	1.77	1.20
2 WAS (Method 2)	<0.0003	0	1.31	1.29	1.15	2.52	1.34	1.82	0.26	0.41	1.91	1.10
3 WAS (Method 2)	0.002	0	1.25	1.16	1.20	2.03	1.11	1.63	0.23	0.37	1.61	1.18

Appendix B. Raw Data : Field Samples

Table B.1 PPCP concentraion Welsville influent

	6/5/2019			8/15/2019		
	Influent (ug/L)			Influent (ug/L)		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Acetaminophen	81.3[1]	98.7[1]	106[1]	129[1]	143[1]	95.5[1]
β-Estradiol	0.15[1]	0.13[1]	0.13[1]	<0.005[2]	<0.005[2]	<0.005[2]
Caffeine	18.8[1]	16.0[1]	14.5[1]	19.1[1]	18.55[1]	18.4[1]
Carbamazepine	0.09[1]	0.08[1]	0.08[1]	0.08[1]	0.10[1]	0.09[1]
DEET	1.27[1]	1.17[1]	1.16[1]	2.14[1]	1.57[1]	2.83[1]
Estrone	1.20[2]	1.17[2]	0.67[2]	<0.013[2]	<0.013[2]	<0.013[2]
Fluoxetine	0.07[1]	0.07[1]	0.07[1]	0.09[1]	0.08[1]	0.08[1]
Gemfibrozil	0.14[2]	0.14[2]	0.10[2]	0.01[2]	0.02[2]	0.004[2]
Progesterone	0.003[2]	0.002[2]	0.001[2]	<0.0002[1]	<0.0002[1]	<0.0002[1]
Sulfomethoxazole	0.27[1]	0.30[1]	0.31[1]	1.83[1]	2.01[1]	1.65[1]
Triclosan	0.12[2]	0.12[2]	0.13[2]	0.07[2]	0.06[2]	0.06[2]
Tris 2 chloroethyl phosphate	<0.004[2]	<0.004[2]	<0.004[2]	0.01[2]	0.01[2]	0.02[2]
[1]Method 1 ; [2]Method 2						

Table B.2 Effluent concentration of PPCPs in Wellsville effluent

	6/5/2019		
	Effluent (ug/L)		
	Sample 1	Sample 2	Sample 3
Acetaminophen	0.001[2]	0.001[2]	0.001[2]
β-Estradiol	<0.005[2]	<0.005[2]	<0.005[2]
Caffeine	0.008[2]	0.010[2]	0.009[2]
Carbamazepine	0.077[1]	0.079[1]	0.082[1]
Deet	0.034[2]	0.035[2]	0.035[2]
Estrone	<0.013[2]	<0.013[2]	<0.013[2]
Fluoxetine	0.001[2]	0.001[2]	0.001[2]
Gemfibrozil	0.167[2]	0.194[2]	0.181[2]
Progesterone	<0.0002[2]	<0.0002[2]	<0.0002[2]
Sulfomethoxazole	0.193[1]	0.196[1]	0.207[1]
Triclosan	<0.006[2]	<0.006[2]	<0.006[2]
Tris 2 chloroethyl phosphate	0.050[2]	0.056[2]	0.050[2]
[1]Method 1 ; [2]Method 2			

Table B.3. PPCPs concentrations in water in Wellsville lagoon cells 8/15/2019

	Cell 1			Cell 2			Cell 3			Cell 4		
	ug/L			ug/L			ug/L			ug/L		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Acetaminophen	0.001[2]	0.001[2]	0.001[2]	<0.0004[2]	<0.0004[2]	<0.0004[2]	0.0004[2]	0.0003[2]	<0.0004[2]	0.078[b]	0.147[b]	0.270[b]
B- Estradiol	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]
Caffeine	3.837[1]	3.860[1]	3.584[1]	0.808[1]	0.779[1]	0.777[1]	0.369[1]	0.378[1]	0.376[1]	0.079[1]	0.078[1]	0.076[1]
Carbamazepine	0.090[1]	0.086[1]	0.085[1]	0.091[1]	0.088[1]	0.092[1]	0.088[1]	0.086[1]	0.093[1]	0.071[1]	0.067[1]	0.067[1]
DEET	0.029[2]	0.017[2]	0.024[2]	<0.001[2]	<0.001[2]	<0.001[2]	0.005[2]	0.005[2]	0.019[2]	0.031[2]	0.033[2]	0.033[2]
Estrone	<0.013[2]	<0.013[2]	<0.013[2]	<0.013[2]	<0.013[2]	<0.013[2]	<0.013[2]	<0.013[2]	<0.013[2]	<0.013[2]	<0.013[2]	<0.013[2]
Fluoxetine	0.030[1]	0.036[1]	0.037[1]	0.019[1]	0.015[1]	0.018[1]	0.019[1]	0.022[1]	0.019[1]	0.012[1]	0.017[1]	0.028[1]
Gemfibrozil	0.126[2]	0.137[2]	0.130[2]	0.129[2]	0.133[2]	0.131[2]	0.124[2]	0.106[2]	0.147[2]	0.082[2]	0.086[2]	0.094[2]
Progesterone	<0.0002[2]	<0.0002[2]	<0.0002[2]	<0.0002[2]	<0.0002[2]	<0.0002[2]	<0.0002[2]	<0.0002[2]	<0.0002[2]	<0.0002[2]	<0.0002[2]	<0.0002[2]
Sulfomethoxazole	0.846[1]	0.866[1]	0.858[1]	0.266[2]	0.262[2]	0.255[2]	0.169[1]	0.175[1]	0.176[1]	0.068[1]	0.066[1]	0.060[1]
Triclosan	<0.006[2]	0.013[2]	0.015[2]	<0.006[2]	<0.006[2]	<0.006[2]	<0.006[2]	<0.006[2]	<0.006[2]	<0.006[2]	<0.006[2]	<0.006[2]
Tris 2 chloroethyl phosphate	0.063[2]	0.048[2]	0.050[2]	0.050[2]	0.054[2]	0.052[2]	0.040[2]	0.042[2]	0.050[2]	0.033[2]	0.037[2]	0.031[2]

[1]Method 1 ; [2]Method; [b]Imputed values

Table B.4 PPCP concentration in Wellsville sewage lagoons duckweed 8/15/2019

	Cell 1			Cell 2			Cell 3			Cell 4		
	ug/g			ug/g			ug/g			ug/g		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Acetaminophen	0.015[2]	0.012[2]	0.018[2]	0.003[2]	0.003[2]	0.0005[2]	0.005[2]	0.005[2]	0.006[2]	<0.0003[2]	<0.0003[2]	0.001[2]
β- Estradiol	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]
Caffeine	<0.0002[2]	<0.0002[2]	<0.0002[2]	<0.0002[2]	0.0003[2]	<0.0002[2]	<0.0002[2]	<0.0002[2]	<0.0002[2]	<0.0002[2]	<0.0002[2]	0.0004[2]
Carbamazepine	0.001[2]	0.001[2]	0.001[2]	0.002[2]	0.002[2]	0.002[2]	0.001[2]	0.002[2]	0.002[2]	0.001[2]	0.001[2]	0.002[2]
DEET	0.002[2]	0.002[2]	0.002[2]	<0.001	0.001[2]	0.001[2]	0.001[2]	<0.001[2]	<0.001[2]	0.002[2]	0.001[2]	0.002[2]
Estrone	<0.01[2]	<0.01[2]	<0.01[2]	<0.01[2]	<0.01[2]	<0.01[2]	<0.01[2]	<0.01[2]	<0.01[2]	<0.01[2]	<0.01[2]	<0.01[2]
Fluoxetine	0.051[1]	0.021[1]	0.016[1]	0.008[1]	0.005[1]	0.006[1]	0.001[2]	0.0002[2]	0.001[2]	0.0002[2]	0.001[2]	0.001[2]
Gemfibrozil	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]
Progesterone	0.011[1]	0.007[1]	0.010[1]	0.008[1]	0.009[1]	0.009[1]	0.008[1]	0.006[1]	0.008[1]	0.006[1]	0.006[1]	0.008[1]
Sulfamethoxazole	0.012[1]	0.005[1]	0.008[1]	0.001[2]	0.001[2]	0.001[2]	0.001[2]	0.001[2]	0.001[2]	<0.0003[2]	<0.0003[2]	<0.0003[2]
Triclosan	0.169[1]	0.078[1]	0.098[1]	0.010[2]	0.013[2]	0.014[2]	<0.005[2]	0.006[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]
Tris-(2-chloroethyl) Phosphate	0.019[2]	0.021[2]	0.018[2]	0.016[2]	0.016[2]	0.017[2]	0.012[2]	0.012[2]	0.016[2]	0.009[2]	0.008[2]	0.015[2]

[1]Method 1 ; [2]Method 2

Table B.5 PPCP concentration in sediments in Wellsville sewage lagoons 8/15/2019.

	Cell 1			Cell 2			Cell 3			Cell 4		
	ug/g			ug/g			ug/g			ug/g		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Acetaminophen	0.001[2]	0.001[2]	0.001[2]	<0.0003[2]	<0.0003[2]	<0.0003[2]	<0.0003[2]	<0.0003[2]	<0.0003[2]	<0.0003[2]	<0.0003[2]	<0.0003[2]
β-Estradiol	<0.004[2]	<0.004[2]	<0.004[2]	<0.004[2]	<0.004[2]	<0.004[2]	<0.004[2]	<0.004[2]	<0.004[2]	<0.004[2]	<0.004[2]	<0.004[2]
Caffeine	0.021[1]	0.019[1]	0.016[1]	0.008[2]	0.004[2]	0.003[2]	0.010[2]	0.008[2]	0.007[2]	0.001[2]	<0.0002[2]	0.002[2]
Carbamazepine	0.014[1]	0.010[1]	0.012[1]	0.009[2]	0.004[2]	0.003[2]	0.009[2]	0.007[2]	0.007[2]	0.001[2]	0.001[2]	0.002[2]
DEET	0.013[2]	0.008[2]	0.0072	0.005[2]	0.003[2]	0.003[2]	0.008[2]	0.007[2]	0.007[2]	0.004[2]	0.004[2]	0.004[2]
Estrone	<0.01[2]	<0.01[2]	<0.01[2]	<0.01[2]	<0.01[2]	<0.01[2]	<0.01[2]	<0.01[2]	<0.01[2]	<0.01[2]	<0.01[2]	<0.01[2]
Fluoxetine	0.069[1]	0.076[1]	0.052[1]	0.016[2]	0.006[2]	0.013[2]	0.014[2]	0.008[2]	0.008[2]	0.002[2]	0.002[2]	0.002[2]
Gemfibrozil	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	0.011	<0.005	<0.005	<0.005	<0.005
Progesterone	0.022[1]	0.022[1]	0.019[1]	0.009[1]	0.006[1]	0.008[1]	0.008[1]	0.005[1]	0.006[1]	0.003[1]	0.002[1]	0.004[1]
Sulfamethoxazole	0.011[2]	0.006[2]	0.007[2]	0.004[2]	0.002[2]	0.001[2]	0.002[2]	0.001[2]	0.001[2]	0.001[2]	<0.0003[2]	0.001[2]
Triclosan	0.852[1]	0.817[1]	0.800[1]	0.126[1]	0.129[1]	0.156[1]	0.109[1]	0.101[1]	0.124[1]	0.026[2]	0.021[2]	0.019[2]
Tris-(2-chloroethyl) Phosphate	0.020[2]	0.014[2]	0.019[2]	0.017[2]	0.011[2]	0.013[2]	0.011[2]	0.013[2]	0.010[2]	0.012[2]	0.009[2]	0.013[2]
[1]Method 1 ; [2]Method												

Table B.6 PPCP concentration in Hyrum WWTP influent

		Hyrum Influent (ug/L)				
		4/1/2019			6/5/2019	
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Acetaminophen	75.0[1]	85.5[1]	80.4[1]	56.6[1]	56.3[1]	55.6[1]
β-Estradiol	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]
Caffeine	16.6[1]	17.62[1]	16.2[1]	19.8[1]	20.8[1]	20.9[1]
Carbamazepine	0.06[1]	0.06[1]	0.06[1]	0.05[1]	0.05[1]	0.05[1]
DEET	0.27[2]	0.55[2]	0.23[2]	3.16[1]	3.13[1]	3.26[1]
Estrone	0.15[2]	0.16[2]	0.13[2]	0.24[2]	0.20[2]	<0.013[2]
Fluoxetine	0.12[1]	0.09[1]	0.10[1]	0.08[1]	0.09[1]	0.10[1]
Gemfibrozil	0.27[2]	0.25[2]	0.21[2]	0.34[1]	0.69[1]	0.66[1]
Progesterone	0.05[1]	0.09[1]	0.07[1]	0.04[1]	0.04[1]	0.04[1]
Sulfamethoxazole	3.15[1]	2.39[1]	2.70[1]	2.95[1]	3.03[1]	3.36[1]
Triclosan	0.11[2]	0.10[2]	0.11[2]	0.12[2]	0.12[2]	0.11[2]
Tris-(2-chloroethyl) Phosphate	0.17[2]	0.61[2]	0.18[2]	0.07[2]	0.08[2]	0.06[2]
[1]Method 1 ; [2]Method						

Table B.7 PPCP concentration in Hyrum WWTP effluent

		Hyrum Effluent (ug/L)				
		4/1/2019			6/5/2019	
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Acetaminophen	0.322[1]	0.326[1]	0.304[1]	0.029[1]	0.033[1]	0.045[1]
β-Estradiol	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]
Caffeine	1.35[1]	1.27[1]	1.30[1]	0.011[2]	0.008[2]	0.022[2]
Carbamazepine	0.055[1]	0.051[1]	0.054[1]	0.062[1]	0.061[1]	0.062[1]
DEET	0.023[2]	0.028[2]	0.022[2]	0.740[1]	0.740[1]	0.750[1]
Estrone	<0.013[2]	<0.013[2]	<0.013[2]	<0.013[2]	<0.013[2]	<0.013[2]
Fluoxetine	0.029[1]	0.026[1]	0.017[1]	0.017[1]	0.013[1]	0.018[1]
Gemfibrozil	0.840[1]	0.720[1]	0.550[1]	0.332[2]	0.363[2]	0.367[2]
Progesterone	<0.0002[2]	<0.0002[2]	<0.0002[2]	<0.0002[2]	<0.0002[2]	0.001
Sulfamethoxazole	3.48[1]	3.26[1]	2.99[1]	2.15[1]	2.16[1]	2.17[1]
Triclosan	0.028[2]	0.034[2]	0.030[2]	<0.006[2]	<0.006[2]	<0.006[2]
Tris-(2-chloroethyl) Phosphate	0.062[2]	0.062[2]	0.053[2]	0.060[2]	0.063[2]	0.076[2]
[1]Method 1 ; [2]Method						

Table B.8 PPCP concentrations in Hyrum WAS

		Hyrum WAS (ug/g)				
		4/1/2019			6/5/2019	
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Acetaminophen	0.001[2]	0.002[2]	0.001[2]	0.002[2]	0.001[2]	0.002[2]
β-Estradiol	<0.004[2]	<0.004[2]	<0.004[2]	<0.004[2]	<0.004[2]	<0.004[2]
Caffeine	0.019[1]	0.019[1]	0.010[1]	0.017[1]	0.017[1]	0.022[1]
Carbamazepine	0.002[1]	0.002[1]	0.002[1]	0.004[1]	0.004[1]	0.004[1]
DEET	0.002[2]	0.002[2]	0.002[2]	0.003[2]	0.002[2]	0.002[2]
Estrone	<0.01[2]	<0.01[2]	<0.01[2]	<0.01[2]	<0.01[2]	<0.01[2]
Fluoxetine	0.025[1]	0.027[1]	0.022[1]	0.021[1]	0.020[1]	0.018[1]
Gemfibrozil	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	<0.005[2]	0.011[2]
Progesterone	0.002[1]	0.002[1]	0.002[1]	0.012	0.015	0.019
Sulfamethoxazole	0.017[1]	0.017[1]	0.011[1]	0.008[1]	0.007[1]	0.007[1]
Triclosan	0.091[1]	0.116[1]	0.139[1]	0.099[1]	0.118[1]	0.095[1]
Tris-(2-chloroethyl) Phosphate	0.014[2]	0.013[2]	0.010[2]	0.011[2]	<0.004	<0.004
[1]Method 1 ; [2]Method						

Appendix C. Raw Data : Anaerobic Digestion of Duckweed

Table C.1 Bromide concentration, TSS, gas production, methane, carbon dioxide and nitrogen gas composition in lab-scale anaerobic reactor

	Date	Br Concentration in Reactor(mg/L)	TSS(g/L)	VSS(g/g)	Gas Production(mL)	Methane %	CO_2 %	N_2 %
Background	9/13/2019	11.1	5.1	0.8	1110.0	72.3	17.4	10.3
	9/16/2019	11.9	11.9	0.8	1160.0	69.2	20.6	10.2
	9/19/2019	10.9	3.7	0.8	1330.0	69.3	20.9	9.8
After PPCP and Br Spike	9/20/2019	202.1	4.1	0.8	500.0	71.5	18.1	10.4
	9/23/2019	153.6	3.3	0.8	1220.0	68.7	19.3	12.0
	9/26/2019	122.8	4.0	0.9	1220.0	68.3	22.5	9.2
	9/29/2019	104.2	2.6	0.9	1160.0	72.4	18.2	9.4
	10/2/2019	78.7	4.5	0.7	1000.0	70.2	18.5	11.3
	10/5/2019	67.8	3.1	0.7	1120.0	71.9	19.3	8.8
	10/8/2019	59.8	1.0	0.8	1110.0	68.8	18.9	12.3
	10/11/2019	55.0	11.4	0.8	1000.0	71.1	18.8	10.1
	10/14/2019	41.5	12.7	0.8	1000.0	69.9	18.0	12.1
	10/17/2019	37.9	10.3	0.6	930.0	69.9	21.1	9.0
	10/20/2019	36.5	5.6	0.8	910.0	70.7	22.1	7.2

Table C.2 PPCP concentration in digestate solids sampled from lab-scale anaerobic digester

			PPCP Concentration on digestate solids (ug/g)												
	Date	Sample	Acetaminophen	β-Estradiol	Caffeine	Carbamazepine	DEET	Estrone	Fluoxetine	Gemfibrozil	Progesterone	Sulfomethoxazole	Triclosan	Tris 2 chloroethyl phosphate
Background	9/13/2019	Sample 1	<0.004	<0.05	0.02	0.06	0.13	<0.14	0.02	<0.05	0.05	0.02	<0.005	0.09
	9/13/2019	Sample 2	<0.004	<0.05	0.11	0.07	0.13	<0.14	0.11	<0.05	0.08	0.05	0.19	0.13
	9/13/2019	Sample 3	0.01	<0.05	0.17	0.18	0.24	<0.14	0.15	0.25	0.14	0.11	0.33	0.18
	9/16/2019	Sample 1	0.01	<0.05	0.07	0.06	0.13	<0.14	0.08	0.08	0.06	0.03	0.14	0.11
	9/16/2019	Sample 2	<0.004	<0.05	0.02	0.03	0.10	<0.14	0.04	<0.05	0.06	0.01	0.10	0.07
	9/16/2019	Sample 3	0.03	<0.05	0.24	0.27	0.30	<0.14	0.29	0.35	0.15	0.15	0.52	0.29
	9/19/2019	Sample 1	0.06	<0.05	0.04	0.04	0.14	<0.14	0.07	<0.05	0.06	0.01	0.22	0.12
	9/19/2019	Sample 2	<0.004	<0.05	0.04	0.03	0.15	<0.14	0.16	<0.05	0.07	0.02	0.36	0.00
	9/19/2019	Sample 3	0.12	<0.05	0.67	1.25	0.90	<0.14	0.40	1.31	0.48	0.52	1.50	1.46
After PPCP and Br Spike	9/20/2019	Sample 1	0.02	<0.05	1.56	2.60	1.74	5.36	3.08	1.90	0.41	0.27	13.27	1.75
	9/20/2019	Sample 2	0.07	<0.05	1.75	2.79	2.14	6.52	2.98	2.30	0.39	0.37	11.76	2.43
	9/20/2019	Sample 3	<0.004	<0.05	1.46	2.58	1.65	3.98	4.95	1.68	0.21	0.20	10.93	1.81
	9/23/2019	Sample 1	<0.004	<0.05	1.19	2.17	1.50	8.17	2.74	1.52	0.34	0.09	8.83	1.40
	9/23/2019	Sample 2	<0.004	<0.05	1.35	2.13	1.76	7.70	4.07	2.59	0.57	0.12	11.70	1.71
	9/23/2019	Sample 3	<0.004	<0.05	1.40	2.02	1.47	6.24	2.89	1.77	0.35	0.09	7.74	1.59
	9/26/2019	Sample 1	0.26	<0.05	1.02	1.59	1.09	6.18	2.23	0.73	0.21	0.03	12.15	1.17
	9/26/2019	Sample 2	0.11	<0.05	1.14	1.71	1.18	5.68	2.03	1.30	0.34	0.09	12.27	1.17
	9/26/2019	Sample 3	<0.004	<0.05	0.98	1.64	1.20	6.51	1.04	1.01	0.19	0.03	13.17	1.12
	9/29/2019	Sample 1	0.06	<0.05	0.99	1.43	1.31	6.97	0.96	0.62	0.21	0.05	3.17	1.23
	9/29/2019	Sample 2	<0.004	<0.05	0.87	1.43	0.98	5.63	1.82	0.64	0.15	0.03	4.28	0.96
	9/29/2019	Sample 3	<0.004	<0.05	0.96	1.40	0.94	3.64	1.43	0.86	0.14	0.00	4.17	1.08
	10/2/2019	Sample 1	<0.004	<0.05	0.49	1.15	0.67	3.28	1.75	0.93	0.15	0.02	3.90	0.68
	10/2/2019	Sample 2	<0.004	<0.05	0.42	1.14	0.64	2.16	0.87	0.84	0.14	0.01	3.65	0.67
	10/2/2019	Sample 3	<0.004	<0.05	0.42	1.10	0.68	2.36	1.35	1.12	0.18	0.01	4.63	0.66
	10/5/2019	Sample 1	<0.004	<0.05	0.30	0.82	0.61	2.85	0.93	1.07	0.09	0.00	1.89	0.52
	10/5/2019	Sample 2	<0.004	<0.05	0.28	0.82	0.54	2.71	0.61	0.68	0.09	0.01	1.99	0.49
	10/5/2019	Sample 3	<0.004	<0.05	0.33	0.82	0.58	2.47	0.75	0.73	0.10	0.02	1.97	0.48
	10/8/2019	Sample 1	<0.004	<0.05	<0.002	<0.002	<0.01	<0.14	<0.001	<0.05	<0.002	<0.003	<0.06	<0.04
	10/8/2019	Sample 2	<0.004	<0.05	<0.002	<0.002	<0.01	<0.14	<0.001	<0.05	<0.002	<0.003	<0.06	<0.04
	10/8/2019	Sample 3	<0.004	<0.05	<0.002	<0.002	<0.01	<0.14	<0.001	<0.05	<0.002	<0.003	<0.06	<0.04
	10/11/2019	Sample 1	<0.004	<0.05	0.15	0.57	0.33	2.59	0.98	0.48	0.05	0.01	2.10	0.29
	10/11/2019	Sample 2	<0.004	<0.05	0.17	0.60	0.36	2.60	0.95	0.59	0.05	0.005	2.24	0.30
	10/11/2019	Sample 3	<0.004	<0.05	0.17	0.67	0.37	2.84	1.00	0.57	0.05	0.01	2.16	0.29
	10/14/2019	Sample 1	<0.004	<0.05	0.12	0.51	0.30	1.74	0.93	0.44	0.06	0.006	1.91	0.28
	10/14/2019	Sample 2	<0.004	<0.05	0.11	0.52	0.31	1.65	1.13	0.52	0.07	0.004	2.08	0.22
	10/14/2019	Sample 3	<0.004	<0.05	0.11	0.47	0.28	1.64	1.05	0.46	0.06	0.004	2.02	0.22
	10/17/2019	Sample 1	<0.004	<0.05	0.10	0.50	0.39	2.69	1.13	0.61	0.04	0.004	2.24	0.27
	10/17/2019	Sample 2	<0.004	<0.05	0.09	0.55	0.29	3.21	0.88	0.36	0.04	0.004	1.87	0.23
	10/17/2019	Sample 3	<0.004	<0.05	0.13	0.56	0.51	3.36	1.21	0.62	0.04	<0.003	2.35	0.46
	10/20/2019	Sample 1	<0.004	<0.05	0.09	0.59	0.30	4.80	0.78	0.55	0.07	<0.003	1.34	0.16
	10/20/2019	Sample 2	<0.004	<0.05	0.07	0.51	0.28	2.55	0.73	0.51	0.06	<0.003	1.35	0.29
	10/20/2019	Sample 3	<0.004	<0.05	0.08	0.55	0.26	2.20	0.96	0.28	0.06	<0.003	1.52	0.24

Table C.3 PPCP concentration in digestate liquid sampled from lab-scale anaerobic digester

			PPCP concentration in digestate liquids (ug/L)											
	Date	Sample	Acetaminophen	β-Estradiol	Caffeine	Carbamazepine	DEET	Estrone	Fluoxetine	Gemfibrozil	Progesterone	Sulfomethoxazole	Triclosan	Tris 2 chloroethyl phosphate
Background	9/13/2019	Sample 1	<0.018	<0.23	0.07	0.16	0.36	<0.67	<0.006	<0.26	<0.01	<0.02	<0.30	0.59
	9/13/2019	Sample 2	<0.018	<0.23	0.08	0.17	0.36	<0.67	<0.006	<0.26	<0.01	<0.02	<0.30	0.58
	9/13/2019	Sample 3	<0.018	<0.23	0.06	0.17	0.38	<0.67	<0.006	<0.26	<0.01	<0.02	<0.30	0.62
	9/16/2019	Sample 1	0.03	<0.23	<0.01	0.14	0.37	<0.67	<0.006	<0.26	<0.01	<0.02	<0.30	0.55
	9/16/2019	Sample 2	0.03	<0.23	<0.01	0.14	0.35	<0.67	<0.006	<0.26	<0.01	<0.02	<0.30	0.58
	9/16/2019	Sample 3	<0.018	<0.23	<0.01	0.15	0.36	<0.67	<0.006	<0.26	<0.01	<0.02	<0.30	0.54
	9/19/2019	Sample 1	0.16	<0.23	0.07	0.31	0.32	<0.67	0.07	<0.26	0.02	0.24	<0.30	0.57
	9/19/2019	Sample 2	0.18	<0.23	0.07	0.32	0.32	<0.67	0.08	<0.26	0.02	0.25	<0.30	0.61
	9/19/2019	Sample 3	0.16	<0.23	0.07	0.33	0.31	<0.67	0.08	<0.26	0.02	0.25	<0.30	0.67
After PPCP and Br Spike	9/20/2019	Sample 1	2.61	2.59	71.73	79.23	78.02	37.49	3.88	53.21	0.02	62.25	0.60	96.93
	9/20/2019	Sample 2	2.57	2.37	72.35	76.88	78.87	35.97	3.75	55.44	0.03	61.80	0.43	94.53
	9/20/2019	Sample 3	2.54	2.32	72.17	78.51	71.47	36.65	4.05	49.04	0.02	61.79	0.58	85.99
	9/23/2019	Sample 1	1.11	2.52	47.50	52.78	53.12	18.28	3.43	36.80	0.05	19.20	2.80	71.46
	9/23/2019	Sample 2	1.35	2.34	46.12	55.05	55.95	17.71	3.43	38.81	<0.01	18.86	0.55	68.40
	9/23/2019	Sample 3	2.62	2.05	45.38	55.56	56.15	20.50	2.81	39.80	0.06	19.27	9.11	73.58
	9/26/2019	Sample 1	0.18	0.69	34.60	44.96	44.61	14.18	1.57	30.41	<0.01	4.53	<0.30	52.56
	9/26/2019	Sample 2	0.14	0.23	35.24	47.66	44.05	16.81	1.68	31.47	<0.01	4.52	<0.30	57.74
	9/26/2019	Sample 3	0.12	0.52	34.58	45.89	44.79	15.03	1.63	33.62	<0.01	4.57	<0.30	52.98
	9/29/2019	Sample 1	0.14	0.35	27.42	38.15	37.93	14.42	1.31	26.57	<0.01	1.07	<0.30	46.08
	9/29/2019	Sample 2	0.11	0.56	27.12	37.63	38.25	11.36	1.24	26.93	<0.01	1.00	<0.30	43.63
	9/29/2019	Sample 3	0.20	0.83	26.26	38.54	37.27	12.21	1.30	27.26	<0.01	1.01	<0.30	43.84
	10/2/2019	Sample 1	0.16	1.58	22.14	32.02	31.94	11.37	1.68	27.38	0.65	2.10	<0.30	36.66
	10/2/2019	Sample 2	0.15	1.76	22.60	33.52	31.37	10.59	1.68	28.26	0.45	2.15	<0.30	35.58
	10/2/2019	Sample 3	0.14	1.48	22.45	32.95	31.82	9.77	1.62	29.03	0.54	2.15	<0.30	36.31
	10/5/2019	Sample 1	0.09	0.87	17.27	29.74	26.94	7.66	1.21	18.39	<0.01	0.09	<0.30	27.41
	10/5/2019	Sample 2	0.12	1.15	17.29	26.51	26.56	8.71	1.21	19.47	<0.01	0.08	<0.30	27.71
	10/5/2019	Sample 3	0.10	1.02	17.06	27.12	25.32	10.37	1.15	19.15	<0.01	0.10	<0.30	29.18
	10/8/2019	Sample 1	0.12	<0.23	13.59	24.66	23.10	7.35	1.05	16.36	<0.01	0.04	<0.30	23.13
	10/8/2019	Sample 2	0.12	<0.23	13.73	24.94	22.59	8.94	1.03	15.84	<0.01	0.02	<0.30	22.70
	10/8/2019	Sample 3	0.14	<0.23	13.42	24.58	22.29	7.95	0.99	15.54	<0.01	0.05	<0.30	22.84
	10/11/2019	Sample 1	0.07	1.13	10.24	21.40	20.89	5.96	1.01	14.42	<0.01	0.04	<0.30	19.51
	10/11/2019	Sample 2	0.09	1.01	10.42	22.40	18.94	6.57	0.99	14.06	<0.01	0.04	<0.30	20.01
	10/11/2019	Sample 3	0.07	1.01	10.41	22.34	19.80	6.80	0.95	15.59	<0.01	0.04	<0.30	18.45
	10/14/2019	Sample 1	0.08	<0.23	9.28	23.87	19.65	6.25	0.97	15.74	0.01	0.14	<0.30	19.84
	10/14/2019	Sample 2	2.01	<0.23	9.36	22.40	19.11	10.05	0.94	13.69	<0.01	0.15	<0.30	19.31
	10/14/2019	Sample 3	0.14	<0.23	9.16	23.03	19.15	6.45	1.03	14.46	0.03	0.15	<0.30	19.21
	10/17/2019	Sample 1	0.11	<0.23	6.45	19.96	15.87	5.94	0.83	12.61	<0.01	0.34	<0.30	15.87
	10/17/2019	Sample 2	0.21	<0.23	6.40	21.08	16.47	5.17	0.83	11.37	0.03	0.55	<0.30	16.84
	10/17/2019	Sample 3	0.10	<0.23	6.33	20.46	16.45	5.88	0.77	11.77	<0.01	0.34	<0.30	16.75
	10/20/2019	Sample 1	0.07	<0.23	3.46	15.81	13.60	2.73	0.51	10.63	<0.01	<0.02	<0.30	11.31
	10/20/2019	Sample 2	0.08	<0.23	3.54	16.81	13.50	3.40	0.50	9.10	<0.01	<0.02	<0.30	12.05
	10/20/2019	Sample 3	0.08	<0.23	3.49	16.94	13.45	3.04	0.50	10.79	<0.01	<0.02	<0.30	11.92

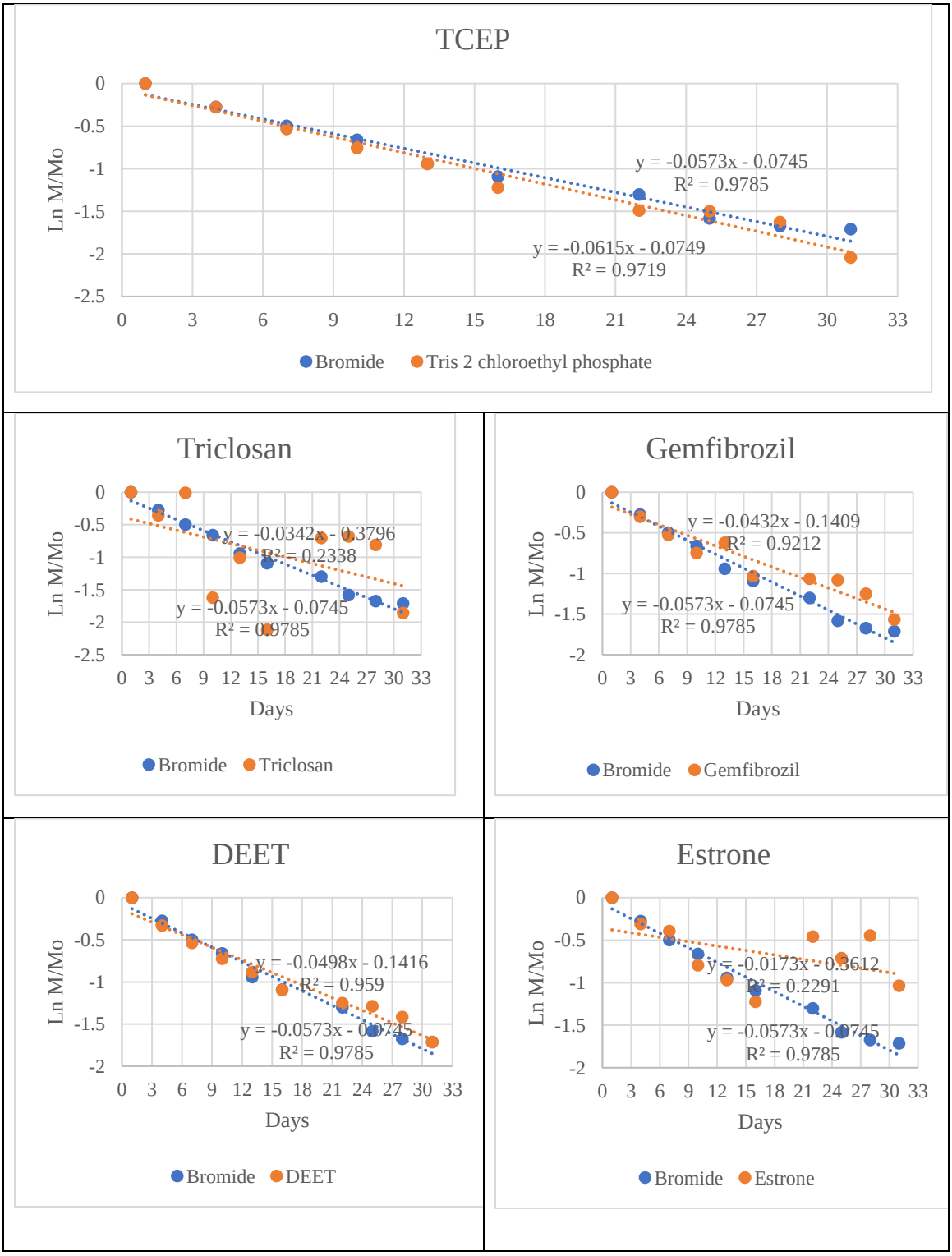


Fig. C.1 First order linear regression of TCEP, triclosan, gemfibrozil, DEET, estrone and bromide washout from lab-scale anaerobic digester

Appendix D. Raw Data and Extra Graphs : Duckweed and WAS Compost

Table D.1 PPCP concentration in duckweed compost

Day	Acetaminophen	β-Estradiol	Caffeine	Carbamazepine	DEET	Estrone	Fluoxetine	Gemfibrozil	Progesterone	Sulfamethoxazole	Triclosan	Tris-(2-chloroethyl) Phosphate
	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g
T0	17.08	6.08	56.31	41.94	66.96	83.27	22.62	49.12	11.69	18.12	31.78	71.97
T0	16.57	5.31	54.96	44.56	64.58	57.99	26.70	49.11	12.55	17.45	42.18	66.10
T0	20.00	6.36	61.88	52.38	76.02	73.56	29.82	54.76	14.08	20.22	36.53	82.04
T3	<0.04	<0.5	24.55	54.47	62.95	30.64	17.85	51.06	6.62	4.00	42.36	74.85
T3	<0.04	6.04	26.05	71.66	65.58	35.79	25.78	55.99	5.54	4.28	39.29	78.49
T3	<0.04	<0.5	25.45	56.38	64.58	39.98	22.09	51.80	8.58	4.25	44.19	74.66
T6	<0.04	<0.5	6.32	56.10	52.69	29.51	16.91	44.96	7.03	2.08	37.82	80.18
T6	<0.04	<0.5	6.22	55.54	51.12	29.31	17.02	48.02	6.61	1.81	38.25	75.54
T6	<0.04	<0.5	6.22	64.65	53.05	36.31	21.64	54.49	8.46	1.26	51.84	82.87
T9	<0.04	<0.5	1.09	59.50	0.93	27.68	21.23	39.62	2.36	0.56	46.93	87.95
T9	<0.04	<0.5	1.47	59.65	1.06	20.68	20.99	42.74	2.77	1.13	54.39	70.15
T9	<0.04	<0.5	1.43	65.46	1.38	28.69	23.33	41.09	2.74	0.99	54.35	92.14
T12	<0.04	<0.5	0.77	65.37	0.70	35.46	15.22	39.49	0.98	0.99	28.79	70.87
T12	<0.04	<0.5	0.44	65.21	0.96	41.10	12.18	33.89	8.14	0.54	73.38	71.18
T12	<0.04	<0.5	0.51	66.69	0.37	32.12	16.26	35.42	1.34	0.51	34.72	68.30
T15	<0.04	<0.5	0.61	60.95	0.49	25.97	14.25	32.16	0.15	0.75	23.45	65.06
T15	<0.04	<0.5	0.72	59.07	0.28	19.42	13.19	29.42	0.20	0.79	26.62	66.73
T15	<0.04	<0.5	0.95	65.30	0.44	24.62	15.23	32.04	0.36	0.80	35.46	72.95
T18	<0.04	<0.5	0.59	41.36	0.62	21.89	10.30	21.75	0.12	0.56	21.76	48.58
T18	<0.04	<0.5	0.58	56.63	0.26	23.46	11.86	23.04	0.04	0.54	18.49	47.47
T18	<0.04	<0.5	0.59	75.64	0.26	24.86	12.49	25.64	0.54	0.60	21.92	52.66
T21	<0.04	<0.5	0.92	37.29	0.19	35.90	10.41	21.95	1.11	0.30	41.66	49.13
T21	<0.04	<0.5	0.88	38.13	0.24	19.38	13.94	22.88	0.07	0.56	25.13	49.59
T21	<0.04	<0.5	1.10	39.12	0.88	24.51	11.30	24.49	0.03	0.32	23.53	51.47

Duckweed Compost

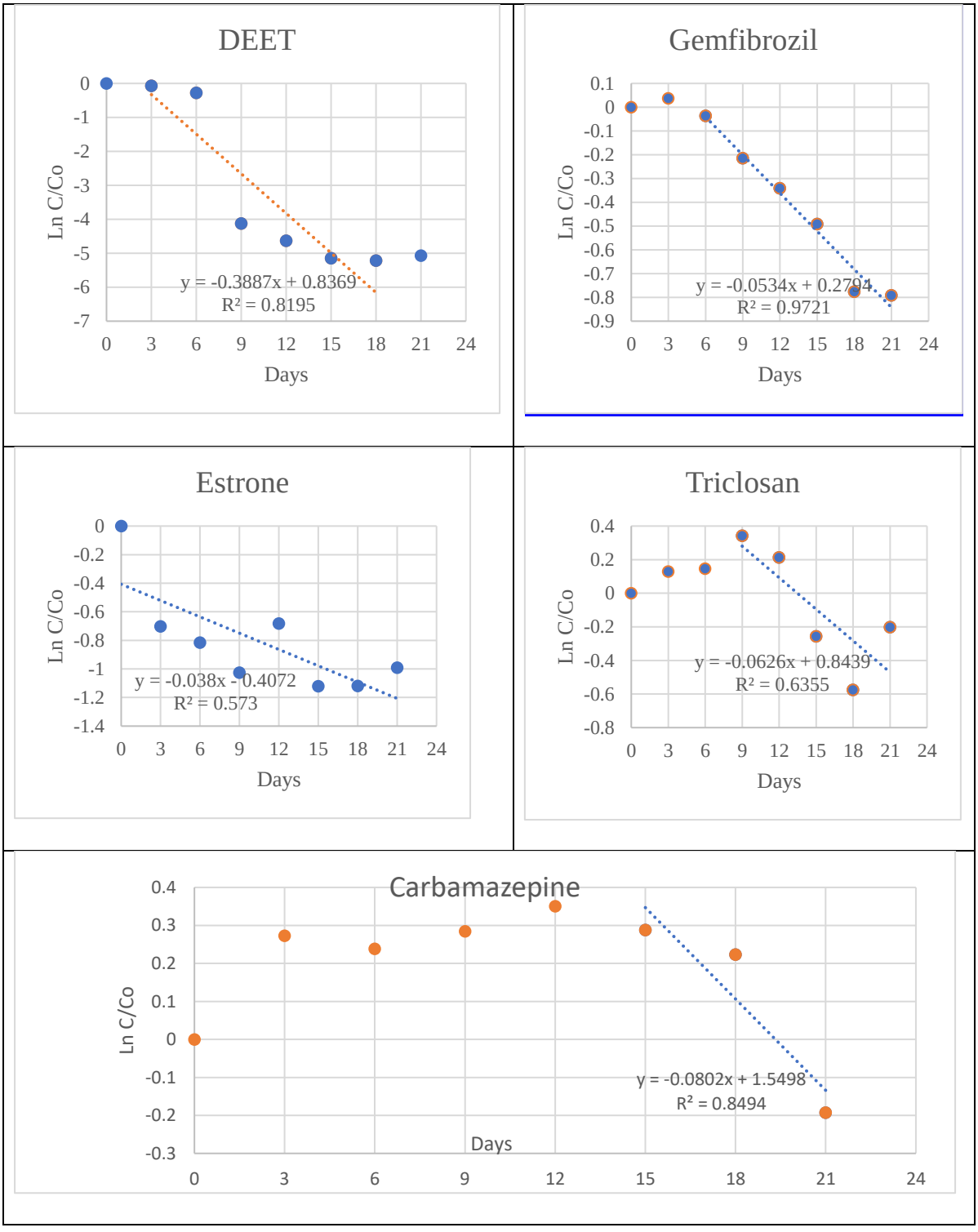


Fig. D.1 First order linear regression of DEET, gemfibrozil, estrone, triclosan and carbamazepine in duckweed composted for 21 days.

Temperature- PPCP concentration graph for duckweed compost

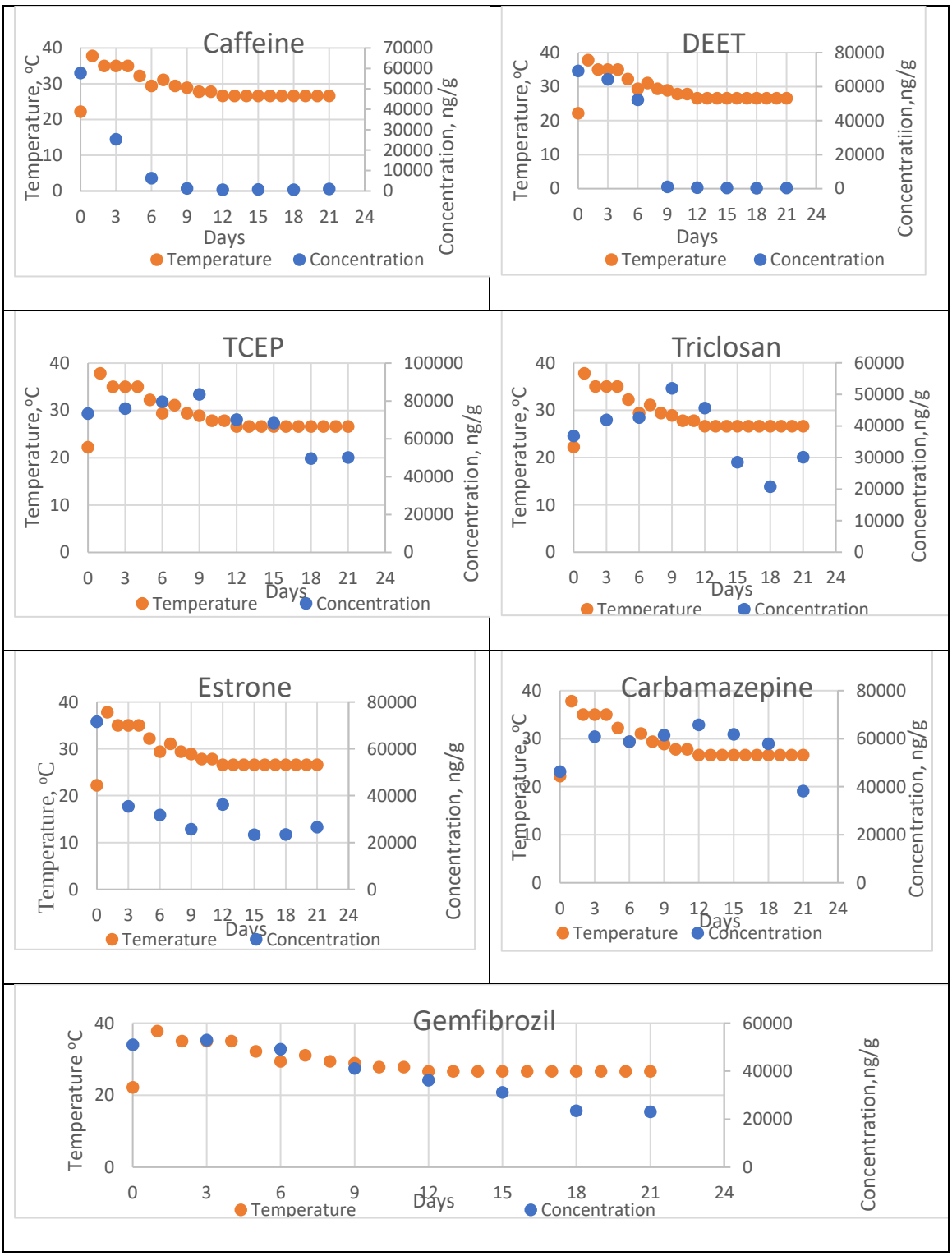


Fig. D. 2 Temperature profile and concentrations of caffeine, DEET, tris-2-chloroehyl phosphate, estrone, carbamazepine and gemfibrozil in compost

Table D.2 PPCP concentrations in Hyrum WAS compost

Day	Acetaminophen	β-Estradiol	Caffeine	Carbamazepine	DEET	Estrone	Fluoxetine	Gemfibrozil	Progesterone	Sulfamethoxazole	Triclosan	Tris-(2-chloroethyl) Phosphate
	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g
T0	6.01	4.55	15.90	17.03	19.29	33.14	7.43	13.38	9.62	10.65	19.95	23.85
T0	5.54	4.63	14.66	15.71	19.02	18.09	7.70	10.44	8.20	10.97	15.00	23.22
T0	5.83	5.02	15.76	15.87	20.17	24.18	5.62	10.68	8.34	11.16	14.79	24.70
T3	<0.04	0.00	20.69	20.88	24.22	29.48	7.49	14.42	0.86	3.64	16.74	31.69
T3	<0.04	2.46	20.31	19.11	24.32	25.41	7.70	11.47	0.27	2.99	15.34	31.94
T3	<0.04	2.00	18.85	16.80	23.04	19.48	5.95	10.16	0.29	2.83	13.51	27.49
T6	<0.04	<0.5	19.75	19.16	17.55	14.10	6.86	12.67	0.09	1.87	15.46	27.83
T6	<0.04	<0.5	15.40	17.12	16.69	15.88	4.67	10.87	<0.02	1.70	13.09	24.21
T6	<0.04	<0.5	18.29	18.78	19.40	17.60	6.79	11.08	0.02	1.93	15.51	27.31
T9	<0.04	<0.5	8.64	17.30	16.27	17.91	4.75	9.11	<0.02	0.97	9.94	28.23
T9	<0.04	<0.5	7.78	15.87	14.48	18.70	5.14	8.01	<0.02	0.93	9.92	25.83
T9	<0.04	1.06	8.02	17.49	14.86	18.45	6.13	8.32	<0.02	1.00	10.51	26.85
T12	<0.04	<0.5	0.66	15.40	9.95	8.13	2.80	8.30	<0.02	0.55	6.37	25.62
T12	<0.04	<0.5	0.84	19.46	10.83	13.93	5.36	8.55	<0.02	0.77	8.10	26.83
T12	<0.04	<0.5	0.67	17.72	10.54	20.23	4.44	7.67	<0.02	0.92	7.69	27.19
T15	<0.04	<0.5	0.42	19.89	4.42	15.21	4.80	9.05	<0.02	0.45	9.49	28.85
T15	<0.04	<0.5	0.44	21.05	4.34	14.62	4.78	8.94	0.05	0.47	8.69	29.43
T15	<0.04	<0.5	0.62	16.01	3.60	10.80	4.34	8.54	0.61	0.39	10.80	22.32
T18	<0.04	<0.5	0.20	13.35	1.68	28.27	3.47	6.39	<0.02	0.24	7.10	18.47
T18	<0.04	0.59	0.25	14.37	1.52	15.87	3.86	6.45	<0.02	0.26	7.58	20.71
T18	<0.04	<0.5	0.27	17.59	1.98	19.19	4.50	8.45	<0.02	0.47	8.56	22.80
T21	0.32	1.55	0.43	15.69	0.87	14.00	4.27	6.14	<0.02	0.21	7.15	21.80
T21	<0.04	<0.5	0.47	14.57	0.89	14.61	3.50	6.10	<0.02	0.23	6.82	21.50
T21	<0.04	<0.5	0.46	13.88	1.28	15.89	3.58	6.19	<0.02	0.21	7.35	19.52

Hyrum WAS Compost

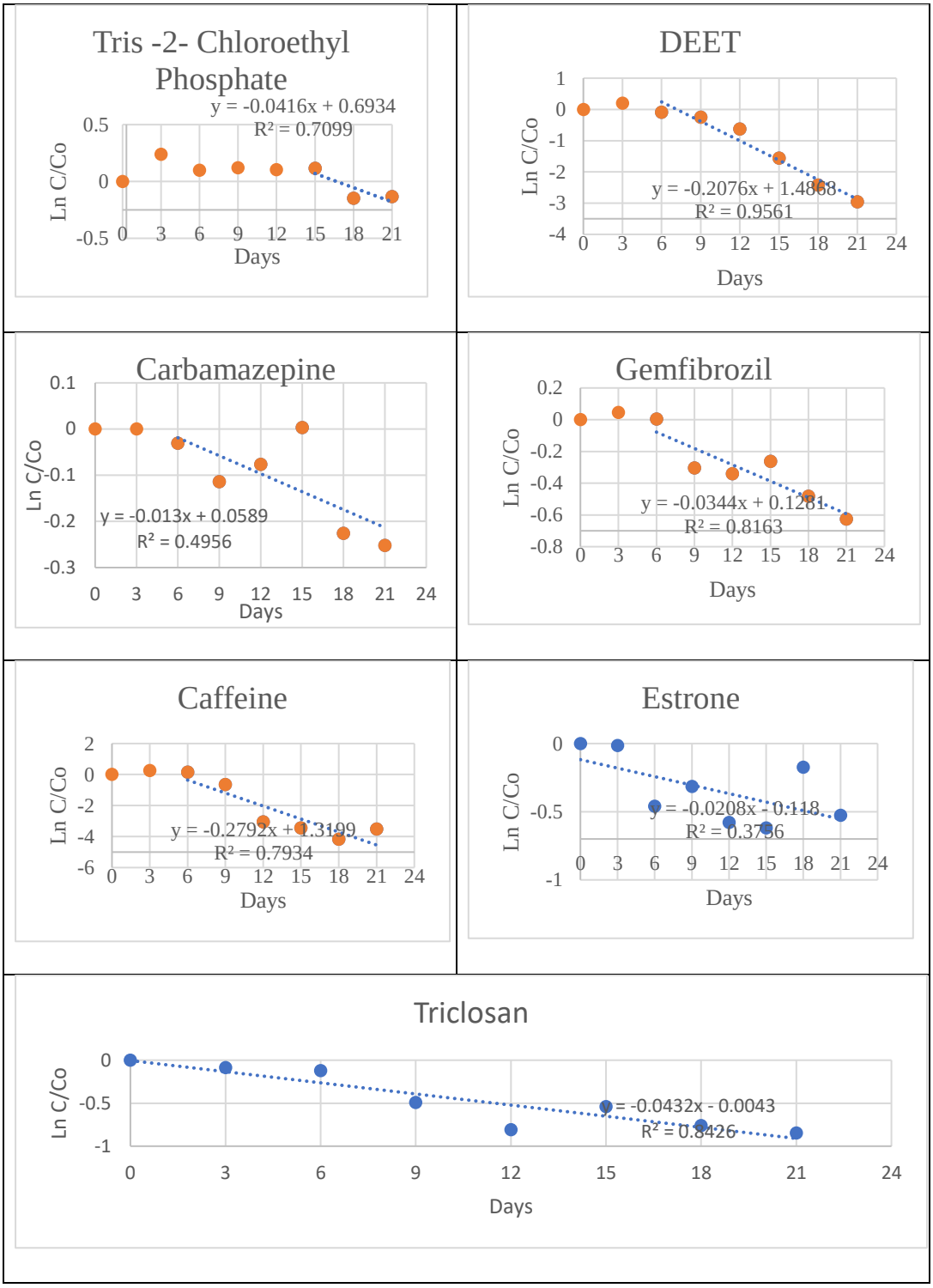


Fig. D.3 First order linear regression of TCEP, DEET, carbamazepine, gemfibrozil, caffeine, estrone and triclosan in Hyrum WAS compost

Temperature-PPCP concentration graph in WAS compost

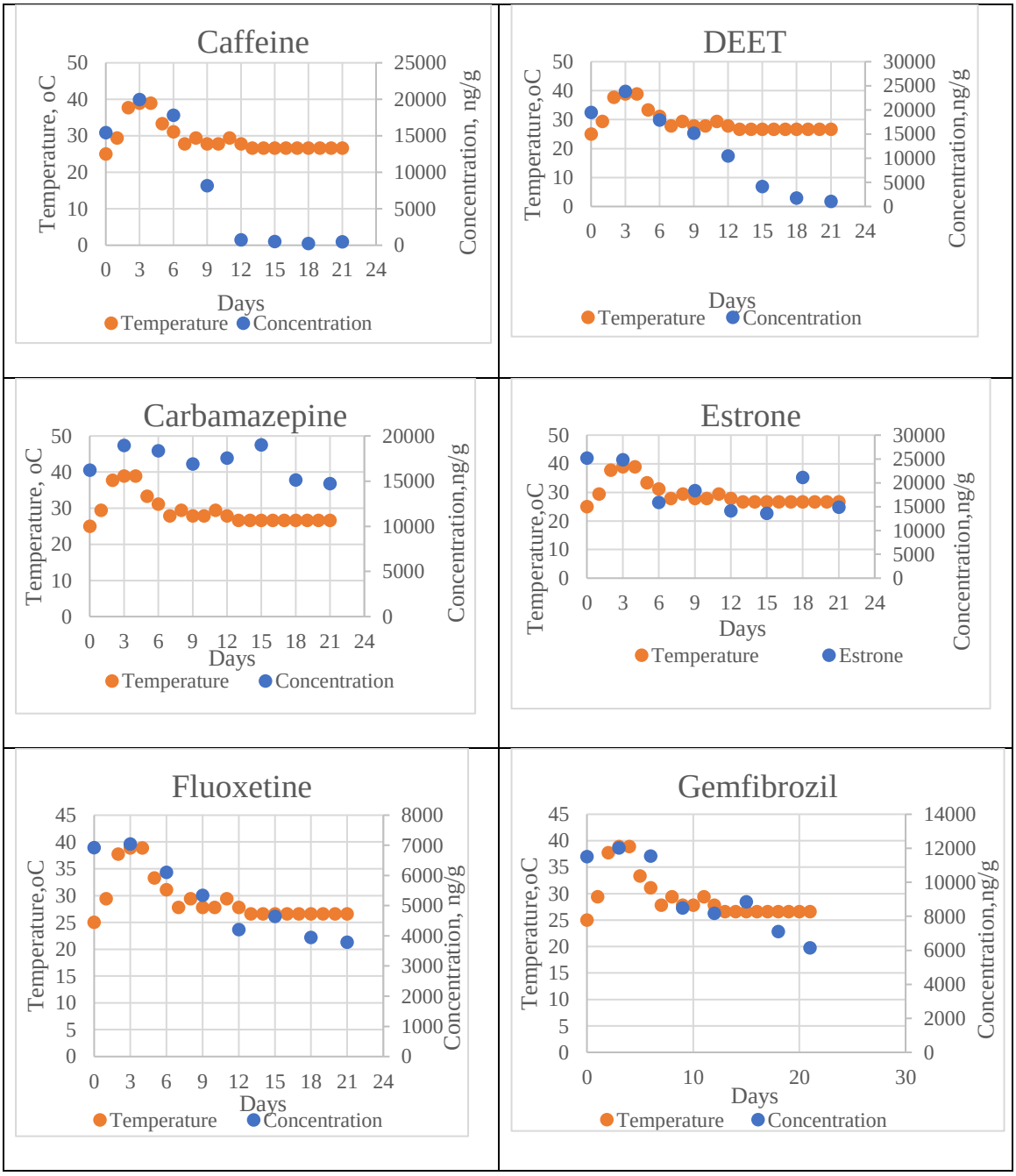


Fig. D.4 Temperature profile and PPCP and concentrations of caffeine, DEET, carbamazepine, estrone, fluoxetine and gemfibrozil in Hyrum WAS compost

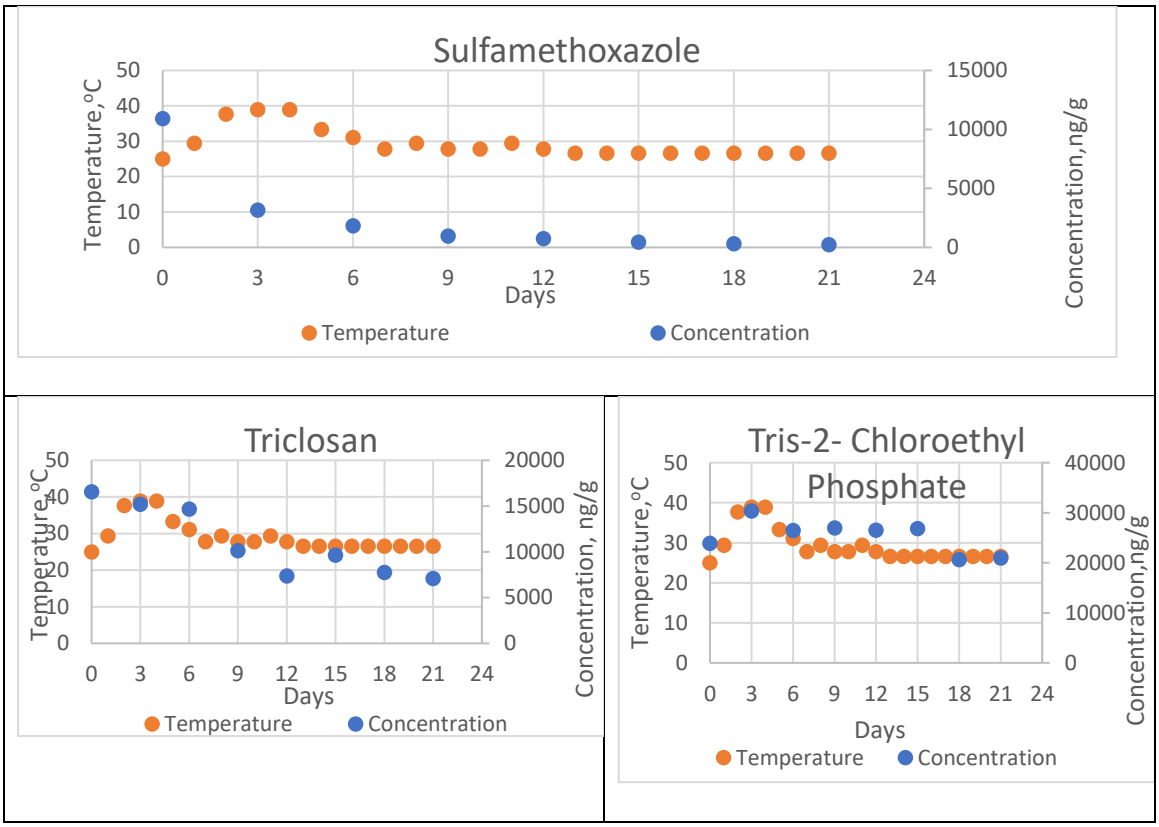


Fig. D.5 Temperature profile and concentrations of sulfamethoxazole, triclosan, and TCEP in Hyrum WAS compost

Table D.3 Temperature and oxygen content duckweed compost and WAS compost

Duckweed Compost			WAS Compost		
Day	Temperature, ºC	Oxygen, %	Day	Temperature, ºC	Oxygen, %
0	22.2	20.5	0	25	20.4
1	37.8	20.6	1	29.4	20.7
2	35	20.7	2	37.7	20
3	35	20.3	3	38.9	20.6
4	35	20.4	4	38.9	20.6
5	32.2	20.5	5	33.3	20.7
6	29.4	20.6	6	31.1	20.7
7	31.1	20.7	7	27.8	20.6
8	29.4	20.4	8	29.4	20.5
9	28.9	20.6	9	27.8	20.5
10	27.8	20.4	10	27.8	20.5
11	27.8	20.6	11	29.4	20.4
12	26.6	20.4	12	27.8	20.4
13	26.6	20.7	13	26.6	20.5
14	26.6	20.4	14	26.6	20.5
15	26.6	20.5	15	26.6	20.4
16	26.6	20.5	16	26.6	20.5
17	26.6	20.5	17	26.6	20.4
18	26.6	20.5	18	26.6	20.5
19	26.6	20.4	19	26.6	20.5
20	26.6	20.5	20	26.6	20.5
21	26.6	20.5	21	26.6	20.5

Table D.4 pH of influent and effluent water samples from Hyrum WWTP and Wellsville sewage lagoons

Sample	pH
Wellsville sewage lagoon influent	6.50
Wellsville sewage lagoon Cell 1	6.79
Wellsville sewage lagoon Cell 2	6.71
Wellsville sewage lagoon Cell 3	6.95
Wellsville sewage lagoon Cell 4	6.79
Wellsville sewage lagoon effluent	6.80
Hyrum WWTP influent	6.93
Hyrum WWTP effluent	6.92

Table D.5 PPCP Concentration Lagoon Duckweed Fed into Lab-scale Anaerobic Digester

PPCP Concentration in Lagoon Duckweed (ug/g)			
	Sample 1	Sample 2	Sample 3
Acetaminophen	<0.004	<0.004	<0.004
B Estradiol	<0.05	<0.05	<0.05
Caffeine	0.008	0.006	0.008
Carbamazepine	0.003	0.003	0.004
DEET	0.044	0.015	0.045
Estrone	<0.14	<0.14	<0.14
Fluoxetine	0.002	<0.001	<0.001
Gemfibrozil	<0.05	<0.05	<0.05
Progesterone	0.018	0.005	0.018
Sulfamethoxazole	<0.003	<0.003	<0.003
Triclosan	<0.06	<0.06	<0.06
TCEP	0.053	<0.04	0.052

Table D.6 Percent removal (± 95% CI) of PPCPs from duckweed and WAS compost (n=3).

	Duckweed (%)	WAS (%)
Caffeine	98.3±0.08	97.0±0.26
Carbamazepine	-	-
DEET	99.4±0.39	94.8±1.16
Estrone	63.4±4.50	-
Fluoxetine	-	44.5±10.5
Gemfibrozil	54.7±0.87	45.9±8.03
Sulfamethoxazole	-	98.0±0.15
Triclosan	-	56.3±8.03
Tris-(2-chloroethyl) Phosphate	31.3±4.93	12.3±8.48

Appendix E. Analysis of Variance Plots

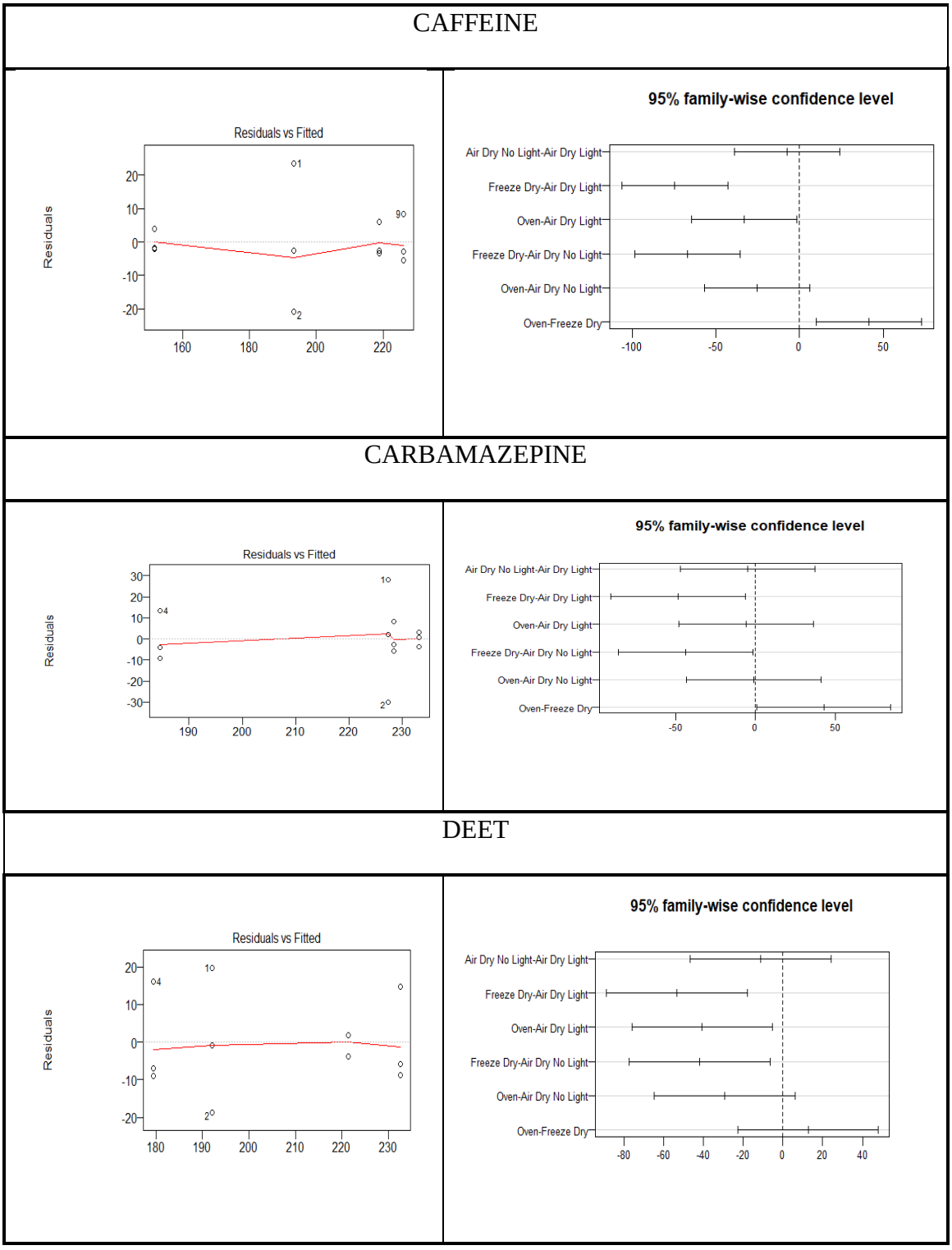


Fig. E.1 Residual plot and Tukey HSD plots comparing effects of drying method carbamazepine, DEET and caffeine concentrations in duckweed

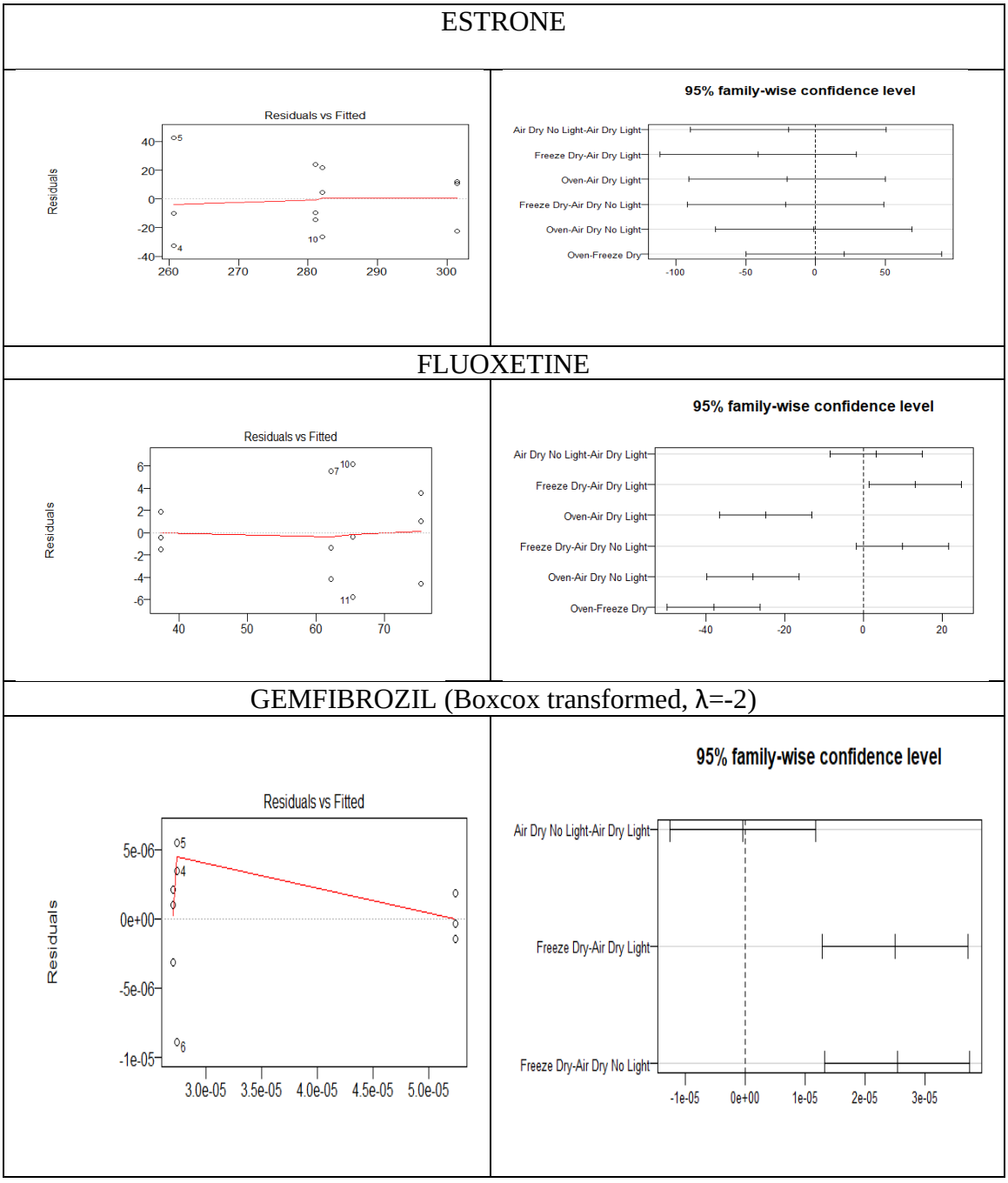


Fig. E.2 Residual plot and TukeyHSD plot comparing effects of different drying methods on estrone, fluoxetine and gemfibrozil concentration in duckweed

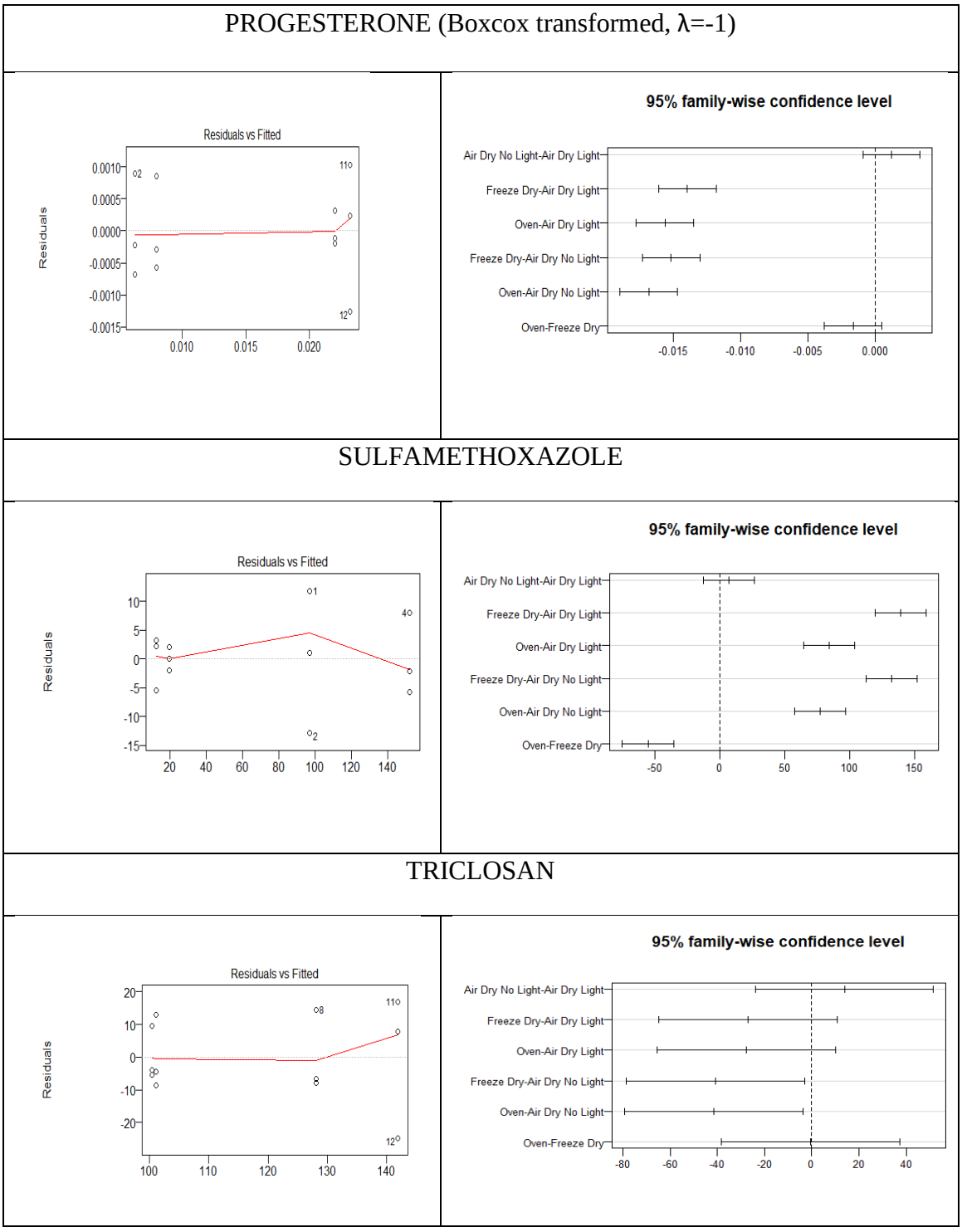


Fig. E.3 Residual plot and TukeyHSD plot comparing effects of different drying methods on progesterone, sulfamethoxazole and triclosan concentration in duckweed

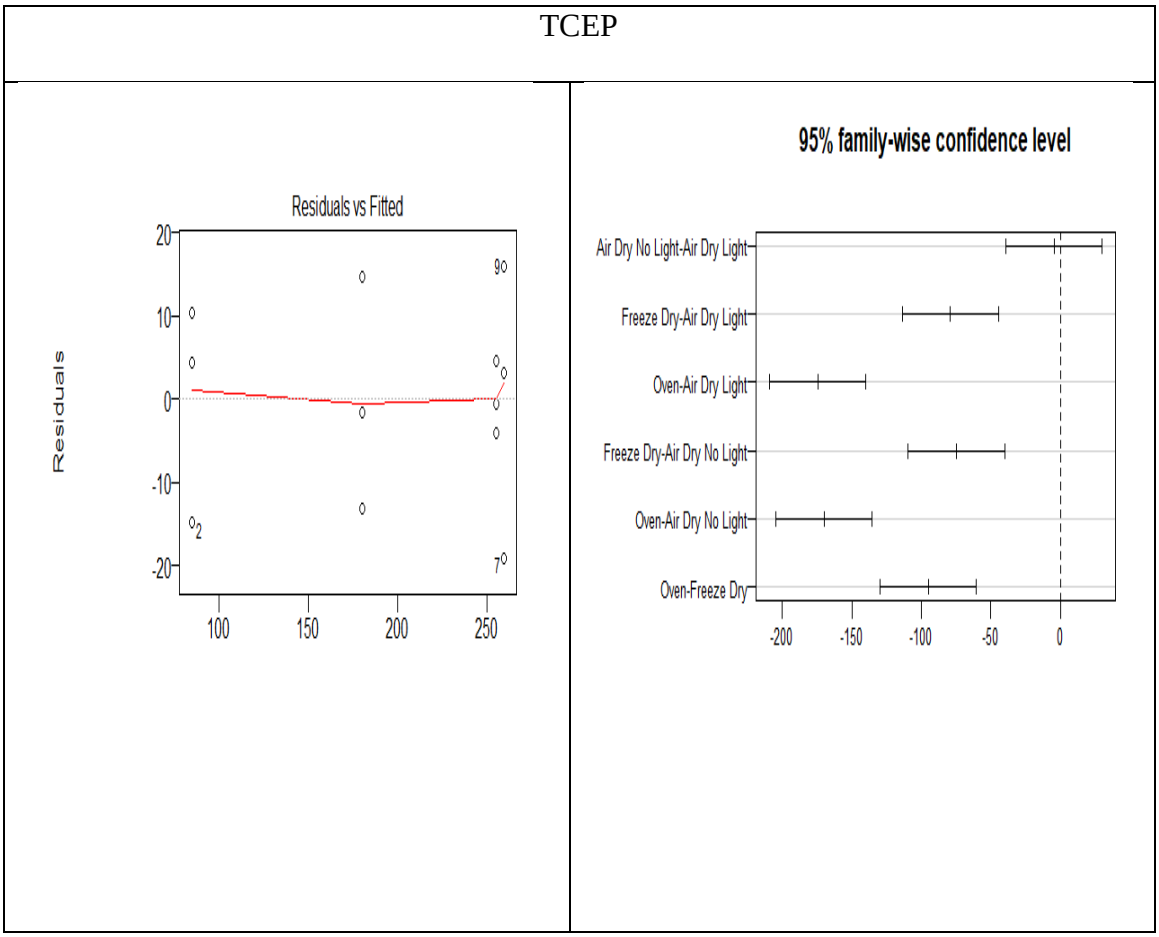


Fig. E.4 Residual plot and TukeyHSD plot comparing effects of different drying methods on TCEP concentration in duckweed

Hyrum WAS

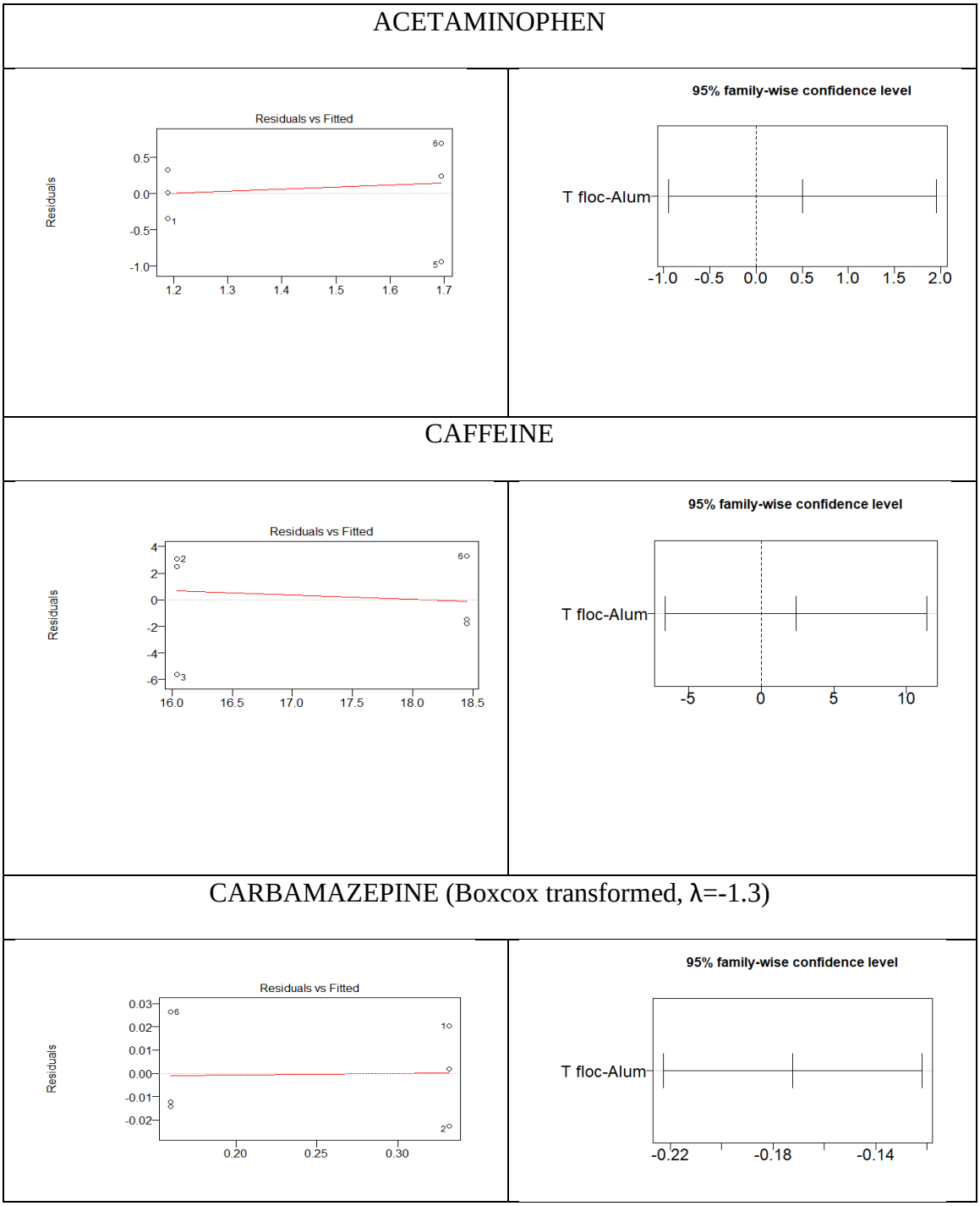


Fig. E.5 Residual plot and Tukey HSD plot comparing effect of chemical addition on acetaminophen, caffeine and carbamazepine concentration on Hyrum WAS

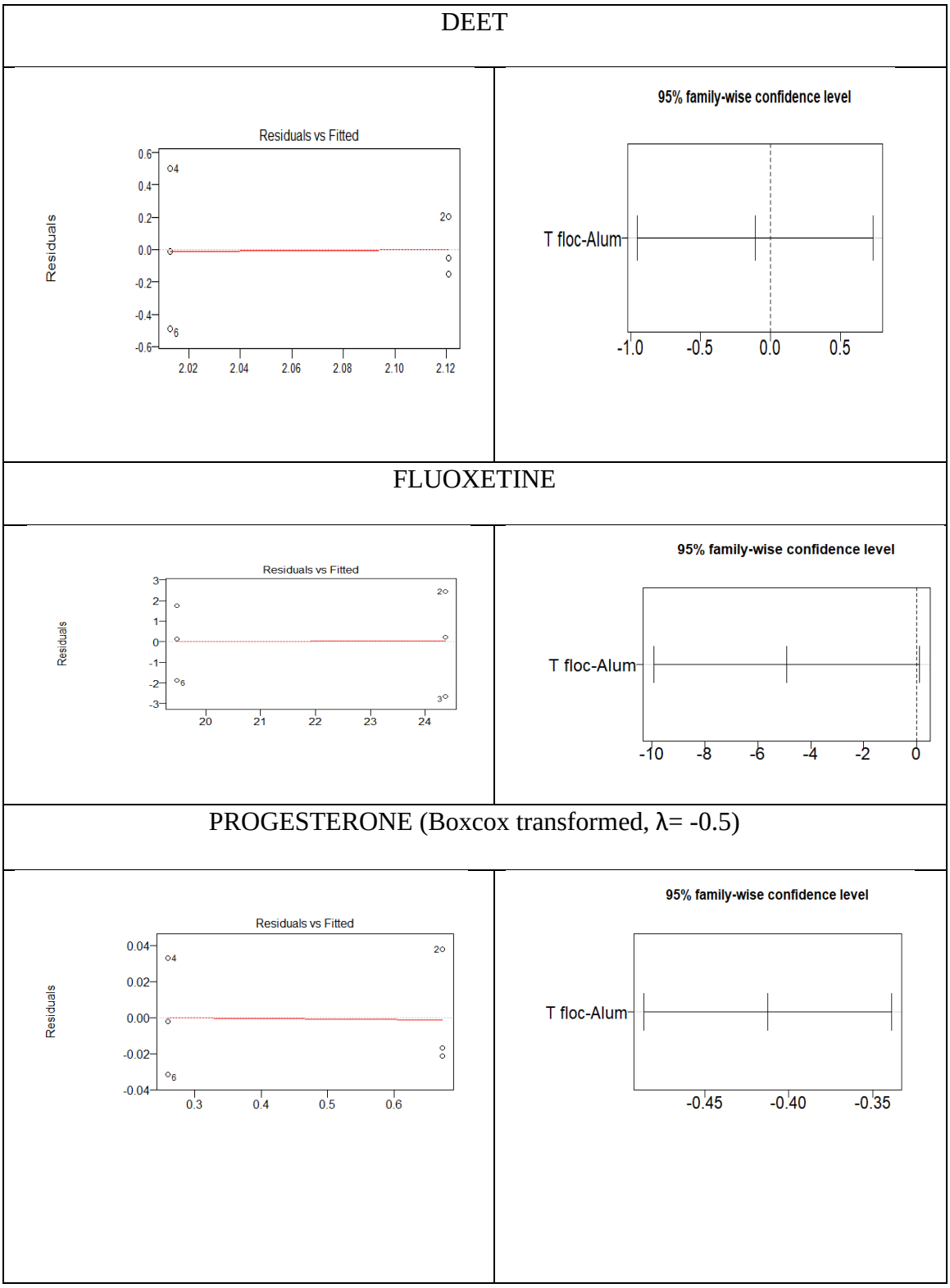


Fig. E.6 Residual plot and Tukey HSD plot comparing effect of chemical addition on DEET, fluoxetine, progesterone, concentration on Hyrum WAS

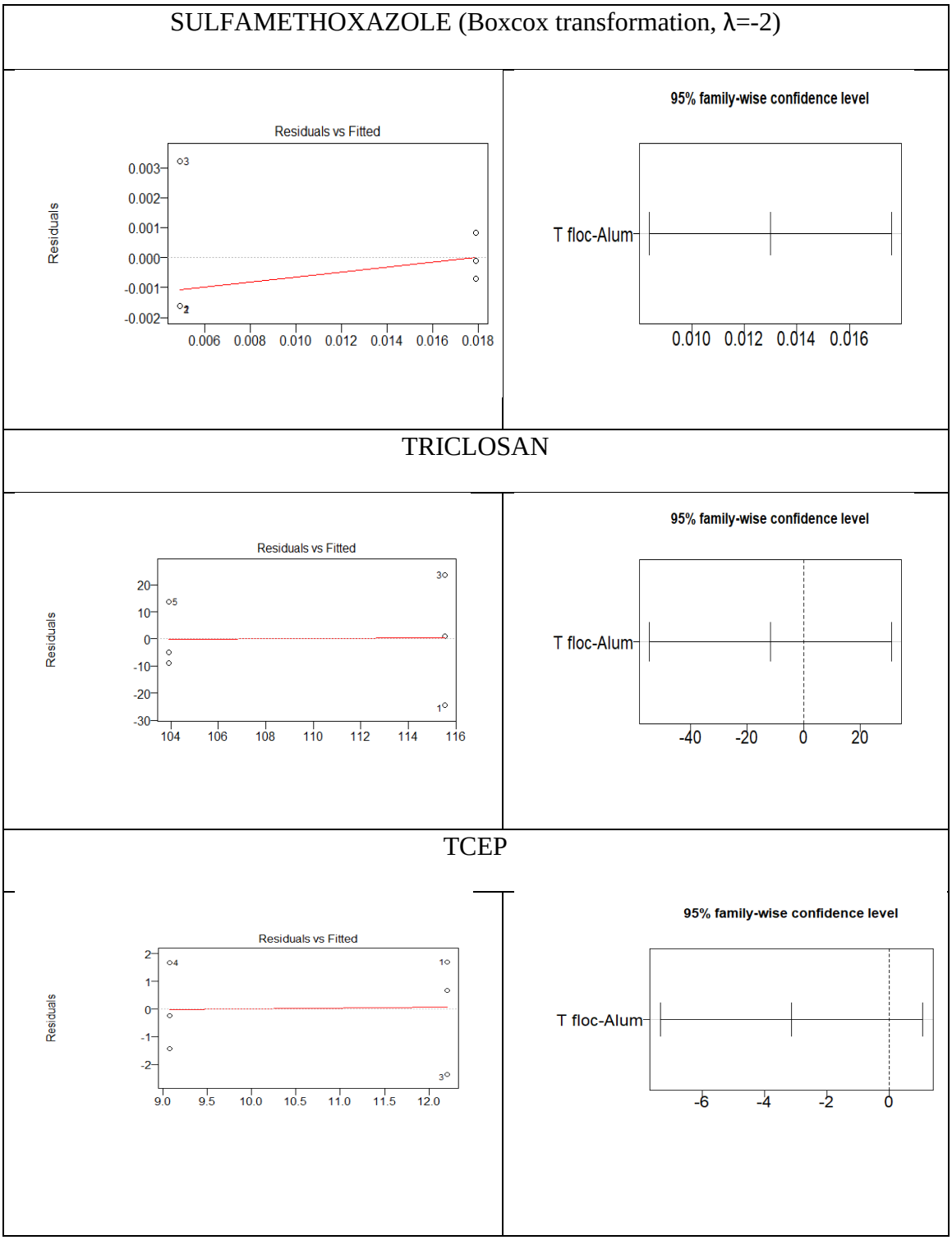


Fig. E.7 Residual plot and Tukey HSD plot comparing effect of chemical addition on sulfamethoxazole, triclosan and TCEP concentration on Hyrum WAS

Hyrum WWTP Effluent

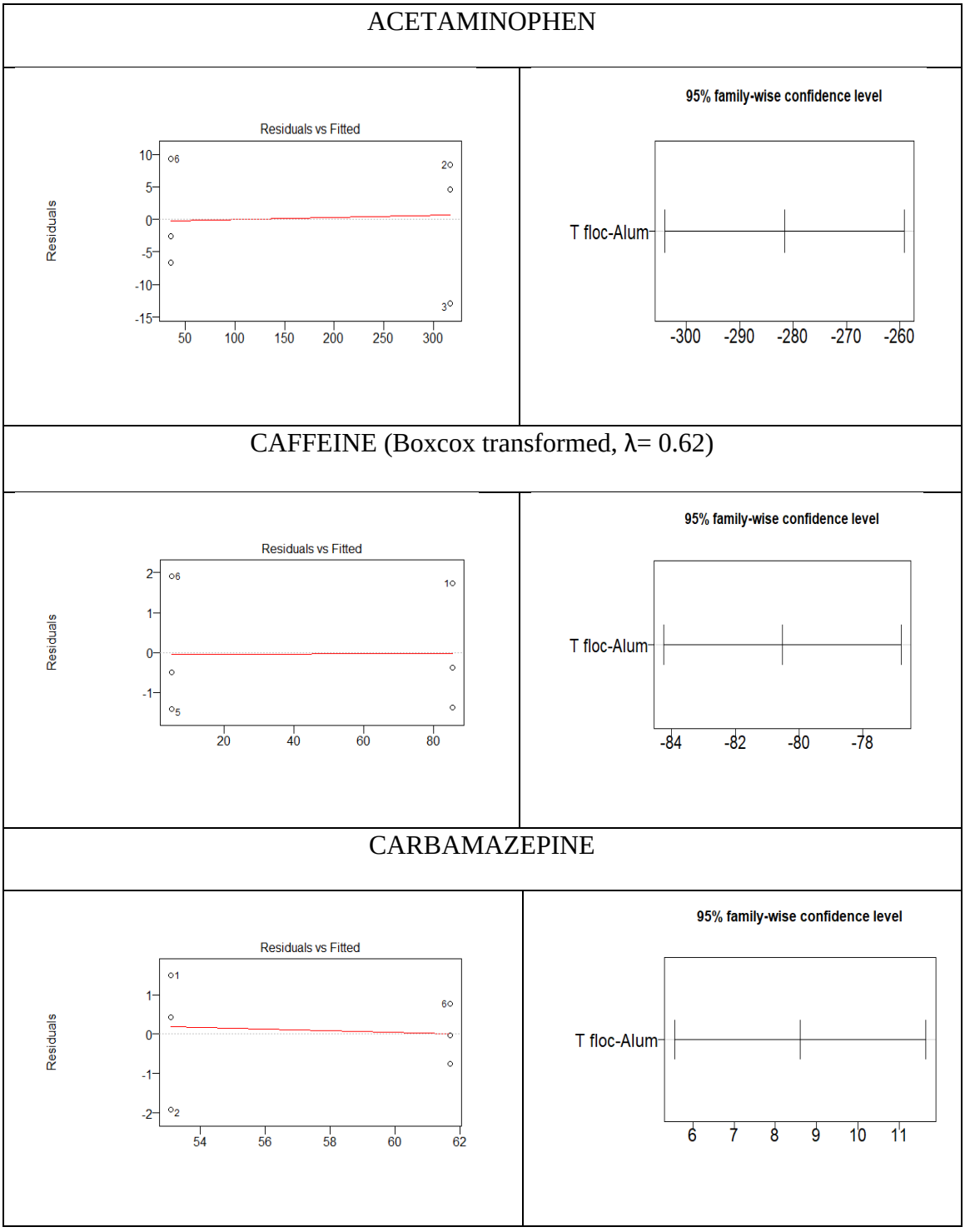


Fig. E.8 Residual plot and Tukey HSD plot comparing effect of chemical addition on acetaminophen, caffeine and carbamazepine concentration on Hyrum WWTP effluent

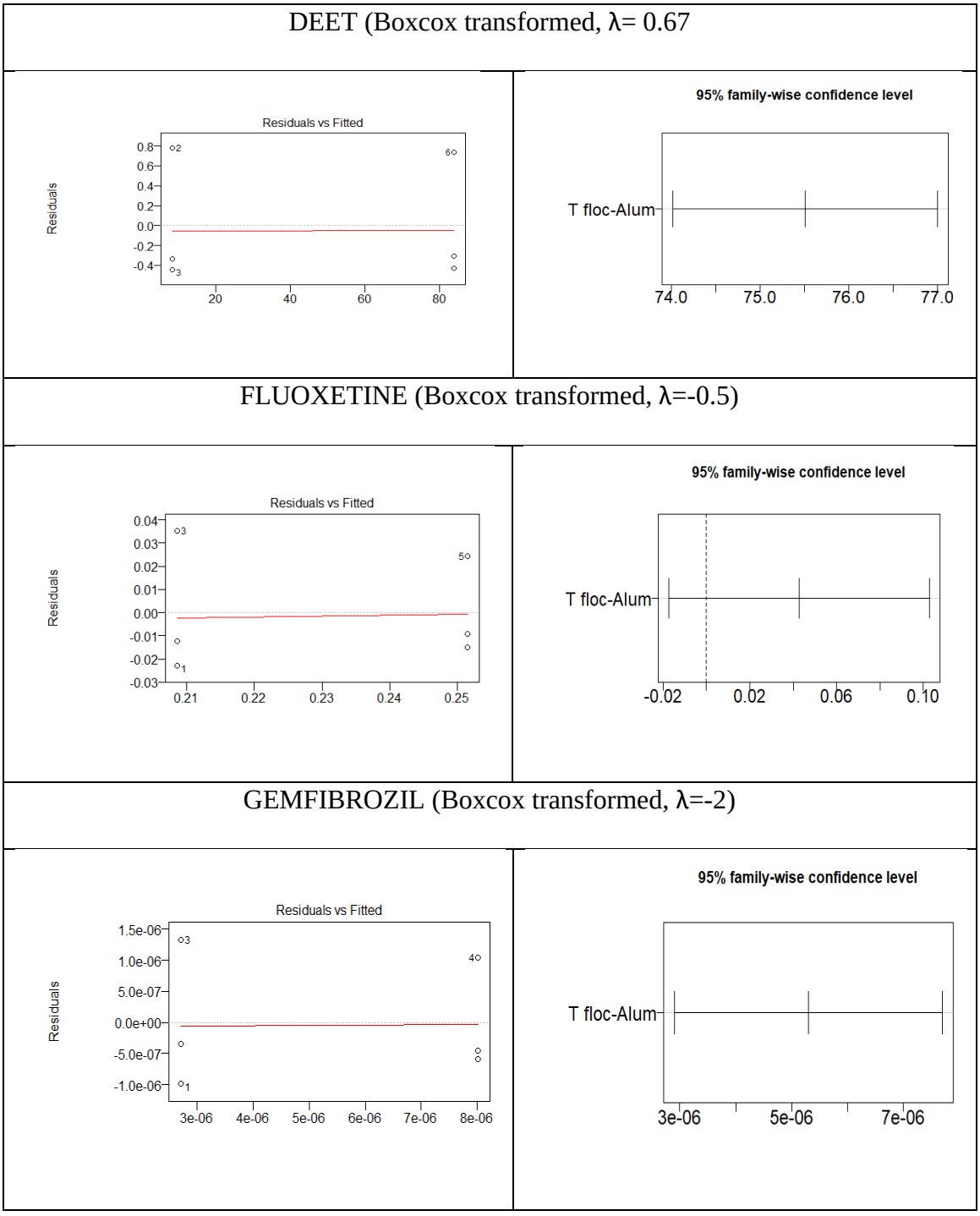


Fig. E.9 Residual plot and Tukey HSD plot comparing effect of chemical addition on DEET, fluoxetine and gemfibrozil concentration on Hyrum WWTP effluent

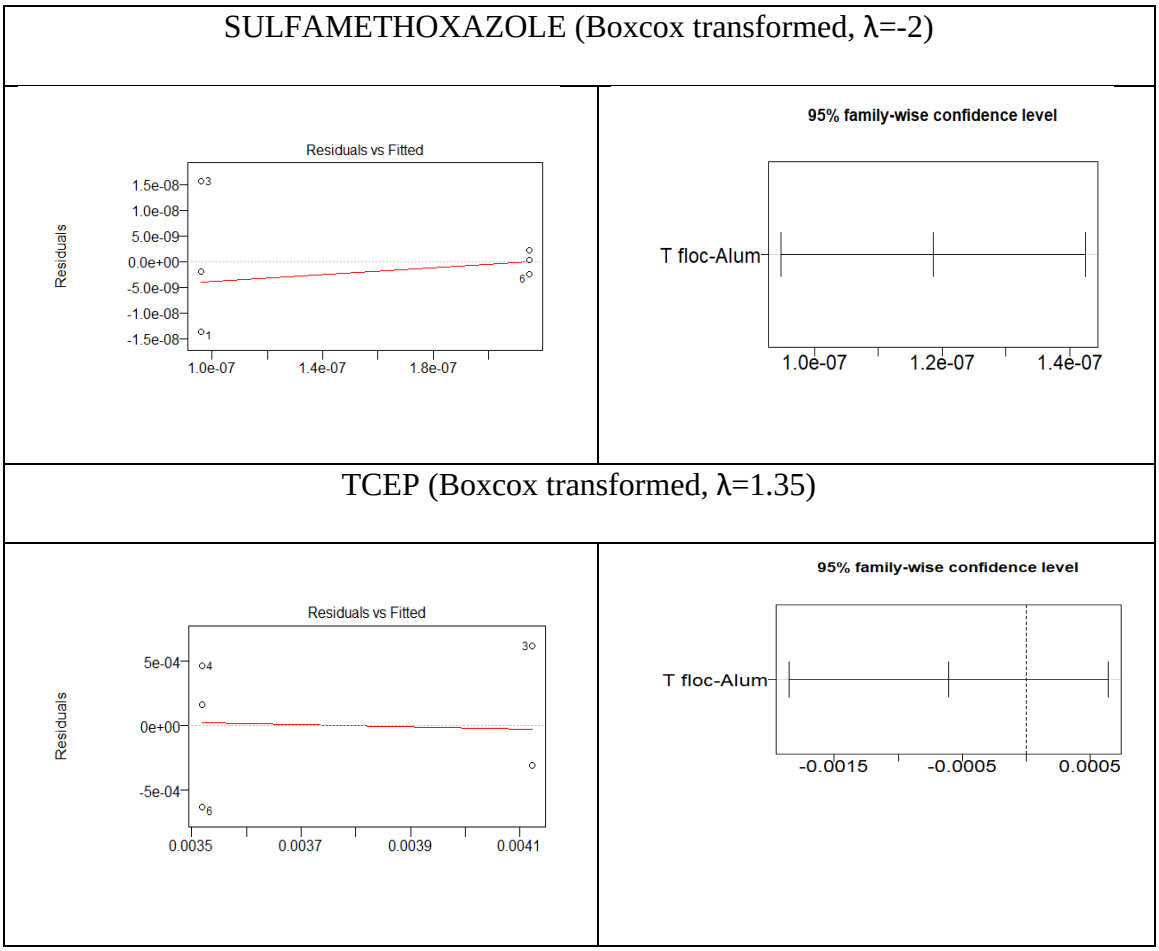


Fig. E.10 Residual plot and Tukey HSD plot comparing effect of chemical addition on sulfamethoxazole and TCEP concentration on Hyrum WWTP effluent

Hyrum WWTP Influent

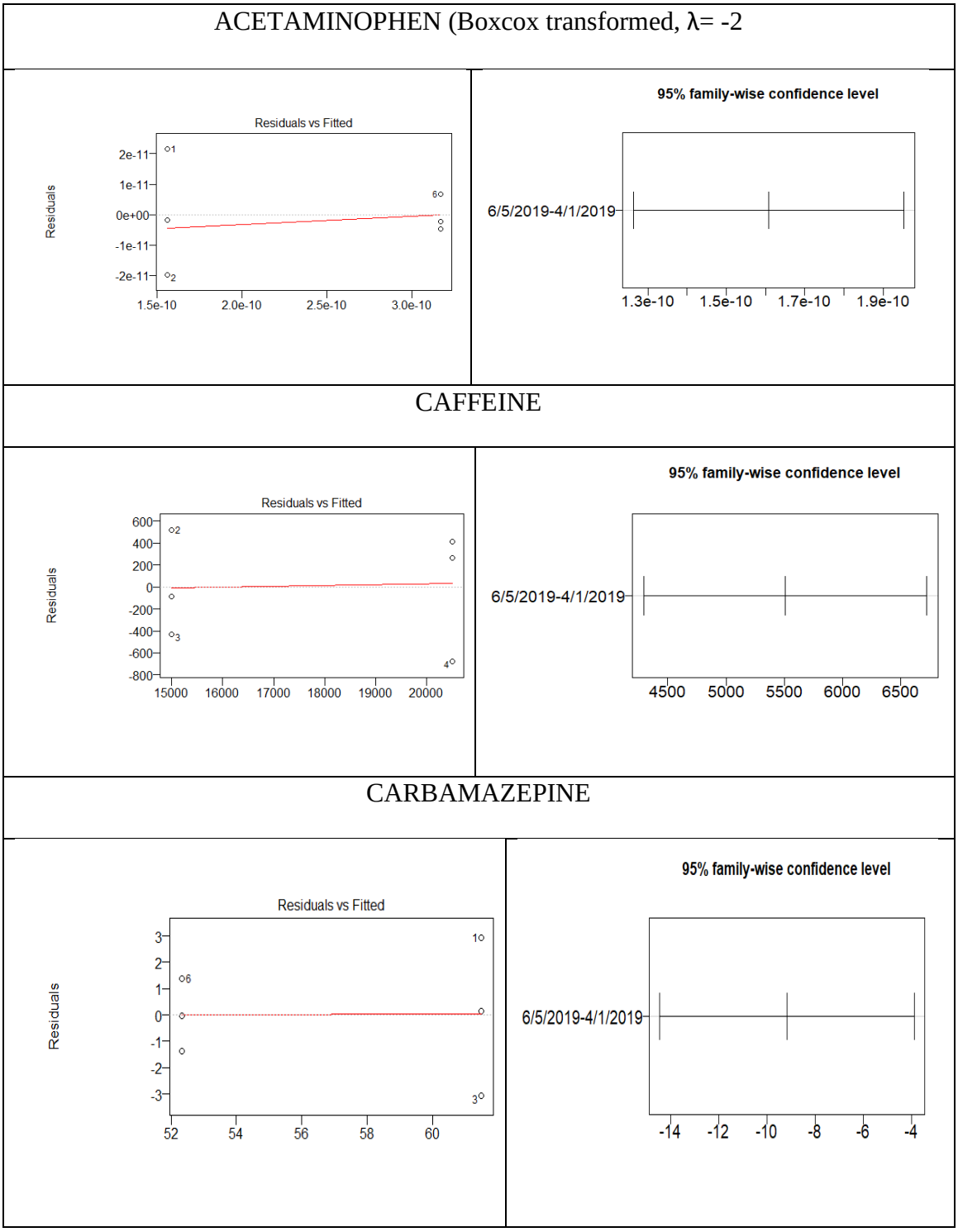
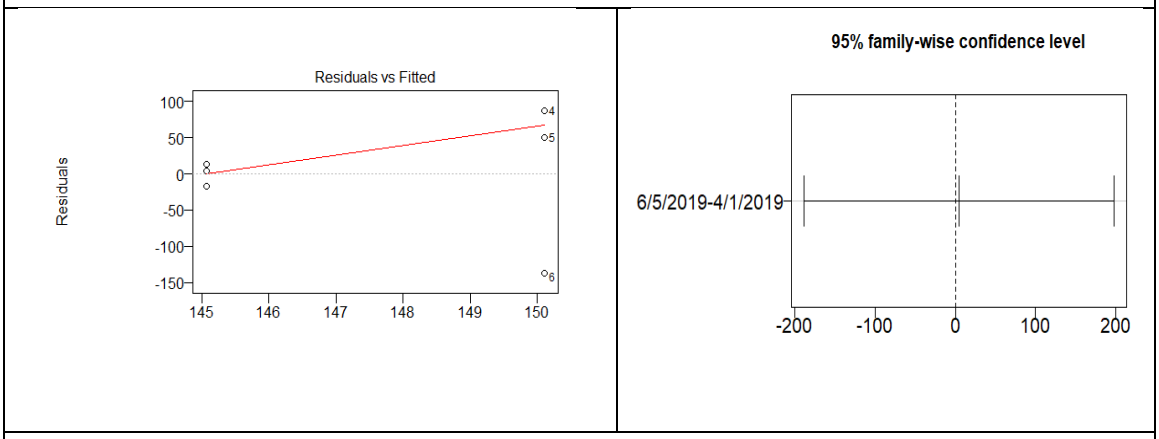
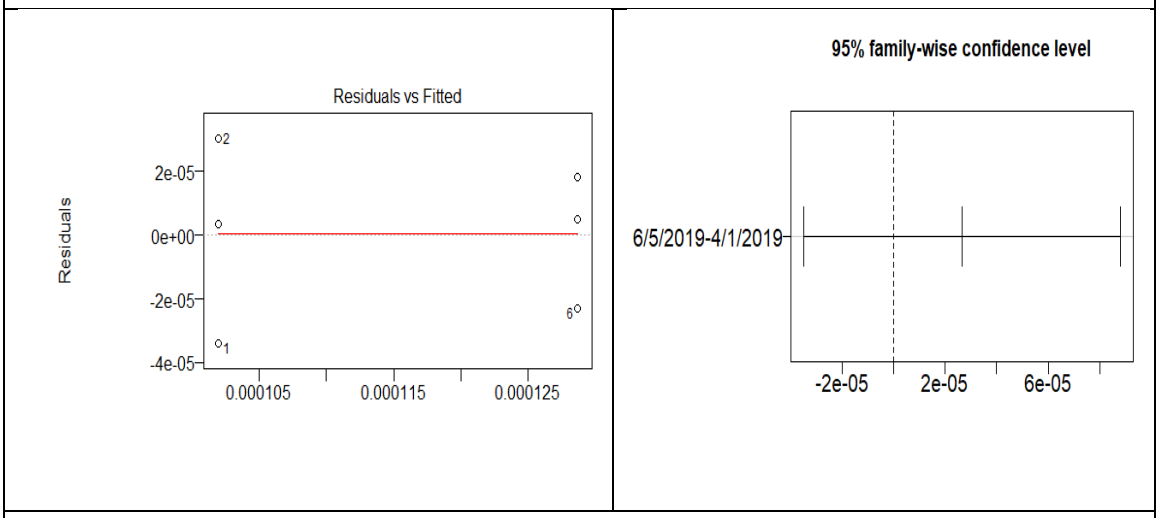


Fig. E.11 Residual plot and Tukey HSD plot comparing concentrations of acetaminophen, caffeine and carbamazepine in influent of Hyrum WWTP on two different sampling dates

ESTRONE



FLUOXETINE (Boxcox transformed, λ= -2)



DEET (Boxcox transformed, λ= -1.4)

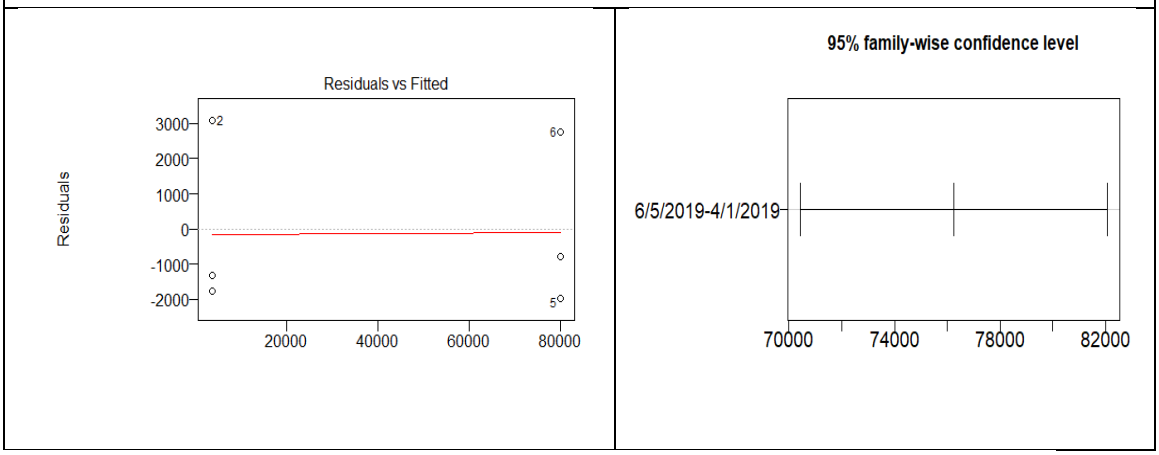


Fig. E.12 Residual plot and Tukey HSD plot comparing concentrations of estrone, fluoxetine, DEET in influent of Hyrum WWTP on two different sampling dates

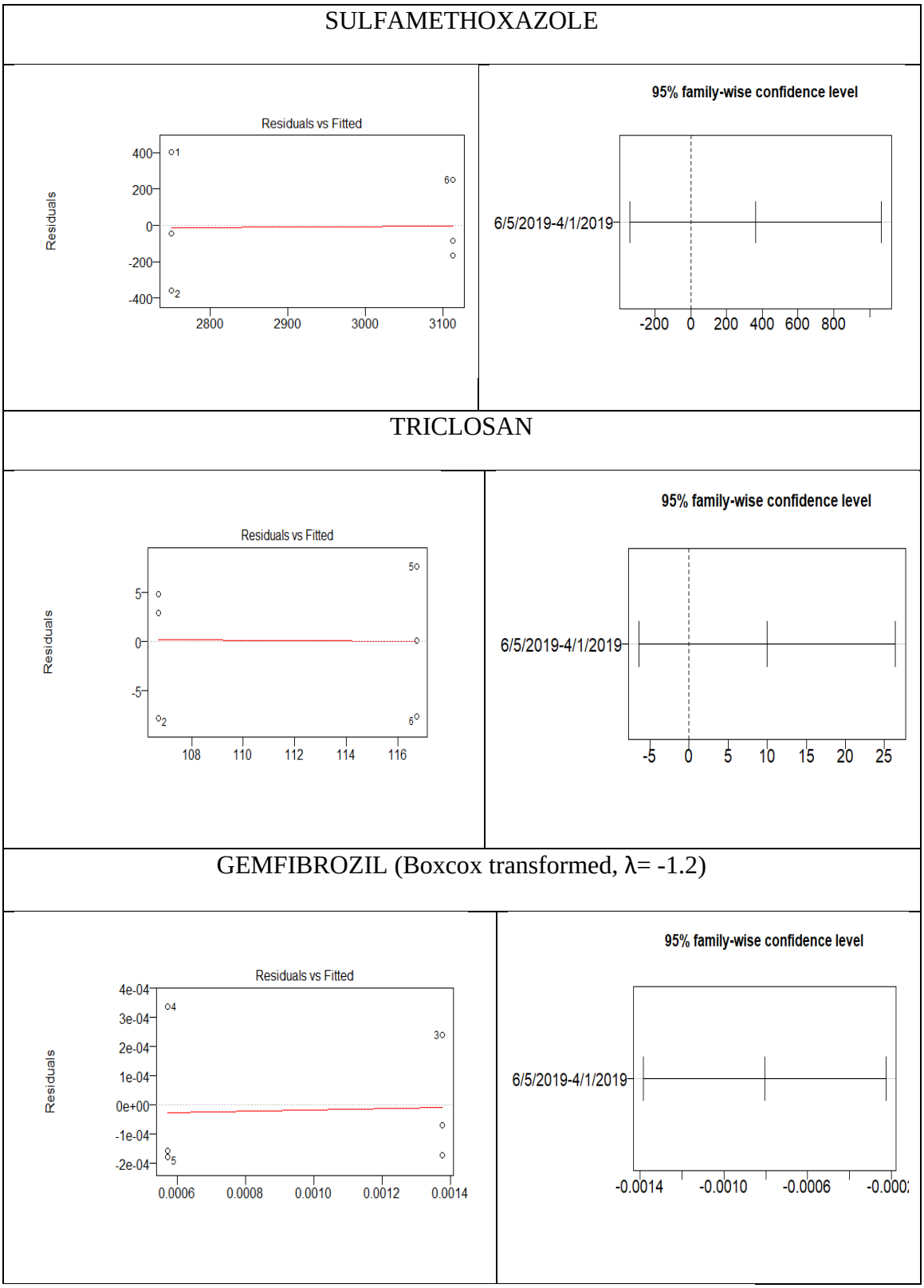


Fig. E.13 Residual plot and Tukey HSD plot comparing concentrations of sulfamethoxazole, triclosan and gemfibrozil in influent of Hyrum WWTP on two different sampling dates.

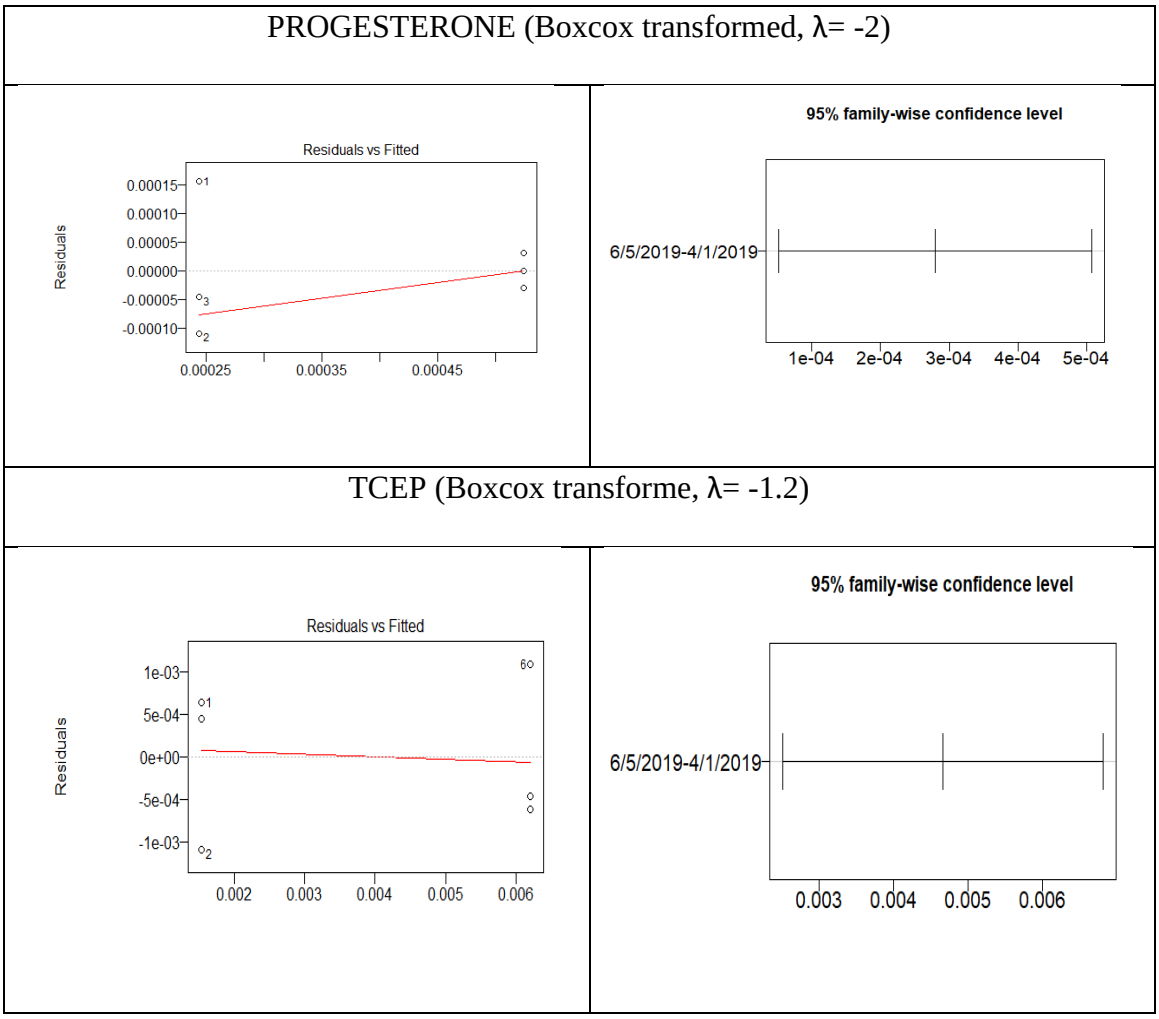


Fig. E.14 Residual plot and Tukey HSD plot comparing concentrations of progesterone and TCEP in influent of Hyrum WWTP on two different sampling dates

Wellsville sewage lagoons duckweed

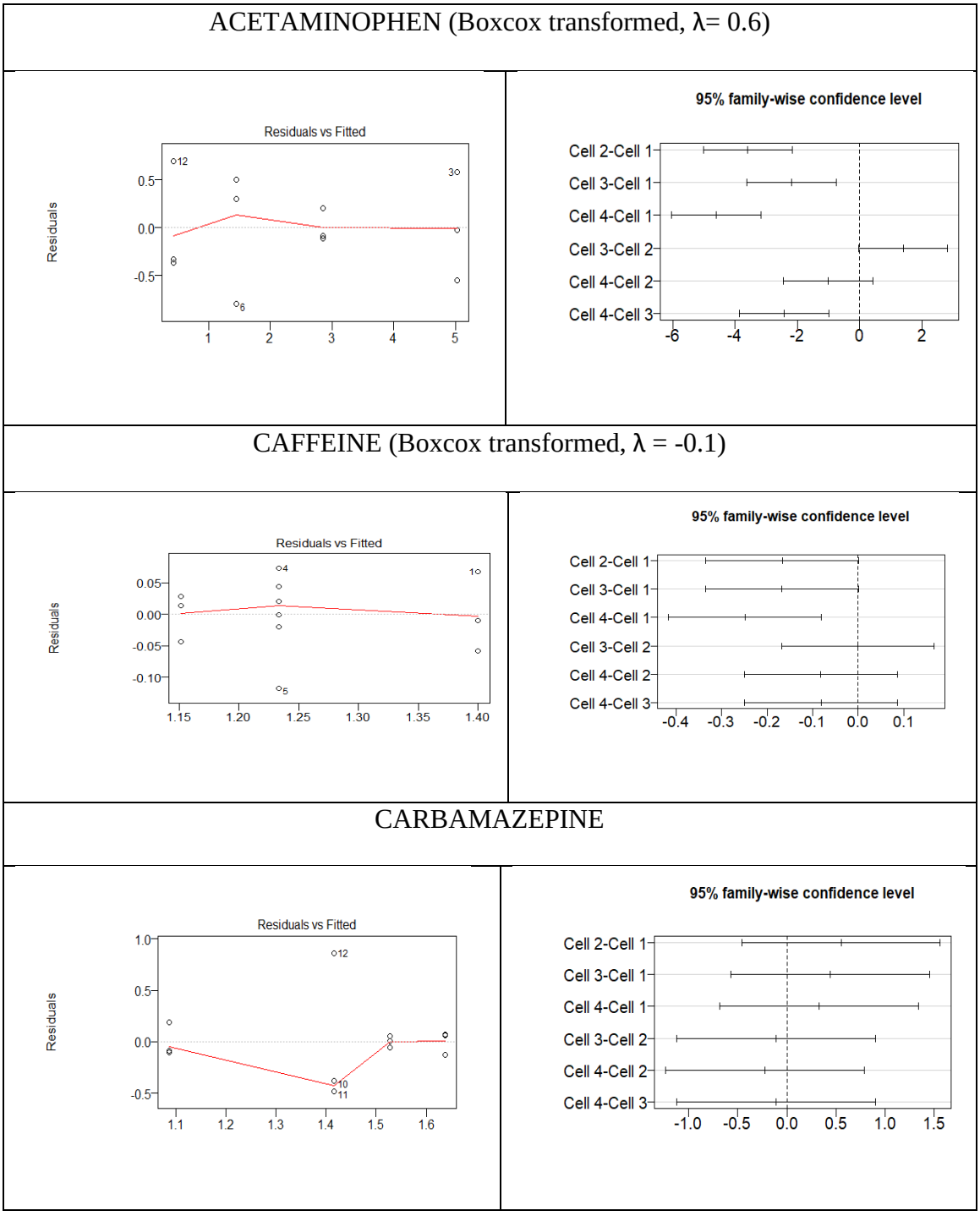


Fig. E.15 Residual plot and Tukey HSD plot comparing concentrations of acetaminophen, caffeine, carbamazepine in Wellsville sewage lagoons duckweed sampled from different cells on 8/15/2019.

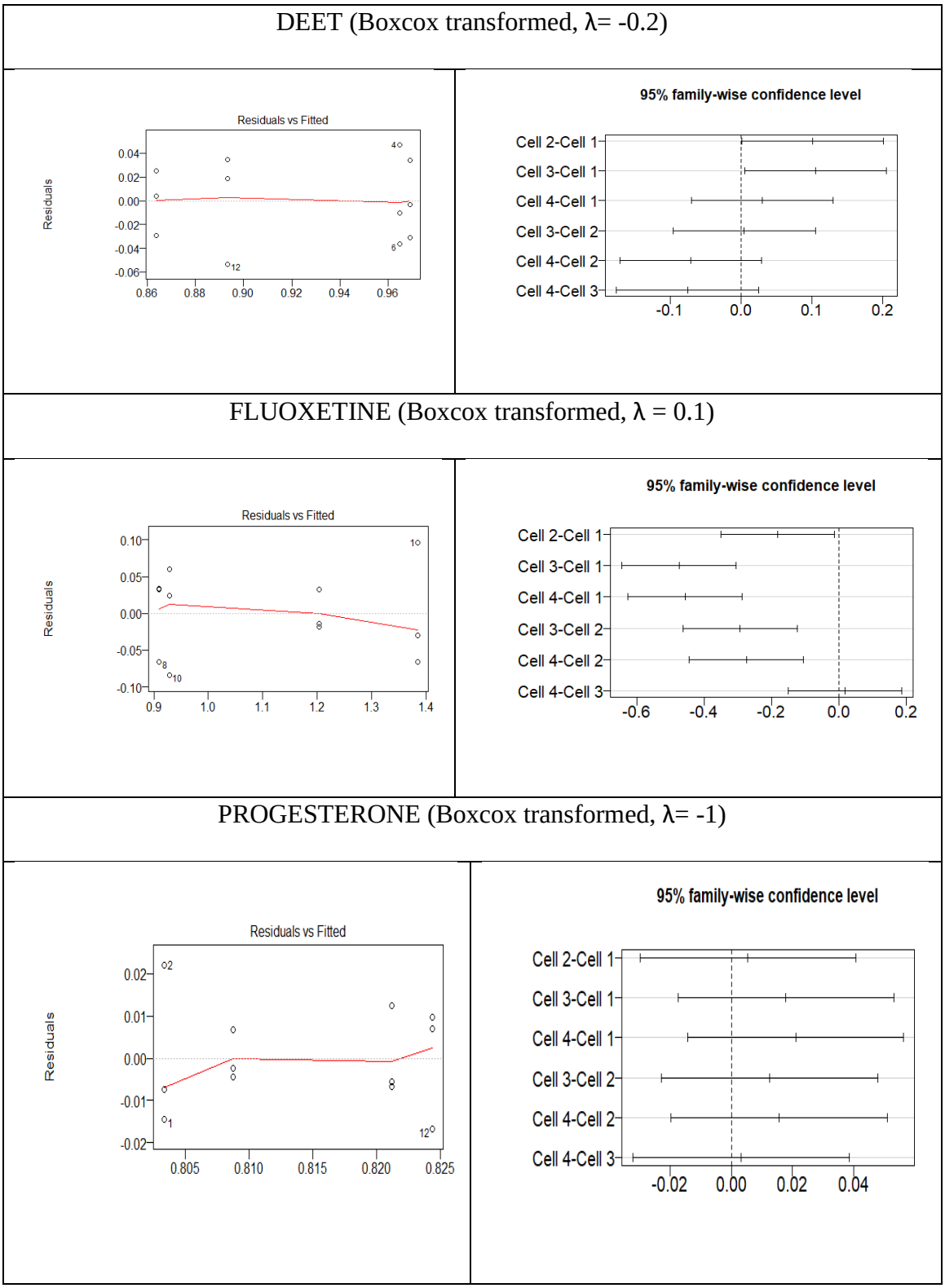


Fig. E.16 Residual plot and Tukey HSD plot comparing concentrations of DEET, fluoxetine, progesterone in Wellsville sewage lagoons duckweed sampled from different cells on 8/15/2019.

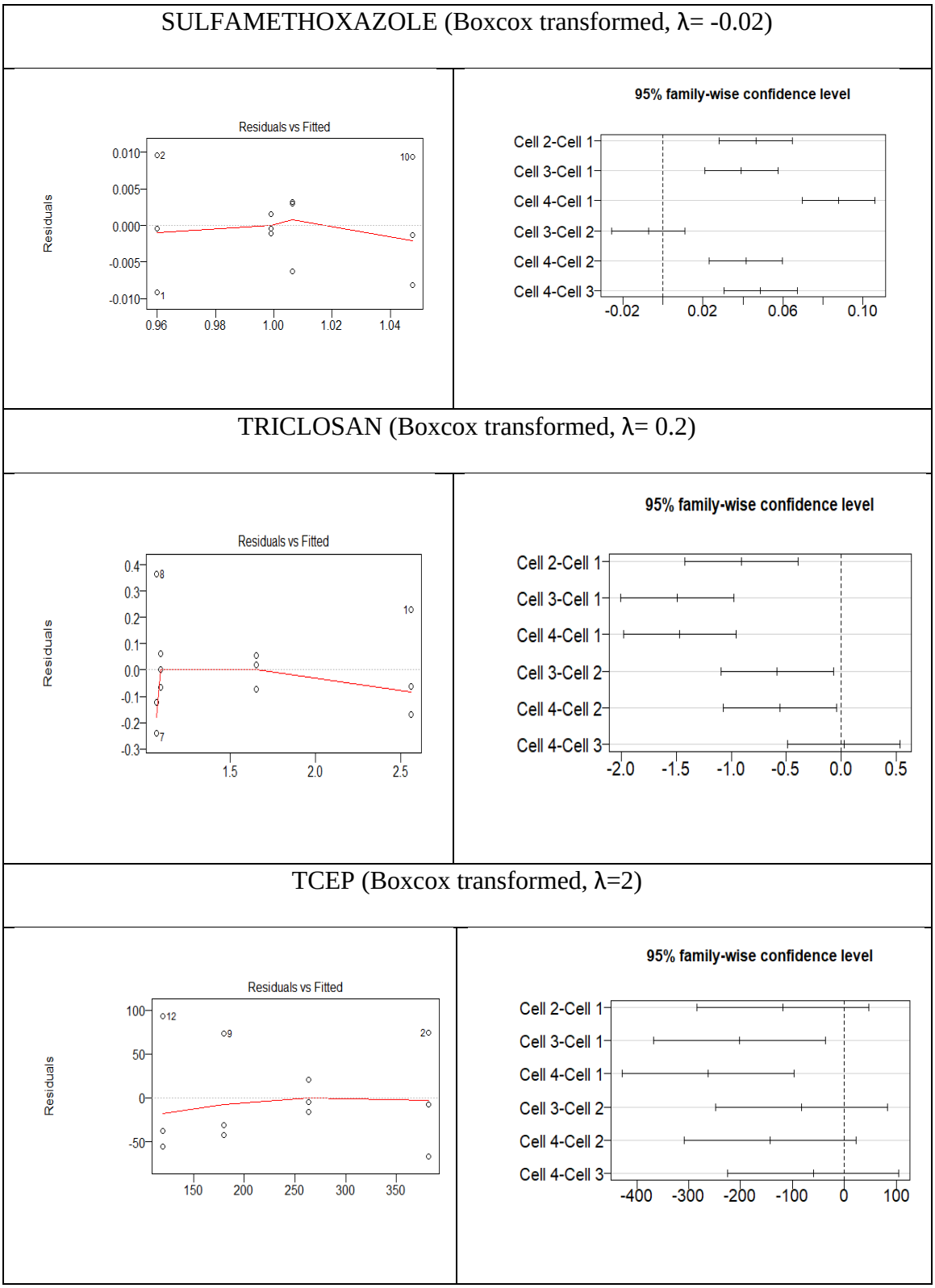


Fig. E.17 Residual plot and Tukey HSD plot comparing concentrations of sulfamethoxazole, triclosan and TCEP in Wellsville sewage lagoons duckweed sampled from different cells on 8/15/2019

Wellsville sewage lagoons sediments

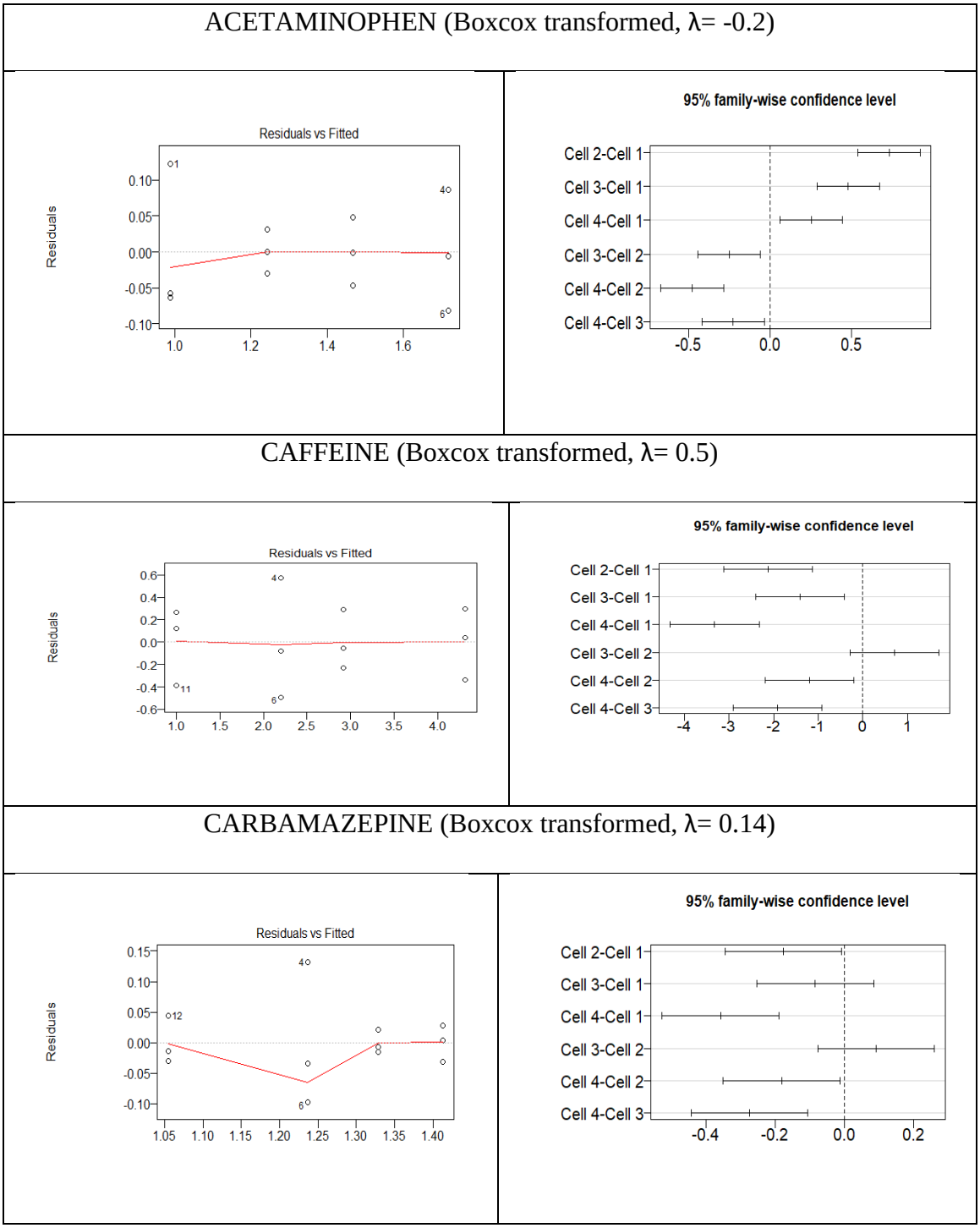


Fig. E.18 Residual plot and Tukey HSD plot comparing concentrations of acetaminophen, caffeine, carbamazepine in Wellsville sewage lagoons sediments sampled from different cells on 8/15/2019.

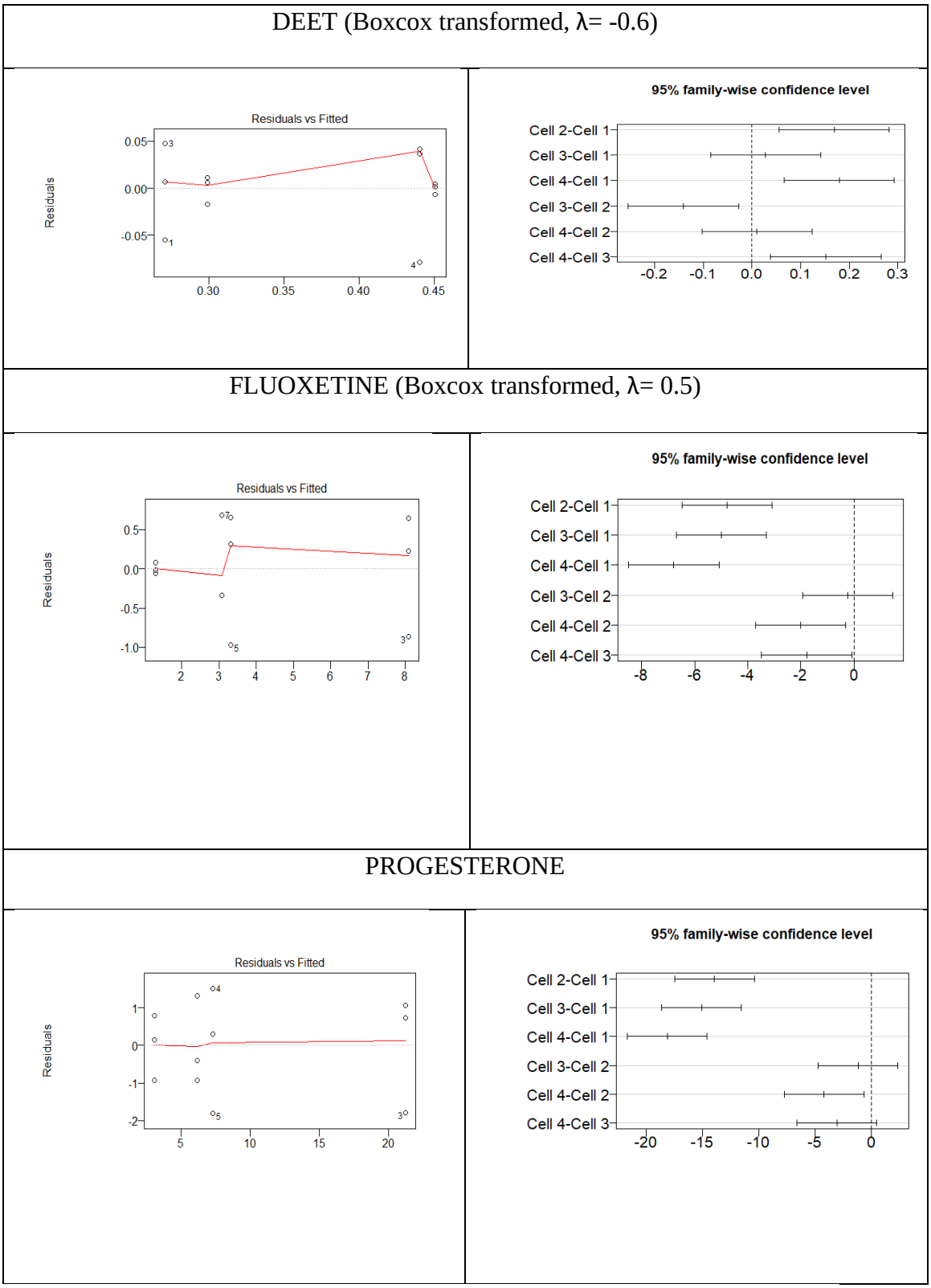


Fig. E.19 Residual plot and Tukey HSD plot comparing concentrations of DEET, fluoxetine, progesterone in Wellsville sewage lagoons sediments sampled from different cells on 8/15/2019.

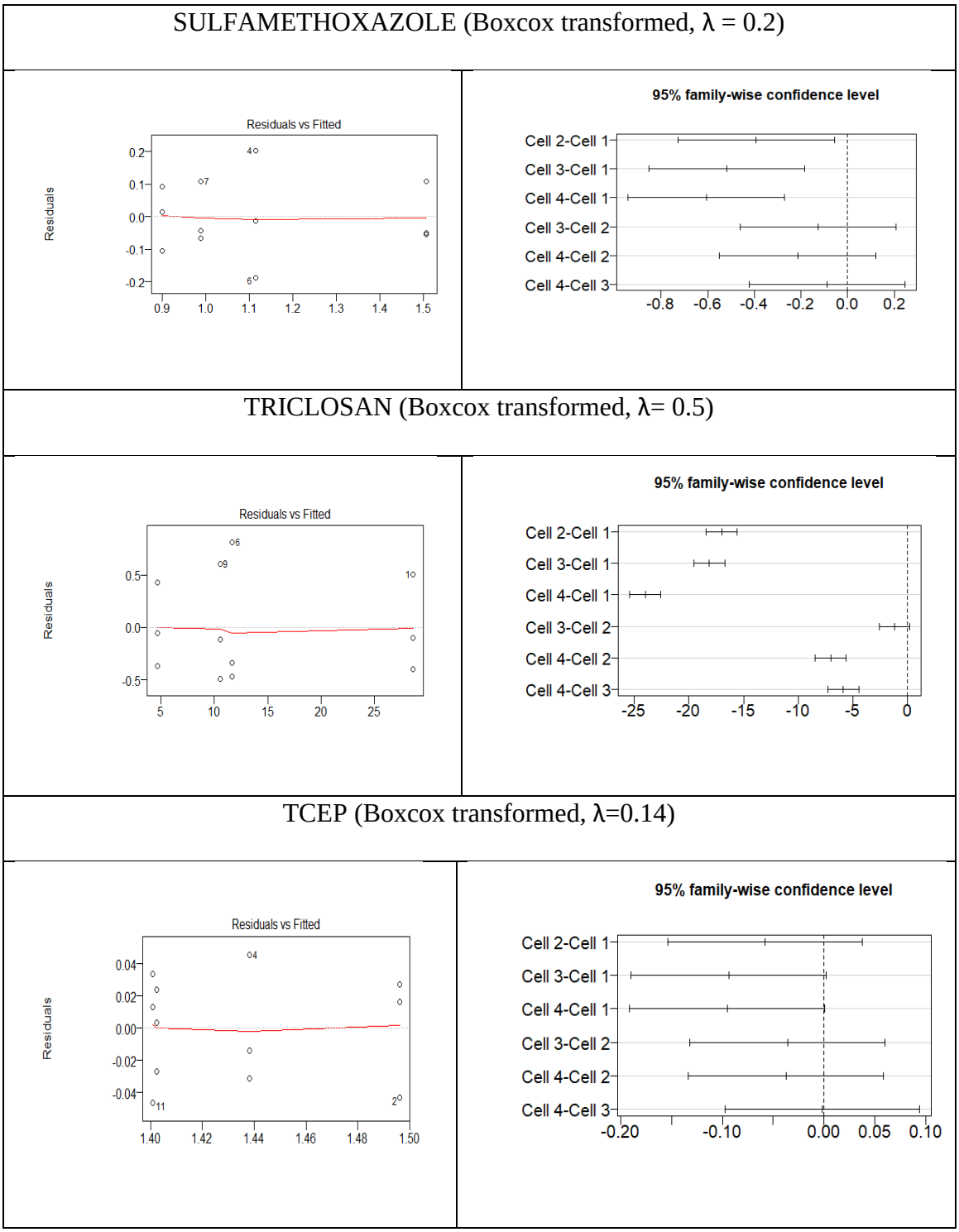


Fig. E.20 Residual plot and Tukey HSD plot comparing concentrations of sulfamethoxazole, triclosan and TCEP in Wellsville sewage lagoons sediments sampled from different cells on 8/15/2019

Wellsville sewage lagoons liquids 8/05/2019

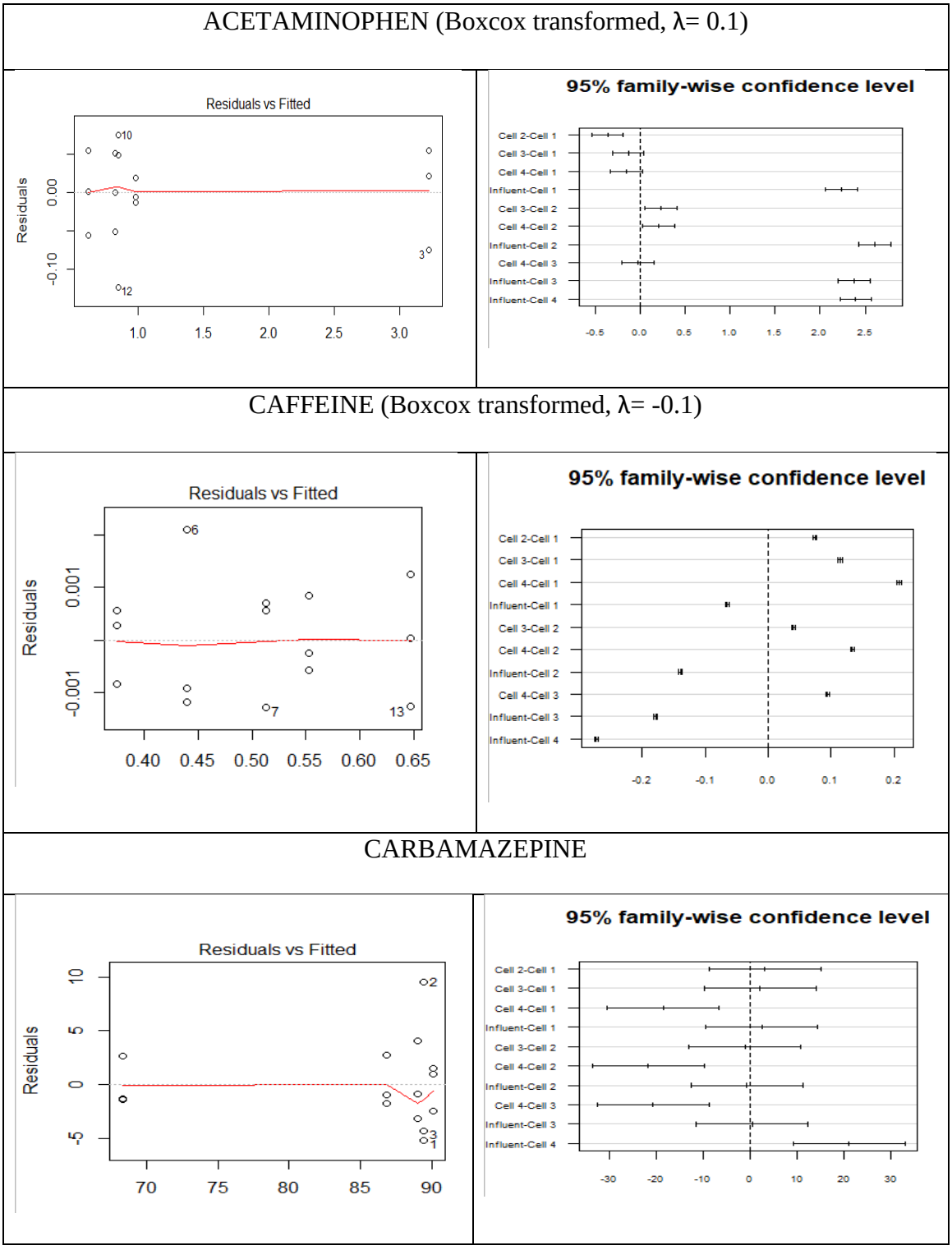
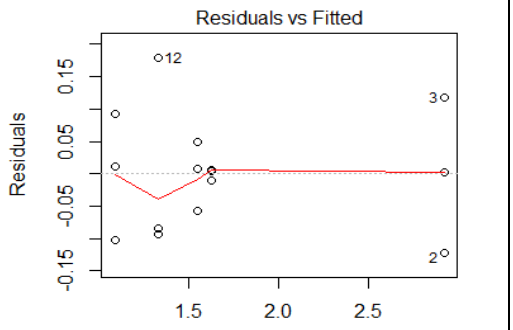
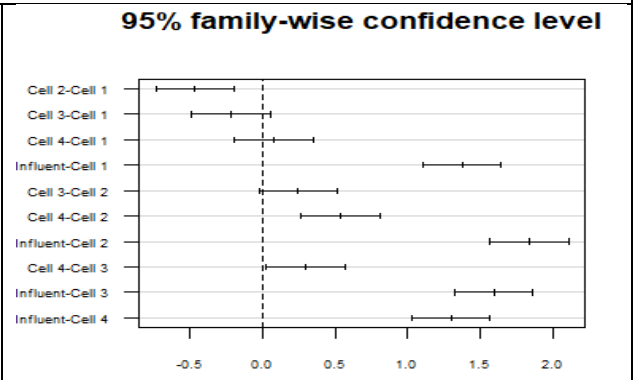


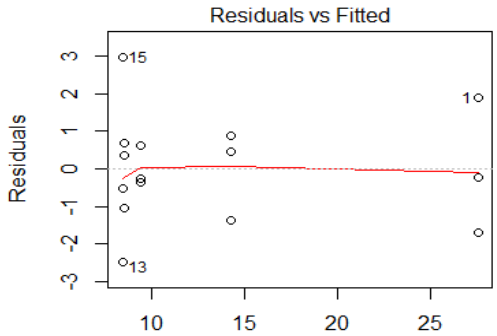
Fig. E.21 Residual plot and Tukey HSD plot comparing concentrations of acetaminophen, caffeine, carbamazepine in Wellsville sewage lagoons liquids sampled from different cells on 8/15/2019.

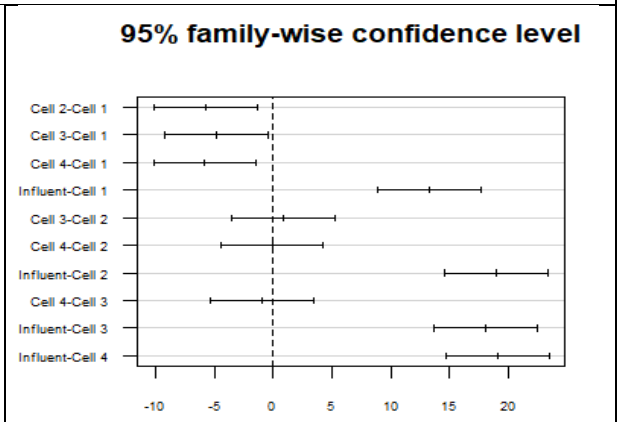
DEET (Boxcox transformed, λ= 0.14)



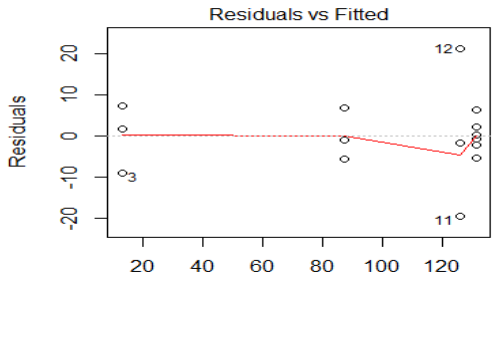


FLUOXETINE (Boxcox transformed, λ=0.75)





GEMFIBROZIL



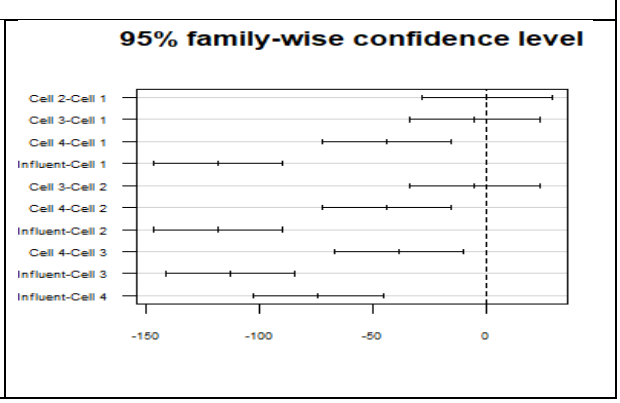


Fig. E.22 Residual plot and Tukey HSD plot comparing concentrations of DEET, fluoxetine, gemfibrozil in Wellsville sewage lagoons liquids sampled from different cells on 8/15/2019

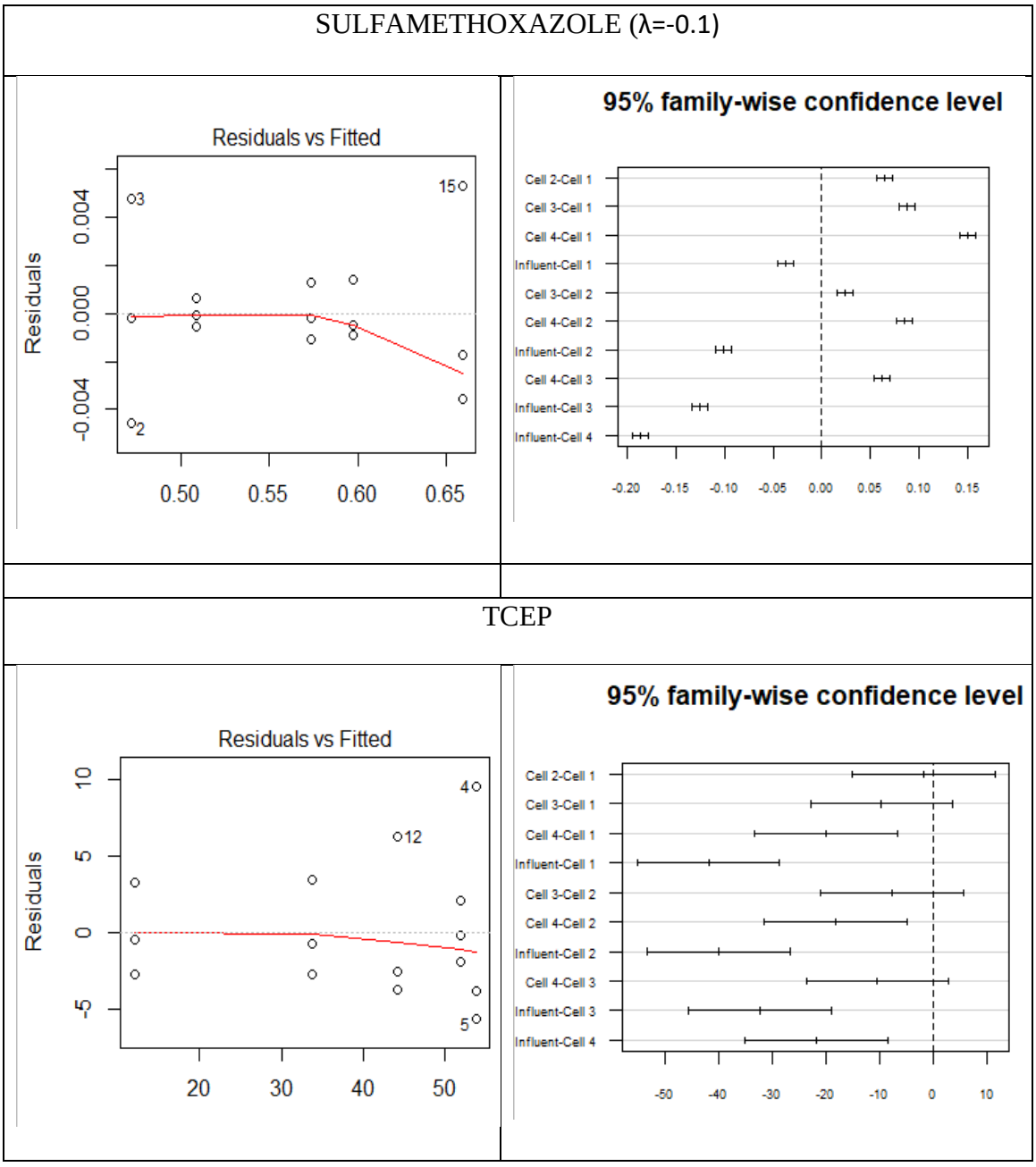


Fig. E.23 Residual plot and Tukey HSD plot comparing concentrations of sulfamethoxazole and TCEP in Wellsville sewage lagoons liquids sampled from different cells on 8/15/2019

Wellsville influent and effluent 6/5/2019

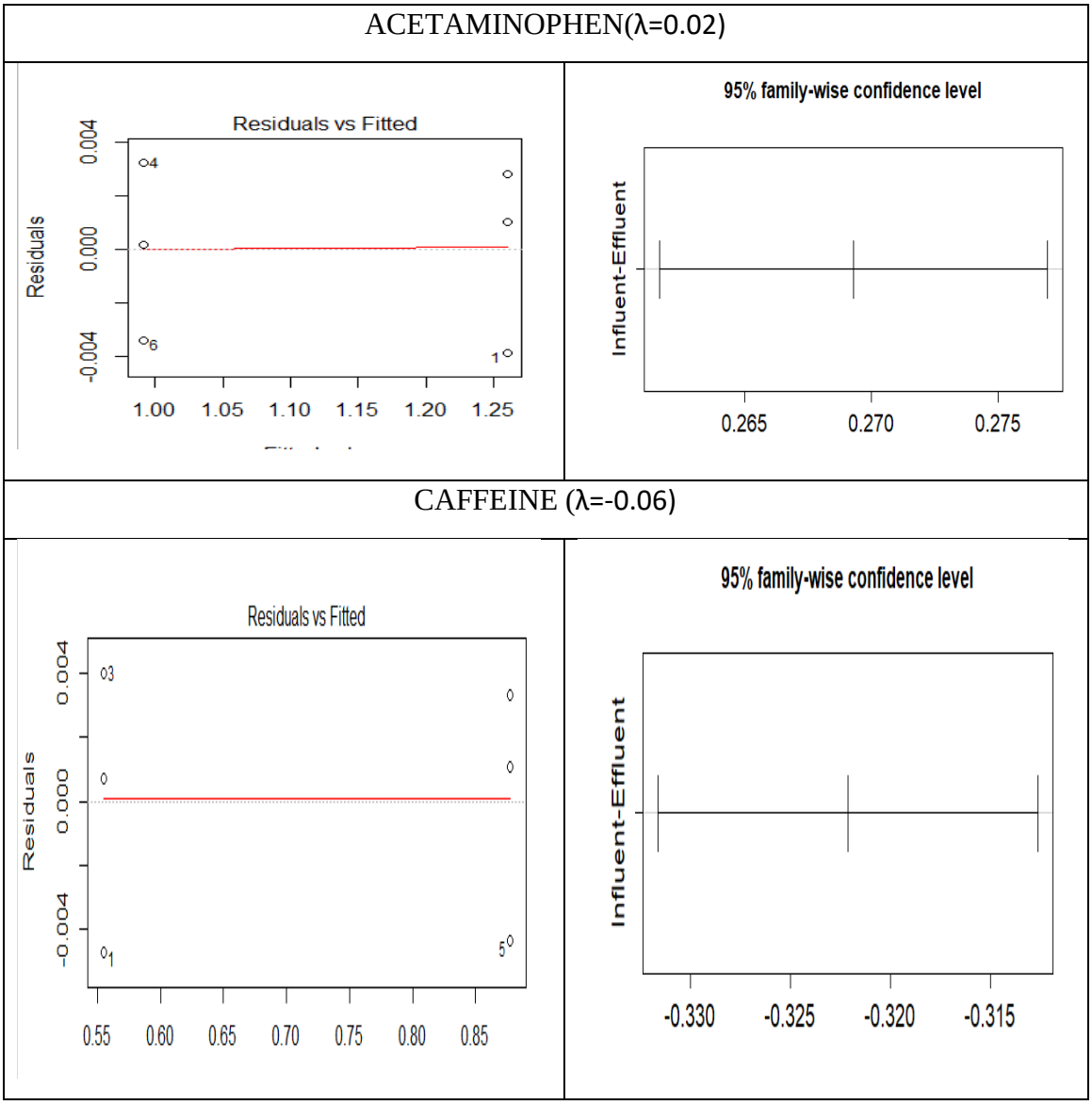


Fig. E. 24 Residual plot and Tukey HSD plot comparing influent and effluent concentrations of acetaminopen and caffeine in Wellsville sewage lagoons liquids collected on 6/5/2019

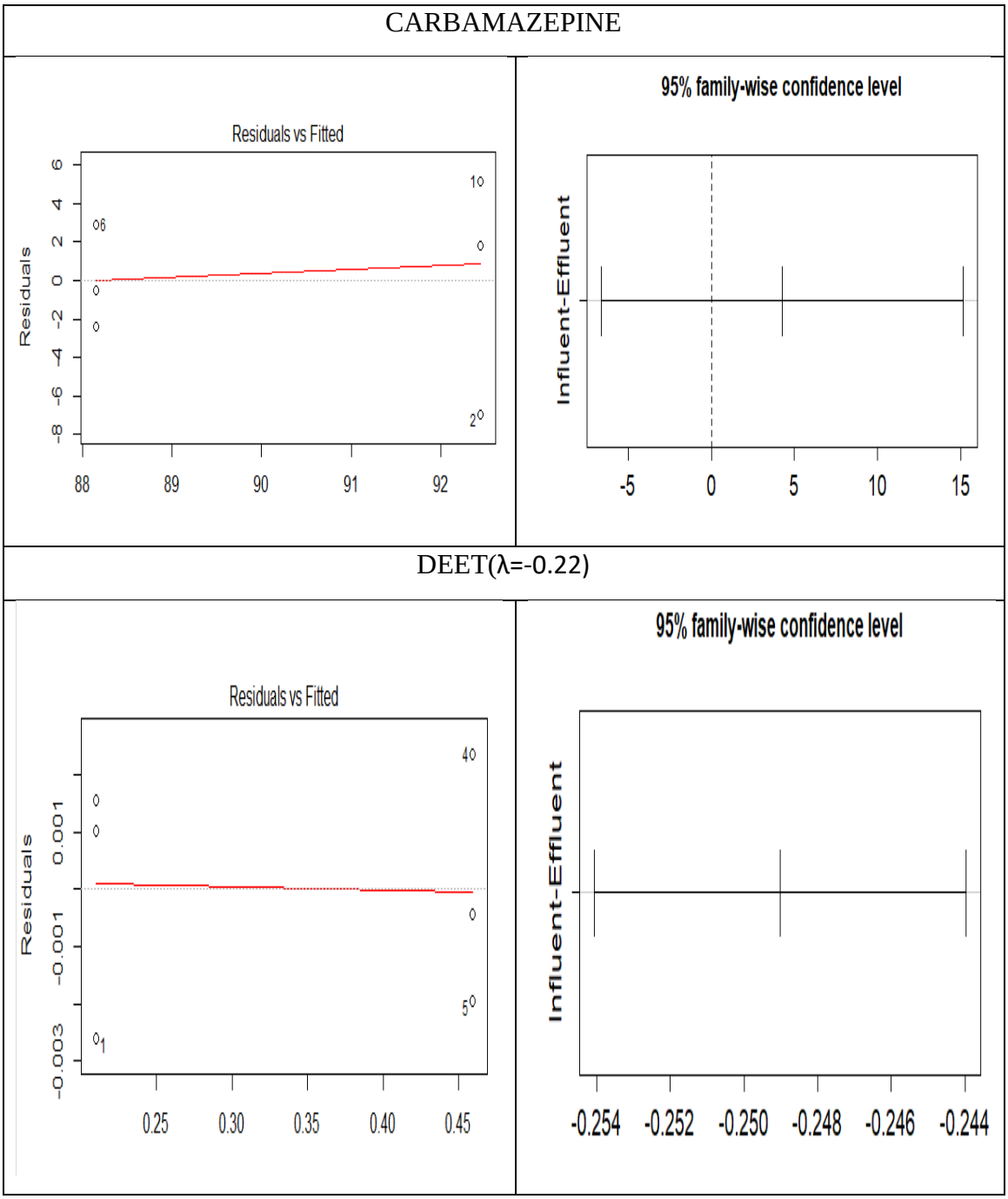


Fig. E. 25 Residual plot and Tukey HSD plot comparing influent and effluent concentrations of carbamazepine and DEET in Wellsville sewage lagoons liquids collected on 6/5/2019

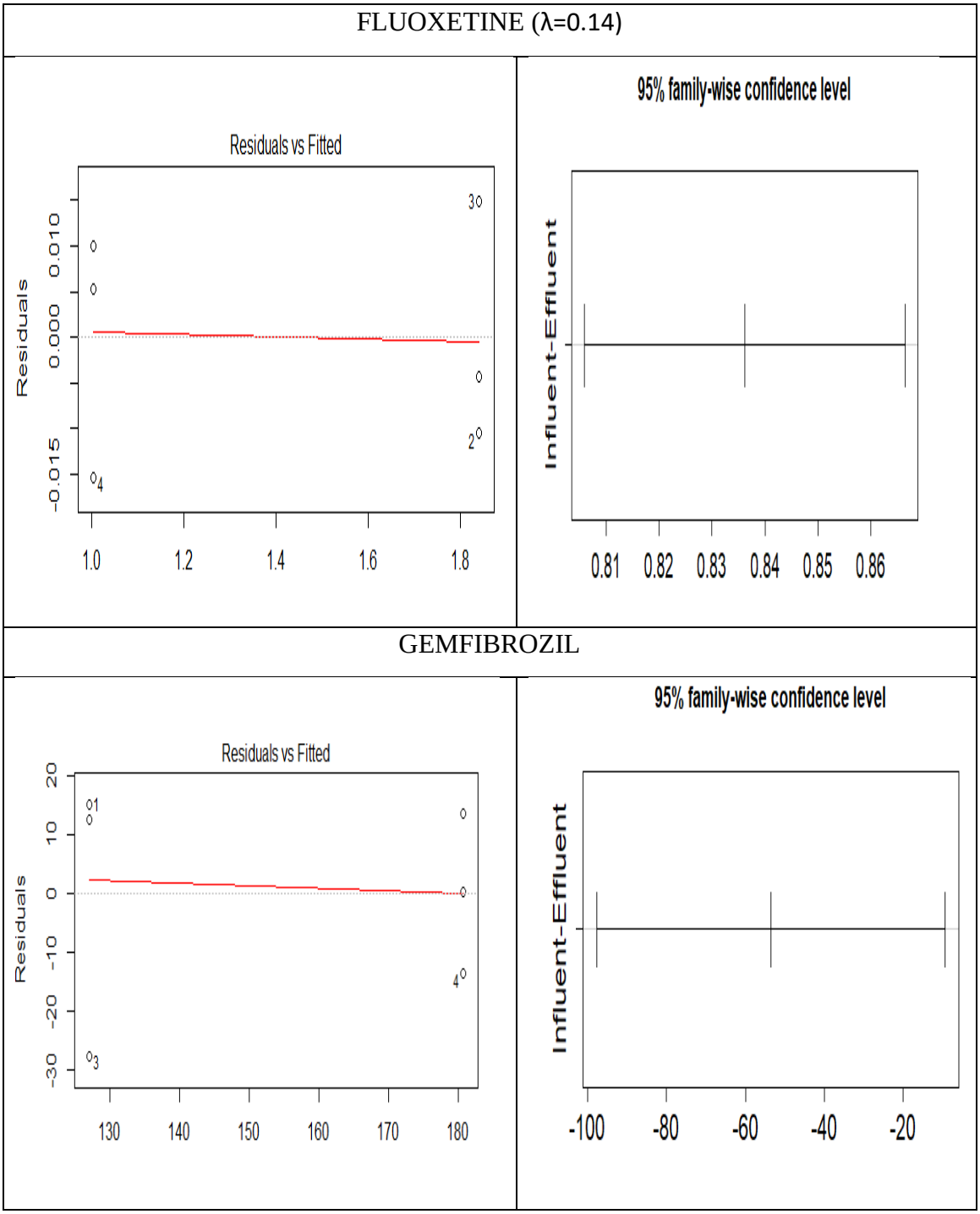


Fig. E. 26 Residual plot and Tukey HSD plot comparing influent and effluent concentrations of fluoxetine and gemfibrozil in Wellsville sewage lagoons liquids collected on 6/5/2019

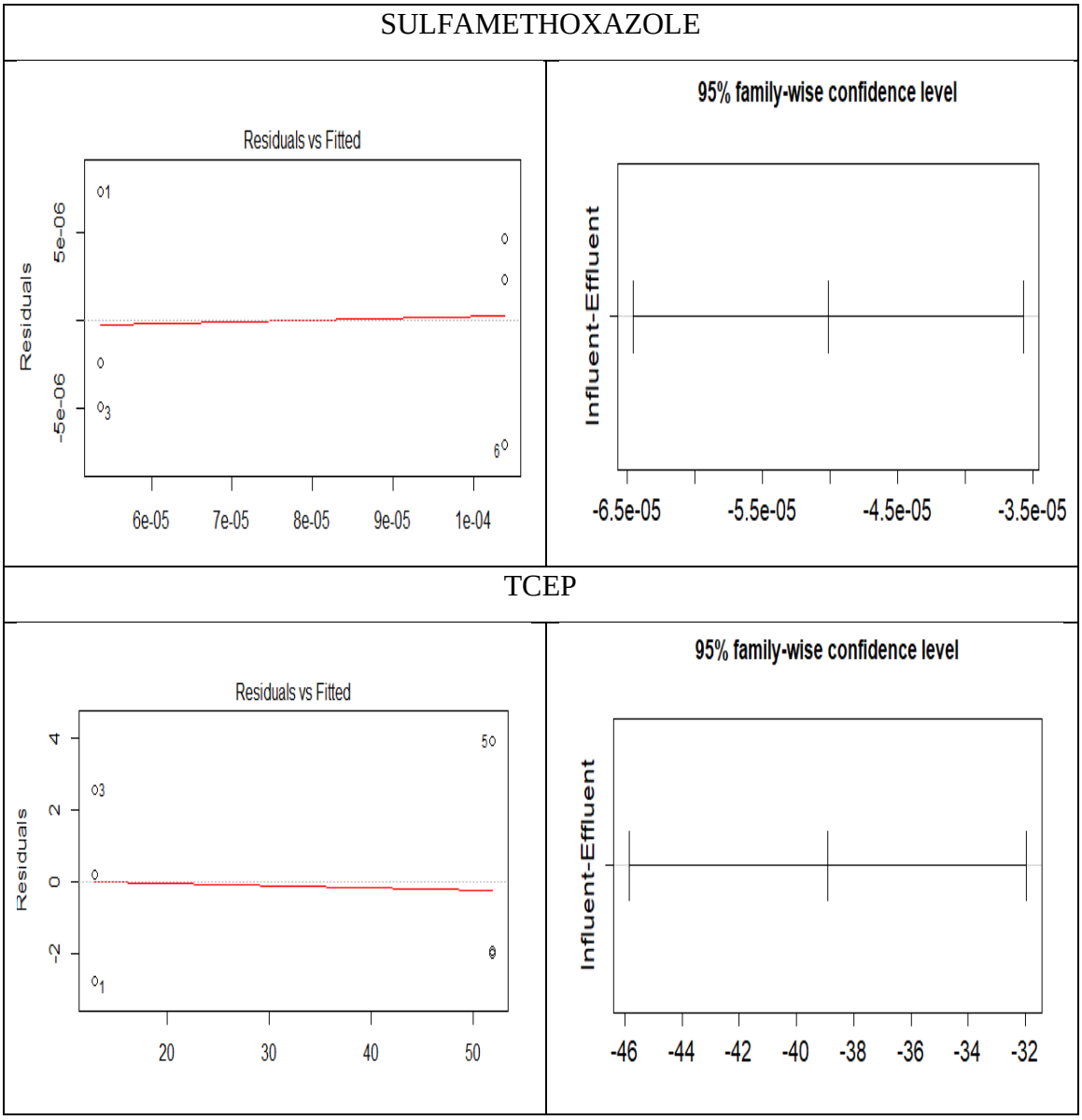


Fig. E. 27 Residual plot and Tukey HSD plot comparing influent and effluent concentrations of sulfamethoxazole and TCEP in Wellsville sewage lagoons liquids collected on 6/5/2019