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Sinkhole Lake Infiltration of Contaminants of Emerging Concern from Onsite Water Treatment Systems

by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
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Table of Contents

List of Tables

List of Figures

Abstract

The advancement in synthetic chemicals commonly referred to as contaminants of emerging concern (CECs) and their application has led to an increase in environmental pollution. The use of septic tanks otherwise referred to as onsite water treatment systems (OWTS), promotes the introduction of CECs into the environment while allowing little in the way of remediation. In order to study the impacts of CECs from OWTS as they infiltrate the environment and the aquifer system, water, sediment, and vegetation samples were collected around a sinkhole lake surrounded by residential housing using OWTSs. The main question of this research project is what is the fate of CECs from OWTSs effluent within the catchment of a sinkhole lake? Liquid chromatograph mass spectrometry was used to analyze the samples for the presence of CECs. It was found that the relative quantity of CECs in the individual constituents is dependent upon 1) the hydrophobicity and polarity of the individual compound, 2) the specific sampling site, 3) the topography gradient, and 4) for vegetation the connectedness of the sample type to the sediment. The implications derived from this study can be applied in environmental engineering, urban and suburban planning, environmental monitoring, and should be considered when residents use well water as their source of potable water.

Chapter One:

Introduction

Groundwater is the world's single largest source of potable water providing 26% of the global population with fresh water resources (Dodgen et al., 2017). However, due to the approximate location of aquifers and anthropogenic activity, aquifers are extremely susceptible to contamination. All aspects of the hydrologic cycle are intertwined making groundwater, rivers, retention ponds, lakes, and even oceans vulnerable to adverse anthropogenic forces.

Advancements in health technology, through the development and application of synthetic chemicals, provide benefits for daily living in society. However, there appears to be a positive correlation between knowledge advancement in synthetic chemicals and contaminants of emerging concern (CECs) in water that is used for human consumption. With the production of a whole range of new chemicals comes an increase in the potential hazards of chemical application and by-products to the environment and human health.

Onsite Water Treatment Systems (OWTSs) are of particular concern due to their poor filtration of certain CECs which can then infiltrate and contaminate aquifers and the environment. OWTSs are wastewater treatment systems commonly used when municipal WWTP sewer lines are unavailable. The typical OWTS consists of three main filtration elements that eliminate waste: the septic tank, a drain field trench, and sediment as seen in Figure 1 (Del Rosario et al., 2014). While some CECs found in waste are eliminated through microbial digestion volatilization, sorption and other naturally purifying factors, the system is incomplete in remediation of all CECs resulting in synthetic chemicals migrating through the subsurface and into aquifers.

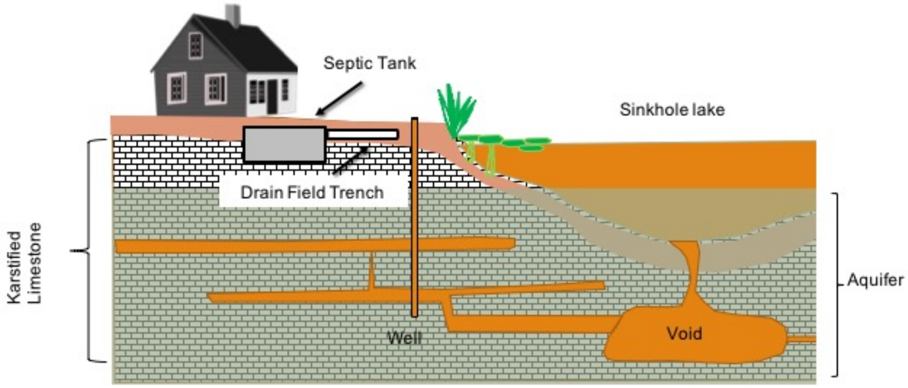

Figure 1. OWTS filtration elements and CEC pathway into the sinkhole lake environment

CEC is an umbrella term referring to a number of chemical classes that include pesticides, pharmaceuticals, personal care products, and other commercial and industrial chemicals as seen in Table 1 (Ma et al., 2018). CECs have been shown to infiltrate aquifers, rivers, and other inland bodies of water (IBWs) through surface runoff, incomplete remediation of polluted water from wastewater treatment plants (WWTPs) and from onsite water treatment systems (OWTSs) commonly known as septic tanks (Dodgen & Zheng., 2016; Dodgen et al., 2017; Blair et al., 2013; Nakada et al., 2016; Aubertheau et al., 2016; Del Rosario et al., 2014; Serra-Roig et al., 2016). Therefore, due to the release of CECs from OWTSs, investigation and understanding of the impact of synthetic chemicals on the environment and on human health is imperative.

Table 1. CEC classification and toxicological effects ranging from N/A to acute toxicity

Compound	Type	Use	Toxicological effect
Acetaminophen	Pharmaceutical	Analgesic	Harmful
Androstenedione	Pharmaceutical	Hormone	Harmful
Atenolol	Pharmaceutical	Antihypertensive	N/A
Atrazine	Pesticide	Herbicide	Harmful to Acute Toxicity
Bisphenol-A	Plasticizer	Plasticizer	Harmful
Caffeine	Pharmaceutical	Stimulant	N/A
Carbamazepine	Pharmaceutical	Antiseizure	Unknown
Cotinine	Pharmaceutical	Antidepressant	Harmful
DEET	Personal Care Product	Insect Repellent	Harmful to Acute Toxicity
Diclofenac	Pharmaceutical	Analgesic	Harmful
Diphenhydramine	Pharmaceutical	Antihistamine	Harmful
Estradiol	Personal Care Product	Hormone	Harmful
Estrone	Personal Care Product	Hormone	Harmful
Equilin	Pharmaceutical	Hormone	Harmful
Fluoxetine	Pharmaceutical	Antidepressant	Harmful to Acute Toxicity
Gemfibrozil	Pharmaceutical	Fibrate	Harmful to Acute Toxicity
Ibuprofen	Pharmaceutical	Analgesic	Harmful to Acute Toxicity
Metoprolol	Pharmaceutical	Beta-Blocker	N/A
Naproxen	Pharmaceutical	Analgesic	Harmful
Primidone	Pharmaceutical	Anticonvulsant	Harmful
Propranolol	Pharmaceutical	Beta-Blocker	N/A
Sucralose	Artificial Sweetener	Sweetener	Harmful
Sulfamethoxazole	Pharmaceutical	Antibiotic	N/A
TCEP	Flame Retardant	Flame Retardant	Harmful to Acute Toxicity
TCPP	Flame Retardant	Flame Retardant	Harmful to Acute Toxicity
Theophylline	Pharmaceutical	Bronchodilator	Harmful to Acute Toxicity

Table 1. Continued

Compound	Type	Use	Toxicological effect
Triclocarban	Personal Care Product	Antimicrobial	Acute Toxicity
Triclosan	Personal Care Product	Antimicrobial	Acute Toxicity
Tylosin	Pharmaceutical	Antibiotic	Harmful to Acute Toxicity

**Compounds chosen based on compounds that will be examined by this project and common CECs found in Chen, Yao, & Zhou, 2015; Conn et al, 2010; Del Rosario et al, 2014; Dinardo & Downs, 2018; Katz et al, 2010; Subedi et al, 2015; Swartz et al, 2006; Wilcox et al, 2009; Yang et al, 2016.*

Certain environments are more vulnerable than others due to their geology and hydrologic properties. For examples, sinkholes are located in areas where dissolution of the underlying calcium carbonate bedrock (karstification) has occurred due to undersaturated, acidic water infiltrating, creating voids for which overlying sediment or bedrock can enter (Kaufmann & Dreybrodt, 2007; Gutiérrez et al., 2014). With little CEC remediation due to the rapid infiltration and high permeability of the karstified carbonate platform and the direct connection of sinkholes to the below groundwater, karst aquifers are highly vulnerable to contamination from OWTSs and surface runoff (Dodgen et al., 2017; Gutiérrez et al., 2014). The anthropogenic introduction of CECs into aquifers, waterways, and IBWs has adverse effects on the environment and on human health at varying degrees depending on the individual contaminant, concentration, and additive effects of the combination of multiple chemicals.

Research Question and Objectives

The aim of this research is to determine the presence and fate of CECs within the water, sediment, and vegetation of a sinkhole environment that receives runoff from OWTSs. As a karstified carbonate platform, Florida contains three aquifer systems, the Surficial, Intermediate and the Floridan. These aquifers, particularly the latter, are important sources of drinking water and irrigation for Florida. Consequently, understanding how the CECs may interact with this environment, particularly these aquifers, is of great importance. My research question is: what is the fate of CECs from OWTSs effluent within the catchment of a sinkhole lake?

To address this question the research objectives of this study are 1) collect water, sediment, and vegetation samples from a sinkhole lake surrounded by OWTSs, 2) analyze the samples for CEC's using liquid chromatograph-mass spectrometry, 3) determine the different CECs in the samples and subsequently the environment, 4) and determine the difference in CEC presence between the three samples types.

Intellectual Merit and Broader Impacts

The role of this research is to determine the potential harm posed by the release of CECs from septic tanks to the environment and subsequently to human health. Assessing the potential effects of CECs is critical to help highlight the need for the mitigation of contaminants, to help create best management practices, and determine differing levels of risk based on the individual chemicals. Humans can experience direct exposure through ingestion, inhalation, or physical contact depending on the chemical compound (Stuart et al., 2012). Concerns about the negative environmental effects of CECs are not only constrained to humans but also for the flora and fauna that occupy the built and natural world. Potential risk posed by contaminants can be assessed by the levels at which CECs resist degradation through the OWTS process, become toxic, and bioaccumulate (Ebele et al., 2017). This research project assesses the presence of CECs in the environment and presents results that can be applied to CEC mitigation in the environment and in the underlying groundwater.

Chapter Two:

Study Area

The study area is located in Riverview, Florida in the Bell Creek Watershed. The Bell Creek watershed is a sub-watershed of the Alafia River located in Hillsborough County, Florida (Haber & Mayfield, 2003). Bell Creek is a tributary of the Alafia River and flows from North to South. The geology of the region is prone to cover-collapse sinkholes which occur when dissolution creates a large void below the surface into which overlying bedrock and sediment containing a significant amount of clay, collapses into the void creating the sinkhole and if the groundwater is close to the surface, a sinkhole lake (Tihansky, 1999).

The sinkhole lake study area is located west-northwest of Lake Grady (Figure 2) and is surrounded by residential homes each using septic tanks as their means of waste treatment. The distance from the homes to the riparian zone is ~ 30-40 meters. The western side of the lake has a greater elevation decline while the eastern side of the lake has a longer, more gentle topographic slope. The land use within the Bell Creek Watershed includes residential, commercial, agricultural, and pastureland (Haber & Mayfield, 2003). Lake Grady is a 184-acre manmade lake created in 1969 by damming Bell Creek to attract buyers to the newly constructed Shadow Run subdivision (Haber & Mayfield, 2003). The closest NOAA weather station is located approximately 20 miles NE of the study area in Plant City, Florida, (Longitude: -82.1422, Latitude: 28.0236). For the period from 1981 through 2010 the precipitation average for the dry season (October to May) is 550 mm while the wet season (June to September) averages 800 mm. The mean average temperature is 22.2 °C.

Figure 2. Study area in Riverview, Florida located at 27°50'09.5"N 82°16'46.4"W. Reprinted from *Study Area in Riverview Florida located at 27°50'09.5"N 82°16'46.4"W*. by Google Maps, 2019. Copyright 2019 by Copyright Holder. Retrieved from https://www.google.com/maps/@27.8351735,-82.2785955,626m/data=!3m1!1e3 Reprinted with permission.

Chapter Three:

Methodology

An extensive review of the literature was conducted to determine the best method of choice for the water, sediment, and vegetation of the project. The methodology is based on Batley et al. (2016) Fairbairn et la. (2015), Ferrer et al. (2010), Hindle (n.d.), Petrie et al. (2017), Rodil et al. (2009), and Wu et al. (2015). In order to obtain results that are comparable, the same methodology was used for all samples with the exception of the homogenization and grinding process which will not be performed for the liquid samples.

Sampling

Samples of water, sediment and vegetation (compartments) were collected on February 13, 2019 to determine the portioning and accumulation of the CECs as a reflection of their source. These samples were collected from sites on opposite sides of the sinkhole lake in order to have a more accurate representation of the research site. Samples sight 1 (SS1) is located on the western side of the sinkhole lake while sample sight 2 (SS2) is located on the eastern side. For the vegetation, *Lemna minor* and *Nymphaea aquatica,* most commonly referred to as Duckweed and Florida Water Lilly, respectively, were examined as they both incorporate CECs into the tissues from the surrounding water/sediments.

The water was collected mid-way between the surface and base of the sinkhole lake using 1 Liter amber glass bottles. The bottles were filled with sinkhole lake water to the top to prevent the exchange of molecules from water to air. The bottles were then placed in a cooler for transport. Sediment was collected using a stainless-steel scoop from the top 5cm of the lake bed and placed in an amber jar to prevent photo decay. Finally, the entire plant (roots, stems, and leaves) of the water lily and duckweed were collected and placed in an amber glass jars. All samples were transported to the University of South Florida (USF) Chemical Purification Analysis and Screening (CPAS) core facility for sample preparation. The water samples were stored at 4˚C until the samples were cleaned and extracted. Sediments and vegetation were stored at -20˚C until sample preparation.

Cleaning and Extraction Preparation

The cleaning process for the lake water began with the filtering of the samples through a vacuum assisted 0.7µm nitrocellulose filter twice to expel any particulate matter within the samples. The vials used for filtration were cleaned with soap and de-ionized water before they were dried with acetone. After the samples were filtered, 500ml of each sample were placed in a -20˚C freezer to be frozen before they were lyophilized. Lyophilization uses sublimation to turn the solid ice to a gas by expelling the moisture out of the sample leaving behind the anhydrous solids.

The sediment samples were brought to room temperature and strained through a 2mm sieve. The samples were drained of excess water before being placed in the -70˚C freezer to be prepared for lyophilization. Upon completion of lyophilization, the sediment was homogenized and ground down using a mechanical blender to maximize the surface area that can undergo extraction.

The vegetation was cleaned for 2 minutes under de-ionized water to remove any impurities that are bound to the roots, stem, or leaf. Due to the vegetation sample's size, the stems leaf and roots were cut into pieces in order to be lyophilized. These samples were then placed in the -70˚C freezer in preparation for lyophilization. As with the sediment, the samples were then ground down using a mechanical blender upon completion of freeze-drying.

Microwave Assisted Extraction

Microwave assisted extraction (MAE) is used to extract the desired compounds from the solid matrix of the vegetation, sediment, and water. For consistency, the water samples underwent MAE before solid phase extraction (SPE). The solvents and quantity used for MAE consist of 6.25ml (25%) of HPLC grade methanol and 18.74ml (75%) of de-ionized water. 0.5g of the target sample were weighed and mixed together with the MAE solvents in a 30ml vessel. A stirring vial was used to properly agitate the mixture. The method was as follows: heat sample to 50˚C for 10 minutes using 800 watts, wait for 30 minutes at 50˚C, then cool sample to 50˚C. The stirring speed maintained a constant speed of 800rpm throughout the extraction process. Finally, the completed MAE samples were then filtered using a vacuum assisted 0.7µm to prevent insoluble contaminants from entering the target solvent used for SPE.

Solid Phase Extraction (SPE)

Prior to SPE, the overall sample concentration of methanol was reduced to 5% by diluting the sample with 100ml of de-ionized water. Hydrophilic-Lipophilic-Balanced (HLB) cartridges, which draw out both polar and nonpolar compounds, were conditioned with 2 ml of methanol followed by 2ml of de-ionized water at a flow rate of 1ml per minute before SPE. After the cartridges were conditioned, the samples were then continually filtered through the cartridge at the same 1ml per minute flow rate. In order to extract the target analytes from the HLB cartridge, 8ml of methanol were poured through the cartridge and into a vial bringing the target contaminants with it. This vial was then blown down using a continual stream of nitrogen. For storing, the sample vials were filled with nitrogen and purged of any other gas to prevent degradation of the contaminants before analysis.

Liquid Chromatography Tandem Mass Spectrometry Analysis

To prepare the samples for analysis, a mixture of 80:20 water and methanol were added to the vial respectively. The total volume was 0.5ml of water making the mixture 0.4ml of water to 0.1ml of methanol. This mixture was then sonicated before being extracted through a syringe and filtered using a 0.45µm polyvinylidene difluoride (PVDF) filter to remove any traces of insoluble material. Liquid chromatography tandem mass spectrometry (LC/MS/MS) was chosen over other forms of analysis due to its sensitivity to detect known analytes and to accurately determine the presence of compounds based on multiple reaction monitoring (MRM) (Rushing et al., 2016). The samples were then analyzed using an Agilent 6460 Triple Quad (QqQ) with the 1260 Infinity HPLC system in electrospray ionization (ESI) positive and negative mode. A Synergi Fusion RP column was used for all of the samples as it is exceptional at separating both polar and nonpolar compounds before the compounds are detected by the QqQ.

The LC/MS/MS conditions and timetable for both forms of analysis are listed in Table 2-4. In positive ion mode, acetonitrile with 0.5% formic acid were used for the non-polar mobile phase while water and 0.5% formic acid were used for the polar mobile phase as shown by solvent B and A respectively in Table 2. In negative ion mode, 65% methanol and 35%

acetonitrile were used for the non-polar mobile phase while 1mM of ammonium fluoride in water were used for the polar mobile phase as shown by solvent B and A respectively in Table 3. The source parameters for both ESI+ and ESI- mode are listed in Table 4 and were used based on a solvent flow rate of 1ml per minute. In conducting a literature review of compounds found in OWTS effluent and compounds analyzed using the chosen methodology, 34 compounds were chosen for investigation in the samples and to determine whether they are present in the environment. These compounds were loaded into the LC/MS/MS software operating system using the compounds precursor ion charge, product ion charge, fragmentor voltage, and collision energy voltage listed in Table 4 and 5 for positive and negative mode respectively. Through the use of multiple reaction monitoring the presence of these compounds were determined using the parameters listed in Table 5 and 6 of the 34 desired compounds. The parameters listed in Table 1-5 are from analysis previously used to detect the compounds listed from Ferrer et al. (n.d.) and Anumol et al. (n.d.).

Table 2. ESI+ Solvent Gradient

Step	Time	Parameter
1	2.00 min	Solvent composition A: 100.0 % B: 0.0 %
2	25.00 min	Solvent composition A: 0.0 % B: 100.0 %
3	28.00 min	Solvent composition A: 0.0 % B: 100.0 %
4	29.00 min	Solvent composition A: 100.0 % B: 0.0 %

Table 3. ESI- Solvent Gradient

Step	Time	Parameter
1	2.00 min	Solvent composition A: 95.0 % B: 5.0 %
2	25.00 min	Solvent composition A: 5.0 % B: 95.0 %
3	28.00 min	Solvent composition A: 5.0 % B: 95.0 %
4	29.00 min	Solvent composition A: 100.0 % B: 0.0 %

Table 4. LC/MS/MS Source Parameters for ESI+ and ESI–

Parameter	**(ESI+)**	**(ESI-)**
Gas Temp (°C)	250	300
Gas Flow (l/min)	8	4
Nebulizer (psi)	35	40
Sheath Gas Heater (°C)	300	375
Sheath Gas Flow (L/min)	10	11
Capillary (V)	4000	4500
VCharging	0	1500
Injection Volume (µl)	20	20

Standards for as many of the individual compounds as possible were procured from the Chemistry and Biology departments. The standards used are as follows: acetaminophen, atenolol, caffeine, DEET, diclofenac, ibuprofen, metaprolol, naproxen, propranolol, sulfamethoxazole, theophylline, and triclosan as seen in Table 6. These samples were prepared using a ratio of 10µg of standard to 1ml of methanol. The standards were run either in positive mode or in negative mode using the same method as the field samples depending on the compound. Each of the standards were prepared in a solution of water and methanol in a ratio of 80:20 respectively. The purpose of the standards is to verify the retention time and presence of the individual compounds within the samples.

Once the results were acquired they were analyzed using Agilent Mass Hunter Qualitative Analysis software. This software is used to analyze quantitative and qualitative chromatograph data. Each of the compounds have distinct retention times and are detectable by MRM. Based on the method previously mentioned the samples were analyzed based on retention time and relative quantity to determine the presence of compounds and to compare and contrast the presence and quantity of the compounds within each sample relative to the other samples.

Table 5. Common contaminants found in septic tanks that are also detected in ESI+ analysis from Ferrer et al. (n.d.) and Anumol et al. (n.d.)

Compound	Precursor Ion	Product Ion	Fragmentor (V)	Collision Energy (V)
Tylosin	916.5	772	110	35
Tylosin	916.5	174	110	35
Sucralose+Na	419	239	110	15
Sucralose+Na	419	**221**	110	15
TCPP	327	99	72	16
TCPP	327	81	72	70
Fluoxetine	310	148	90	5
Trimethoprim	291	261	110	25
Trimethoprim	291	230	110	25
Testosterone	289.2	109.1	116	24
Testosterone	289.2	97.1	116	20
Androstenedione	287.2	109.1	107	24
Androstenedione	287.2	97.1	107	20
Metaprolol	268	116	100	17
Metaprolol	268	56	136	29
Atenolol	267	190	134	13
Atenolol	267	145	134	21
Propranolol	260	116	100	17
Propranolol	260	56	122	29
Imidacloprid	256.1	219.1	125	11
Imidacloprid	256.1	175.1	125	17
Diphenhydramine	256	167	70	15
Diphenhydramine	256	152	70	35
Sulfamethoxazole	254	156	110	15
Sulfamethoxazole	254	92	110	25
Carbamazepine	237	194	110	15
Carbamazepine	237	179	110	35
Primidone	219.3	162.1	70	9
Primidone	219.3	91.1	70	25
Atrazine	218	176	140	15
Atrazine	218	174	140	15
Caffeine	195	138	110	15
Caffeine	195	110	110	25
DEET	192	119	110	15
DEET	192	91	110	30
Theophyline	181	124	90	15
Theophyline	181	99	90	15

Table 5. Continued

Compound	Precursor Ion	Product Ion	Fragmentor (V)	Collision Energy (V)
Cotinine	177	80	90	25
Acetaminophen	152	110	90	15
Acetaminophen	152	65	90	35

Table 6. Common contaminants found in septic tanks that are also detected in ESI- analysis from Ferrer et al. (n.d.) and Anumol et al. (n.d.)

Compound	Precursor Ion	Product Ion	Fragmentor (V)	Collision Energy (V)
Triclocarban	313	160	100	10
Triclocarban	313	126	100	25
17-Ethynylestradiol	295.2	145	139	36
Diclofenac	294	250	75	4
Diclofenac	294	214	75	16
Estriol	287.2	171.2	159	36
Estriol	287.2	145	159	44
Triclosan	287	35	75	5
Triclosan	287	35	75	5
TCEP	285	223	95	10
TCEP	285	223	95	10
17b-Estradiol	271.2	183.2	171	40
17b-Estradiol	271.2	145.1	171	44
Estrone	269.1	145	136	40
Estrone	269.1	143.2	136	56
Equilin	267.1	265.1	136	20
Equilin	267.1	143.1	136	40
Gemfibrozil	249	121	100	5
Naproxen	229	170	75	5
Naproxen	229	169	75	25
Bisphenol A	227	212	115	11
Bisphenol A	227	133	115	19
Ibuprofen	205	161	75	5

Chapter Four:

Results

LC-MS/MS analysis was conducted to determine the presence of CECs in the samples. CECs were found to be present in all samples and subsequently classified based on whether a standard is run and whether the target compound peak within the sample aligns with the peak of the standard at a similar retention time (high confidence) or no standards were available (moderate confidence). Standards are pure compounds of CECs that undergo the same LC/MS/MS analysis to obtain a peak and retention time to compare to the sample. The peak height within a chromatogram is the proportional quantity of the compound relative to the sample quantity in the chromatogram at any given time. Retention time is the time it takes for a compound to pass through the Synergi Fusion RP column that was chosen for this specific research project. Clearly identified CECs are those compounds detected within the sample that have a peak with the same retention time +/- 0.5 minutes as the known standard. The CECs that could be known compounds but for which there was not a comparable standard are those detected within the samples and show a peak height count of $>10^2$.

The distribution coefficient (log k_{ow}) is the ratio of an ionized or un-ionized compound's concentration between the two immiscible solutions, water and octanol (Mohsen-Nia et al., 2012). The log k_{ow} of a compound determines its solubility with lower values being more hydrophilic and higher values being more lipophilic. The distribution coefficient of the individual compounds will have implications for the presence and fate of the CECs in question.

For example, lower log k_{ow} values suggest more biodegradation will occur along the pathway and throughout the environment (Goswami et al., 2018). Inversely, the greater the log k_{ow} value the greater potential for environmental bioaccumulation (Pedersen et al., 2014). The log k_{ow} values are presented for both the "high and moderate confidence" compounds were obtained by experimental measures from Dalrymple (2005), Ebrahimi et al. (2013), Groshart et al. (2001), Hansch et al. (1995), Jiskra et al. (2002), Mohsen-Nia et al. (2012), Plácido et al. (2018), Tollefsen et al. (2012), World Health Organization (1998), Zhang et al. (2019), and Zhou et al. (2019).

Presence of CECs – High Confidence

12 standards were run to determine the whether their presence existed in the samples: acetaminophen, atenolol, caffeine, DEET, diclofenac, ibuprofen, metaprolol, naproxen propranolol, sulfamethoxazole, theophylline, and triclosan (Table 7). Of the 12 standards run through LC-MS, only five were identified in the samples based on peak height and retention times: atenolol, caffeine, DEET, theophylline, and triclosan as show in Table 8. The remaining seven standards were compared to the samples and there were no comparable consistent retention times, or no detectable peaks of the compounds suggesting their absence within the samples. The five compounds identified were present in each of the samples though their relative quantities varied by sample type and by sample location. The relative quantity is the concentration of an individual compound within a given sample relative to the compound concentration found in another sample.

Table 7. Standards ran under the same ESI +/- mode as the samples, their respective retention times, and whether or not presence of the compound is found in the samples

Compound	Retention Time (Minutes)	Mode (+/-)	Presence (/X)
Acetaminophen	11.3	+	X
Atenolol	9.14	+	
Caffeine	12.08	+	
DEET	18.57	+	
Diclofenac	22	-	X
Ibuprofen	23.76	-	X
Metaprolol	11.27	+	X
Naproxen	21.68	-	X
Propranolol	12.86	+	X
Sulfamethoxazole	15.67	+	X
Theophylline	11.14	+	
Triclosan	27	-	

** Presence – known presence ( ), unknown presence (X).*

Atenolol is a polar, hydrophilic compound. The atenolol standard has a retention time of 9.14 minutes which corresponds with peaks in all of the samples within ~0.1 minutes of the standard. However, the relative quantities are different for each sample type and different based on sampling location. The greatest relative quantity of all the sample types is found in the vegetation, followed by the water, and lastly the sediment. The highest average quantity of atenolol were in the WL and DW respectively at sampling site 2 (SS2). With the exception of vegetation, the quantities of the water and sediment were greater at sampling site 1 (SS1) than at SS2.

Like atenolol, caffeine is a polar, hydrophilic compound. The retention time for the caffeine standard is 12.08 minutes which corresponds with peaks within each of the sample within ~0.1 minutes of the standard. The overall quantity is higher at SS2 than at SS1 with the exception being to the LW at SS1 which is the most concentrated sample overall. Due to the hydrophilic nature of caffeine, there is a higher average amount of caffeine in the water than there is in the vegetation and sediment at both sites. The LW has the highest overall quantity followed by DW.

Of the nonpolar compounds DEET was found in all samples and in the greatest quantity. The retention time for the DEET standard is 18.57 minutes. The sample peaks appear within 0.54 minutes or less of the standard peak retention time. This time is right o the border of whether or not we consider the DEET to be a high confidence compound. However, the large relativesample quantity may play a factor in the peak location in that its width is from 18.9 to 19.46. As a result it is included in the high confidence compounds SS1 has more of this compound than the samples at SS2. Of the samples analyzed, the WL at SS1 has the greatest quantity of DEET followed by the sediment at SS1. Furthermore, the discrepancies between the WL at SS1 and SS2 is more than 4 times the quantity while the sediment is more than 20 times the amount at SS1 than at SS2. The relative quantity of DEET in the water at SS1 is ~ 50% higher than in the LW at SS2. The DEET presence in DW is the smallest among the sample types the quantity is more than half of the next smallest quantity of any of the samples with the exception to the sediment at SS2 which contains the smallest quantity overall.

Theophylline is a hydrophilic, polar compound and using this study's analytical procedures has a retention time of 11.14 minutes. Corresponding theophylline peaks were measured within the samples to within ~0.1 minutes of the target standard retention time of the standard for this compound. The average relative quantities from greatest to least are: WL, DW, LW, and sediment. The highest to lowest presence of the individual samples are as follows: the LW at SS1, the WL at SS2, the WL at SS1, the DW at SS2, and the DW at SS1, sediment at SS2, LW at SS2, and sediment at SS1. Comparing the sample sites, SS2 represents a higher relative quantity of theophylline than SS1 with the exception to the LW at SS1 which consists of the highest quantity of all of the samples.

Triclosan is a nonpolar compound most commonly used as an antimicrobial in hygienic products such as soaps, shampoos, and toothpastes. The retention time for the triclosan standard of this project's LC/MS analysis is 27.0 minutes. Presence of triclosan is seen in the sample's peaks within ~0.1 seconds of the standard retention time concluding that the compound is present in the samples in relative quantities. The vegetation samples show trace amounts of triclosan and represent the smallest quantities overall among sample types and at the two sample sites. The greatest quantities are found in the LW followed by the sediment. The relative quantities from largest to smallest are as follows: the LW sample at SS1, the sediment sample at SS2, the LW sample at SS2, and the sediment sample at SS1.

Table 8. CECs with high confidence of their presence within the samples

High Confidence												
Compound	**Retention Time**	**Δ RT**	**Log k_{ow}**	**Mode (+/-)**	**Sample Site 1**				**Sample Site 2**			
					LW	WL	DW	S	LW	WL	DW	S
Atenolol	9.14	0.17	0.16	+	2	3	1	4	3	1	2	4
Caffeine	12.08	0.06	-0.07	+	1	3	2	4	3	4	1	2
DEET	18.57	0.54	2.97	+	3	1	4	2	1	2	3	4
Theophylline	11.14	0.04	-0.89	+	1	2	3	4	4	1	2	3
Triclosan	27.0	0.05	4.76	-	1	4	3	2	2	3	4	1

**Polarity – polar (P), and nonpolar (NP); lake water (LW); vegetation – water lily (WL), duckweed (DW); sediment (S). Relative Quantity – Ranking 1-4, with the highest concentration being 1 and the lowest being 4.*

Figures 3-6 are the chromatograms of the four sample types where the red represents SS1 and the blue represents SS2. The five known contaminants atenolol (A), caffeine (C), DEET (D), theophylline (TH), and triclosan (TR) are represented by peaks in the chromatogram. The largest peak of the multiple reaction monitoring was chosen to represent the known compound presence in the figures.

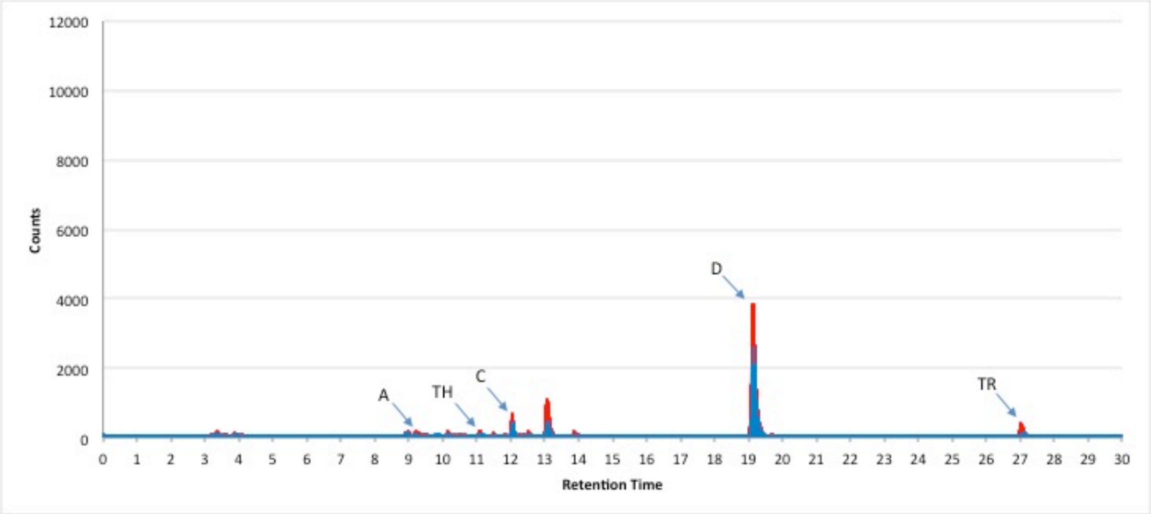

Figure 3. Total Ion Chromatogram depicting the five known CECs in the lake water samples at both sample sights

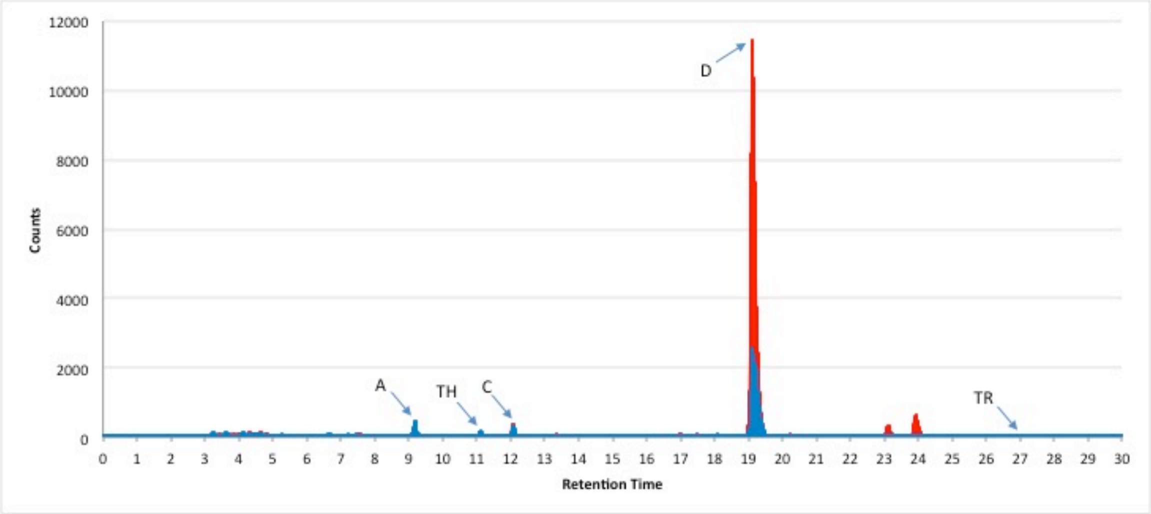

Figure 4. Total Ion Chromatogram depicting the five known CECs in the water lily samples at both sample sights

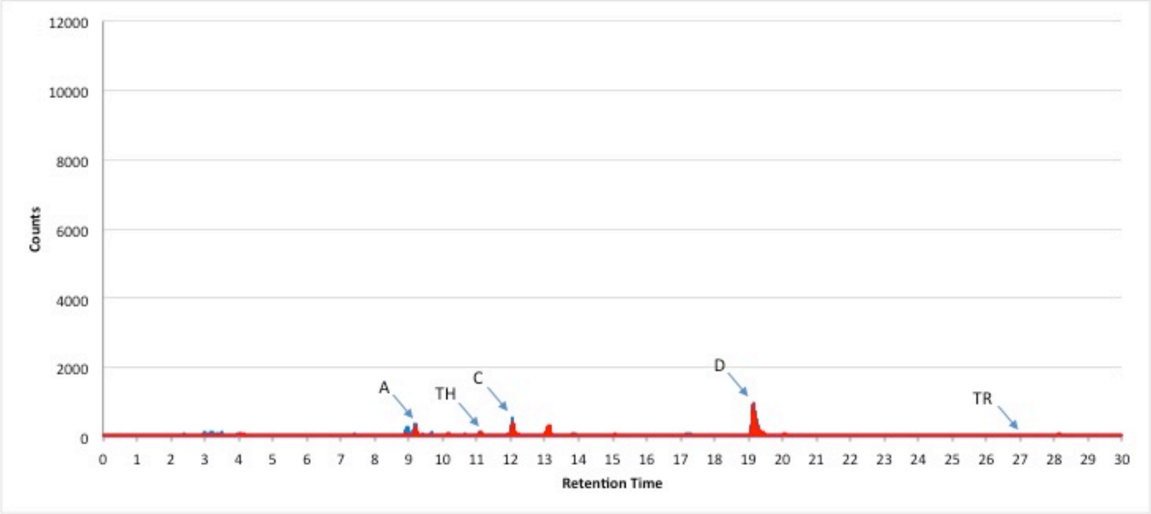

Figure 5. Total Ion Chromatogram depicting the five known CECs in the duckweed samples at both sample sights

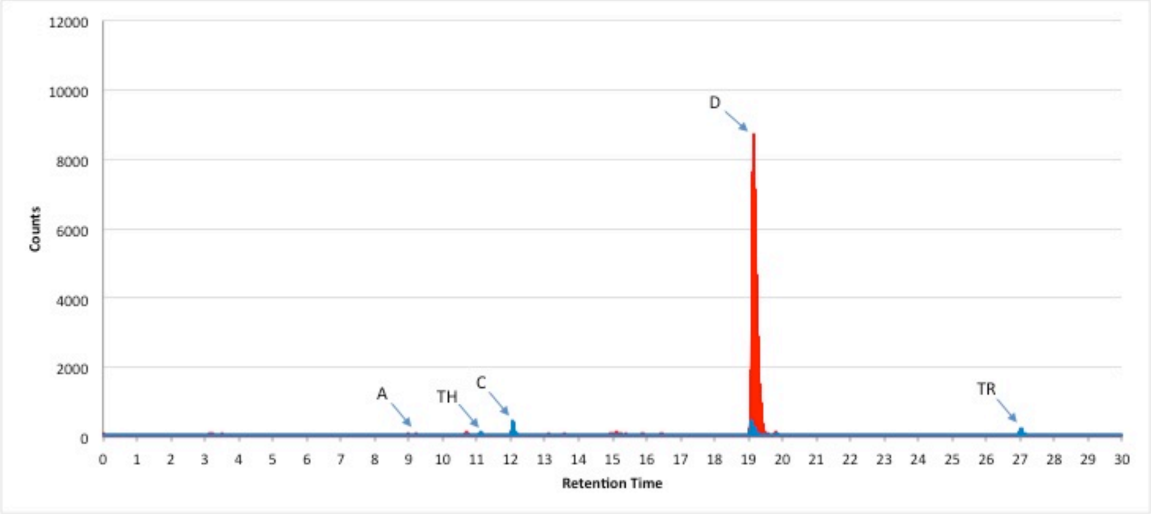

Figure 6. Total Ion Chromatogram depicting the five known CECs in the sediment samples at both sample sights

Presence of CECs – Moderate Confidence

CECs, shown in Table 9, with peaks in the samples that are potentially present include: sucralose, TCPP, fluoxetine, testosterone, androstenedione, diphenhydramine, carbamazepine, primidone, and atrazine in ESI+ mode where a voltage is applied to the sample spray to produce positive ions which are then detected by the mass spectrometer. Other potential CECs that may be present with the samples in ESI- mode include: TCEP, estriol, estrone, gemfibrozil, and bisphenol-A.

Table 9. CECs with moderate confidence of their presence within the samples

Moderate Confidence		
Compound	**Log k_{ow}**	**Mode (+/-)**
Androstenedione	2.75	+
Atrazine	2.16	+
Bisphenol-A	3.4	-
Carbamazepine	2.45	+
Diphenhydramine	3.11	+
Estriol	2.45	-
Estrone	3.13	-
Fluoxetine	4.05	+
Gemfibrozil	4.77	-
Primidone	0.91	+
Sucralose	-0.49	+
TCEP	1.7	-
TCPP	2.59	+
Testosterone	3.37	+

Chapter Five:

Discussion

CECs have been shown in literature and in this study to infiltrate environments through OWTS. The results presented are congruent with similar studies conducted by Bloomfield et al. (2006), Carrara et al. (2008), Del Rosario et al. (2014), Godfrey et al. (2007), and Prosser & Sibley (2015), in which contaminants flow from OWTSs, on top of and through the carbonate platform by way of preferential flow and infiltrate the environment. The uptake of contaminants from vegetation is consistent with Schnoor et al. (1995) in which *Lemna minor* or duckweed is a hyperaccumulator and helps to mitigate the contamination of aquatic environments.

Atenolol is a synthetic compound used in products to treat high blood pressure and to reduce the risk of heart attacks. The results of this study and of other OWTS effluent studies show that Atenolol can persist through the OWTS filtration process and the compound therefore enters the downstream environment (Subedi et al., 2015). Atenolol's log k_{ow} value is 0.16 showing its hydrophilic tendencies. This property allows the compound to transpire into the tissue of the aquatic vegetation and may bioaccumulate (Goswami et al., 2018). This could provide an explanation for why the vegetation samples contain the highest quantity of the compound. Because atenolol is transported by the OWTS's effluent and is hydrophilic, the LW samples have higher concentrations than the sediment.

Caffeine is a stimulant commonly found in food and drink products. It is easily soluble in water and is frequently consumed by humans. It is commonly found in OWTS effluent as shown by this and other similar studies (Conn et al., 2010; Del Rosario et al., 2014; Katz et al., 2010). Due to its high solubility and hydrophilicity (log k_{ow} value is -0.07), the greatest quantities are seen in the water relative to the vegetation and sediment. Vegetation contains the second highest quantity once again due to caffeine's soluble nature. An explanation for the higher levels at SS1 may by greater consumption from those home owners who live on that side of the sinkhole lake and as a result produce greater output from the OWTSs near SS1. Another possible explanation is the steeper hydraulic gradient at SS1 than at SS2. The increased gradient creates a more pronounced hydraulic head of the water table thereby increasing the flow of contaminated water from the OWTs into the lake. According to Phillips (1989) the slope gradient, hydraulic conductivity of the underlying soil, and buffer zone greatly impact pollutant transport. Seeing as SS1 is relatively closer to the sinkhole lake waterline and has a steeper slope, the CECs would have less time to degrade and would enter into the lake environment in greater quantity. This would result in greater relative quantity of caffeine at SS1 than at SS2. Furthermore, the DW that lives on top of the water contains the second highest caffeine relative quantity due to its direct connectedness to the water and its separation from the inorganics of the sediment. That being said, the WL also contains the presence of caffeine but slightly less than the DW drawing on the conclusion that the polarity and solubility of caffeine is easily absorbed by organics.

DEET is a compound commonly found in wastewater effluent and is seen by the results shown (Del Rosario et al., 2014; Weeks et al., 2012; Singh et al., 2010). DEET, being non-polar and lipophilic with a log k_{ow} of 2.97 would not be expected to be present in the organic constituents. However, that is not the case as seen in by the WL. The reason for the large quantities at SS1 and small quantities at SS2 can be explained by the 1) input of DEET from OWTSs and preferential flow from OWTSs near and around SS1, 2) uptake of DEET by vegetation directly connected to the soil, and 3) organics in the sediment. Inputs from houses in closer proximity to SS1 may play a significant factor in the difference between the relative quantities of DEET at SS2. Humans near SS1 may be expelling more of the compound through their waste after which it then flows into the sinkhole lake. Like caffeine, the steeper hydraulic gradient at SS1 than at SS2 may also contribute to a reduction of sediment remediation resulting in a greater quantity of DEET at SS1 than at SS2. As the WL has roots in the soil it is most likely that the non-polar DEET would be found in the inorganic sediment but not absorbed by the WL due to its log k_{ow} of 2.97. This is however contrary to the results in which the WL at SS1 has the greatest relative quantity of DEET. In analyzing the physiochemical properties of DEET we find that there is an inverse relation between root adsorption of DEET and the log k_{ow} (Wu et al., 2013; Weeks et al., 2012). Upon collecting the sediment samples at the sample locations the top 4 cm of sediment at SS1 was organic matter while only the top 1 cm of sediment at SS2 was organic. Thus it is likely that the structure of DEET is adsorbing to the WL root and other organics in the soils and being absorbed by the plant via a hydrophilic transport (Wu et al., 2013). This is also shown by the lack of DEET in the DW which floats on the water surface and is not directly connected to the sediment. It is possible that the dying WL could retain the DEET and would then be reabsorbed into the living WL. However, it is more likely that the inputs and topography at SS1 are directly impacting the initial quantity and causing adsorbance to the sediment.

Theophylline is a polar compound most commonly used in bronchodilator products to treat asthma. Theophylline is a compound that is structurally similar to caffeine with one less methyl group. Caffeine can be broken down into theophylline naturally in the body and in the environment. Because caffeine breaks down into theophylline one would expect there to be a higher proportion of caffeine in the environment than theophylline, a finding seen in the data. The log k_{ow} of theophylline is -0.89 providing further evidence of its high potential of biodegradation. The vegetation and the water contain the highest levels of theophylline due to its hydrophilic nature. An interesting observation is that SS2 has a greater presence of the compound relative to SS1. However, like the caffeine samples, the SS1 LW sample contains the greatest quantity of theophylline overall. Meanwhile the theophylline at SS2 is found in a reduced quantity in the LW possibly due to past uptake from vegetation and degradation of the compound within the water. As previously mentioned, the increased hydraulic gradient at SS1 may play a factor in that theophylline has less time to biodegrade due to the increased flow from the steeper slope. With the exception to the water from SS1, the next four highest quantities consist of all of the vegetation which is to be expected from the high hydrophilicity of theophylline.

The common occurrence of triclosan in anthropogenic hygienic products makes it an excellent tracer for OWTS effluent as seen in a number of studies (Svenningsen et al., 2011; Conn et al, 2010; Singh et al., 2010). Evidence of the pathway from source to final receptacle of CECs is most evident with the presence of triclosan due to its anthropogenic nature, its resistance to degradation, and its lack of potential inputs into the sinkhole lake from outside sources other than through OWTS effluent. Due to triclosan's non-polar, lipophilic (log k_{ow} value of 4.76) nature one would not expect to see the presence of this compound in the LW as the results suggest. However, due to the percolation and overland flow waters being the main source of transport for this chemical we would expect it to be highest in the LW until it binds itself to other non-polar, lipophilic compounds. As mentioned previously, the sediment at SS1 is high in organic matter relative to the sediment at SS2 which from observation has a higher sand fraction.

The polarity of organic matter may discourage any interaction of triclosan with organics which explains the reduced levels in the vegetation and subsequently with the organic-rich sediment at SS1. The inorganic sediment at SS2 is preferential for the non-polar triclosan resulting in the binding of the compound to the sediment resulting in the large quantity found in the sample. In addition, the anthropogenic use of triclosan at SS2 may be higher than at SS1 providing greater quantity in the sediment. Not only would greater relative quantities of triclosan at SS2 provide larger quantities overall but would be exceptionally prolific in the inorganic sediment at SS2 as seen in the results. The current levels of the antimicrobial triclosan seen in many areas in the United States are toxic to aquatic bacteria and should be evaluated in the sinkhole lake environment (Ricart et al., 2010).

Regarding the presence of CECs in the samples (moderately confident, see Table 8) it is only possible to tentatively suggest that the chemicals are present in that they have similar molecular masses and structures to those found in the samples. The reason for this tentativeness is that other compounds may fragment into the product ion (listed in the methodology parameters) of the CEC that is identified. An example, in Figure 3 the peak with a retention time of ~13.1 minutes is shown to have similar MRM results as caffeine. However, when comparing the caffeine standard to the DW SS1 sample, we can conclude that the only actual location of caffeine is the one shown at the 12.08 minute retention time. The determining factor is the retention time based on standards of the pure compound which filters out any of the peaks that are not the CEC in question. What determines the retention time is the polarity of the compound. While the peak at ~13.1 minutes may have a similar structure to caffeine and as a result fragment as caffeine does, there is no conclusive evidence that the peak is caffeine. The reason being that the compound’s polarity found at ~13.1 minutes is distinguishably different from the polarity of the caffeine standard that gives us a retention time of 12.08 minutes. All of the compounds identified with moderate confidence have been seen in OWTS effluent in literature which is the reason for their inclusion in this study.

As CECs degrade, they change their molecular structure. The impact of the degraded compounds could also impact the environment adversely. An example is that of caffeine and theophylline in which theophylline contains one less methyl group than caffeine and can result in further contamination of sinkhole environments. Research into the impacts of the degraded compound structure should be considered based on the precursor compounds' willingness to breakdown into the new compound and the product compounds potential to harm the environment and humans.

CECs Impact on the Surrounding Areas

The sinkhole lake is a closed drainage system with possible input from the aquifer systems which may be connected to the lake. A study done by Haber and Mayfield (2003) found that sinkhole lakes in the area were connected to the aquifer so contamination from sources such as sinkholes does place a significant risk to the water quality of the area. However, other sources include surface water infiltration, runoff from roads and other impermeable surfaces, or from Lake Grady which sits in relatively close proximity to the sinkhole lake sample site. Infiltration into the aquifer system is potentially harmful to the residents of the community due to their dependence on well water for potable water.

Bioaccumulation of CECs is a distinct possibility based on the results of this study. The vegetation has shown to uptake CECs in different quantities based on the physiochemical nature of the individual compounds and of the biological nature of the vegetation. DEET and Triclosan have the chemical structure to resist degradation but may or may not degrade depending on the vegetation and physiochemical properties of the sinkhole lake and the surrounding environment. Additionally, contaminants may be problematic for the aquatic animal species which feed on plants and organisms within the lake. Conversely, one could draw the conclusion that vegetation is beneficial for remediating CECs in the environment and should be cultivated to promote a healthier environment and cleaner water.

Chapter Six:

Conclusion and Future Outlook

The presence of CECs in the environment are clearly evident in this study's carbonate environment. The potential impact of CECs infiltration into aquifers such as the one in this area must be considered especially for those who use well water for potable use. Percolation from the surface to the subsurface may result in contamination of potable well water causing human harm. Consequently, one important outcome of this study is the importance of water monitoring and the removal of septic tanks as the main method of waste water treatment. Contaminant infiltration must be determined before the consumption and usage by humans and also determine what CECs are most likely to infiltrate the environment. Furthermore, infiltration of CECs may differ in sinkhole lakes based on inputs and the topography surrounding the sinkhole lake environment.

The results of this study are useful for environmental engineering, urban planning, and suburban planning. For example, one outcome of this research is how the design of stormwater ponds that drain OWTS must consider the importance of maintaining aquatic vegetation and a healthy riparian zone because of the ability of vegetation to remove CECs from the water. In addition, steeper slopes can increase hydraulic gradients that can increase the concentrations of CECs in the water which then enters the ponds. Consequently, slope contours of constructed ponds may play a significant role in the rapid infiltration of CECs and should be considered in urban and suburban planning regulations.

When conducting future research other factors could be included such as 1) seasonal weather variability, 2) multiple lakes, 3) connectivity of the underlying carbonate matrix, the underlying aquifer, and surrounding water bodies, 4) determination of hydraulic conductivity between the OWTS and the lake, 5) determination if different CECs are mitigated in different portions of vegetation, 6) which types of vegetation are best at mitigating contamination, 7) how long CECs persist in the environment based on the lakes hydrologic physiochemical properties, 8) survey conducted to determine how many full time residents reside around the sinkhole lake and in which houses, 9) survey to determine the management and state of septic tanks as they are maintained by the home owners, and 10) the quantification of the compounds to determine the hazards of the concentrations in the environment.

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Appendices

Appendix A: Known CECs Auxiliary Data Within the Samples

Table A1. Peak height, peak area, and retention times of known compounds found in samples

Atenolol	MRM: 267 – 145			MRM: 267 – 190		
	Peak Height	Peak Area	Ret. Time	Peak Height	Peak Area	Ret. Time
DW SS1	277	1123	9.205	99	497	9.202
DW SS2	348	1534	9.187	225	1175	9.186
WL SS1	83	851	9.178	17	52	9.309
WL SS2	439	2550	9.179	347	1661	9.176
LW SS1	83	356	9.189	133	539	9.193
LW SS2	32	167	9.173	71	276	9.186
S SS1	51	295	9.222	52	226	9.201
S SS2	22	80	9.205	20	146	9.175
Caffeine	MRM: 195 – 110			MRM: 195 – 138		
	Peak Height	Peak Area	Retention Time	Peak Height	Peak Area	Ret. Time
DW SS1	90	420	12.035	365	2000	12.064
DW SS2	69	495	12.02	497	2351	12.05
WL SS1	29	132	12.071	321	1977	12.071
WL SS2	78	418	12.064	384	1841	12.078
LW SS1	100	678	12.066	671	3562	12.045
LW SS2	58	693	12.024	375	1897	12.071
S SS1	47	294	12.042	174	975	12.048
S SS2	79	363	12.059	411	2078	12.067

Table A1. Continued

DEET	MRM: 192 -> 91			MRM: 192 -> 119		
	Peak Height	Peak Area	Retention Time	Peak Height	Peak Area	Ret. Time
DW SS1	459	4486	19.128	911	7318	19.145
DW SS2	841	7054	19.149	922	8416	19.146
WL SS1	8607	82393	19.11	11417	109477	19.116
WL SS2	1969	24470	19.122	2503	31690	19.126
LW SS1	2991	24949	19.136	3815	33046	19.134
LW SS2	1909	15221	19.159	2576	20756	19.159
S SS1	6651	61300	19.155	8684	81846	19.152
S SS2	238	1987	19.128	428	3555	19.084
Theophylline	MRM: 181 -> 99			MRM: 181 -> 124		
	Peak Height	Peak Area	Retentiont Time	Peak Height	Peak Area	Ret. Time
DW SS1	75	277	11.116	105	453	11.108
DW SS2	29	160	11.15	110	732	11.17
WL SS1	-	-	-	118	665	11.108
WL SS2	-	-	-	158	823	11.122
LW SS1	-	-	-	169	930	11.098
LW SS2	-	-	-	69	237	11.096
S SS1	-	-	-	61	384	11.143
S SS2	-	-	-	85	476	11.109

Table A1. Continued

Triclosan	MRM: 287 -> 35		
	Peak Height	Peak Area	Retention Time
DW SS1	36	163	26.968
DW SS2	17	49	27.049
WL SS1	10	51	27.037
WL SS2	27	131	27.001
LW SS1	363	2594	27.02
LW SS2	111	731	27.118
S SS1	39	154	26.989
S SS2	203	1022	27.038

**MRM is the multiple reaction monitoring with the precursor ion displayed first followed by the product ion. Peak height and peak area are displayed in counts from the Mass Agilent Hunter chromatogram data. Theophylline fragmentation from the precursor ion charge of 181 to the product ion charge of 99 has counts below the minimum detectable limit and are therefore not shown.*

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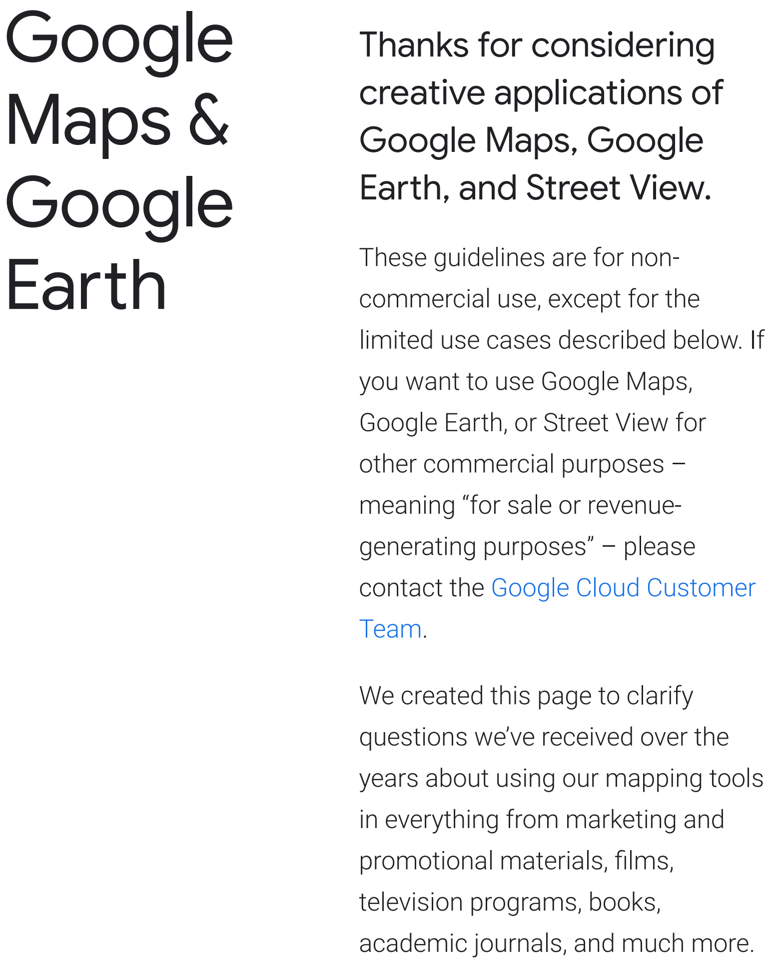

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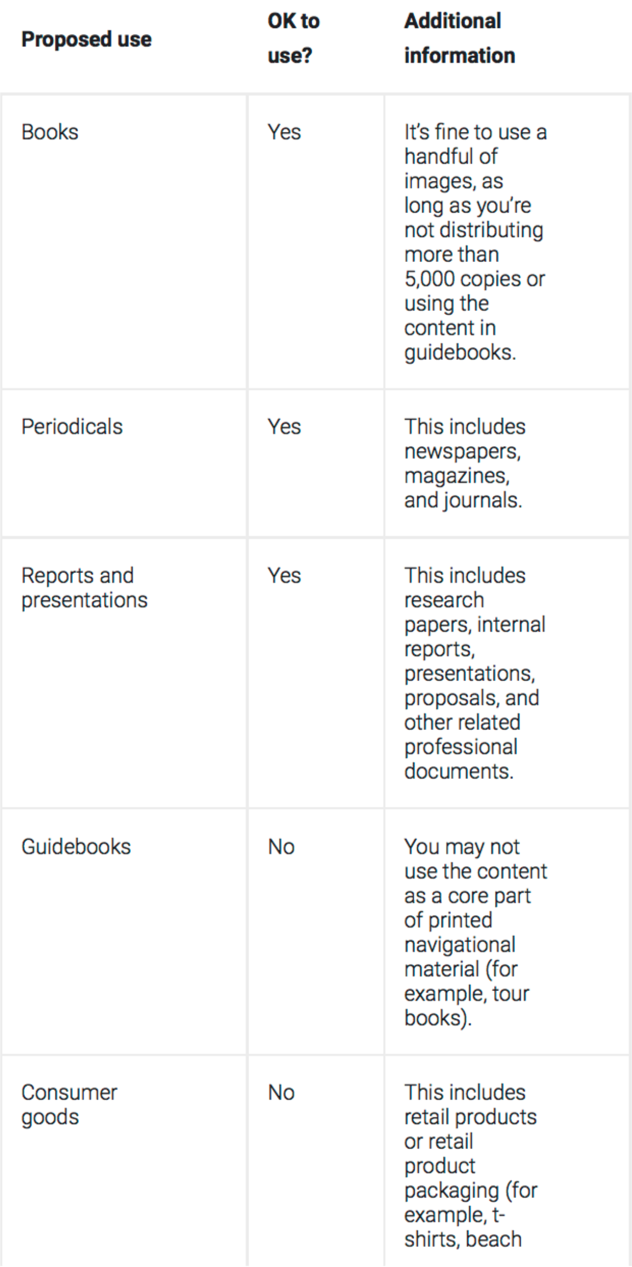

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