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Developing Rapid Indicators to Detect Micropollutants in Coastal Blackwater Rivers in South Carolina

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DEVELOPING RAPID INDICATORS TO DETECT MICROPOLLUTANTS IN COASTAL BLACKWATER RIVERS IN SOUTH CAROLINA

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ABSTRACT

Micropollutants in water sources are found in higher concentrations in areas with high levels of urban and agricultural land. These pollutants can be introduced into water sources during storms and rain events, through water treatment outputs, and commercial or residential waste. These events increase the levels of both nutrients and micropollutants in these water sources. With increasing levels of micropollutants in rivers, this study's goal was to develop an alternative detection method or an indicator test for the levels of micropollutants in water. We first proposed the use of amicrobial fuel cell (MFCs) operating as a biosensor could be utilized as an in-situ detection method. An MFC system was developed with the potential to detect micropollutants introduced into the system, specifically sulfamethoxazole (SMX). This detection was shown by a decrease in current output from the system as SMX concentration increases. However, it took far higher of a concentration of SMX for detection to be indicated than that which could typically be found in natural waters, rendering this system impractical for our uses. We then proposed that an enzyme-linked immunosorbent assay (ELISA) could have the potential to provide quantitative analysis of the micropollutants in water, as they are generally used to detect antibiotic presence in medical and veterinary applications. Some drawbacks to this method are only being able to detect one micropollutant or class of micropollutants at a time and it is relatively expensive. In early testing we determined the dissolved organic carbon (DOC) levels found in natural waters adversely interfere. Our third experiment proposed that the presence of organic halides (specifically chlorine and bromine,) in a water source could be correlated to the percentage of urban land use in the immediate surrounding area and used as an indicator test for other micropollutants that may be present. This correlation was proposed due to the increase of these nutrients in urban areas, especially after rainfall events, and that urbanized areas also tend

to have higher levels of these micropollutants. Nitrogen levels are also expected to increase in areas with relatively high percentages of agricultural land use as nitrogenous fertilizers leach into the surrounding water table. Samples from rivers across the state of South Carolina were measured for the presence of total organic halides, dissolved total nitrogen, and other water quality parameters. These were compared to land use percentages for natural land, agricultural land, and urban land. We found no significant correlations between these measures. Further testing is needed to determine if land use percentage is a viable indicator for organic halide presence in waters. Current recommendations are to continue using primary detection methods for micropollutants in surface waters.

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CHAPTER ONE

INTRODUCTION

Rapid urbanization and deforestation for urban and agricultural land use have led to increased concentrations of micropollutants in water sources in more urban and agricultural areas due to the introduction of herbicides and pharmaceuticals, wastewater treatment runoff, and other forms of commercial and residential waste (Strokal et al., 2021). Many micropollutants can be considered toxic and pose risks to the environment, wildlife, and human health. Currently, there is no active water monitoring program for these micropollutants in South Carolina because there is a wide variety of pollutants and analytical procedures used in their detection, which are both labor and cost-intensive (Wittmer et al., 2015).

Pollutants in water sources are seen to increase shortly after rainfall events due to water run-off entering bodies of water (Chaudhry & Malik, 2017). In addition to the continuation of urbanization across the state of South Carolina, climate change has shown a considerable increase of storm events and rainfall which are projected to continue to increase. This increase of rainfall coupled with rapid urbanization will result in the introduction of micropollutants into water sources at both a higher and more frequent rate (Greer et al., 2008). Due to the reduction of trees and other land cover which can provide the land with better water absorption, deforestation can lead to increased run-off during storms and in wet seasons. Clearing land for agriculture, housing, highways, and many other anthropogenic uses increases both the introduction of nutrients and of micropollutants to water sources. With micropollutants becoming increasingly present in our waterways, detection and mitigation efforts are needed to monitor and regulate levels (Gholami, 2013; Kavian et al., 2014; Zhao et al., 2022). Current micropollutant detection methods such as LC-MS, GC, and IC are time consuming and costly. The primary goal of this study is to develop

a rapid indicator test for micropollutants to assist in detection efforts. This study utilized three different experimental designs to find a suitable rapid detection method for micropollutants; exploring biological testing with a microbial fuel cell, biochemical testing with enzyme linked immunosorbent assay, and water chemistry analysis of organic halides correlated with land use analysis.

Microbial fuel cells have shown promise to function as in-situ monitoring for micropollutants in rivers and streams which would allow for almost immediate detection (Yang et al., 2015). In this study a microbial fuel cell (MFC) was set up to use the bio-available current to show the presence of an antibiotic, a common pollutant in water sources. The current should decrease in the presence of an antibiotic because the biofilm viability would be impacted. Sulfamethoxazole, an antibiotic, would be intermittently introduced to the system, which was continuously monitored for any changes in current output.

Enzyme-linked immunosorbent assay (ELISA) was used to detect the presence of an antibiotic. ELISA is an assay commonly used in pharmaceutical and veterinary senses as a means of detection and quantification of proteins, peptides, antibiotics, and many other indicators in biological samples (Crowther, 2002). Primary experiments were conducted to determine viability for use in environmental research prior to testing water samples. Tests plates of predetermined concentrations of both dissolved organic carbon (DOC) and Tetracycline were analyzed to determine if the presence of DOC found in natural waters would interfere with the ELISA test.

Organic halides are micropollutants regularly found in natural waters, originating from a variety of anthropogenic sources including wastewater treatment, sewage, and landfill leaches. Agricultural fertilizers are also found routinely in natural waters, usually found in forms of dissolved total nitrogen as it is the main component in most modern fertilizers. Both types of

micropollutants are linked to a particular type of land use, urban and agricultural respectively (Zahn & Grimm, 1993). Water samples were assessed for the presence of organic halides and dissolved total nitrogen to correlate the presence of micropollutants with land use percentages so that land use could serve as an indicator test for micropollutants.

Using these experimental designs, we hope to understand how land use change can impact the presence of micropollutants and deterioration of water quality to provide considerations when determining land use planning and water management practices as well as increase our knowledge that the role urbanization, agriculture, and population increase has on water management practices.

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CHAPTER TWO

MICROBIAL FUEL CELL FOR USE AS A BIOSENSOR FOR MICROPOLLUTANTS

Abstract

A wide variety of micropollutants are found in water sources across the state of South Carolina, leading to difficult, time consuming, and cost prohibitive means of detection. This experiment aimed to develop a detection method capable of detecting many different types of pollutants by the same means, therefore being a more cost-effective method. A microbial fuel cell (MFC) is a biological system consisting of an anode, cathode, and biofilm which produces a current and has the capability to function as a biosensor. This biofilm would be negatively impacted by the presence of toxins present in the system, shown in detectable changes in current. Our study aimed to show this change in current and quantify the level of micropollutant needed to have a detectable effect on the system. To assemble the microbial fuel cell, the anode and cathode chambers were separated by an anion exchange membrane and connected to the circuit via a graphite fiber brush, a Pt/carbon cloth, and a resistor to form a dual-chamber reactor. The biofilm was inducted into the system with a bacterial sludge from a Greenville, SC wastewater treatment facility and the anode and cathode were filled with distilled, deionized water and a nutrient-rich solution and the bacterial sludge, respectively. After the fuel cell was assembled and the biofilm established: sulfamethoxazole, a common antibiotic, was introduced to the system via the cathode and current was observed for any detectable decrease. Sulfamethoxazole was introduced as concentrations of 10ug/L, 100ug/L, 1mg/L, and 10mg/L at intervals of approximately 240 hours to allow for system cycling. The current output decrease of the fuel cell was only observed after the injection of sulfamethoxazole at 10mg/L.

Introduction

In recent years, many have begun to research the capabilities of microbial fuel cells (MFCs) for use in many applications, including wastewater treatment, bioenergy production and as biosensors (Bakonyi et al., 2018; Gajda et al., 2018; Gude, 2016; Jadhav et al., 2014). Though microbial fuels cells have not yet shown the capability for bioenergy production, they do produce a high enough energy output to be self-sustaining (Abrevaya et al., 2015; Yang et al., 2015).This ability to self-sustain shows promise for use of MFCs as in-line pollution biosensors. Biosensors are devices that are used to detect analytes in different mediums, depending on the type, and can detect presence or concentration of an analyte (M. Kim et al., 2007). This study attempts to develop a microbial fuel cell as a biosensor for micropollutants commonly found in the blackwater rivers of South Carolina.

To develop a microbial fuel cell capable of acting as a biosensor, we must first understand the basics of how these systems operate. Microbial fuel cells consist of both an anode and a cathode, usually in the form of chambers. These anodes and cathodes are formed due to an anaerobic biofilm in the anode chamber. This biofilm, which could be further described as a body of microbial cells in an extracellular substance matrix, will digest an electron donor to extract an electron (Dávila et al., 2011; Donlan, 2002; Ter Heijne et al., 2007).The electron donor in microbial fuel cells is generally a form of carbon, with acetate commonly reported to be used for the carbon source in other attempted MFC biosensors (Dávila et al., 2011b; Rismani-Yazdi et al., 2008; Shanmuganathan P & Rajasulochana P, 2018). Electrons will then pass from the anode to the cathode via an external load, or a resistor that is applied to the circuit between the anode and cathode. The external load is used in conjunction with the internal resistance of the unit to achieve optimal power output (Koók et al., 2020, 2021; Oh & Logan, 2006). The cathode

chamber receives the electrons after they pass through the external load and they are used in a reduction reaction of an oxidant, which is typically oxygen. Reduction of at atom occurs when the atom gains an electron, thus resulting in a small energy output. The energy of this reaction is harvested by the external load and can be seen as a current output from the system (Logan et al., 2006; Logan & Regan, 2006).

When a toxin is introduced to an MFC, the biofilm within the anode will attempt to digest the toxin, leading to the destabilization of the biofilm due to cell death. As the biofilm is unable to digest the toxin for electron extraction, this then in turn prevents the rest of the reaction, including the electron transfer from the anode to the cathode. When this transfer does not occur, energy is not produced and the current output decreases (Bradley, 2019). Furthermore, the biofilm is weakened by the toxin and its overall capabilities for electron extraction, and thus energy output, are diminished and completely disrupted in some cases. The decrease in current output can be seen and correlated with the presence of a toxin; and in this case the microbial fuel cell is acting as a biosensor (Di Lorenzo et al., 2014; Gonzalez Olias et al., 2019).

Methods and Materials

MFC Configuration

Dual-chamber reactors were composed of an equal volume (0.2 L, holding 0.18 L solution) of anode and cathode chambers and a rinsed 16 cm^2 anion exchange membrane (International Membrane Co., Ringwood, NJ, USA). The MFC circuit contained a rinsed graphite fiber brush (Mills-Rose Co.) with surface area of 0.22 m^2 as the anode, a 10 cm^2 10%-Pt/carbon cloth as the cathode, and a 10000-ohm resistor (**Figure 2-1**) (Hutchinson et al., 2011).

Sludge Sampling

The bacterial sludge used in this microbial fuel cell study was collected from Greenville Water's Wastewater Treatment plant in Greenville, SC on 4/30/2019. Greenville Water collected the bacterial sludge from their wastewater system, bottled it in a 1-gallon plastic jug and refrigerated it at 4 degrees Celsius until retrieved. Once these samples had been obtained, they were transferred in an ice cooler and stored at 4 degrees Celsius at L.G. Rich Lab in Anderson, SC. Microbial fuel cell assembly and testing were also performed at the L.G. Rich Lab.

Solution Preparation

The anoxic chamber of the microbial fuel cell contained several solutions: a buffer to function as electrolytes, a basic nutrient solution, and a complex nutrient solution. The potassium buffer was made by mixing 9.34g of K_2HPO_4 and 6.31g of KH_2PO_4 (EMD Chemicals, ACS Grade) in 800mL of Distilled De-Ionized water (DDI) until completely dissolved and adding DDI until a volume of 1L. The basic nutrient solutions consisted solely of Sodium Acetate Trihydrate crystals in DDI to a 5g/L concentration. The complex nutrient solution consisted of $CH_3COONa{\cdot}3H_2O$ (J.T.Baker, USP, FCC, ASC Grade), NH_4Cl (Mallinckrodt, ACS Grade), K_2HPO_4, KH_2PO_4 (EMD Chemicals, ACS Grade), $NaHCO_3$ (VWR Analytical, ASC Grade), $CaCl_2{\cdot}2H_2O$ (Sigma- Aldrich, ACS Grade), and $MgSO_4{\cdot}7H_2O$ (EMD Chemicals, ACS Grade). All solutions were stored at 4 degrees Celsius prior to use in the fuel cell.

Results

MFC Start-up

To start up the replicate dual-chamber MFCs, we filled distilled and deionized water (DDI) to anode chamber, added ~130 mL 5 g/L sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$)

to cathode chamber, and added 15 mL anaerobic digestion sludge from the ReWa municipal WWTP (Greenville, SC) to cathode chamber. After 120 h, the outputs of currents from these two replicates MFCs were close to zero (**Figure 2-2**). This may possibly be due to the poor exchange of electrons between the cathode and anode chambers.

We made the following changes: 1) activating the exchange membrane by placing in 10 g/L NaCl for at least 24 h before using; 2) filling the cathode chamber with potassium phosphate buffer pH 7 (PMS; 9.343 g/L K_2HPO_4 and 6.309 g/L KH_2PO_4); 3) using the mixture of 5 g/L $CH_3COONa\ 3H_2O$ [dissolved organic carbon (DOC): 5 g/L / 136.0796 g/mol x 12 x 2 = 881.8 mg/L), 0.7 g/L NH_4Cl [total nitrogen (TN): 0.7 g/L / 53.491 g/mol x 17 = 222.5 mg/L], 93.4 mg/L K_2HPO_4, 63.1 mg/L KH_2PO_4, 2.2035 g/L $NaHCO_3$, 30 mg/L $CaCl_2\ 2H_2O$, and 25 mg/L $MgSO_4\ 7H_2O$ as artificially prepared nutrients in the another chamber. And then we restarted the replicate dual-camber MFCs. The stable currents were obtained from both dual-chamber MFCs after around 96 h (**Figure 2-3**), indicating the successful start-up of MFCs.

When the performance of the replicate MFCs became stable, we measured their internal resistance by changing the external resistance from 300 ohm to 900 ohm by using the 10000-ohm resistor. After plotting voltage versus current, we obtained the linear regression of $y=-82.59x + 551.65$ ($R^2=0.996$; $p<0.001$) and $y=-74.38x + 560.78$ ($R^2=0.995$; $p<0.001$) (y – voltage; x-current) (**Fig 2-4**). The internal resistances were 82.6 and 74.4 ohm for these two replicate MFCs, indicating low internal resistances in our dual-chamber MFCs.

Nutrient Solution Concentration Determination

To maintain an appropriate cycle time within the microbial fuel cell, concentration of the nutrient solution used had to be optimized. The cycle of the cell should have a sustained current for at least 1 hour, with a drop in current at the end of that time indicating the depletion of

nutrients in the anode. If the nutrient solution has too high of a concentration, the drop in current will not occur within a reasonable time. Alternatively, if the nutrients are present in the anode at too low a concentration, the fuel cell will never reach a stable current plateau. A stock solution was made for the complex nutrient solution containing these concentrations; 5 g/L $CH_3COONa{\cdot}3H_3O$, 0.7 g/L NH_4Cl, 93.4 mg/L K_2HPO_4, 63.1 mg/L KH_2PO_4, 2.2035 g/L $NaHCO_3$, 30 mg/L $CaCl_2{\cdot}2H_2O$, and 25 mg/L $MgSO_4{\cdot}7H_2O$. 30ml of solution was used at this concentration for an initial test of the nutrient load on the fuel cell. After 160 hours, the current was still stable and did not show any indications of dropping. The anode was then drained and reinoculated with buffer. The nutrient stock was diluted to 1:20 of the original concentration and 30ml was added back to the anode chamber. This cycle showed the potential for a stable current, so after 240 hours the anode was drained and started back up for a second cycle with nutrients at the same concentration. This second cycle showed only a peak reaching approximately 0.3 mA and then a drop in current, showing that the nutrient concentration was too low to sustain a current for any amount of time (**Figure 2-5**). It is possible that the first cycle at this concentration showed a small plateau due to small amount of nutrients being left behind from the initial concentration cycle. Realizing 1:20 dilution left the concentration of nutrients in the anode too low, a new dilution of 1:10 was made for the next cycle. The cycle of 1:10 nutrient solution showed a small plateau in one anode and a peak in the other, both reaching approximately 0.45 mA. Both the small plateau and the increase in current showed that the concentration was close to preferable but would still need to be higher.

The fifth and final test cycle was conducted with the nutrient concentration at 1:5 of the initial concentration. This cycle reached a current over 0.5 mA and a plateau of approximately 30 hrs. This nutrient solution concentration showed both a sufficient current level and a sustained

current for 30 hrs. would be used for the rest of the experiment. The total concentrations for this nutrient dilution for total Carbon, total nitrogen, and total phosphorus were 176.37 mg/L, 44.49 mg/L, and 6.2 mg/L respectively (**Figure 2-6**).

Fuel Cell Assembly

The dual chamber fuel cells were assembled with provided bolts with a 6x6cm anion single sheet ion exchange membrane (ResinTechInc, AMB-SS) between the large middle connection point. This membrane was soaked in 10g/L NaCl (EMD Millipore, ACS Grade) solution for 24 hours prior to assembly. Carbon sheet (Royal Carbon ½") was secured to carbon rod plugs with cotton string and inserted in place into both chambers, making sure to achieve a water-tight seal with sealant tape if necessary. The designated aerobic chamber was filled with approximately 120-150mL of DDI and an air input was placed into the top of the chamber. The anoxic chamber was then loaded with 160mL of potassium buffer at a pH of 7 and purged with 30% CO_2/ 70% N_2 gas (Airgas) for 15 min.

Once the chamber was purged 1.6 mL of either the basic or complex nutrient solution and 15mL (with basic nutrient solution) or 30mL (with complex nutrient solution) of the collected bacterial sludge were added to the anoxic chamber and sealed. Tubing was attached to the anoxic chamber and inserted via needle into a non-permeable container. This container had another tube inserted and the opposite end of the tubing was placed under approximately 200mL of DDI. This system was designed to relieve pressure of the anoxic container as the bacteria produced CO2 and ensure that the chamber remained anoxic. After approximately 120 hours, the anoxic chamber would be carefully drained making sure not to lose any bacterial sludge or biofilm formed and would be reloaded with the buffer and nutrients to encourage cycling.

Current(mA) Cycling

The cycling of both microbial fuel cell units, R1 and R2, are shown in **Figure 2-7**. This figure shows the depletion of nutrients through falling current(mA) and the addition of more nutrient solution to maintain the microbiota developing within the fuel cells. Each nutrient depletion cycle would take about 120 hours total.

SMX Injections

After the current cycling of the two microbial fuel cells had steadied, injections of Sulfamethoxazole (SMX) were started to test the systems for antibiotic resistance or sensitivity, shown as the steady or early dropping of the current in a cycle. Injection times and concentrations can be seen in **Table 2-1**. Injection concentrations were increased when no current dropping was detected, indicating antibiotic resistance. Once injection concentrations far exceeded concentrations that may be found in the natural environment, injections were concluded.

Antibiotic Effects Testing

Once the microbial fuel cell was able to maintain a consistent cycle, we began our antibiotic experiment. A solution of 17 mg/L sulfamethoxazole (SMX), an antibiotic, was prepared prior to this point and was stored at 4 degrees Celsius until use. Consistent cycling occurred at approximately 720 hours. After draining the anoxic chamber, 120ml of buffer were added as per procedure. 5 mL of the SMX solution was mixed with 25 mL of the complex nutrient solution and was loaded to the anoxic chamber as well, then let cycle as normal. SMX injections occurred at 720, 960, 1200, and 1440 hours, or every other cycle. A cycle without SMX occurred between each cycle with SMX to let the bacterial community recover.

Concentrations of SMX injected increased with each injection 10ug/L, 100ug/L, 1mg/L, 10mg/L to test the system for sensitivity.

Discussion

No antibiotic sensitivity was detected during the first and second injections at 10ug/L and 100 ug/L, the likely range for sulfamethoxazole to be found in natural waters. Injections at higher concentrations, 1mg/L, showed slight sensitivity but were able to recover before the end of the nutrient cycle and the natural current dropping due to nutrient consumption. The 10mg/L injection showed further antibiotic sensitivity, with two significant drops before the end of the cycle completion and no recoveries in between. Though the 10mg/L injection showed promise in limiting the system's current producing power and thus indicating the presence of an antibiotic, 10mg/L is far outside the concentrations that may be found in the natural environment (Kergoat et al., 2021). These injections showed that our microbial fuel system would not be sensitive enough to detect micropollutants, specifically antibiotics, in natural waters. Though we only tested one antibiotic in this experiment, we felt sensitivity would not differ enough for other antibiotics for our purposes.

Conclusion

Armed with the knowledge that microbial fuel cells have the capabilities of being biosensors, we set out to develop a system with the ability to detect micropollutants (specifically herbicides, pesticides, and pharmaceuticals) in natural surface waters as well as water treatment outputs. Though our system was able to detect the micropollutant that we introduced at intervals, it was incapable of detection at the levels one could find in natural waters, thus rendering this

system impractical for our purposes. Further testing of this system was not performed due to time and budget constraints, however, there are many possibilities discussed to improve the efficiency of this system. The use of an automated/continuous flow of nutrients may help to establish a more stable baseline than when the system is drained, and nutrients are replaced that way (Gil et al., 2002). This would also allow for a gradual addition of the micropollutant. In addition, a sludge with a different and known microbial composition might respond better to different micropollutants (Stein et al., 2012). Combinations of microbial compositions and micropollutants should be tested to find optimal reactions.

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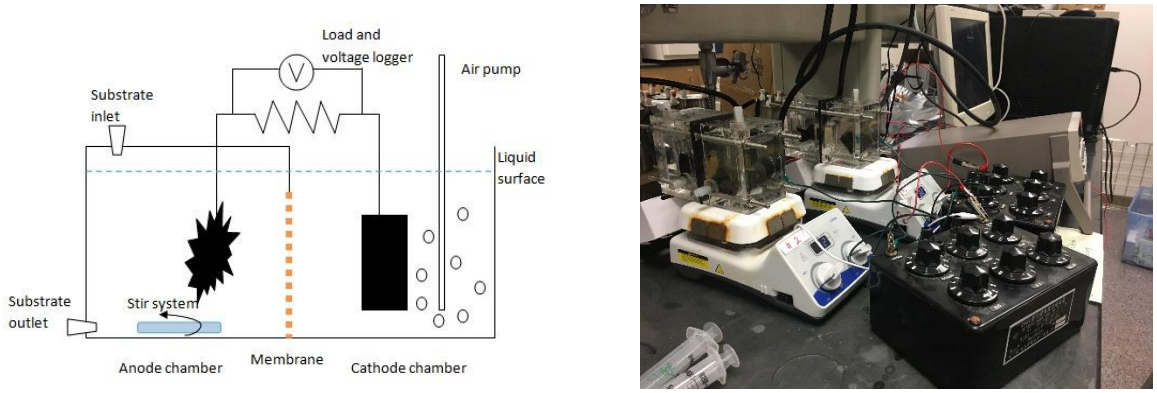

Figure 2.1: Configuration of the dual-chamber microbial fuel cell. Graphite brush and Pt-coating catalytic carbon cloth were used as anode and cathode, respectively. Air was pumped in the cathode chamber. One thousand ohms resistor was used to complete the circuit. Voltage was recorded every 3 minutes.

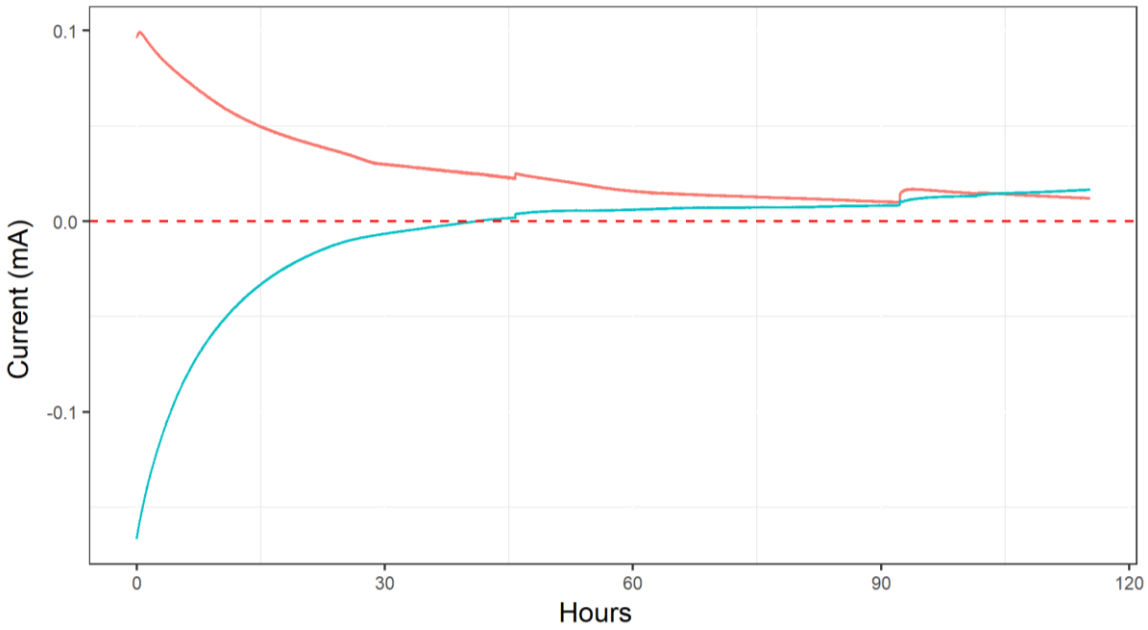

Figure 2.2: Temporal changes of current from two replicate dual-chamber microbial fuel cells when using distilled and deionized water (DDI) in the cathode chamber.

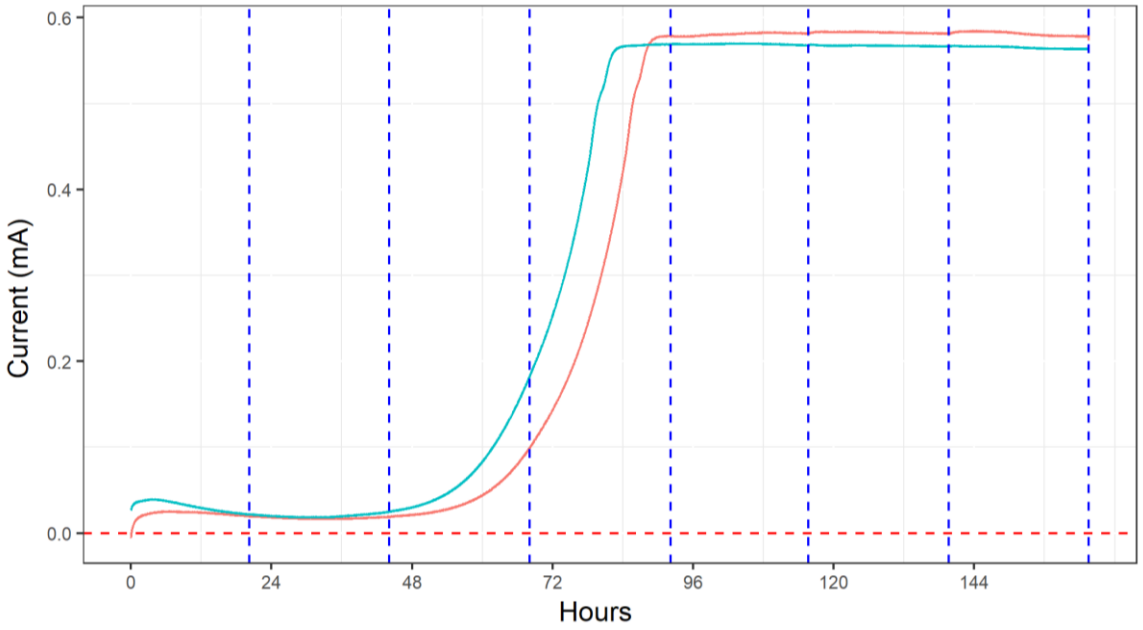

Figure 2.3: Temporal changes of current from two replicate dual-chamber microbial fuel cells when using potassium phosphate buffer pH 7 (PMS; 9.343 g/L K_2HPO_4 and 6.309 g/L KH_2PO_4) in the cathode chamber.

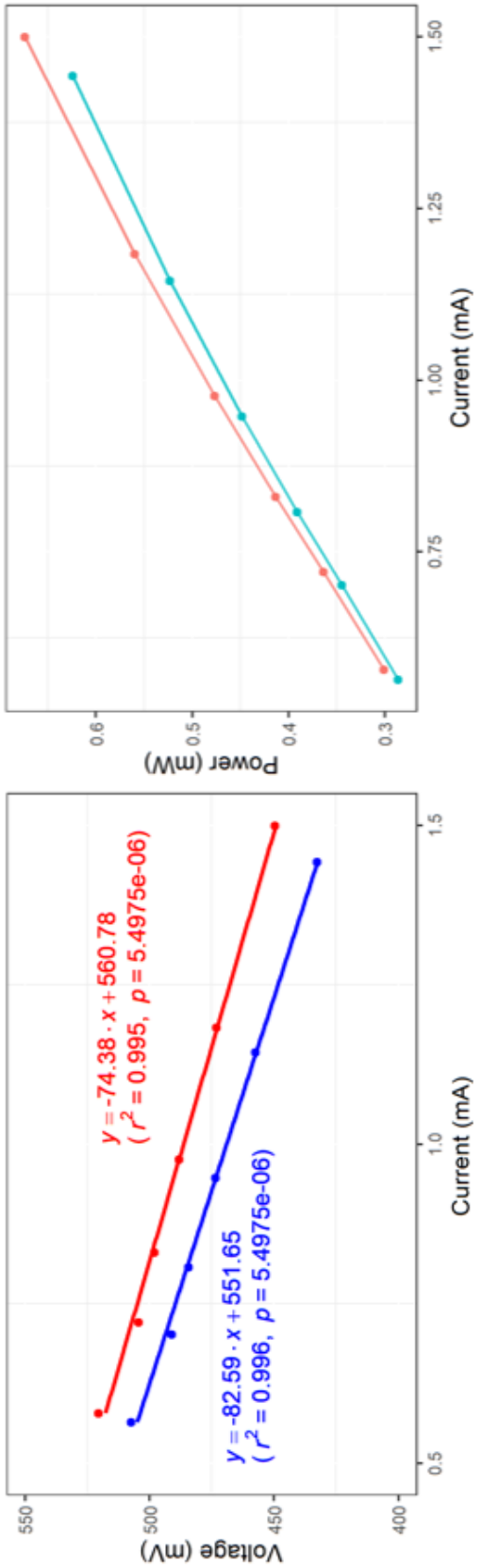

Figure 2.4: Internal resistances of the replicate dual-chamber microbial fuel cell.

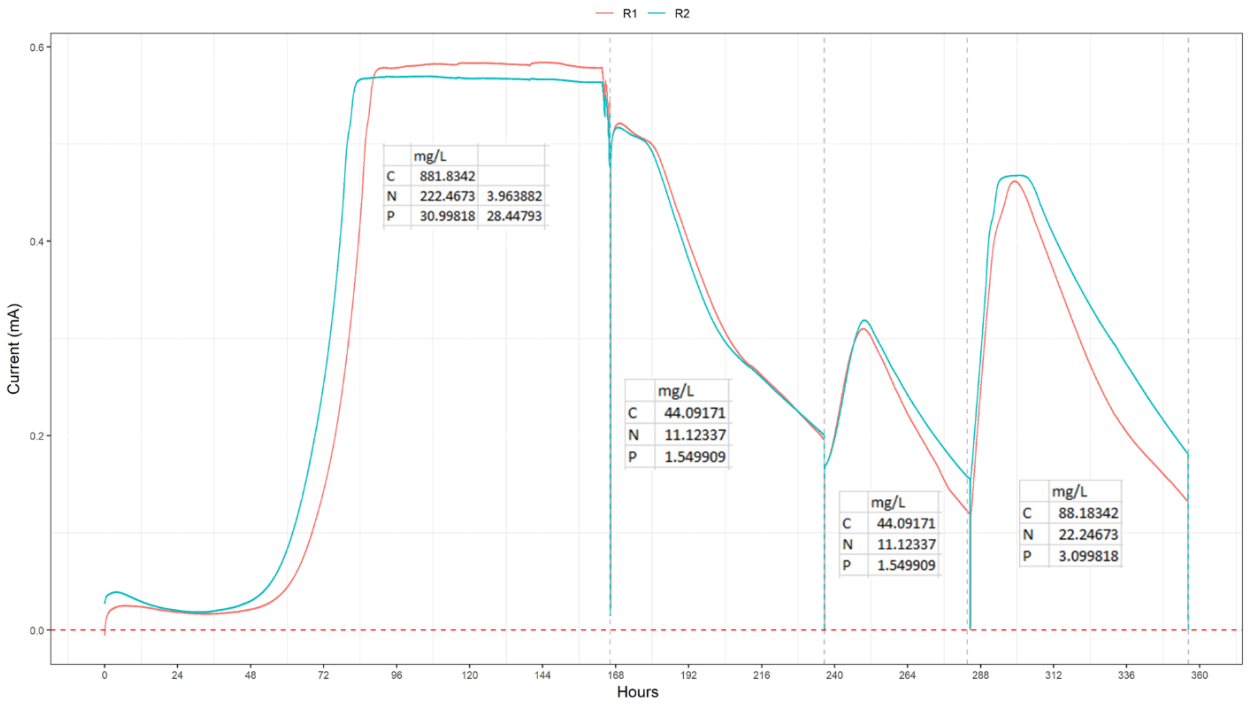

Figure 2.5: Current (mA) of the replicate dual-chamber microbial fuel cells under various concentrations of nutrients.

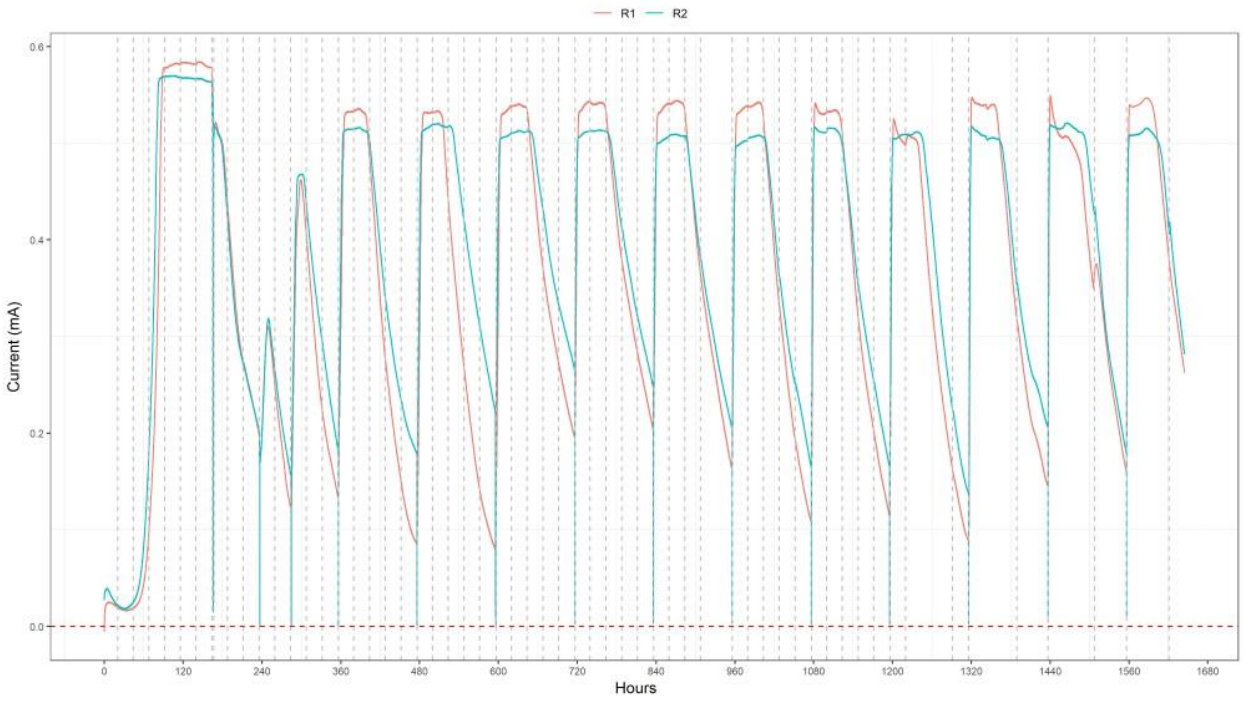

Figure 2.6: Current (mA) of the replicate dual-chamber microbial fuel cells under the long-term operation with the optimized nutrient levels. Total Carbon, total nitrogen, and total phosphorus concentrations were 176.37 mg/L, 44.49 mg/L, and 6.2 mg/L.

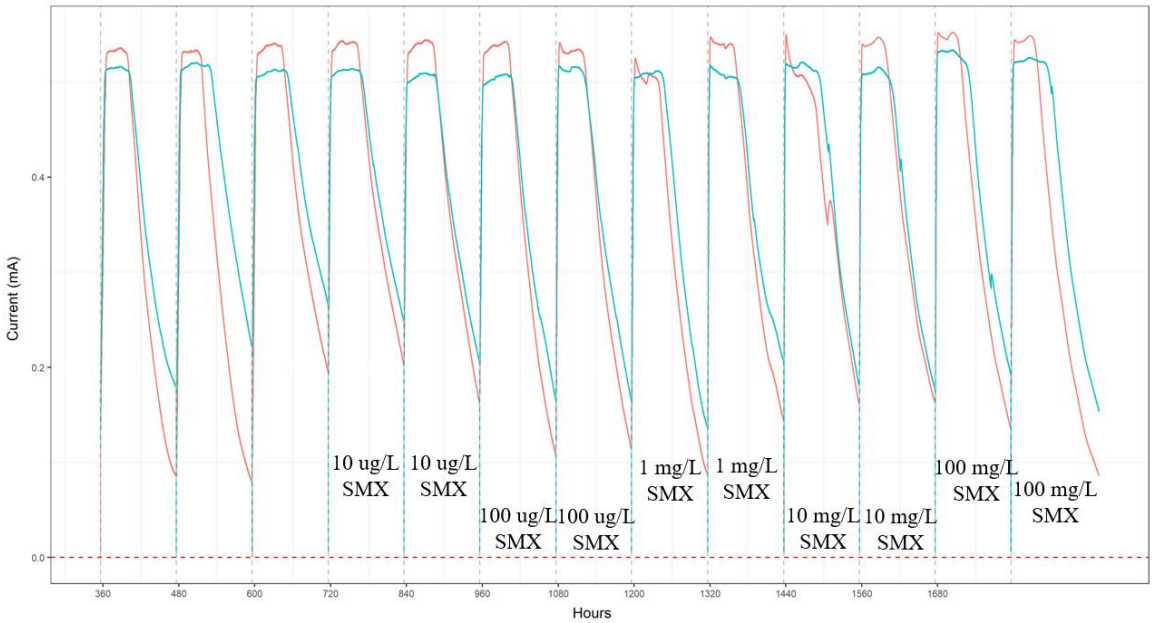

Figure 2.7. Temporal changes of currents (mA) from the replicate dual-chamber microbial fuel cells when spiked the nutrients with the increased concentration of sulfamethoxazole (SMX) from 10 µg/L to 100 mg/L.

Injection Time (Hours)	SMX concentration
720	10 ug/L
960	100 ug/L
1200	1 mg/L
1440	10 mg/L

Table 2.1. Injection Times and Sulfamethoxazole concentrations injected into the microbial fuel cells for antibiotic resistance testing.

CHAPTER THREE

ENZYME-LINKED IMMUNOSORBENT ASSAY VIABILITY FOR USE IN ENVIRONMENTAL RESEARCH

Abstract

Many detection methods for micropollutants in water sources are time-inefficient, having both involved sample preparation procedures, long instrument run times, and complex analyses. Enzyme-linked immunosorbent assays can provide results in a matter of a couple of hours for multiple samples, from sample prep to results. This simple and time-efficient method would be an attractive method for micropollutant detection, and this experiment aimed to determine if it would be useful in environmental detection. ELISA is a method of detection that is generally used for biological samples and kits can be found for a wide variety of antibiotics, hormones, and protein samples. For our purposes, we utilized a tetracycline kit from Eurofins Abraxis. Because ELISA kits are designed to be used for biological samples, prior to sample testing, we conducted a preliminary experiment to find the viability of our kit with natural water samples. Using distilled, deionized water and pine leaf litter, to make a stock solution of water with dissolved organic carbon. Additionally, humic acid stock was made to acidify our stock DOC water to mimic acidic water conditions and a stock of tetracycline was made from an analytical quality tetracycline powder. Samples containing different concentrations of diluted DOC stock and humic acid stock were first added to an uncoated 96-plate to test the viability of the plate reader to determine different concentrations of DOC. After viability was confirmed, tetracycline stock and DOC stock in different concentrations, as well as freshwater samples and the tetracycline stock were added to

the coated tetracycline plate. This preliminary test showed that both the DOC stock and the natural freshwater samples interfered with the ELISA kit's ability to detect the presence of tetracycline.

Introduction

Pharmaceuticals are a common micropollutant in surface and ground waters and concentrations found can be detrimental to the ecology of the watershed and to human health (Elliott et al., 2018). ELISA or enzyme-linked immunosorbent assay is a common method of detection and quantification typically used in medical settings for many peptides, proteins, antibodies, and hormones, there are a wide number of pharmaceutical and antibiotic specific plates available (Lequin, 2005). In this study, we use tetracycline as our target micropollutant due to the wide availability of tetracycline specific plates in addition to the threat that tetracycline poses to the environment when present in surface waters (Eurofins Abraxis, 2020; Pogue et al., 2017). While there are multiple ELISA methods including direct, indirect and sandwich, they all use the same basic idea for detection in which the antigen that which is being tested for is immobilized on a plate and then coupled by an antibody labeled with a reporter enzyme (Engvall, 2010). The substrate on the plate and the antibody label are generally compounds that are specific to the detectable enzyme. Most commonly the reporter enzyme used is horseradish peroxidase (HRP), as it is active over a wide range of pH and allows for the selection of an optimal pH for the reaction within the range (Boguszewska et al., 2019; Crowther, 2002). The amount of the reporter enzyme measured corresponds to the detection amount of the antigen. The signal detection of the reporter enzyme can be detected by spectrophotometer, fluorometer, or luminometer. ELISA methods are sensitive, specific, rapid, and generally easy to use. In addition, ELISA kits tend to provide all solutions needed for the assay and do not require any specific equipment, making them a

comprehensive testing method (Aydin, 2015). For these reasons, though they are generally used with biomedical samples, we hoped to be able to use ELISA to detect and quantify different micropollutants in surface waters.

Methods and Materials

Stock solution preparation

Several stock solutions were produced for use throughout this experiment. The first stock solution was pine litter leachate. This was produced by adding 3.0977g of dried pine litter to 225mL DDI and shaking the solution vigorously for 15 min. After the 15 minutes, the pine litter was strained, and the resulting liquid was a transparent, brown solution. Stock was stored at 4C when not in use.

A humic acid solution was made to be a quantifiable dissolved organic carbon solution. The stock solution was made by adding 0.1021g of humic acid (Aldrich Chemical Company) to 200ml of DDI (Wallace, 2003). This solution was placed on a stir plate and stirred for approximately 30 minutes. Stock was stored at 4C when not in use.

The final stock solution produced was a tetracycline solution for use in higher concentrations than the standards provided by the ELISA kit. The analytic tetracycline powder that we had was anhydrous, it would not be able to mix directly into water. A 71% ethyl alcohol was produced by mixing 7.8mL of 91% ethyl alcohol with 2.2 mL DDI. After the ethyl alcohol was made, 5.2mg of the tetracycline powder was added and mixed on a stir plate for approximately 30 minutes. Stock solution was approximately 5 mg/ml and was stored at 4C until needed for further dilution.

Preliminary testing

To determine the dissolved carbon concentration of the humic acid stock solution, it was run in multiple dilutions on a total organic carbon (TOC) analyzer (Shimadzu). The solution was run in duplicate for each of the dilutions – 1:1, 1:2, 1:3, 1:4, and 1:10 (**Table 3.1**).

Sample runs

Samples would be run on a 96-well plate reader. This microplate reader can read absorbance at single/multiple wavelengths and can also scan the samples with a user-defined range. The first run on the plate reader was performed using an uncoated 96-well plate with the pine leachate to determine if the microplate reader would be suitable for our purposes. Pine leachate was added to 16 wells (A1- 2H) and ran at 450nm.

After determining that the microplate reader would work to detect the color changes that were expected from the ELISA plate, we needed to determine if the DOC concentrations of the water we would be testing would affect the tetracycline coated ELISA plate and cause inaccurate readings. The plate used for the rest of the sample testing was a Eurofins-Abraxis Tetracyclines Plate ELISA kit. The 0.8ng/ml tetracycline standard from the ELISA kit was run in duplicate, as well in combination with a 5mg/L DOC humic acid solution and a 100mg/L DOC humic acid solution in wells A1-H1. A further test was run with 100mg/L DOC humic acid solution alone and surface water samples. Samples were collected from a creek in the SC Botanical Gardens (34.66, -82,82), Lake Hartwell (34.68, -82.85), and a creek in Nettles Park (34.68, -82.77) and ran in duplicate. These samples were placed in wells A2-H2.

In addition to these samples, higher concentrations of tetracycline were tested in combination with DOC humic acid solutions. The tetracycline stock was diluted to create 5ng/mL, 10ng/mL, 20ng/mL and 50ng/mL solutions. These were run in duplicate with 10mg/L DOC humic

acid and 50mg/L DOC humic acid. These samples were in wells A3-H4. All samples were processed using the methods provided in the Eurofins-Abraxis Tetracyclines Plate ELISA kit (Tetracyclines/Epimers Plate Kit Part # 52254BA, 2020). For any samples that had both a tetracycline solution and a DOC humic acid solution in a single well, 50ul of each was added to the well instead of the 100ul of a single solution.

Results

Preliminary study

Preliminary testing of the humic acid solution showed a great linear progression, and an approximate value of 33mg/L DOC **(Figure 3.1)**.This solution would later be diluted appropriately to make lower concentrations of DOC run in conjunction with tetracycline in our tetracyclines coated ELISA plate.

Sample Runs

Tests with the uncoated 96-well plate and the pine leachate showed that the plate reader we had obtained would be able to detect subtle changes in color and saturation of the solutions placed in the wells **(Table 3.2)**.

After determining the concentration and developing a good linear correlation with the humic acid solutions, we were able to accurately dilute the solution to produce lower concentrations to be run with the tetracycline standards provided in the ELISA kit. The results obtained showed that there was DOC interfering with the analysis due to the inaccurate tetracycline concentration results obtained from samples run with DOC. Further, the surface water samples appeared to have a higher concentration of tetracycline present than might be expected **(Table 3.3)**.

Discussion

The tetracyclines ELISA kit is dependent on color changes and saturations on, so there was a concern that the higher level of DOC is typically associated with a darker colored water would interfere. We performed a preliminary test to ensure the higher-level DOC waters we would be testing would not affect the efficacy of the ELISA plate. Absorbance from the plate gave the unreasonable results for samples containing DOC, such as lower concentrations of tetracycline than that of the standard in the well, as the well containing only the 0.8ng/mL standard had a result of 0.724ng/mL tetracycline present, however wells containing the same standard in combination with a DOC humic acid solution resulted tetracyclines in approximately 0.4ng/mL tetracycline present. In addition, the well that contained only DOC had an absorbance that showed an inaccurate result of 0.4mg/mL present. Even the surface waters tested, which were clear in color to the eye even though they contained DOC, showed a higher level of tetracyclines present than would be expected. These results show the DOC in combination with the tetracycline resulted in almost identical values regardless of tetracycline concentration and did in fact seem to be interfering with the capabilities of the ELISA plate.

Conclusions

While we hypothesized that using ELISA might be a quick method of micropollutant detection, testing disproved this. The DOC content found in surface waters interferes with the specific absorbance of the wells. Because the calculations of the final concentrations of tetracyclines are based on the absorbance of the wells, the DOC ultimately affects the results of the plate. Due to this, using this plate with water samples that contained the higher levels of DOC that we were expecting would not be possible. Further sample clean-up, extraction, and sample

concentration should be considered as a possible next step (Jaria et al., 2020; Krall et al., 2018). Potentials for other testing with different plate types were not explored due to the time and cost restraints of the project but should be considered for future research.

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Concentration of 100ppm solution	DOC mg/L
HUMIC0.1	3.6
HUMIC0.1	3.4
HUMIC.25	7.7
HUMIC.25	7.7
HUMIC.3	10.2
HUMIC.3	10.3
HUMIC.5	15.5
HUMIC.5	15.4
HUMIC1	32.6
HUMIC1	33.3

Table 3.1: Different concentrations of Humic acid solution run on TOC to determine DOC levels of solutions.

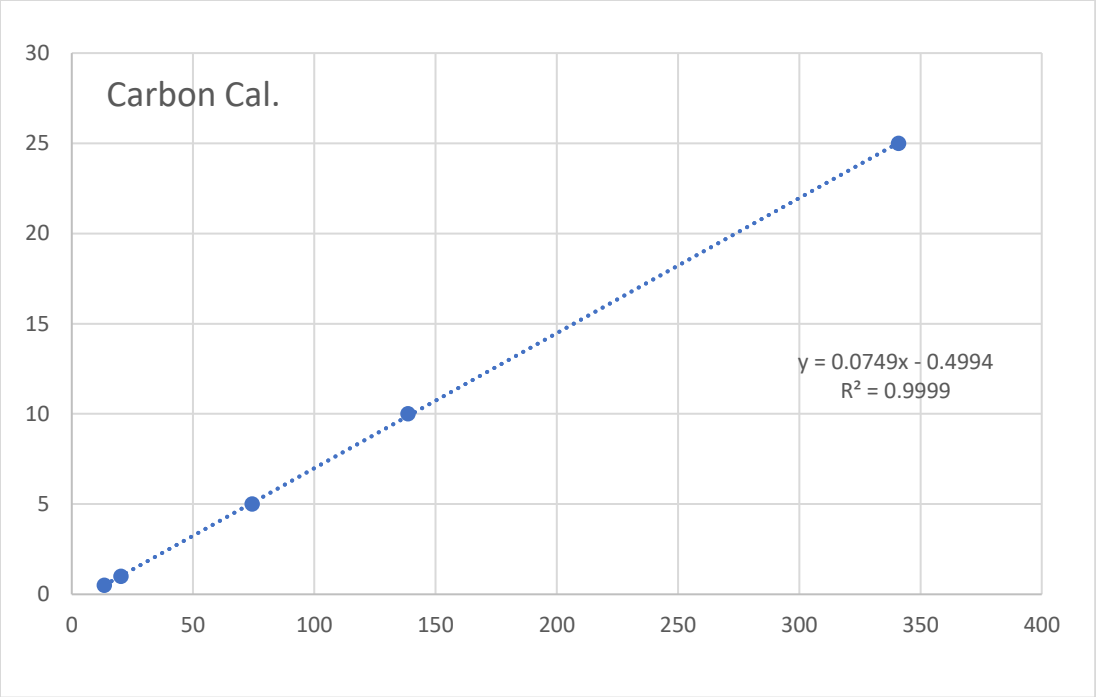

Figure 3.1: Calibration of DOC values from humic acid.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.071	0.065	0.048	0.048	0.055	0.05	0.048	0.048	0.048	0.049	0.051	0.048	450
B	0.064	0.065	0.048	0.049	0.048	0.049	0.048	0.049	0.049	0.054	0.054	0.048	450
C	0.063	0.064	0.048	0.051	0.05	0.048	0.048	0.048	0.048	0.048	0.048	0.048	450
D	0.062	0.064	0.048	0.048	0.048	0.048	0.048	0.048	0.049	0.048	0.048	0.048	450
E	0.062	0.063	0.048	0.048	0.048	0.048	0.048	0.048	0.048	0.048	0.048	0.048	450
F	0.063	0.063	0.048	0.048	0.048	0.048	0.048	0.049	0.052	0.048	0.048	0.048	450
G	0.065	0.048	0.048	0.05	0.048	0.048	0.048	0.048	0.048	0.048	0.051	0.048	450
H	0.067	0.078	0.048	0.058	0.048	0.048	0.048	0.048	0.048	0.048	0.049	0.048	450

Table 3.2: Uncoated 96-well plate with pine litter leachate in columns one and two tested at 450nm in plate reader. Consistent readings in wells B1-F2 show no DOC interferenc

Enter descriptions and absorbance values of each sample and control into the grey boxes						
Sample/Control	Absorbances	Std Dev	%CV	Sample Concentration (ng/ml)	Sample Concentration Avg (ng/ml)	Comments
Blank	2.008			0.068		LESS than Standard 1, outside of quantifiable range
Blank	2.189	0.128	6.099	Invalid	Invalid	
Tetra 0.8ng/ml	0.644			0.802		
Tetra 0.8ng/ml	0.805	0.114	15.713	0.646	0.724	
Tetra 0.8ng/ml & 5mg/L DOC	1.199			0.399		
Tetra 0.8ng/ml & 5mg/L DOC	1.200	0.001	0.059	0.398	0.399	
Tetra 0.8ng/ml & 100mg/L DOC	1.120			0.439		
Tetra 0.8ng/ml & 100mg/L DOC	1.178	0.041	3.569	0.409	0.424	
100mg/L DOC	1.248			0.375		
100mg/L DOC	1.422	0.123	9.216	0.299	0.337	
SC Botanical Gardens	1.530			0.256		
SC Botanical Gardens	1.443		0.000	0.290	0.273	
Lake Hartwell	1.506			0.265		
Lake Hartwell	1.703	0.139	8.682	0.190	0.228	
W.C Nettles Park	1.538			0.253		
W.C Nettles Park	1.556	0.013	0.823	0.246	0.249	

Table 3.3: Absorbances and Sample concentration of both tests showing similar concentration/inaccurate concentration results.

CHAPTER FOUR

TOTAL ORGANIC HALIDES AND WATER QUALITY PARAMETERS WITH LAND USE AS A REPRESENTATIVE INDICATOR

Abstract

Micropollutants are found in natural waters across the state of South Carolina and cause many negative environmental, health, and economic impacts. These pollutants are introduced into the natural environment as runoff from pesticides and fertilizer from agriculture; disinfectants and cleaners from industry; leaches from landfills and sewage; and many other anthropogenic sources. Organic halides are commonly found as micropollutants in relatively high quantities in natural waters due to them being present in disinfectants, pesticides, and sewage and landfill waste. Current detection methods are time consuming and costly and regular testing can be sporadic: potentially leading to more testing than necessary. Correlating land use with the presence of these micropollutants can help to direct detection efforts. Areas with high percentages of urban land use have been shown to generally have higher levels of micropollutants than areas with low percentages of urban land use. Samples were collected from natural waters across the state and analyzed for the presence of organic halogens. Sample locations were then analyzed to determine the percentage and land use category within a 10km radius surrounding the sampling point. Concentrations of total dissolved nitrogen were also measured to determine if there would be any correlation with percentage of agricultural land use, as nitrogen is commonly found in many agricultural fertilizers. In addition to land use, statistical analysis was performed for distance between sample location and wastewater treatment plants as wastewater commonly contain organic halides as they are used as disinfectants.

Introduction

Chlorine, bromine, and other organic halides are Group 7A elements and are found in natural waters as micropollutants. Chlorine, in particular, can be found in high quantities as it is commonly used as a disinfectant in wastewater treatments plants, private and public pools, and commercial and in homes as cleaning products (Fass et al., 2003; Ghernaout & Elboughdiri, 2020). Bromine is also found in natural waters in high quantities as the result of waste waters, sewage leaks, pesticides, and landfill leaching (Vanbriesen, 2019; Winid, 2015). Due to these reasons, they are thought to be anthropogenic indicators, with concentrations increasing in water sources near more urban areas (Zahn & Grimm, 1993). While all pollutants have risks associated with their presence, organic halides specifically have the potential to form disinfectant by-products when they react with organic matter such as humic and fulvic substances found in natural waters (Hu et al., 2022). Chronic exposure to disinfectant by-products, or DBPs, can lead to increased cancer risk and can be ingested through drinking water, absorbed through the skin during activities like swimming, bathing, and dish washing, and can also be breathed in through the air during these activities (*Polychlorinated Biphenyls (PCBs) | US EPA*, n.d.; *Polychlorinated Biphenyls (PCBs): Environmental Impact, Biochemical and Toxic Responses, and Implications for Risk Assessment. - PubMed - NCBI*, n.d.). The serious implications of these by-products make it important to monitor and limit the levels of organic halides that are found in our waters (Bond et al., 2014; Bryant et al., 1987). Current analysis for organic halide detection and removal is costly and time extensive, making it impractical to test many water sources continuously and indiscriminately (Noma et al., 2001). While alternate testing methods have not been found to be reliable, we hope to help direct efforts in testing to be more efficient by determining patterns of where we might expect high levels of organic halides to be present (Warton et al., 2006).

Rapid urbanization is the spread of urban areas as more people move from rural to urban area. While urbanization can be beneficial economically, increased urban areas leads to an increase in urban waste and rapid urbanization can lead to current city systems' inability to keep up with demands of a larger population. Continued and rapid urbanization has been shown to add more micropollutants to ground and surface waters in the surrounding areas (Chaudhry & Malik, 2017; Strokal et al., 2021). These factors lead us to believe that total organic halide (TOX) concentration could be correlated to land use of an area, proposing that a relatively high TOX concentration could be linked to increased human population/activity and thus preform as an indicator for the presence of other micropollutants. In addition to land use, we propose that TOX should increase at sample locations that are close to an upstream wastewater discharge output, regardless of surrounding land use due to the introduction of anthropogenic pollutants from the wastewater treatment plant (Milh et al., 2020; Ong et al., 1996).

Many micropollutants also come from agricultural sources, such as fertilizers and pesticides. A combination of runoff and leaching introduces these pollutants into the surrounding waters. These pollutants are rich in nitrogen and are often found in the water supply as dissolved nitrogen(Evans et al., 2019; Savci, 2012). Due to this, dissolved total nitrogen should be found in higher quantities in areas with a higher percentage of agricultural land.

Surface water samples were collected from freshwater sources across South Carolina in 2020 and 2021 and analyzed for total organic halide concentration and dissolved total nitrogen, as well as several water quality parameters including dissolved organic carbon, conductivity, pH, and fluorescence. Statistical analyses of urban and agricultural land use and wastewater discharge data for the state of South Carolina were performed and compared to total organic halide (TOX) and total dissolved nitrogen (DTN) data.

Methods and Materials

Sampling Sites and Time

Surface water samples were collected bimonthly from multiple boat landing sites across South Carolina following the general guidance described in the US EPA Method 533 (Wendelken & Epa, 2018). This collection method was selected as samples would be used for several different studies, including a study testing for the presence of per- and polyfluoroalkyl substances. This method allowed for the testing of chlorine, carbon, nitrogen, and other natural ions as well. Samples were collected by Dr. Huan Chen, Rachael Berger, and Meryem Soyluoglu. Eleven (11) samples were collected in September 2020 on two trips. These trips occurred from 9/14/2020-9/16/2020 and 9/21/2020-9/23/2020. In November, fifty-five (55) samples were collected from 11/9/2020-11/12/2020 and 11/16/2020-11/17/2020. Forty-five (45) samples were collected in January 2021 from 1/17/2021-1/22/2021. The final collection dates were in March of 2021. Fifty-four (54) samples were collected 3/15/2021-3/20/2021. Sample collection sites include locations such as boat landing sites along the Pee Dee River, the Savannah River, Broad River, and many other rivers and streams across the state **(Figure 4.1)**.

Sampling Collection & Storage

Samples were collected in 250-mL Thermo Scientific™ Nalgene wide-mouth polypropylene bottles at depth of approximately 0.2 m until approximately full volume. Prior to initial use the Nalgene bottles were rinsed three times with deionized, distilled water (DDI) and were allowed to dry completely. Clean nitrile gloves were used for each sample collection to lower the possibility of contamination. The collected samples were placed inside the cooler on ice and transferred into refrigerator at 4°C immediately after arriving back to L.G. Rich Laboratory.

Nalgene bottles were re-used for each sampling trip and were cleaned thoroughly using the following protocol: bottles were rinsed with DDI three times, filled with DDI and sonicated for 30 minutes twice. The bottle would then be dumped and refilled with approximately 50mL DDI and placed on a bottle tumbler for 48 hours. Following the tumbling, the bottles would be filled completely with DDI and sonicated for 30 minutes twice again. The bottles would then be dumped, rinsed with DDI three times, and set to air dry completely.

Water Quality Analysis

Prior to any analyses were conducted, collected water samples were filtered through Supor® 0.45-µm polyether sulfone membrane filters (PALL Corporation) to remove any particulate that may interfere with instrumentation.

Water quality parameters that were tested included ultraviolet absorbance at 254nm (UV_{254}), dissolved organic carbon (DOC) in the form of Non-Purgeable Organic Carbon (NPOC), total nitrogen (TN), specific ultraviolet absorbance at 254 nm ($SUVA_{254}$), pH, and Electrical Conductivity (EC). These specific measurements were chosen to allow for the ability to compare samples from the same location between different seasons and to give multiple parameters to compare to the land use data of the state.

Disolved Organic Carbon (DOC) and Total Nitrogen (TN) analysis were preformed on a Shimadzu TOC-$V_{CSH/CSN}$ instrument. The detection limits for this unit are 0-20000mg/L total carbon and 0-4000mg/L total nitrogen. Prior to testing for DOC and TN, samples were run on a Shimadzu UV-1800 for UV visible spectrometry at 254nm (cm^{-1}). Samples with a UVA value greater than 1 were diluted before being run to ensure that the sample would be within the detection limit of the instrument. DOC and TN standards were made from reagent grade potassium hydrogen phthalate and reagent grade potassium nitrate respectively, and run at 0.5mg/L, 1mg/L, 2mg/L,

5mg/L and 10mg/L for each run on the instrument in order to determine the standard curve for the samples on the run. Approximately 20ml each of sample and standard was used.

In addition to determining the need for sample dilution prior to sample run for DOC, UV was also used to determine $SUVA_{254}$. UV_{254} values were recorded when run and later processed to determine the $SUVA_{254}$ values of the samples. SUVA254 is described as specific absorbance (254nm) divided by DOC and expressed in unit (L/mg-C/m).

TOX analysis

Total water-soluble organic Cl (TOCl) levels were determined with a MultiX 2500 TOX Analyzer (Analytikjena, Jena, Germany) coupled with an off-line ion chromatography (ICS-2100, Dionex, Sunnyvale, CA), following a method described in (Hua & Reckhow, 2007) and (Ersan et al., 2019). Specifically, 80ml of sample would be acidified with 2mL concentrated sulfuric acid ($pH \leq 2$) and adsorbed on two granular activated carbon (GAC) columns using a sample adsorption unit (APU2; Analytik Jena, Germany). The GAC columns were then washed with a 20-ml aqueous sodium nitrate solution (6.85 g/L NaNO3) to remove inorganic halides. Both GAC columns would then be burned together at 950 ℃ for 20 min. The off-gas was bubbled in 20-ml distilled, deionized water (DDI). Approximately 6-ml of this off-gas DDI water per sample were analyzed by ICS-2100 with the minimum reporting limit of 5 μg/L for Cl (TOCl). The remaining off-gas DDI water was retained and stored in the refrigerator at 4°C for later use in the case of needing to re-run a sample.

Preparation of standards was done for each run on the IC to form an accurate calibration curve. A mixed standard was made from an Analytical IC Grade Nitrate Standard solution (1000mg/L), Analytical IC Grade Nitrite Standard solution (1000mg/L), Analytical IC Grade Sulfate Standard solution (1000mg/L), Analytical IC Grade Chlorine Standard solution

(1000mg/L), and an Analytical IC Grade Bromide Standard (1000mg/L). All standard solutions were acquired from Fluka Analytical with the exception of the Br standard solution which was acquired from Specpure. 50µl of each standard solution was combined with 50ml of DDI to make a working stock solution of 1000ppb (µg/L). The stock was run and diluted to different strengths in order to form a calibration curve. The dilutions of 5 µg/L, 10 µg/L, 25 µg/L, 50 µg/L, 100 µg/L, 250 µg/L, 500 µg/L, and 1000 µg/L were placed at the beginning of each run on the instrument prior to the samples.

Samples were analyzed for the presence of chlorine to later form potential correlations between the concentrations of chlorine found present in the water with human activity in the area via land use data.

Results

Water Chemistry

To show potential correlations between dissolved organic materials and micropollutants, DOC, DTN, and DOC/DTN were analyzed for each of our samples. DTN was found in low concentrations in many of the samples. The linear regression between dissolved organic carbon and organic halides had an R^2 value of 0.0126, showing little to no correlation between the two **(Figure 4.9)**. UV absorbance at wavelength 254, or UV_{254} (cm^{-1}), was measured for each sample. UV_{254} is linearly proportional to the concentration of organic matter present in the water. UV_{254} was measured to show the overall organic load present in the samples, which includes DOC, DTN, and TOX. Specific ultraviolet absorbance (SUVA) is calculated by dividing UV254 (cm^{-1}) by the dissolved organic carbon (mg/L). This measurement shows the disinfection by-product (DBP) formation potential **(Figure 4.10)**.

National Land Cover Data Analysis

National Land Cover Database (NLCD) was obtained from the Multi-Resolution Land Characteristics (MRLC) Consortium (*Multi-Resolution Land Characteristics Consortium*, 2021). The edition was NLCD 2011 Land Cover (2011 Edition) as this was the most recent edition available at the start of this project. Once obtained the NCLD was applied as a .tif file to ArcGIS Pro (2020) **(Figure 4.1)**. Using ArcGIS, we determined a 10km radius around each sample location and analyzed the data within those parameters for the specific land usage around each sample point.

After land usage for each sample location was found, the percentage of 'Urban', 'Agricultural', and 'Natural' land surrounding each sample point was calculated. 'Urban', 'Agricultural', and 'Natural' land were found by combining multiple Land Cover Class Code Values as provided by the NLCD. 'Urban' land was defined as land having the class codes of Developed, Open Space (21), Developed, Low Intensity(22), Developed, Medium Intensity(23), and Developed, High Intensity(23) **(Figure 4.7)**. 'Agricultural' land was defined as land having the class codes of Pasture/Hay (81) and Cultivated Crops(82) **(Figure 4.8)**. 'Natural' land was defined as land containing the class codes of Open Water (11), Barren Land(31), Deciduous Forest(41), Evergreen Forest(42), Mixed Forest(43), Shrub/Scrub(52), Grassland/Herbaceous(71), Woody Wetlands(90), and Emergent Herbaceous Wetlands(95) **(Figure 4.6)**. Descriptions of these class codes can be found in the appendix under National Land Cover Database Class Legend and Description.

The percentage of Urban, Agricultural, and Natural lands within the 10km radius of the sample location were analyzed against the Total Organic Halides (TOX) for each respective sample to determine if there were any correlations. The linear regression between Natural land percentage

and TOX had an R^2 value of 4E-06, showing no correlation between the two **(Figure 4.3)**. The linear regression between Urban land percentage and TOX had an R^2 value of 0.0019, showing little to no correlation between the two **(Figure 4.4)**. The linear regression between Agricultural land percentage and TOX had an R^2 value of 0.0042, also showing little to no correlation between the two **(Figure 4.5)** .

The percentage of Agricultural land within the 10km radius of the sample location was compared with the Total Nitrogen (TN) data collected for the respective water samples. The linear regression between agricultural land percentage and TN had and R^2 value of 0.0041, showing little to no correlation between the two for our data set **(Figure 4.11)**.

National Pollutant Discharge Elimination System Permits

National Pollutant Discharge Elimination System (NPDES) Permits for the state of South Carolina were accessed through the South Carolina Department of Health and Environmental Control's Interactive Maps and Geospatial Data (Jeannie Eidson, 2021). Datasets were specified as NPDES Permits and NPDES General Permits, both were obtained for our use. Permits were sorted for only Sewage System End pipes.

Nearest upstream wastewater treatment plant end pipes were found for each sample location and the distance between each was calculated using ArcGIS Pro. Distances were found to be between 0.4 km and 53.4 km, with the median being 14.4 km. A linear regression was calculated between the concentration of total organic halides at each sample point and the corresponding distance between that sample point and the nearest upstream wastewater treatment output. The R^2 value for this regression was 0.0002, showing no correlation between the data **(Figure 4.2)**.

Discussion

There is a plethora of factors that contribute to water pollution, including point source pollution from drains and pipes, agricultural pollutants, urban storm water runoff, and more (Chaundry et al). The commonality between most pollution factors is that they originate from an anthropogenic source. Urbanization has and will continue to cause increasing levels of micropollutants in rivers across the world. These increases have been shown through modeling and sample analysis over time (Strokal et al., 2021). While it is difficult to narrow down most specific micropollutants to one source, as they typically originate from many sources, groupings of micropollutants can be tied to areas and sources. Area distribution maps are useful in determining these correlations amongst micropollutant groups (Cecinato et al., 2021).

Organic halides, sometimes referred to as organohalides, are organic compounds that can be found in soil and water. Organic halides originate from a variety of natural and anthropogenic sources, with most compounds having been shown to have been derived through industrial means (Grimvall et al., 1991). This organic group of micropollutants has been linked to several sources, including wastewater treatment, domestic and municipal sewage, landfill leachates, and various factories and plants. Chlorine, bromine, and iodine are organic halides that are often used as disinfectants in wastewater treatment (Kim et al). Another micropollutant group includes herbicides, pesticides, and fertilizers that are commonly used in agriculture. These micropollutants are high in phosphates and nitrates. Use of nitrogen-based fertilizers leads to an excess of nitrogen in the surrounding rivers through leaching, drainage, and runoff flow (Savci, 2012). Other studies have linked nitrates and chlorides with areas of urban and agricultural land use (Zahn & Grimm, 1993). Due to the nature of the micropollutants sources, our study aimed to link organic halide

concentration with urban land use percentage and to link dissolved total nitrogen with agricultural land use percentage.

As shown in other studies, chlorine was the predominate organic halide present in water samples [43.1-5295.9 ug/L, median: 260.3 ug/L] (D. Kim et al., 2020). Bromine concentration varied greatly but was always found in lower concentrations than chlorine [1.4-1773.2ug/L, median: 23.8ug/L]. Iodine was not present in detectable levels in any samples.

No significant correlations were found between any of the proposed parameters. Total organic halide concentration present in samples was expected to positively correlate with the percentage of urban land found in the area surrounding sample location. When land use percentage was used in a linear regression with total organic halide concentration the R^2 value was under 0.01 for all land types; natural, agricultural, and urban. Additionally, total dissolved nitrogen was expected to positively correlate with the percentage of agricultural land surrounding sample location, but these variables had a R^2 value of 0.004. These results show that land use percentage alone cannot predict total organic halide or nitrogen presence in water.

This is potentially due to not applying other factors that may contribute to pollution such as hydrology, rainfall, and drainage patterns. More strategic sample collection may also help to show correlation. Further, the most recent land data available at the time of analysis was from 2011, which is likely to be outdated as urban areas have continued togrow and expand in the last decade. This could potentially cause inaccurate urban land percentages used in this study. Additionally, only the GPS location data was used for both sample locations and wastewater treatment plant outputs. The direction of output flow or output quantity was not available, and it is possible that the output from wastewater plants is diverted away from bodies of water and streams, which is where samples were collected from. If this is true, the potential contaminants

from the plants would not reach the sample locations and thus the distance between sample location and wastewater treatment plants would not be a useful metric in determining micropollutant concentration.

Conclusions

Rapid urbanization has led to higher levels of micropollutants found in waters in recent years, with projections estimating further increases in the future(Strokal et al., 2019). Detection and mitigation of micropollutants becomes incredibly important, but current methods for detection are costly and time consuming. Efforts to develop an indicator test for these micropollutants would aid direct detection efforts and minimize cost and time. Organic halides and nitrogenous compounds are common micropollutants that are from many sources including wastewater treatment, sewage and landfill waste, and pesticides and fertilizers used in agriculture (Bond et al., 2011). Both types of micropollutants are heavily associated with a specific type of land use, leading to the possibility to use percentage of land use as a predictive measure of the potential for increased quantities of micropollutants found.

Multiple linear regressions were preformed to determine correlations between urban land use and total organic halides, agricultural land use and dissolved total nitrogen, and distance between wastewater treatment facilities and sample location and total organic halides. No correlations were found between any variables through these linear regressions.

Further testing is needed to determine the viability of land use for an indicator of micropollutants in South Carolina. Hydrology, rainfall data, and drainage patterns are necessary to include as these variables affect concentrations of micropollutants present in rivers. Sample planning would also provide a better understanding of micropollutant patterns. Sampling

immediately before and after expected rainfall, and within specific distances from urban and agricultural areas is recommended.

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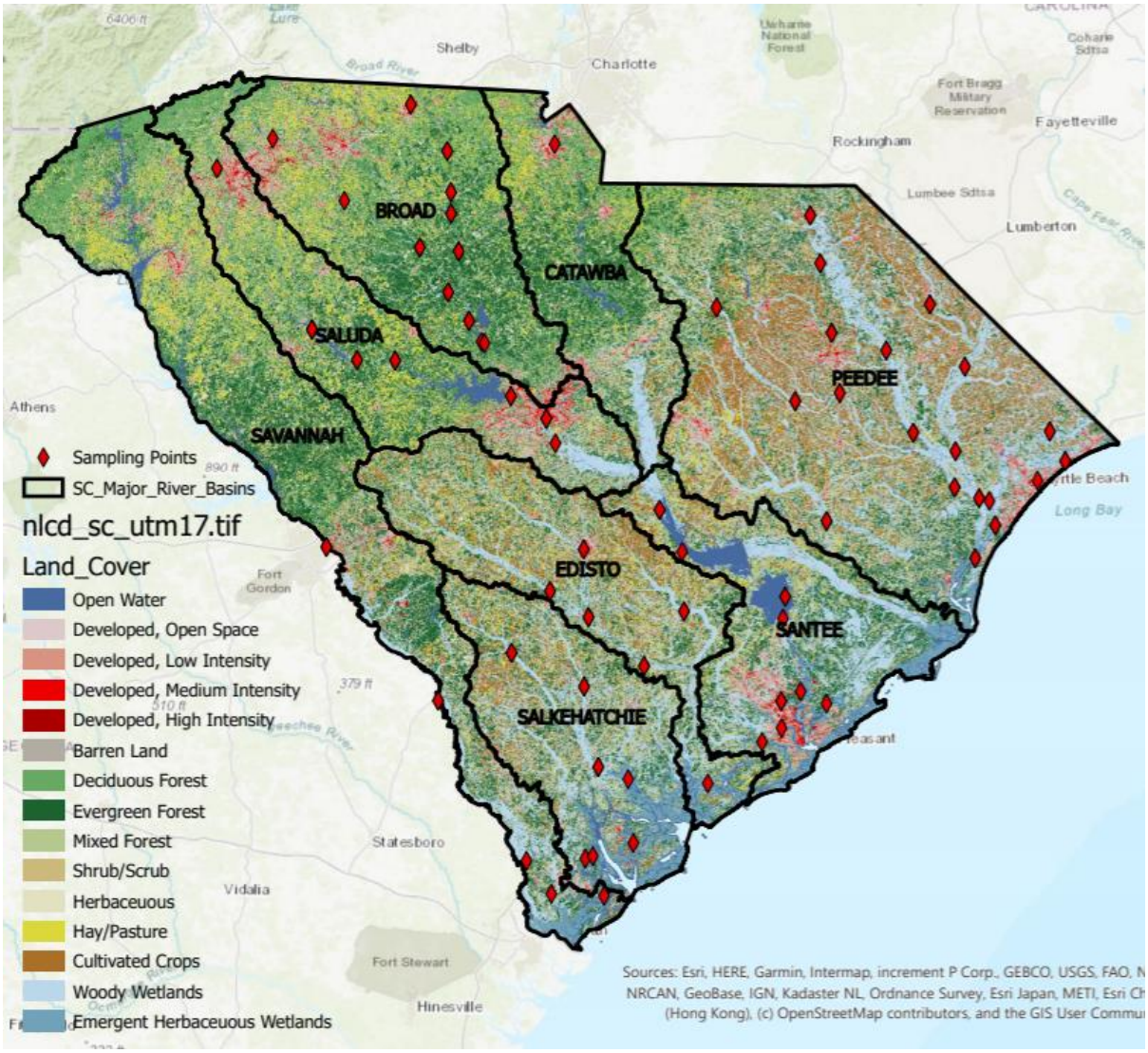

Figure 4.1: Map of South Carolina labeled with sampling points and land use data.

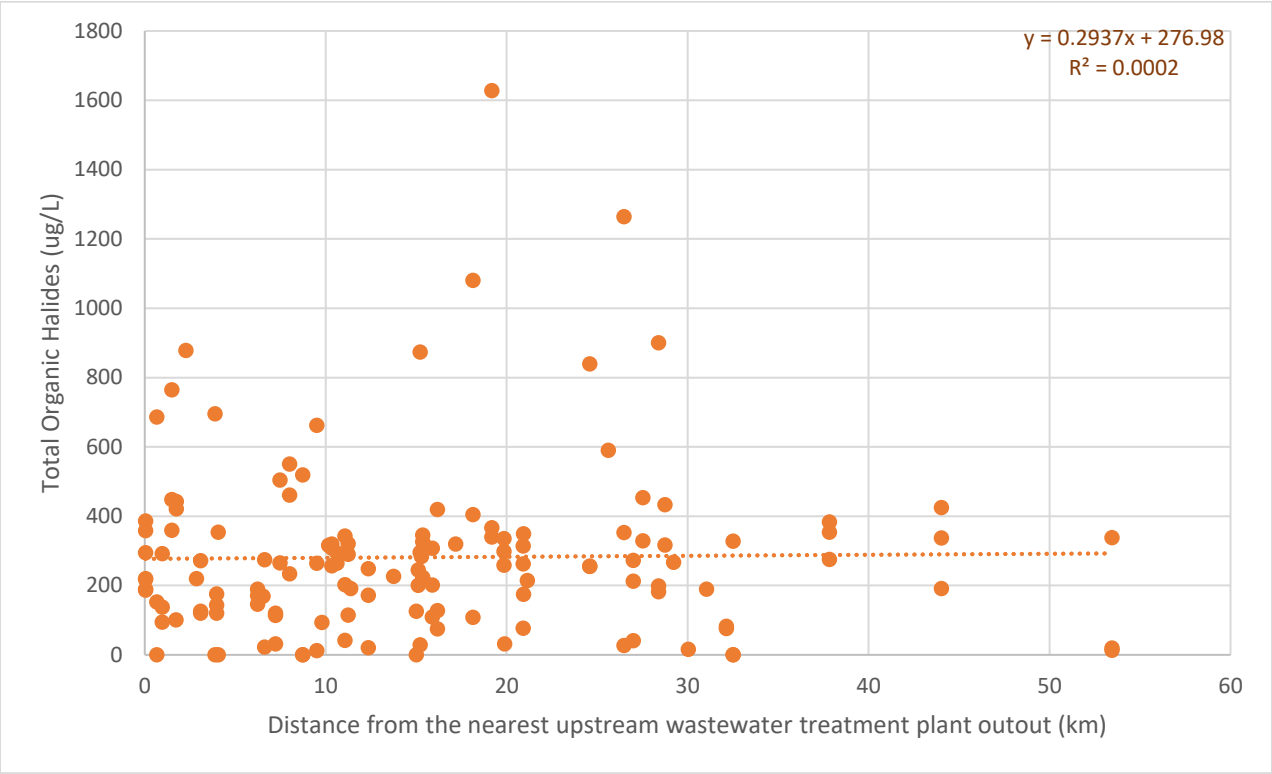

Figure 4.2: Total organic halides and wastewater treatment plant distance.

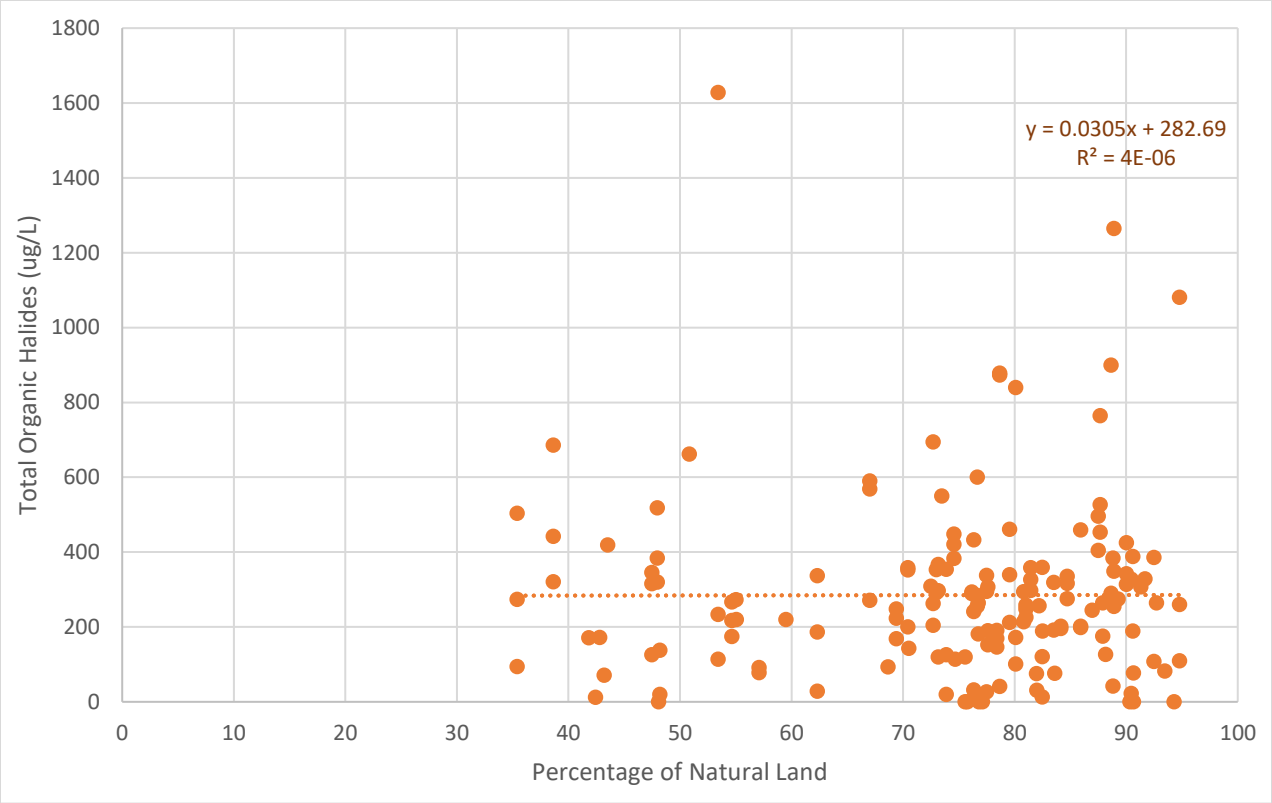

Figure 4.3: Total Organic Halides and percentage of natural land. The percentage of natural land was defined as a 10km radius around each sample location.

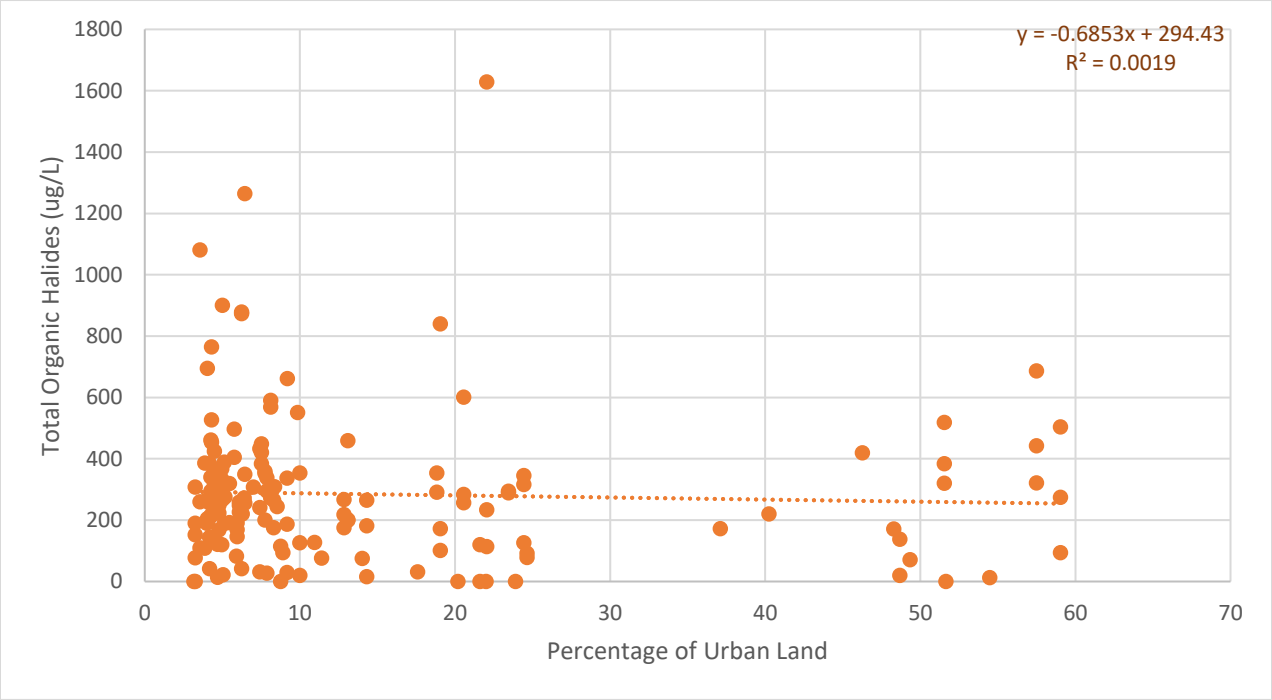

Figure 4.4: Total Organic Halides and percentage of urban land. The percentage of urban land was defined as 10km radius around each sample location.

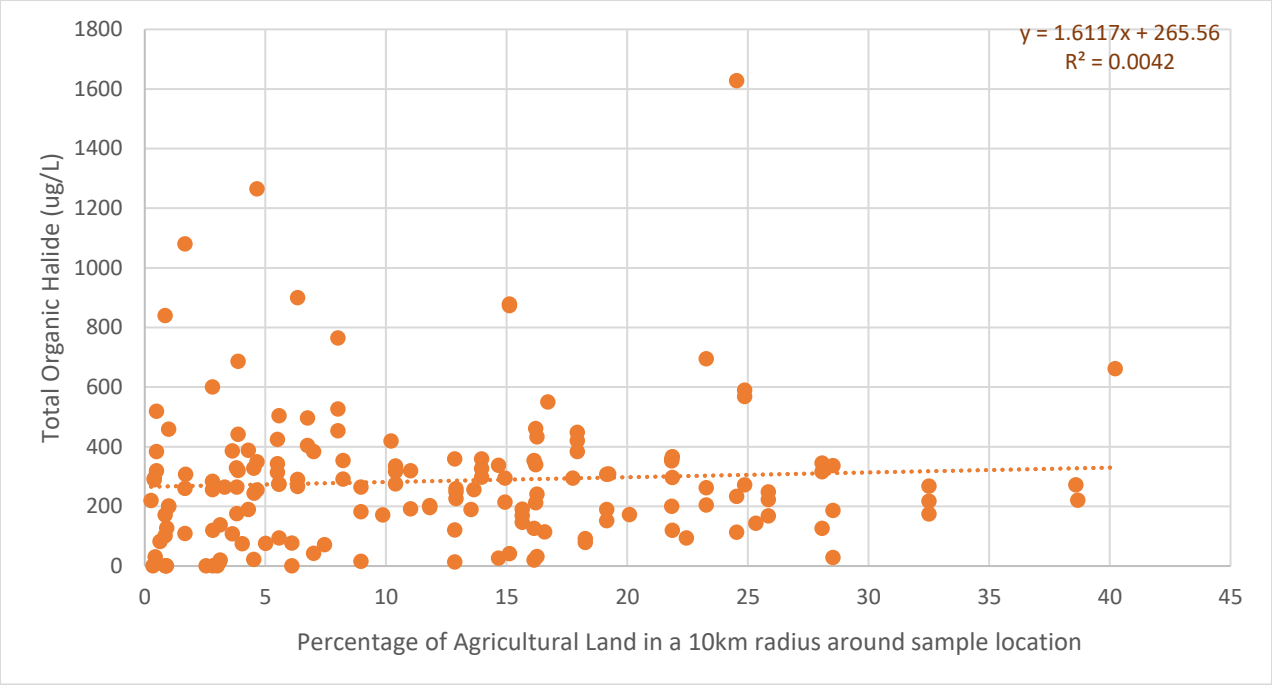

Figure 4.5: Total Organic Halides and percentage of agricultural land. The percentage of agricultural land was defined as a 10km radius around each sample location.

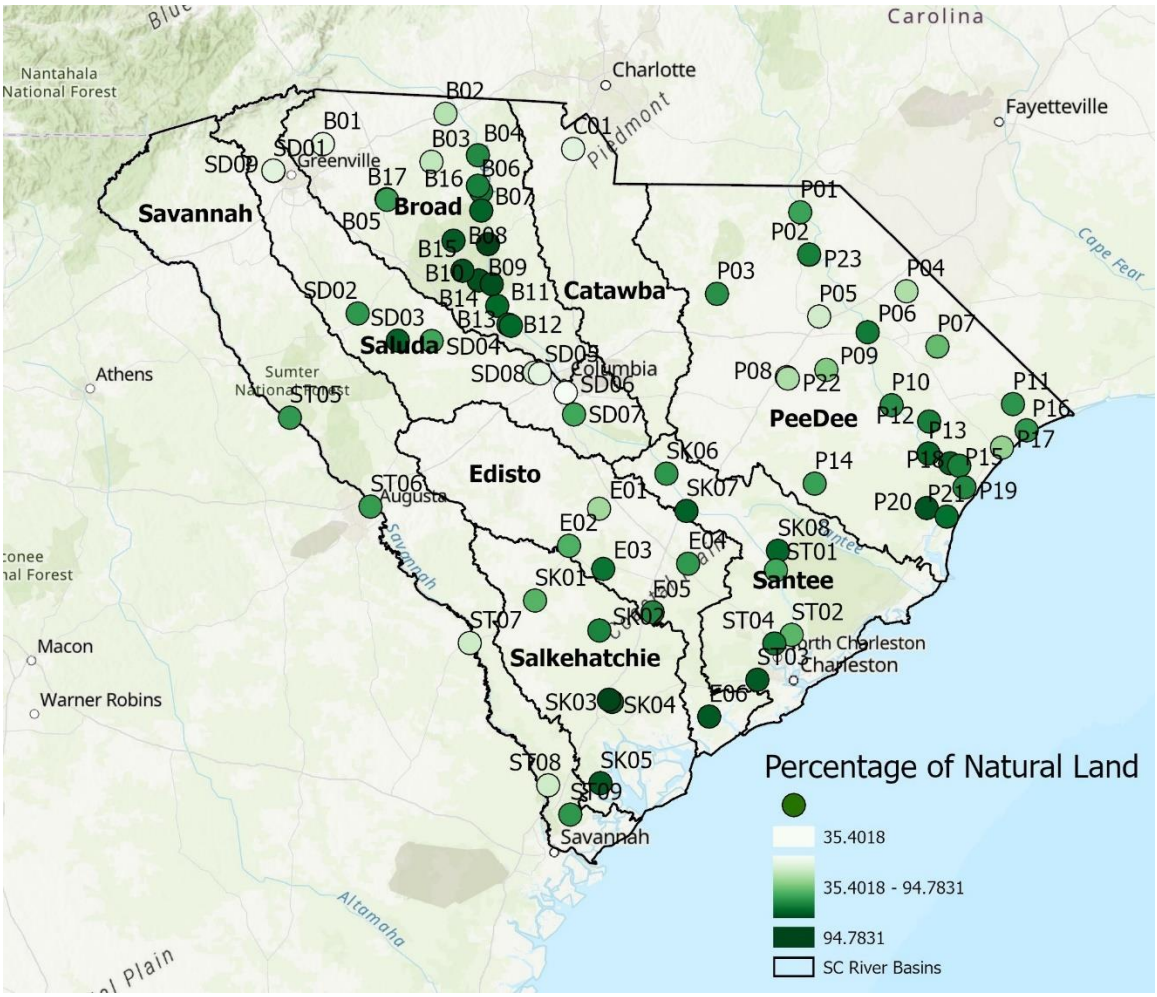

Figure 4.6: Percentage of natural land for each sample displayed on map of South Carolina. The percentage of natural land was defined as a 10km radius around each sample location.

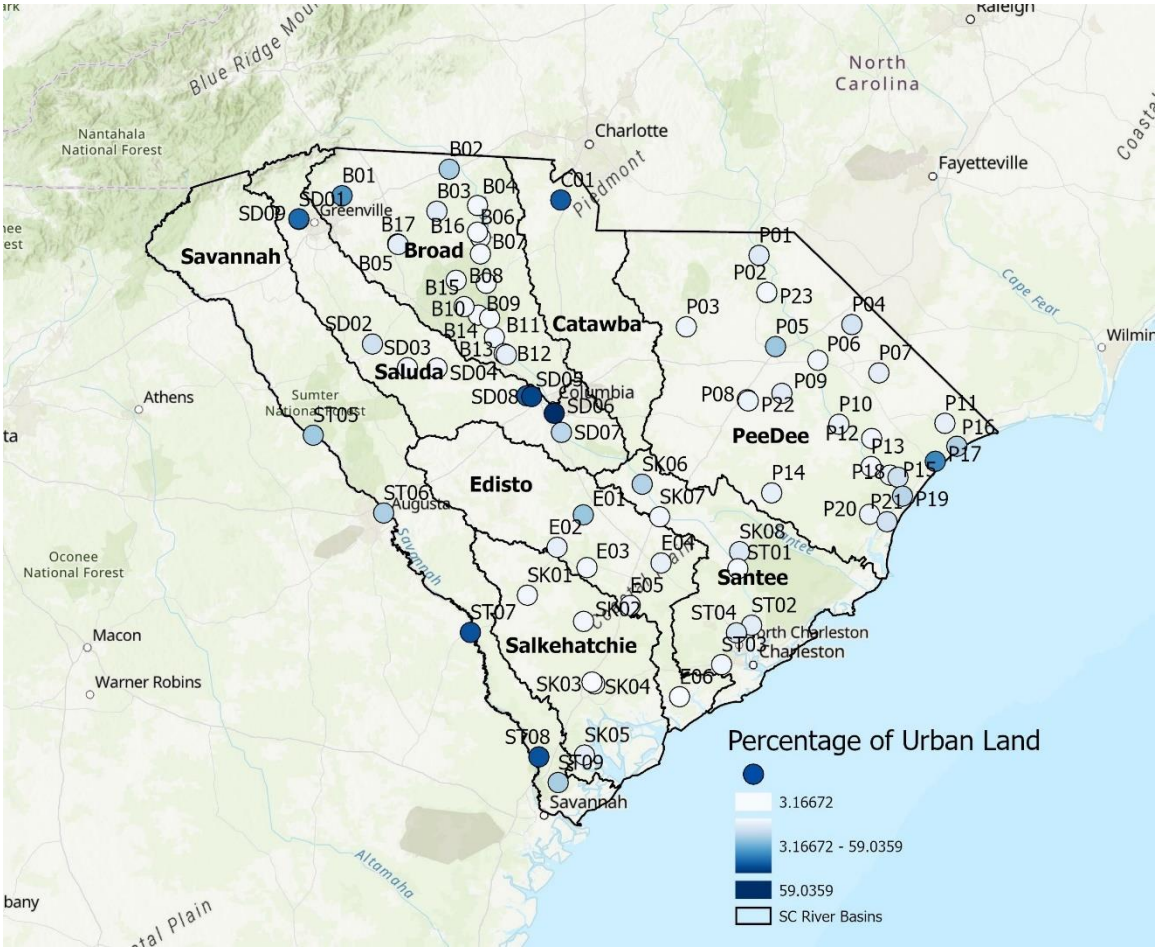

Figure 4.7: Percentage of urban land for each sample displayed on map of South Carolina. The percentage of urban land was defined as 10km radius around each sample location.

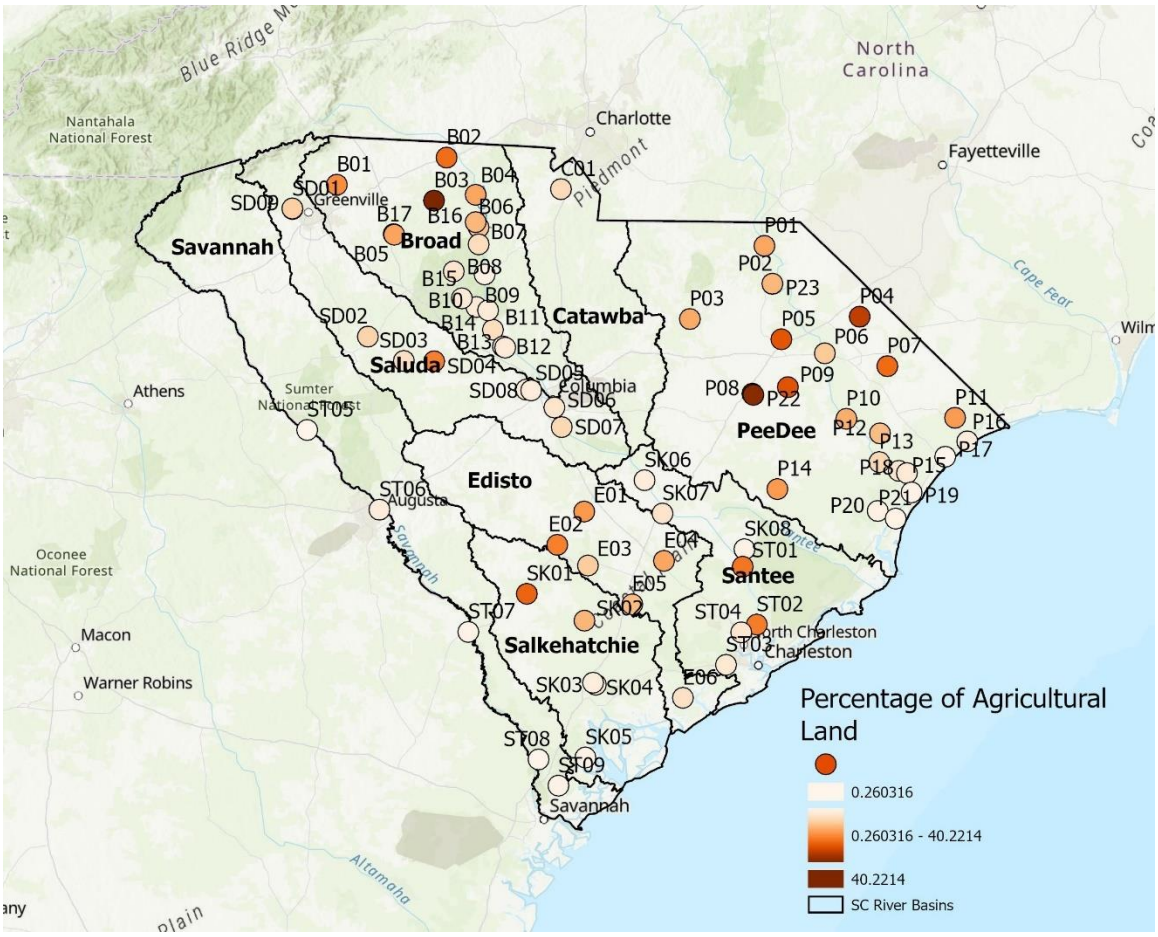

Figure 4.8: Percentage of agricultural land for each sample displayed on map of South Carolina. The percentage of agricultural land was defined as a 10km radius around each sample location.

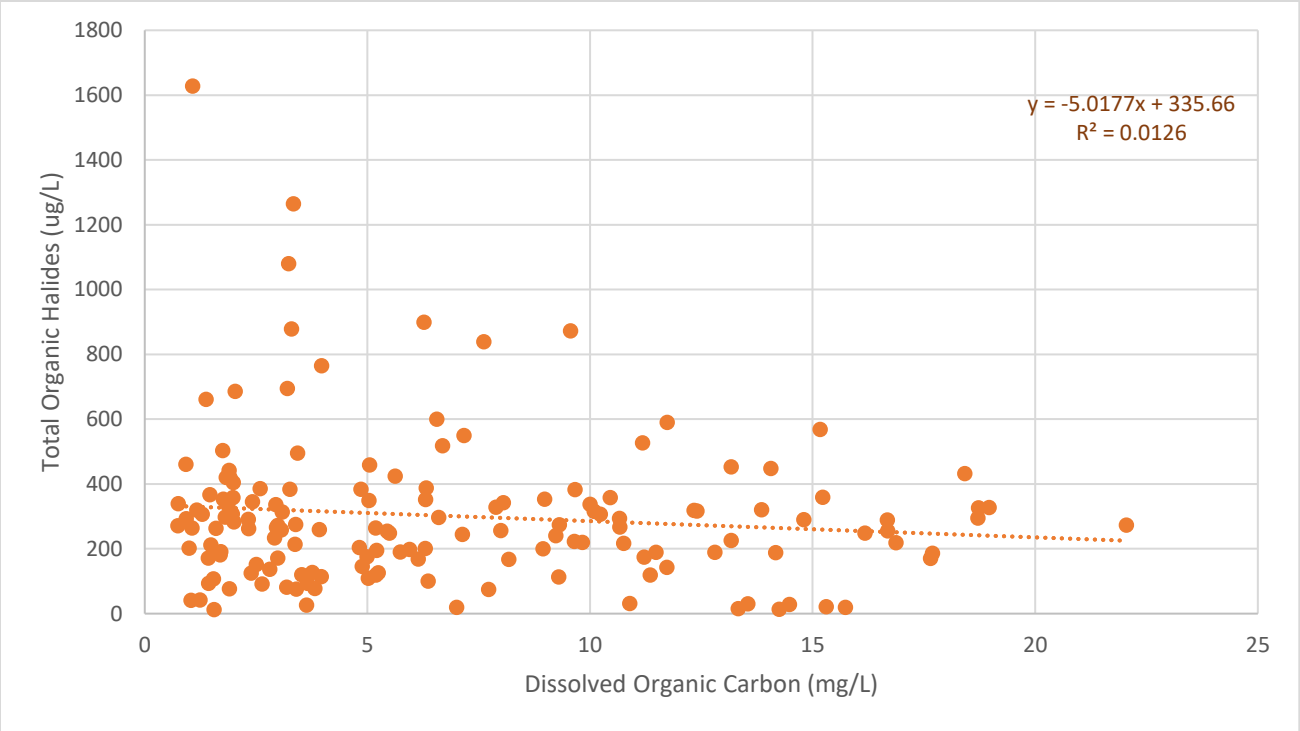

Figure 4.9: Total organic halides and dissolved organic carbon.

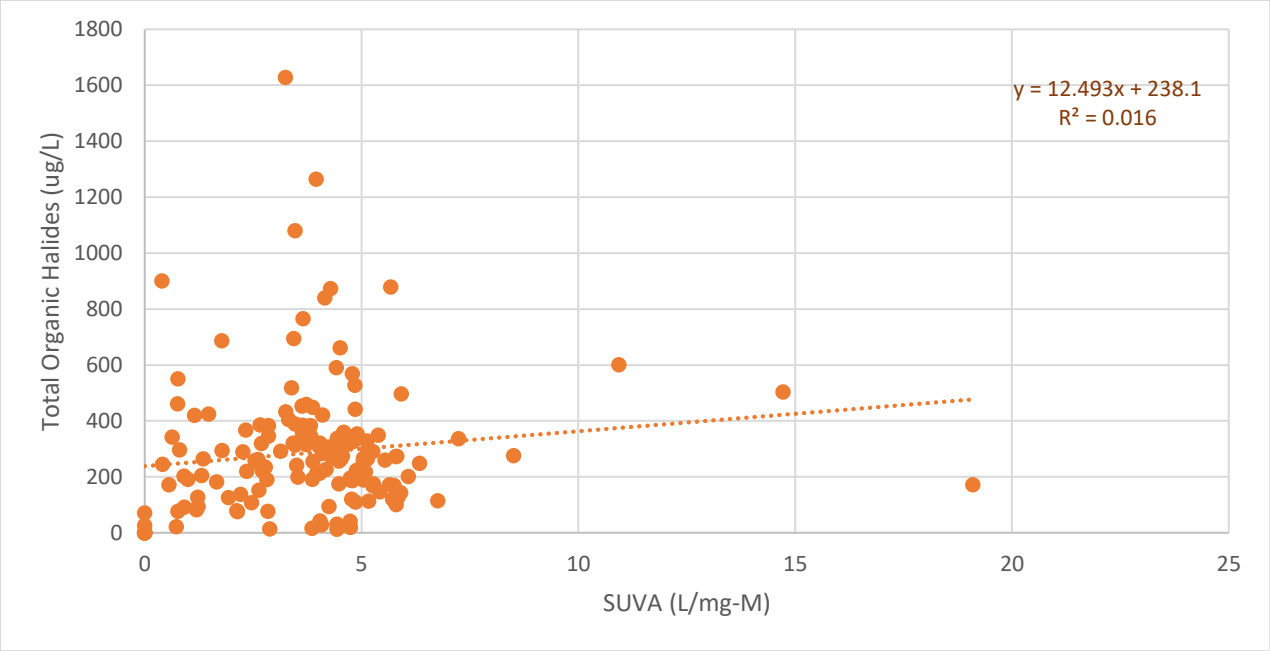

Figure 4.10: Total organic halides and SUVA.

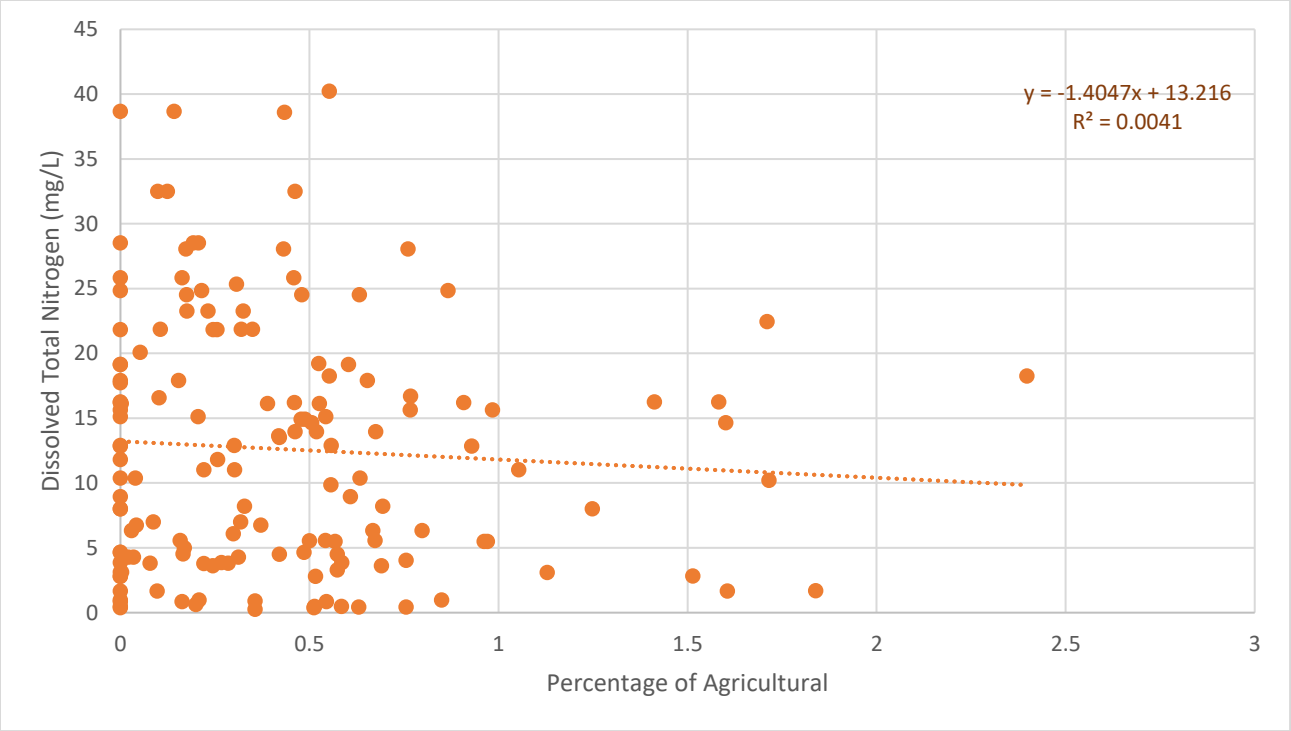

Figure 4.11: Dissolved total nitrogen and percentage of agricultural land. The percentage of agricultural land was defined as a 10km radius around each sample location.

CHAPTER FIVE

CONCLUSIONS

Our goal for this study was to test methods of detection for micropollutants in surface and runoff waters. Micropollutants in surface waters pose health risks to the public and are found in increasingly higher concentrations rivers worldwide (Best, 2019; Strokal et al., 2019). Current testing methods are time intensive and expensive. In hopes to alleviate this issue, we tested three methods that could potentially work as surrogate detection methods.

Microbial fuel cells have shown the potential to operate as biosensors in recent research, producing an output of current that decreases/diminishes with the addition of a toxin to the system (Chouler & Di Lorenzo, 2015). We tested this, creating a biosensor with a microbial community obtained from the wastewater system of Greenville Water's water treatment site. After establishing a steady baseline current, we introduced our toxin, sulfamethoxazole, to the system in increasing doses to determine the point at which the system becomes inhibited. Though the system showed inhibition of the current output, it was observed at higher concentrations than would be found in the samples used in this study. Further testing was halted due to time constraints.

ELISA (enzyme-linked immunosorbent assay) is a comprehensive method that is rapid and easy to use (Engvall, 2010). Because of this we proposed that, though generally used in medical testing, we would be able to use this method to detect micropollutants in surface water. This hypothesis was not met however, due to the levels of DOC in natural waters changing the color of the water and thus affecting the efficacy of the method.

Finally, we hoped to form a connection between the levels of micropollutants present in the collected water samples and the land use of the area surrounding the sample location. Total organic halides and dissolved total nitrogen were used at the micropollutants in this study because they are highly associated with specific land use types. Organic halides are commonly associated with urban land use due to their use in wastewater treatment. Nitrogenous fertilizers are often used in agriculture, leading to dissolved total nitrogen being associated with agricultural land use (Chaudhry & Malik, 2017). In addition, we tested other water quality parameters including pH, DOC, SUVA, and others to potentially compare these values to the levels of pollutants in the samples as well. We were unable to make any significant connection between any of these parameters.

Though we tested three methods with great potential for development into a new method of micropollutant detection, we have been unsuccessful thus far. In future research we may include hydrology and rainfall data in our analysis of land use of South Carolina, as these factors influence micropollutant discharge rates and directionality of micropollutant movement.

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APPENDICES

APPENDIX A – NATIONAL LAND COVER DATABASE CLASS LEGEND AND DESCRIPTION

Class\ Value	Classification Description
Water	
11	**Open Water**- areas of open water, generally with less than 25% cover of vegetation or soil.
12	**Perennial Ice/Snow**- areas characterized by a perennial cover of ice and/or snow, generally greater than 25% of total cover.
Developed	
21	**Developed, Open Space**- areas with a mixture of some constructed materials, but mostly vegetation in the form of lawn grasses. Impervious surfaces account for less than 20% of total cover. These areas most commonly include large-lot single-family housing units, parks, golf courses, and vegetation planted in developed settings for recreation, erosion control, or aesthetic purposes.
22	**Developed, Low Intensity**- areas with a mixture of constructed materials and vegetation. Impervious surfaces account for 20% to 49% percent of total cover. These areas most commonly include single-family housing units.
23	**Developed, Medium Intensity** -areas with a mixture of constructed materials and vegetation. Impervious surfaces account for 50% to 79% of the total cover. These areas most commonly include single-family housing units.
24	**Developed High Intensity**-highly developed areas where people reside or work in high numbers. Examples include apartment complexes, row houses and commercial/industrial. Impervious surfaces account for 80% to 100% of the total cover.
Barren	
31	**Barren Land (Rock/Sand/Clay)** - areas of bedrock, desert pavement, scarps, talus, slides, volcanic material, glacial debris, sand dunes, strip mines, gravel pits and other accumulations of earthen material. Generally, vegetation accounts for less than 15% of total cover.
Forest	
41	**Deciduous Forest**- areas dominated by trees generally greater than 5 meters tall, and greater than 20% of total vegetation cover. More than 75% of the tree species shed foliage simultaneously in response to seasonal change.
42	**Evergreen Forest**- areas dominated by trees generally greater than 5 meters tall, and greater than 20% of total vegetation cover. More than 75% of the tree species maintain their leaves all year. Canopy is never without green foliage.

43	**Mixed Forest**- areas dominated by trees generally greater than 5 meters tall, and greater than 20% of total vegetation cover. Neither deciduous nor evergreen species are greater than 75% of total tree cover.
Shrubland	
51	**Dwarf Scrub**- Alaska only areas dominated by shrubs less than 20 centimeters tall with shrub canopy typically greater than 20% of total vegetation. This type is often co-associated with grasses, sedges, herbs, and non-vascular vegetation.
52	**Shrub/Scrub**- areas dominated by shrubs; less than 5 meters tall with shrub canopy typically greater than 20% of total vegetation. This class includes true shrubs, young trees in an early successional stage or trees stunted from environmental conditions.
Herbaceous	
71	**Grassland/Herbaceous**- areas dominated by gramanoid or herbaceous vegetation, generally greater than 80% of total vegetation. These areas are not subject to intensive management such as tilling, but can be utilized for grazing.
72	**Sedge/Herbaceous**- Alaska only areas dominated by sedges and forbs, generally greater than 80% of total vegetation. This type can occur with significant other grasses or other grass like plants, and includes sedge tundra, and sedge tussock tundra.
73	**Lichens**- Alaska only areas dominated by fruticose or foliose lichens generally greater than 80% of total vegetation.
74	**Moss**- Alaska only areas dominated by mosses, generally greater than 80% of total vegetation.
Planted/Cultivated	
81	**Pasture/Hay**-areas of grasses, legumes, or grass-legume mixtures planted for livestock grazing or the production of seed or hay crops, typically on a perennial cycle. Pasture/hay vegetation accounts for greater than 20% of total vegetation.
82	**Cultivated Crops** -areas used for the production of annual crops, such as corn, soybeans, vegetables, tobacco, and cotton, and also perennial woody crops such as orchards and vineyards. Crop vegetation accounts for greater than 20% of total vegetation. This class also includes all land being actively tilled.
Wetlands	
90	**Woody Wetlands**- areas where forest or shrubland vegetation accounts for greater than 20% of vegetative cover and the soil or substrate is periodically saturated with or covered with water.
95	**Emergent Herbaceous Wetlands**- Areas where perennial herbaceous vegetation accounts for greater than 80% of vegetative cover and the soil or substrate is periodically saturated with or covered with water.