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The Potential of Indian River Lagoon Muck to act as a Reservoir for Emerging Organic Contaminants

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by

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Abstract

Pharmaceuticals, pesticides, and personal care products are frequently found in sediments and waterways around the world. These chemicals, collectively known as Emerging Organic Contaminants (EOCs) can harm the environments in which they are deposited by killing or stressing the local flora and fauna. These EOCs also can be released back into the water column when these substrates are disturbed. Such sediments and their associated EOCs are particularly problematic in urbanized areas. However, the particular composition of urbanization-associated sediments as well as their potential to harbor EOCs needs to be evaluated in the context of their environment, to ensure proper mitigation efforts.

In this study, water and sediment samples were taken from the Sykes Creek Area of Florida's Indian River Lagoon (IRL), which has a highly restricted waterflow. This restricted flow means that pollutants entering the IRL, especially in the north where there are fewer inlets, remain in the system for extended periods. The samples taken for this study were run through liquid chromatography-mass spectrometry (LC-MS) to identify and quantify eight common EOCs: Diazepam, Oxybenzone, Sulisobenzone, Acetaminophen, Atrazine, Chloramphenicol, Ibuprofen, and Sucralose. Information on the concentration and location of these EOCs was then used, in combination with additional information about pollutants at each site provided by the Ocean Resource and Conservation Association (ORCA), to attempt to create a predictive model for each EOC using RStudio.

The EOC data collected indicated that muck does not actually act as a significant reservoir for EOCs in the IRL. The vast majority of EOCs quantified in this study were found in the water column. The modeling in RStudio showed only water depth and Ammonia measured from the water of the sample sites were able to predict the presence of any EOC, while Ammonia, Sulfur, and Mercury were the only parameters measured from the muck of the sample sites capable of predicting the presence of any EOC.

Table of Contents

List of Figures

List of Tables

Chapter 1

Introduction

Urbanization is a major factor in habitat fragmentation and destruction, impacting both the land as well as the surrounding waters (Rudershausen et al., 2019). Urbanization is an especially serious concern along coastal areas, where populations, and urban-land coverage are increasing faster than inland areas (Neumann et al., 2015). This increase in human population and development puts stress on organisms and natural resources due to higher levels of pollution (Neumann et al., 2015). Habitats altered by human activities, such as urbanization, have lower organism density and diversity compared with unaltered habitats (Peterson & Lowe, 2009). In one study rip-rap shorelines had ≈20% less density in benthic fauna when compared with natural marsh, whereas bulkhead shorelines showed a reduction in density of ≈80% (Seitz et al., 2006). The nearshore habitats threatened by urbanization along the coasts represent over $100 billion in annual income in the United States, ~50% of which comes from saltwater fisheries that are heavily dependent upon estuaries (Peterson & Lowe, 2009).

Ironically, some of the measures put in place by local governments to make coastal areas more appealing, such as beach renourishment programs, often result in a marked decline in habitat suitability for native fish and other marine animals (Peterson & Lowe, 2009). Exacerbating the problem is the attitude toward a multitude of polluting and disruptive factors in many coastal urban zones around the world. Specific examples include but are not limited to: (i) agricultural and industrial waste runoff into waterways, (ii) dredge and fill projects, which disrupt benthic and shoreline habitat, and (iii) general reduced water quality from various human activities.

The development of urban areas near the coast frequently involves the creation of multiple canals, connecting previously separated waterways, and merging multiple small streams into one or more larger bodies (Dellapenna et al., 2015). This increases water discharge rates into rivers, as well as the watershed size for both rivers and estuaries. Increased connectivity between these bodies of water can significantly expand watershed size and change the flow of materials within this body. In many cases, these canals redirect drainage to one or more locations within the urbanized watershed, rather than following their natural paths to outflows along the coastline (Figure 1). This causes an increase in flow into the urbanized area, as water that would normally bypass the region is now funneled into it by the very waterways created by development (Figure 1). That water can carry suspended material, including particles, microorganisms, and pollutants which enter from upstream (Dellapenna et al., 2015; Peeler et al., 2006).

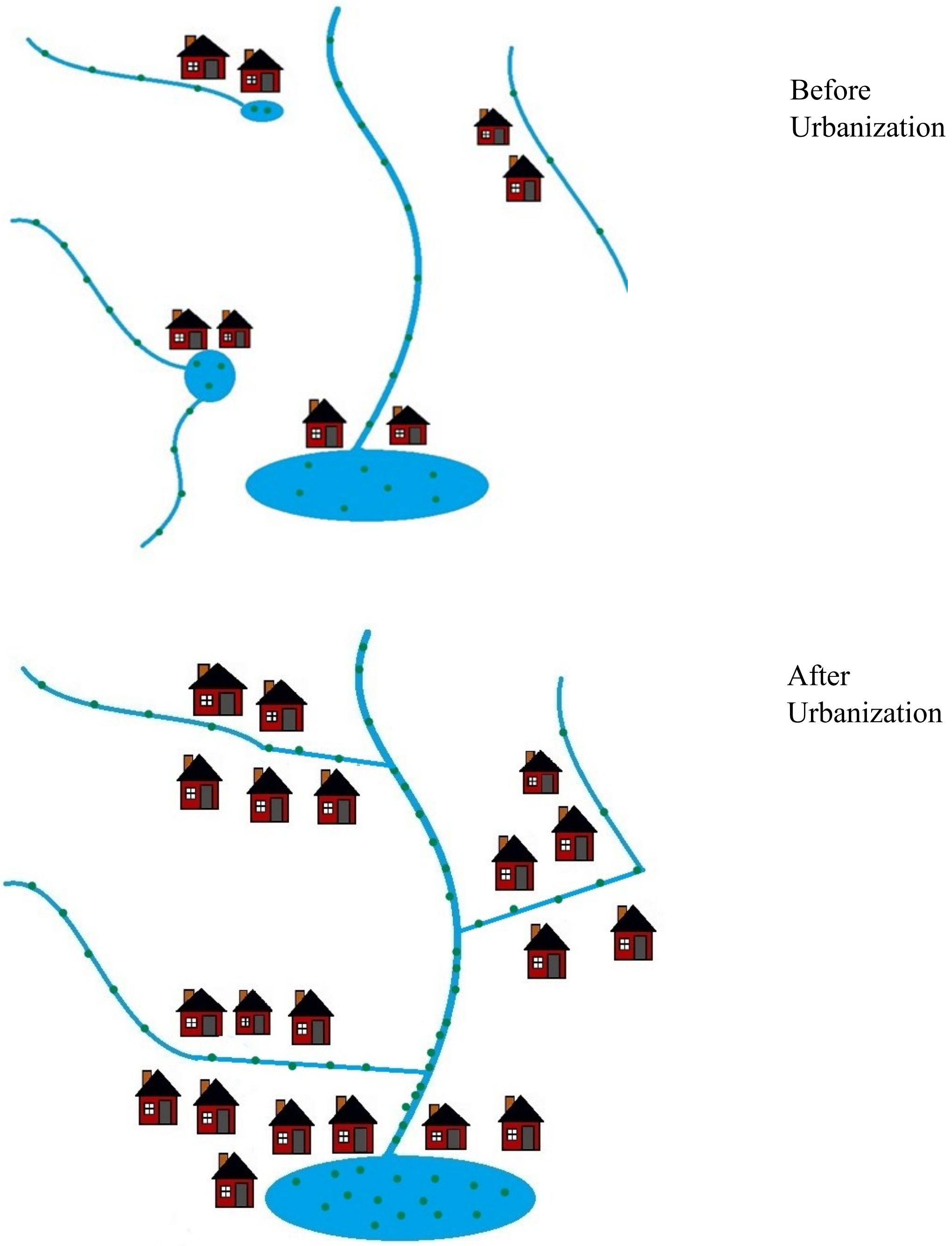


Figure 1: Simple cartoon showing increased pollution, represented by green dots, in urban waterways connected by canals. TOP: natural waterway state, with low levels of pollution in each waterway. BOTTOM: Connected urban waterways concentrate pollution from each stream into one increasing pollution entering the final outflow.

In addition to the increased input from outside sources, the numerous construction projects continually underway in growing urban environments also contribute to increased suspended loads in streams and waterways. For example, the removal of trees and other plants, whose roots help hold sediment in place, often results in the deposition of large quantities of sediment (Foster et al., 2018). To summarize, the inputs from sources both inside and outside a specific site leads to the rapid and increased deposition of sediment and the eventual deterioration in quality of the site.

What is Muck?

In bodies of water with restricted flow rates, urbanization can lead to an even greater degree of deposition of silt and other fine sediments, as well as organic materials. This mixture of fine-grain sediment with a high organic content, is often referred to as ‘muck’ in many locations (Dellapenna et al., 2015). Muck is not just a sulfurous and goopy affront to the eyes and nose, but it is also a dangerous sediment to the organisms in the ecosystems where it accumulates. Muck is high in nitrogen and phosphorus content, which are detrimental to the root systems of aquatic and semiaquatic plants at these concentrations (Wigand et al., 2014). Nitrogen levels in muck samples can be >1.5% of the total mass, whereas phosphorus can reach concentrations as high as 0.1% (Wigand et al., 2014). The high levels of these specific nutrients can also reduce soil strength, a measure of how well the soil resists erosion by various environmental factors, e.g., weather or flow from adjacent waterways (Wigand et al., 2014).

Figure 2: A sample of muck from the Indian River Lagoon.

In addition to its impact on the benthic environment of estuaries, muck is also responsible for a variety of negative impacts on organisms. Some of these negative impacts can be the result of resuspension of muck, which occurs frequently in certain habitats due to strong winds and resuspension of sediment from boat wake. Under sustained high winds, muck can stay suspended for several weeks at a time, reducing light and thereby reducing the depth at which aquatic plants can grow (Foster et al., 2018). This leads to a loss of habitat for plants such as seagrasses and potentially reduces photosynthesis and oxygen (O_2) production (Kjelland, et al., 2015).

Muck can also sequester nutrients and then release them when disturbed. These nutrients can contribute to eutrophication and harmful algal blooms (Foster et al., 2018; Fox & Trefry 2018). Suspension of muck in the water column also facilitates the release of microorganisms and chemicals associated with these sediments, which can then contact humans and wildlife (O'Mullan et al. 2019) (Figure 3). High concentrations of decomposing organic matter in the sediment, means that muck also tends to have a high O_2 demand due to activity of decomposers. This potentially removes O_2 from the surrounding water, creating an anoxic "dead zone" (Foster et al., 2018).

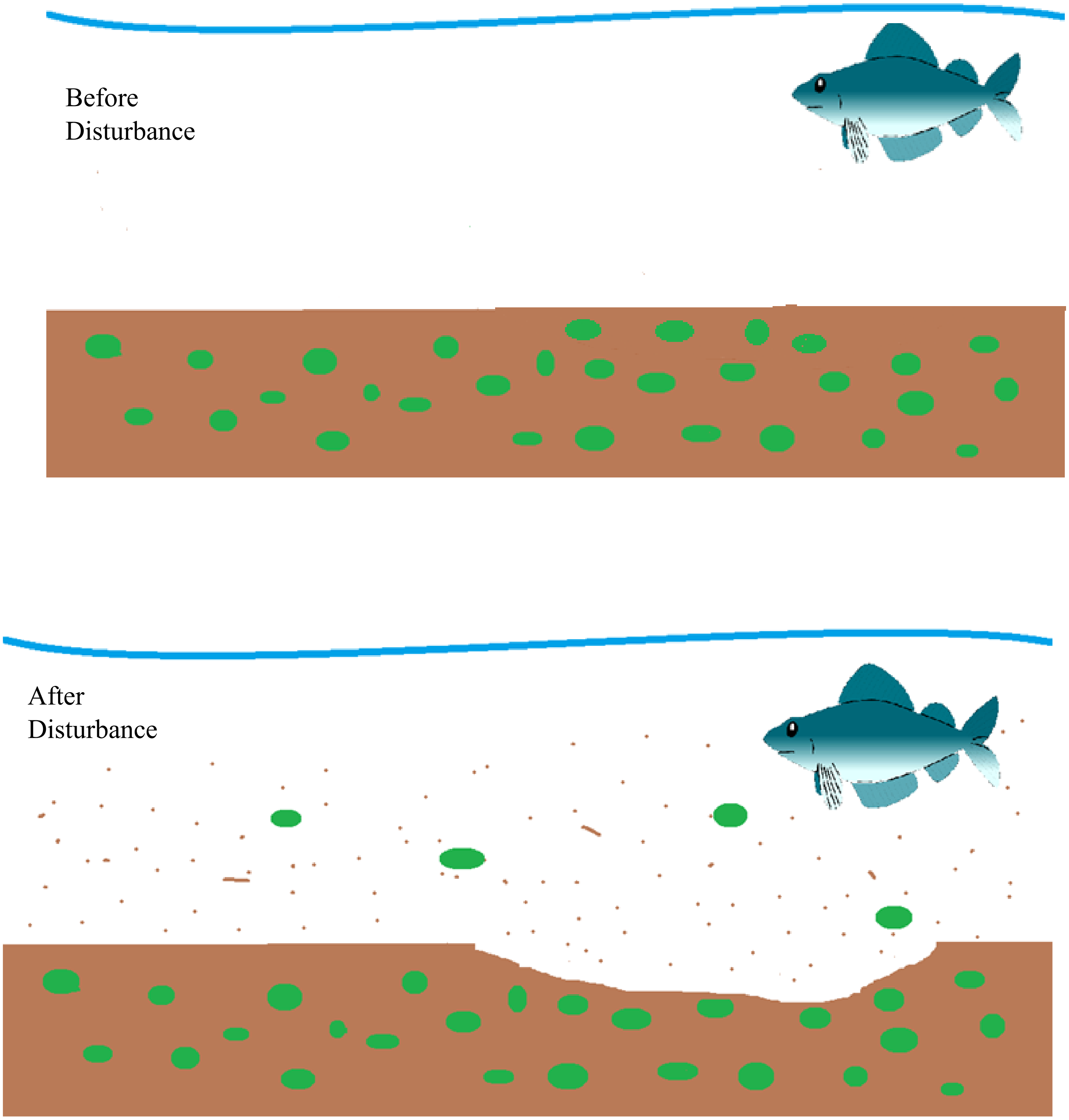


Figure 3: Simple cartoon showing a muck deposit before disturbance (Top) and after a disturbance (Bottom) to demonstrate how disturbance of muck can suspend pollutants in the water column, making them available to wildlife and human populations. Green dots represent pollutants, brown dots in the after disturbance image represent suspended sediments.

The potential problems associated with the physical disruption of muck sites are important to consider when planning remediation, such as dredging, an activity that will stir up the surface muck and leave it suspended in the water column (Fox & Trefry, 2018). Muck from the surrounding area can quickly fill this space, making the long-term benefits of this method unclear. Finally, muck removed during the dredging process is often made available for other applications, such as fertilizing crops (Foster et al., 2018). While such 'recycling' initially seems like a good use of the material, it could inadvertently introduce unknown contaminants into the food supply or other watersheds. Such concerns have previously been raised for biosolids, derived from wastewater treatment facilities (Sorinolu et al., 2020). Moreover, the potential for repeated contamination of the environment via anthropogenic organic compounds like antibiotics and pesticides. could have far-reaching impacts on human and ecosystem health but have been rarely evaluated in muck.

Emerging Organic Contaminants in Aquatic Systems

Emerging organic contaminants (EOCs), a class of pollutants including pharmaceuticals, pesticides, and personal care products may also be preserved in these sediments. Specific examples of EOCs such as Acetaminophen, Caffeine, and Ibuprofen are nearly ubiquitous in wastewaters around the world (Lapworth et al., 2012). Many EOCs are poorly eliminated from wastewater and frequently end up in watersheds. Designed to have effects at low doses, EOCs may have harmful impacts on wildlife in concentrations as low as several parts per billion (Lapworth et al., 2012). These compounds have been shown to affect morphology, mating, and cause mutations in exposed organisms (Shenoy, 2012). Here I will briefly review different EOC classifications as well as their potential threats.

PHARMACEUTICALS One of the major categories of EOCs is pharmaceutical waste. Despite generally having high rates of degradation in the environment, pharmaceuticals are considered semi-persistent pollutants due to their regular input via sewage and wastewater (Serra-Compte et al., 2018). Ibuprofen, diclofenac, sulfamethoxazole, and carbamazepine are among the most common pharmaceutical contaminants around the world, but pharmaceuticals of almost every drug class have been found in waterways, including antibiotics, analgesics, β-blockers, contraceptives, hormones, and antiseptics (Palmer et al., 2008; Serra-Compte et al., 2018). Exposure to these chemicals can reduce production of Palmitic acid, a saturated fatty acid important in energy storage. Ibuprofen, carbamazepine, and sulfamethoxazole have also been shown to decrease lysophosphatidic acid, a major component of biological membranes, leading to toxicity from changes in membrane structures (Serra-Compte et al., 2018). Ibuprofen has also been shown to reduce reproductive capacity in crustaceans and inhibit growth of mollusks (Sung et al., 2014). This problem is likely to become a larger threat to aquatic organisms in the future as populations around the world, especially the elderly, increase, driving up the demand for many of the different classes of pharmaceuticals (Palmer et al., 2008).

One of the most infamous pharmaceutical pollutants in the environment is Acetaminophen. The abundance of Acetaminophen in the environment has led to it being listed as one of the top 20 pharmaceuticals of high risk to the environment, despite the relatively high concentrations required for it to have negative effects on organisms (Kataoka et al., 2019). Concentrations in rivers across the US reached 10 µg/L and as high as 65 µg/L in rivers in Europe. Acetaminophen has also been found in concentrations up to 1.4 mg/kg dry weight in biosolids used in agriculture (Li et al., 2015). Despite a short half-life, of just a couple days, in the environment, Acetaminophen can reach high concentrations due to constant input as well as slower biodegradation in sediments, due to high levels of organic carbon (Kataoka et al., 2019; Li et al., 2015). Hepatotoxic effects (liver damage) from this drug have been shown in zebrafish and other aquatic organisms, as well as increased mortality in zebrafish embryos at under 5 µg/L, significantly lower than the concentrations found in environments around the world (Huang et al., 2017; Kataoka et al., 2019).

Another category of pharmaceutical waste that is considered a potentially hazardous pollutant is antibiotic waste. The antibiotic Chloramphenicol, used by veterinaries and agriculture, has been shown to increase oxidative stress in the model aquatic invertebrate *Daphnia magna*. Chloramphenicol has also been restricted in some countries due to its link to bone marrow and blood disorders including aplastic anemia, thrombocytopenia, leukopenia, and more, though there is evidence of farmers continuing to use it illegally to deal with disease outbreaks in livestock (Yuxuan et al., 2019).

Antibiotic pollution in the environment places selective pressure on environmental bacteria, potentially leading to an increase in antibiotic resistant strains. With 162,000 tons of antibiotics used in 2013 in China alone and with much of that amount eventually deposited into waterways, there are plenty of opportunities for antibiotic resistance to appear in environmental bacterial populations (Yuxuan et al., 2019). Discarded antibiotics into the environment (Schaefer et al., 2009) add additional selective pressure. Treated wastewater has been shown to contain antibiotic residues ranging from ng/L to µg/L, with raw wastewater containing even higher levels (Voigt et al., 2020). Bacteria entering waterways from human sources via wastewater have the potential to spread antibiotic resistance to natural populations.

A study of a single oyster farm found over 280 strains of bacteria with resistance to at least three separate antibiotics, with several strains resistant to at least nine of the ten antibiotics tested (Wang et al., 2014). Several of these antibiotics, such as Furazolidone and Chloramphenicol, have been banned from use in aquaculture based on this research and others. Additionally, farmers are attempting to reduce the incidence of disease and are thereby accelerating the rate of antibiotic resistance in the environment (Wang et al., 2014). While both the wastewater treatment plant and the oyster farm examples deal with waterborne bacteria, it is reasonable that similar levels of resistance would be seen in sediments such as muck. Indeed, it may be even more common in muck due to the preference of bacteria for sediments leading to a greater population density and therefore a greater exchange of traits among individuals.

Anxiety and depression medications, designed to impact the central nervous system at low concentrations, are also of concern pollutants in waterways (de Araujo et al., 2019). Benzodiazepines, for example, are one of the least investigated classes of compounds in surface waters despite recent increases in production, and their effects on aquatic organisms are not well understood (Abreu et al., 2014; de Araujo et al, 2019). However, what research that has been done has shown that they are able to persist through conventional sewage treatment systems and enter the environment. Benzodiazepines have been found in wastewater, drinking water, surface water, and groundwater at concentrations ranging from as low as 0.14 ng/L all the way up to 840,000 ng/L (Chen et al., 2021). Benzodiazepine drugs, such as Diazepam, have also been shown to be stable in seawater, where it can be rapidly taken up and accumulated by benthic filter feeders such as oysters, though they are able to quickly eliminate it once exposure stops (Gomez et al., 2012). These drugs have also been shown to decrease the stress response in zebrafish, which can result in increased mortality in fish due to the inability to react properly to stressors such as predation (Abreu et al., 2014).

SUNSCREENS UV-filters, the active ingredients in sunscreens, are the most common personal care products found in environmental sediments (Semones et al., 2017). Oxybenzone, a common UV-filter used in many sunscreens as well as soaps, lip balms, hair products, and more has been shown to be an Endocrine Disruptor (ED) in multiple organisms, including corals, and was identified as the causative agent of estrogenic activity in infaunal organisms near wastewater outfalls (e.g., sites where wastewater drains to a larger body) (Downs et al., 2016). Oxybenzone containing sunscreens have also been banned from multiple tropical tourist destinations due to their potential link to coral bleaching (Narla & Lim, 2020). ED compounds have also been linked to human diseases such as prostate and breast cancer, altered thyroid activity, and alterations to both male and female reproductive systems. The effects of these compounds are believed to be more potent in aquatic organisms due to accumulation in the sediments (Omar, et al., 2018). Another common UV-filter that can act as an ED is Sulisobenzone. Oxybenzone and Sulisobenzone both have low rates of photodegradation as may be expected from their use as sunscreens, which helps contribute to their long half-lives in the environment (Semones et al., 2017). Sulisobenzone is more soluble in water than Oxybenzone, but both have been found in various waterways around the world in concentrations ranging from ng/L to µg/L (Semones et al., 2017).

HERBICIDES. It should be no surprise that herbicides can cause problems when released into the environment, after all they are designed to kill plants.

However, numerous studies confirm that plants are not the only organisms that can be harmed by herbicides in the environment. The herbicide Atrazine, another ED, is widely used in countries around the world, and can have a half-life of up to 1 year under the right environmental conditions (Jian Lu et al., 2021). It has been shown to impact the ornamentation and reproductive behavior of male guppies (*Poecilia reticulata)* at concentrations that have been found in nature ranging from ~20–200 parts per billion, and reduce fecundity in affected populations (Shenoy, 2012). Atrazine has also been associated with reduced ovarian growth in the estuarine crab species *Neohelice granulata* (Jian Lu et al., 2021). For future reference throughout this document, the phrase 'environmentally relevant' will refer to EOC concentrations, which have been observed in samples isolated from nature.

Numerous herbicides, in addition to Atrazine, cleared for both commercial and domestic use have been detected in waterways (Annett et al., 2014). For example, Glyphosate, the active ingredient in Roundup® is a common environmental contaminant. A popular herbicide in the United States and elsewhere since the 1980s, Glyphosate became the most used herbicide in the world in 2002 (Annett et al., 2014). The popularity of Glyphosate in commercial uses increased significantly with the development of "Roundup Ready®" crops which are resistant to this herbicide. The expiration of Monsanto's patent on Glyphosate also allowed for other herbicides to use it as their active ingredient, leading to multiple companies creating their own Glyphosate-based herbicides (Annett et al., 2014).

Glyphosate kills plants by interfering with the activity of the enzyme 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSPS), a necessary part of the shikimic acid pathway, a biosynthetic pathway only found in plants, and was believed to limit impacts on non-target organisms such as mammals or birds (Annett et al., 2014). Studies have shown, however, that Glyphosate can act as an ED in human cells and exposure in animals can lead to oxidative stress, double stranded DNA breaks, and inhibition of acetylcholinesterase all of which can be lethal (Annett et al., 2014; Gasnier et al., 2009). Chronic exposure to Glyphosate can also cause gill and liver damage in fish. In addition, many Glyphosate herbicides contain surfactants, which help the herbicide to cling to plants and cross the waxy cuticle, killing targeted organisms quickly. These surfactants can be a key factor in toxicity as they make it easier for organisms to absorb contaminants from the environment (Annett et al., 2014).

As more studies have established the potential toxicity of Glyphosate, the usage of this herbicide has become increasingly controversial. In 2015, Glyphosate was deemed a probable human carcinogen by the International Agency for Research on Cancer (IARC), and it has been banned or restricted in over 20 countries (Meftaul et al., 2020). Monsanto has also lost court cases regarding cancer being caused by Roundup®, including one in 2018 where they were ordered to pay out $289 million to the victim ("Monsanto Ordered to Pay US$289 Million to Cancer Patient: Company Vows to Appeal Decision.," 2018). Despite this, there remain questions about the actual toxicity of Glyphosate itself. Several studies, including some conducted by the United States Environmental Protection Agency (USEPA) found Glyphosate to have little to no toxicity in many higher animals (Meftaul et al., 2020). Additionally, other studies have found that a surfactant commonly used in Glyphosate products, polyethoxylated tallow amine, is significantly more toxic than Glyphosate itself, potentially indicating that the surfactants in the herbicide mixes may not be simply increasing the toxicity of Glyphosate but may be the cause of toxicity (Meftaul et al., 2020). Even if Glyphosate use were to stop today, the herbicide can persist in waterways for up to two months, and its primary degradation product, aminomethylphosphonic acid, can have a half-life of up to 985 days in waterways and has similar characteristics to Glyphosate (Feng et al., 2020).

ARTIFICIAL SWEETENERS The artificial sweetener Sucralose, known commercially as Splenda®, is approved for use in over 80 countries and is present in over 4000 products worldwide (Tollefsen et al., 2012). Sucralose is highly stable in acidic environments, and as a result over 90% of Sucralose that is ingested by humans is excreted unchanged and enters the wastewater treatment system. A study in Brazil looking at 5 different types of treatment plants found that four of the five were able to remove less than 20% of the Sucralose entering the system, with the fifth type of treatment plant still only removing 55% (Alves et al., 2021). This results in Sucralose entering the environment in concentrations of up to 119 µg/L; however, studies have shown that it bears no real threat to wildlife at concentrations of less than 1100 mg/L with many organisms showing no adverse effects even from concentrations significantly higher than that. Sucralose is important to study, however, because it is an artificial chemical and therefore can only come from human sources. Due to its stability and water solubility it can be used as a proxy to determine what environmental areas may be impacted by wastewater outflows (Alves et al., 2021; Tollefsen et al., 2012).

In summary, EOCs are a wide-ranging category of pollutants with an equally wide-ranging list of impacts on the environments in which they are found. Some of these compounds break down quickly in the environment, but are constantly being renewed, others can last for years before being broken down into other compounds, which then also must break down over time, potentially continuing to harm organisms for extended periods, even after their use has been reduced or even completely halted. These problems are amplified in urban areas where larger quantities of these chemicals enter the environment. Flow rates also impact the effects of EOCs, with restricted-flow environment showing greater negative impacts than fast-flowing water with a short residence time.

The Legacy of Anthropogenic Contaminants in Restricted Flow Environments

The impacts of coastal urbanization are similar to those seen at other sites where manmade structures impede flow/drainage. Pollutant buildup is a frequent and serious problem in systems with low drainage, such as the reservoir that forms behind a dam along a river or stream (Wang et al., 2019). Dams trap sediment and pollutants from upstream industrial areas for extended periods, with some dating back to the American industrial revolution (Cantwell et al., 2014). The pollutants trapped in the sediments behind these dams were often given little thought, until recently, with most research focusing on the sediments themselves (Cantwell et al., 2014). These sediments have been found to contain organic contaminants, including polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), which can be resuspended in the water column when the sediment is disturbed (Cantwell et al., 2014). This is analogous to the resuspension of muck in restricted lagoons and can have serious impacts on the downstream environment. One such example is the Niagara Mohawk dam which was removed from the upper Hudson River in 1973, releasing PCB-laden sediment downstream, resulting in these areas being designated as an USEPA superfund site, which was still being remediated over forty years after the event (Cantwell et al., 2014).

The Indian River Lagoon – A Case Study in Urbanization and Muck Accumulation in a Restricted Lagoon

The restricted flow encountered in urbanized coastal areas is a serious ecological concern, one that may ultimately introduce unintended risks to residents. It is therefore likely that bodies of water already subject to limited rates of water flow may experience these impacts more acutely. Manmade structures such as dams provide some insight into sediment and pollutant deposition in low-flow environments and suggest that the same should be true of coastal areas. Indeed, one of the many coastal areas affected by urbanization is Florida's Indian River Lagoon (IRL) (Figure 4), one of the most biodiverse estuaries in North America (Lapointe, et al. 2015). The 251 km long lagoon is located within a transition zone between temperate and subtropical biomes, allowing species from both environments to inhabit the area (Lapointe et al., 2015). This lagoon system is home to over 1.6 million people and continues to grow annually, with no projected end in sight (Bilskie et al., 2019). Exacerbating the impact of this heavily urbanized environment is the fact that the IRL is a restricted lagoon with only four inlets along its entire length. This results in an exceptionally low-flow rate for the large portions of the lagoon, which are not near these inlets (Colvin, et al., 2018). This low-flow rate causes suspended particles to fall out of the water column quickly. This can cause adjacent areas to be different in terms of sediment, especially for natural landscapes near urban ones. Additionally, the lagoon reaches a maximum width of only 4 km, with many sections $\leq$ 2 km, which limits fetch and wave buildup, allowing sediment to build up (Colvin et al., 2018).

Muck levels in the IRL have increased gradually from essentially none in 1950, to an estimated 5.3 million cubic meters, covering 1.63 km^2 of benthic habitat in the lagoon by 2016 (Foster et al., 2018). Major factors for this sediment deposition have been identified as poor soil retention in construction projects, agriculture, and other anthropogenic activities near the lagoon (Foster et al., 2018).

Muck removal has been widely regarded as an important component of IRL restoration and dredging is the most popular option, but there are still serious concerns about the potential threats of this approach. Decisions made about the relative safety of muck removal or allowing it to stay in the IRL, as well as repurposing the muck for other applications, requires a better understanding of the contaminants potentially associated with these sediments. *I hypothesize that IRL muck serves as a reservoir for Emerging organic contaminants* (*EOCs), concentrating these potentially harmful chemicals to levels well above the surrounding water.* In the present study, to test the hypothesis, I investigated the concentrations of several EOCs in the muck at specific sites in the IRL and examined the water directly above the sediment. Based on these results, and the use of existing monitoring data for the lagoon, I created a model to predict locations that are likely to contain high levels of EOCs. I also examined the Toxicity Units measurement used by ORCA to describe the danger each sample presents to its surrounding environment, and whether this toxicity unit can be used by itself to predict EOC contamination at a site. My findings may be able to help make informed decisions regarding the relative risk of specific sites for dredging.

Figure 4: Google Earth image of the Indian River Lagoon with pins marking the locations of its four inlets

Chapter 2: Mass Spectrometry based detection of Emerging organic contaminants (EOCs)

Introduction

Liquid Chromatography-Mass Spectrometry to Identify EOCs

The potential negative impacts of EOCs are extensive, ranging from reduced reproductive capacity and embryonic mortality to feminization and cancers in adults. However, knowledge of the impacts of various anthropogenic contaminants on the environment is of limited use without the ability to detect their concentrations readily and accurately within a given sample. Many EOCs have a negative impact on organisms at extremely low concentrations and require a method that can accurately detect them below these concentrations before they become a concern. Mass spectrometry, especially when coupled with liquid or gas chromatography, has become the most common tool for the detection and quantification of EOCs in environmental samples. Indeed, all the information in the previous section regarding the concentrations of EOCs in environmental samples was obtained by a mass-spectrometry (MS) technique.

Previous studies within the IRL have used mass spectrometry (MS) to identify and quantify rare earth elements (REEs) from groundwater collected from beneath and around the lagoon (Johannesson et al., 2011). It has also been used to quantify concentrations of perfluoroalkyl and polyfluoroalkyl substances from the plasma of dolphins living found in the IRL (Lynch et al., 2019). However, MS has not been used to search for EOCs within the lagoon, especially for those in muck, despite their potential as a reservoir for dangerous chemicals like EOCs. This study used liquid chromatography-mass spectrometry (LC-MS) to study EOCs in the Indian River Lagoon for the first time.

The detection of EOCs in environmental samples via mass spectrometry is typically facilitated by passing the dissolved sample through either a liquid or gas chromatography (LC or GC) column prior to entering the mass spectrometer (de Araujo et al., 2019; Lapworth et al., 2012; Palmer et al., 2008; Serra-Compte et al., 2018). Within the column, samples migrate at different rates depending on their interaction with the material comprising the column as well as the carrier solvent or gas. This allows complex mixtures to be separated so that only individual compounds reach the detector at a time (de Araujo et al., 2019; Serra-Compte et al., 2018). The ability of both LC-MS and GC-MS to effectively separate complex mixtures as well as detect low concentrations of organic compounds makes it an ideal technique for identifying EOCs in the environment (Tran et al., 2018). The limits of detection (LDs) for mass spectrometry vary based on the analyte, the method of ionization, as well as the detector, but they frequently exceed 5 ng/L (de Araujo et al., 2019).

Uniform methods for the isolation of EOCs have proven challenging until recently, due to the introduction of solid phase extraction (SPE) strategies. SPE strategies, which are designed to “clean up” and concentrate environmental samples, also remove common contaminants in organic extractions, like heavy metals, that may damage a GC/LC-MS or interfere with the results (de Araujo et al., 2019). This procedure allows for samples several hundred milliliters in volume to be concentrated to just one or two milliliters. By concentrating samples 2-3 orders of magnitude, it makes detection of the materials present in those samples at low concentrations significantly easier. The SPE process is relatively simple and allows multiple samples to be prepared for analysis simultaneously. This permits even complicated and potentially toxic samples, such as muck from the IRL, to be safely run on a mass spectrometer.

Given the potentially high heterogeneity between the muck samples, I selected an SPE-based cleanup followed by LC-MS for the identification of potential EOCs in the muck samples provided by ORCA. After selecting a specific subset of EOCs, representing a variety of the categories mentioned in Chapter 1, I performed a series of sample extractions using muck samples spiked with known concentrations of these EOCs (Table 1). Extraction efficiencies were determined using pure compounds as reference standards. Finally, the LC-MS results of muck and water extractions are presented and their significance to the potential health hazards of muck are discussed.

Table 1: Table showing the effects of 7 EOCs of interest on flora and/or fauna in the environment.

Contaminant	Effects
Diazepam	CNS Depression, Decreased Stress Response
Sulisobenzone	Increased Estrogenic Activity
Oxybenzone	Increased Estrogenic Activity, Coral Bleaching/Mortality
Acetaminophen	Liver Damage, Increased Embryonic Mortality
Atrazine	Reduced Reproductive Capacity
Chloramphenicol	Oxidative Stress, Bone Marrow and Blood Disorders
Ibuprofen	Reduced Energy Storage, Weakened Membranes, Reduced Reproductive Capacity, Reduced Growth

Methods

Site Selection

In order to properly assess the EOC content in IRL muck, we sought an area which had not previously been subjected to extensive dredging, minimizing anthropogenic disruption at the site. However, the area in question must also have experienced on-going urbanization to ensure EOC accumulation was likely. The Sykes Creek area (Figure 3) is highly urbanized, except for the Ulumay Wildlife Sanctuary on the northern half of its eastern shore. The Sykes Creek area also has one of the lowest flow rates within the IRL and has not previously been subjected to muck dredging. These factors make it a prime site for the investigation of EOCs within the muck. One hundred muck and water samples from this area were collected by the Ocean Resource and Conservation Association (ORCA) over the summer of 2018 and made available for our analysis (Figure 5).

Extensive EOC analysis of 100 sites is both cost prohibitive and time intensive; we therefore sought to evaluate a subset of these samples. As our ultimate goal was to determine if there is a correlation between EOC and potential health threats, we limited our sample choices to 30 sites based on a bioluminescence toxicity assay previously performed by ORCA (Schiewe et al., 1985). In this technique, luminescence is inversely correlated with toxicity, providing a highly sensitive method for detecting samples with potential biological hazards. This assay has previously been used as a proxy for other harmful contaminants with the assumption that a higher level of contamination by various pollutants, like EOCs would result in a higher toxicity value. If this assumption was true, then we could expect the high toxicity sites to have higher quantities of EOCs present than low toxicity sites.

The 100 sites were then binned into three categories based on their relative toxicity to controls: low ($\leq$30%), medium (51-80%), and high ($\geq$90%) toxicity. We then selected 10 sites from each of these groups for our study for a total of 30 sample sites. These 30 sites are referred to throughout this paper by their original ID# from the full list of 100 samples, rather than being renumbered. Original ID#'s have been preserved for ease of referencing their location and other relevant data from the full profiles provided by ORCA.

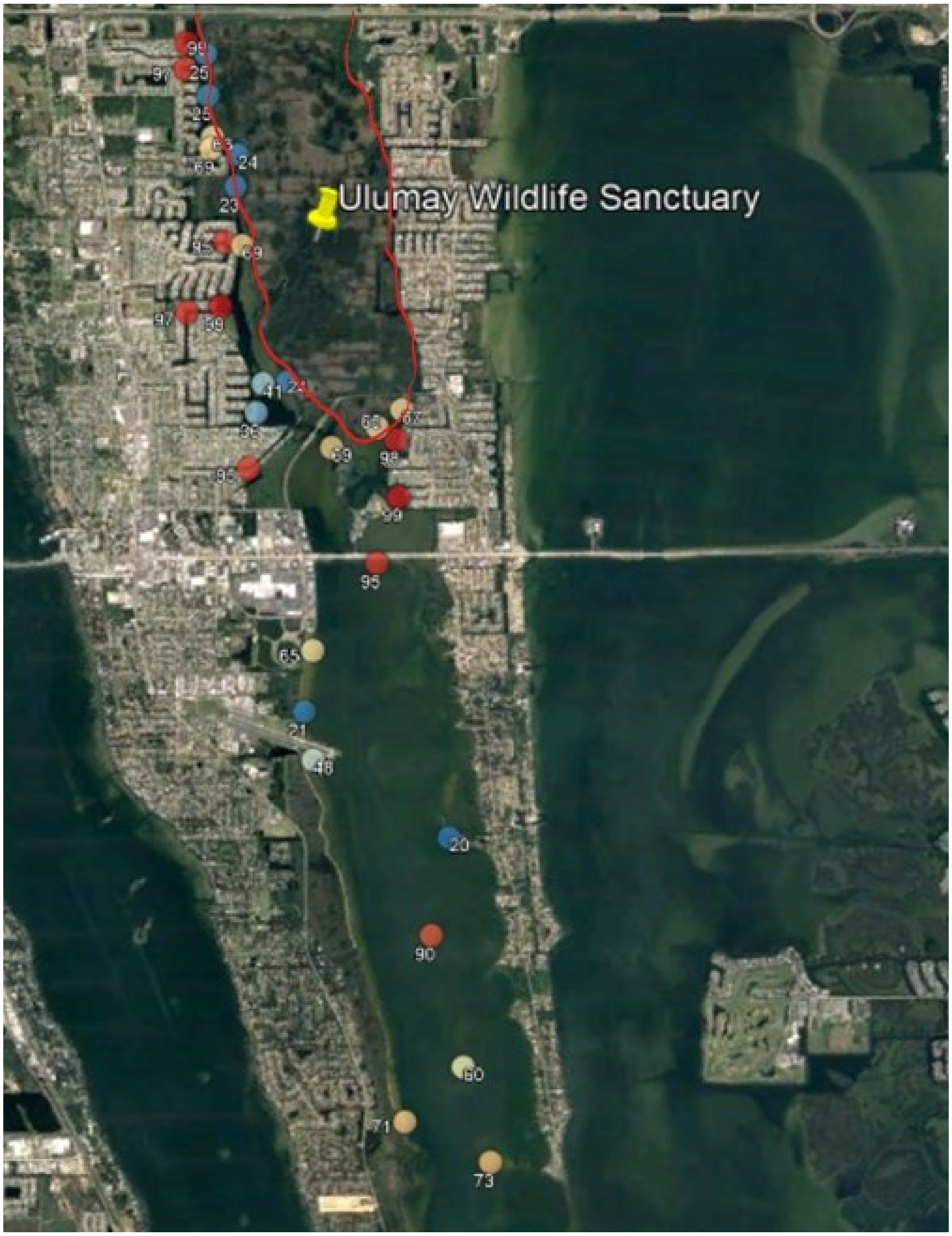


Figure 5: Google Earth image of the entire Sykes Creek study site marking the locations where each of the thirty samples used in this study were acquired. Sites are color coded according to the ORCA toxicity assay with warmer colors indicating higher toxicities. The nearby Ulumay Wildlife Sanctuary is marked with a pin and its approximate borders are outlined in red.

Solid Phase Extractions

Muck samples were retrieved from a -80°C freezer and allowed to thaw at room temperature before the entire sample was placed in a 250 mL beaker and massed (Figure 6A-B). After recording the mass of a sample, 100 mL of a 1:1 hexane (HPLC grade): ethyl acetate (HPLC grade) solution was then added to the muck (Figure 6C). A stir bar was added to the beaker and the beaker was placed on a stir plate and set to mix vigorously. In samples with coarser, and heavier grains of sediment that settled more solidly at the bottom of the beaker, 10-20 mL of dH_2O was added to loosen the sediment and allow the stir bar to stir the sample without getting stuck. The sample was mixed vigorously for another ten minutes on a stir plate (Figure 6D). Samples were then transferred into glass tubes and centrifuged at 2,000 RPM for five minutes to separate the hexane: ethyl acetate from the water and sediment in the sample (Figure 6E-F). The organic phase was then pipetted off and placed in a separatory funnel (Figure 6G). The remaining contents of the centrifuge tubes were emptied back into the beaker and the extraction steps repeated with 50 mL of hexane: ethyl acetate two additional times (Figure 6H). The organic fractions were combined in a separatory funnel, excess water was removed, and then evaporated to dryness with a rotary evaporator at 38°C (Figure 6I).

The dried sample was rehydrated in 200 mL of deionized water (dH_2O) for clean-up via solid phase extraction (SPE) using manufacturer instructions (Figure 6J). An Oasis HLB SPE filter cartridge was loaded onto an SPE manifold, and a vacuum pump was used to create suction. 1 mL of 100% methanol (HPLC grade) was used to wet the cartridge and allowed to sit for five minutes under low vacuum. After five minutes, the methanol was evacuated, and the cartridge washed with 1 mL dH_2O (HPLC grade). The sample was then loaded onto the cartridge and allowed to completely flow through, followed by the addition of 1 mL of 5% methanol (HPLC grade) in dH_2O (HPLC grade). This step removes chemicals which may be weakly bonded to the SPE cartridge but are not chemicals of interest. The waste was then removed and transferred to an aqueous waste container and a test tube was placed beneath the cartridge. 2 mL of HPLC grade methanol was added to the cartridge and allowed to mix for 3 minutes. The methanol was then eluted through the cartridge, placed in a glass vial, and stored at -20°C until the rest of the samples were complete.

Water samples were retrieved from a -20°C freezer and allowed to thaw. Once thawed, 200 mL of each sample was transferred to a centrifuge bottle and spun at 4700 RPM for 5 minutes to pellet solid debris from the samples. The change in speed (RPM) from the muck samples was due to the debris in the water samples requiring a higher spin rate to pellet out and remain at the bottom of the container as the water is added to the SPE column. The water samples were also centrifuged in larger containers than the muck samples, due to increased agitation of a sample that was all liquid. The entirety of each sample was then run through an Oasis HLB column in the same manner as the muck samples.

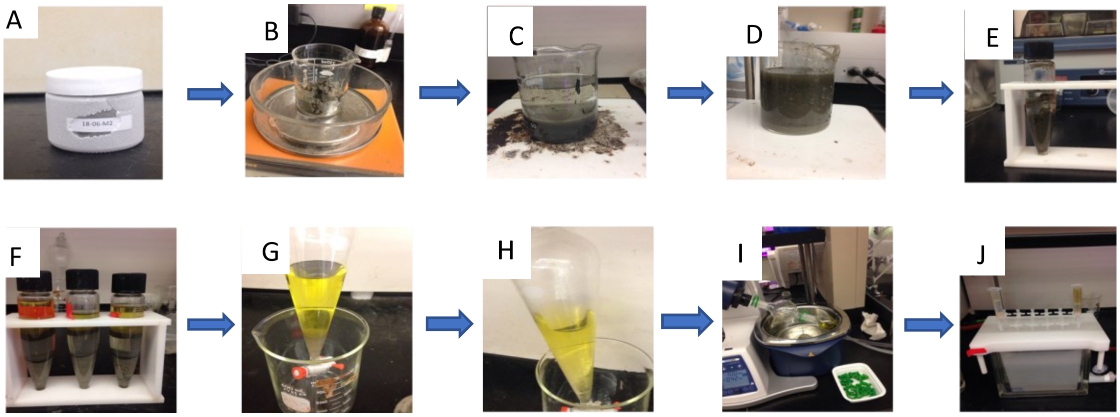


Figure 6: Flowchart showing the steps of the muck extraction procedure. **A.** Muck is taken from the -80^{oC} storage freezer and thawed. **B.** The muck is massed in a beaker. **C.** 50 ml each of hexane and ethyl acetate are added to the muck sample. **D.** The sample is mixed at maximum speed on a stir plate for 10 minutes. **E.** The sample is separated into six centrifuged tubes and centrifuged for 5 minutes at 2000 rpm. **F-G.** The hexane-ethyl acetate mixture rises to the top of the centrifuge tube and is pipetted off into a separatory funnel. **H.** The process is repeated twice more with 25 ml each of hexane and ethyl acetate which is pipetted into the same separatory funnel and any water or sediment in the funnel was removed. **I.** The hexane-ethyl acetate mixture is evaporated off in a rotary evaporator. **J.** The sample that is left behind is rehydrated in 200 ml of deionized water and run through a Solid Phase Extraction (SPE) procedure.

Liquid Chromatography-Mass Spectrometry

In order to estimate EOC concentrations in samples, standardization curves were made using pure samples of Acetaminophen, Chloramphenicol, Ibuprofen, Sucralose, Oxybenzone, Sulisobenzone, Atrazine, Glyphosate, and Diazepam. These standards were then run through liquid chromatography-mass spectrometry (LC-MS). The results from LC-MS runs with samples were then compared to the standards in order to determine whether any of these compounds were present in the sediments at the sample sites.

Standards (1 mg/mL) were prepared in HPLC grade methanol, with the exception of Diazepam, which was delivered as a 0.1 mg/mL standard. All standards underwent a series of 5 dilutions in methanol (HPLC grade) down to a concentration of 1 µg/mL and subjected to LC/MS for the formation of compound-specific calibration curves. Diazepam, Oxybenzone, Atrazine, Acetaminophen, and Sucralose still showed a relatively high number of counts at the lowest concentration and so underwent additional tenfold dilutions until each reached the detection limit of the machine. For Acetaminophen and Sucralose this was achieved with one extra dilution, while Diazepam, Oxybenzone, and Atrazine required two additional tenfold dilutions.

Samples were run through a 3 mm x 50 mm Agilent Poroshell 120 EC-C18 column and analyzed on an Agilent 1260 Infinity HPLC coupled to an Agilent 6120 quadrupole mass spectrometer. Samples were run through a 10-minute method using methanol and water as mobile phases. The method began with an 80:20 mix of water to methanol which then ramped to a 20:80 mix over the course of five minutes. The mobile phase remained at the 20:80 water/methanol mix for 3 minutes before switching back to 80:20 for the last two minutes. The mobile phase moved through the column at a rate of 0.5 mL/min.

In order to determine the recovery rate for the EOCs of interest in this study, three water samples and two muck samples were each spiked with 100 µl of each contaminant. The water samples were spiked with 100 µl of the 1000 µg/ml standards, except for Diazepam which was a 100 µg/ml concentration, and the muck samples were spiked with 100 µl of a 1 µg/ml concentration of each standard. The spiked samples were then run through the extraction process for either water or muck samples, as appropriate. This was then followed by a run through the LC-MS procedure and the recovery was calculated using the calibration curves created earlier.

Results

Developing Standard Elution Conditions and Evaluating Standards

The samples used in these experiments were taken from the relatively undisturbed bottom of Sykes Creek within the IRL, with water samples being taken from the water column directly above the sediment samples. The highly urbanized shoreline and multiple canals in the area likely lead to large amounts of pollutants entering the waterway, whereas the low flow rate of water in the Sykes Creek area means that the pollutants dwell in the area and have sufficient dwell time to settle out of the water; which provides a perfect environment for muck deposits to form. This highly urbanized shoreline is sharply contrasted by the largely preserved area of the Ulumay Wildlife Sanctuary on the eastern shore, which contains more sandy sediment and significantly less muck due to the low flow rate of the area not carrying pollutants to the opposite shore before they fall out onto the bottom. In fact, the sites along the Ulumay Wildlife Sanctuary have a maximum muck depth of 30 cm, well below the overall average depth of all sites, which was approximately 90 cm.

The specific LC-MS method used in this study successfully yielded unique retention times for each EOC noted in Figure 7 and Table 5. This ten-minute method used a solvent gradient mix of methanol and water to deliver individual samples to the quadrupole mass spectrometer using Electrospray Ionization (ESI) in positive mode. Knowing the retention times and the masses of these EOCs are reproducible under these conditions with this particular instrumentation supports the ability to identify the signature of these compounds in environmental samples and allows me to create calibration curves to help quantify any compounds found in those samples (Figures 8-15). From these standards, it was possible to determine the lowest concentrations at which each of the EOCs was detectable (Table 2).

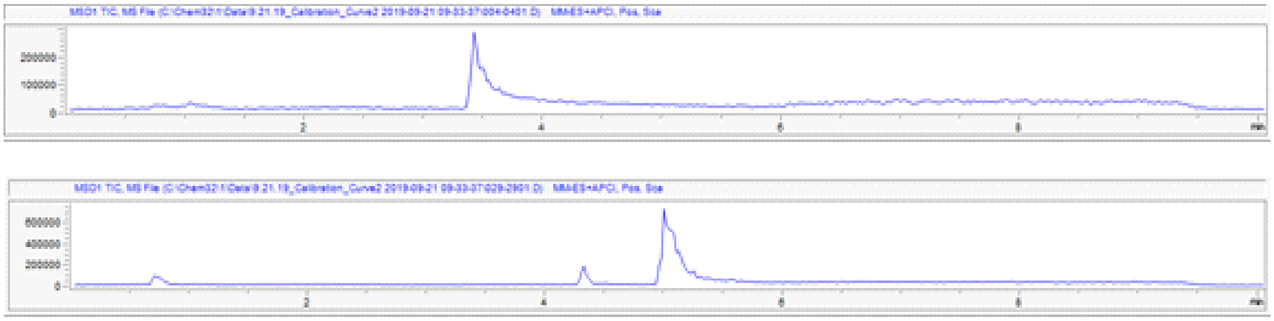


Figure 7: LC-MS chromatographs from 10-minute general method showing Chloramphenicol (top) and Atrazine (bottom). Retention time of each on the x-axis in minutes.

Table 2: All 8 EOCs included in this study. Retention times and mass to charge (m/z) ratios are shown for each of the nine EOCs of interest used along with the limit of detection (LOD) for each from the LC-MS protocol used.

EOC	Retention Time (min)	m/z	Class	LOD (ng/ml)
Acetaminophen	1	151	Pharmaceutical	10
Sucralose	2.5	397	Food Product	100
Sulisobenzone	3.25	229	Sunscreen	1000
Chloramphenicol	3.5	323	Pharmaceutical	1000
Atrazine	5	216	Herbicide	>10
Diazepam	5.6	290	Pharmaceutical	>10
Oxybenzone	6.25	229	Sunscreen	>10
Ibuprofen	6.5	161	Pharmaceutical	1000

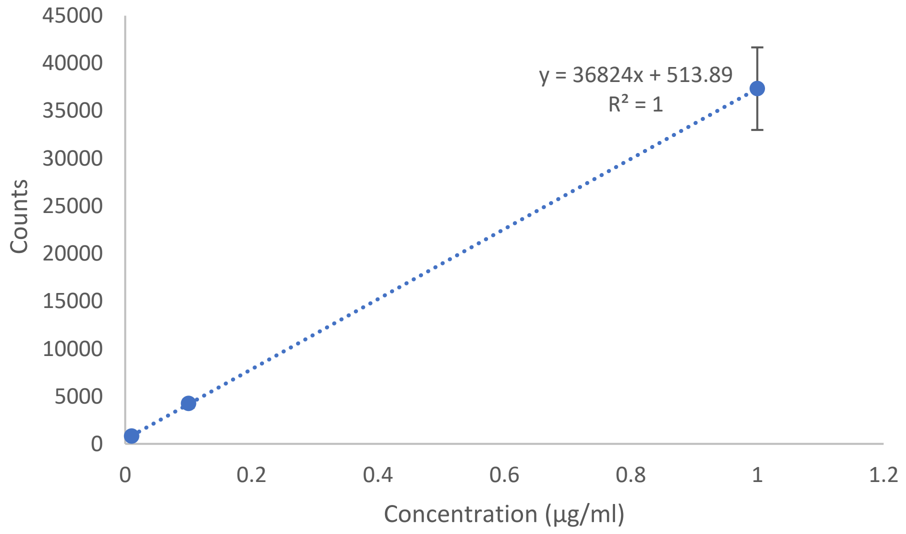


Figure 8: LC-MS calibration curve for Diazepam. Results are expressed as the average of three runs with error bars representing standard error.

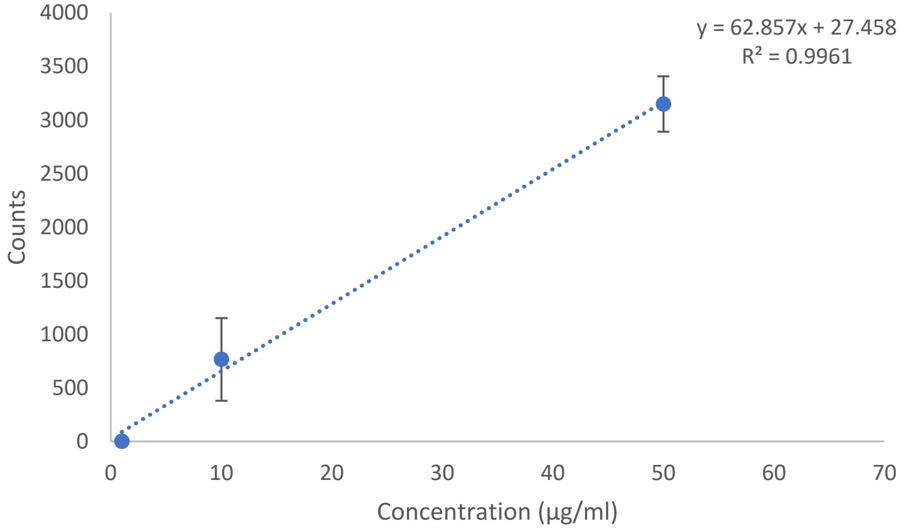


Figure 9: Calibration curve for Sulisobenzone created using average results from three runs with error bars representing standard error.

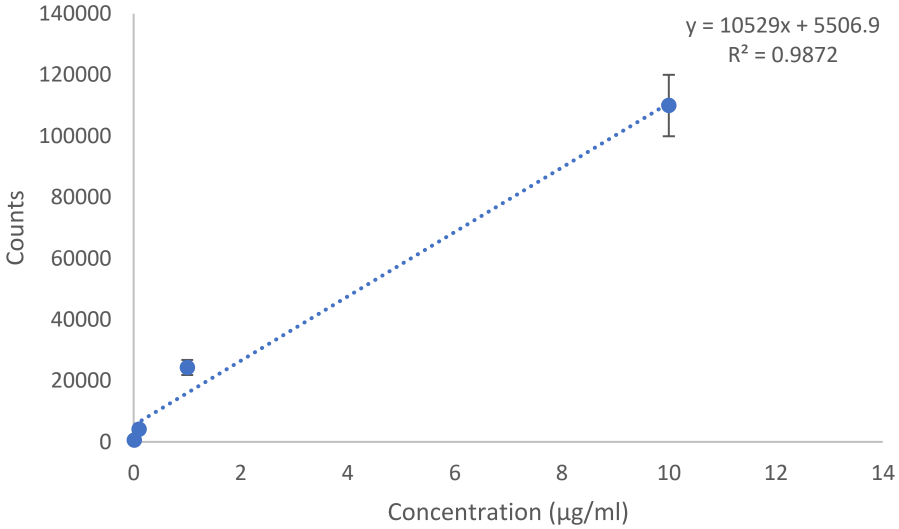


Figure 10: Calibration curve for Oxybenzone created using average results from three runs with error bars representing standard error.

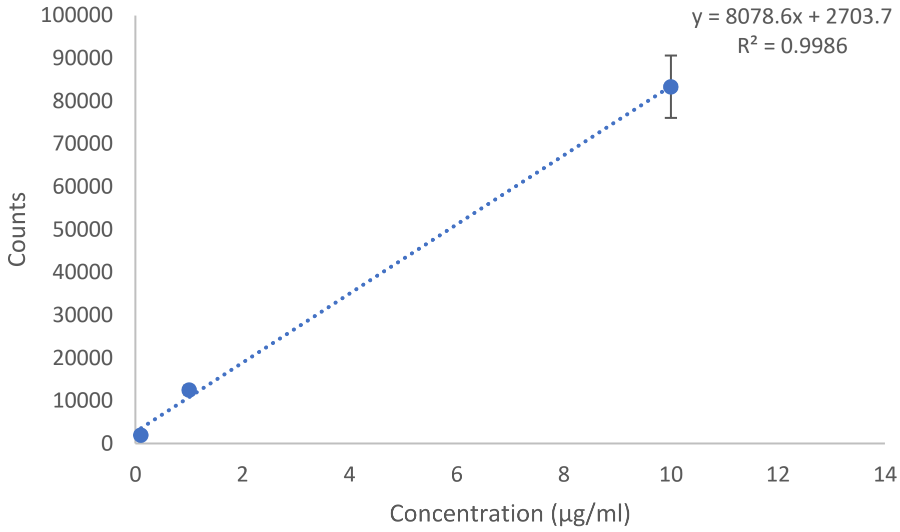


Figure 11: Calibration curve for Acetaminophen created using average results from three runs with error bars representing standard error.

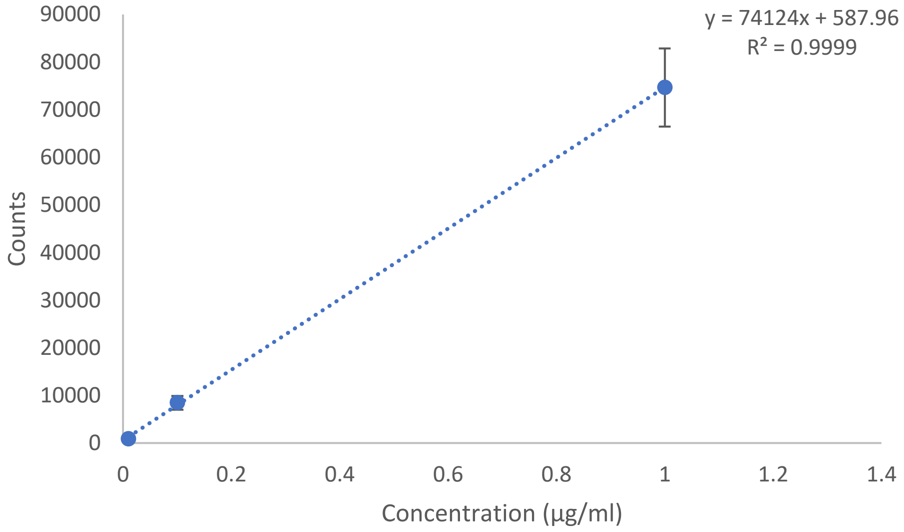


Figure 12: Calibration curve for Atrazine created using average results from three runs with error bars representing standard error.

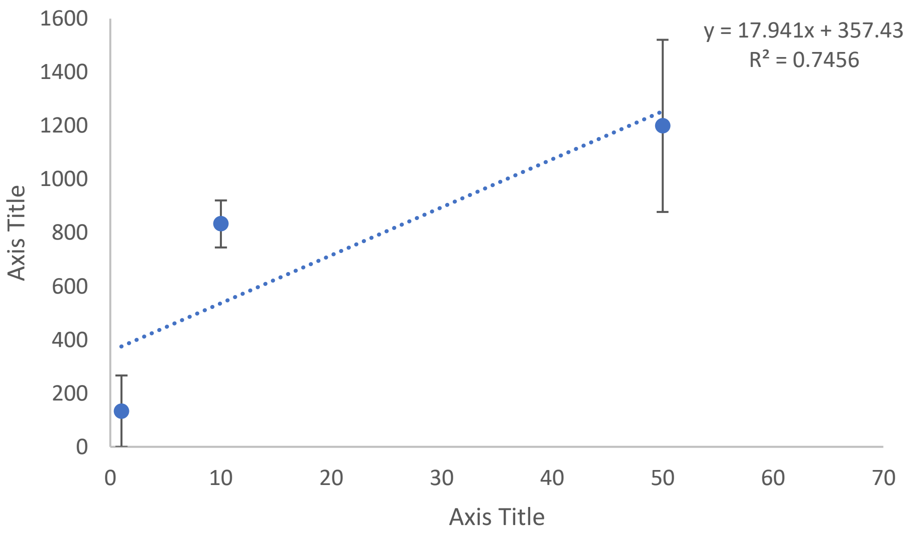


Figure 13: Calibration curve for Chloramphenicol created using average results from three runs with error bars representing standard error.

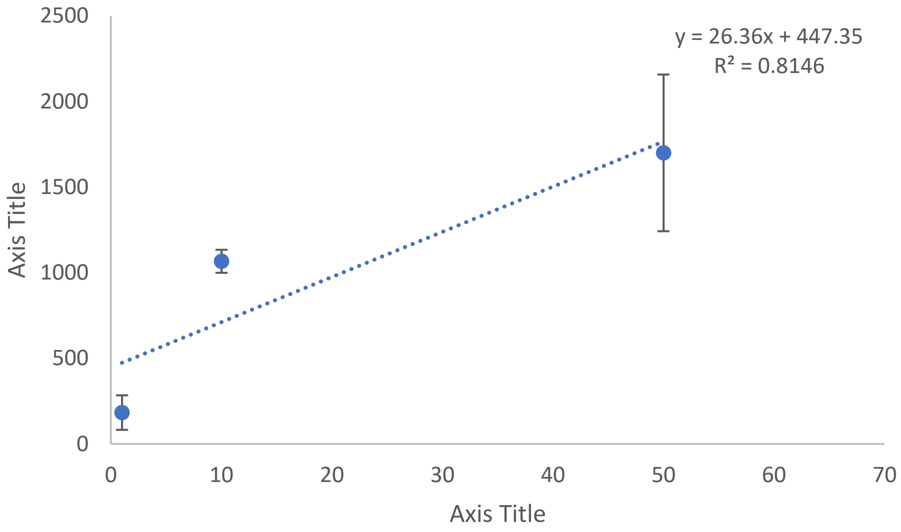


Figure 14: Calibration curve for Ibuprofen created using average results from three runs with error bars representing standard error.

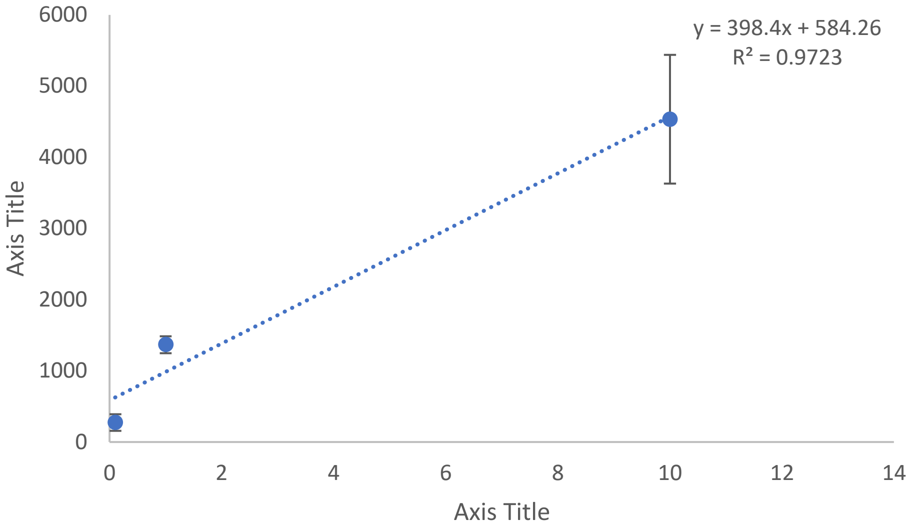


Figure 15: Calibration curve for Sucralose created using average results from three runs with error bars representing standard error.

Recovery of EOC Standards from Muck

The generalized method used for this study was not able to isolate all EOCs of interest from both muck and water samples. Oxybenzone could not be recovered from either muck or water samples. Atrazine could not be recovered from water samples, and Acetaminophen and Ibuprofen could not be recovered from muck samples. The average recovery rates of standards that could be recovered from the extraction and mass spectrometry processes ranged from 0.24% (Diazepam) to 1.26% (Ibuprofen) for water samples. This is in stark contrast to muck samples, which ranged from 6.89% (Diazepam) to 1,230% (Sulisobenzone), with Chloramphenicol also reaching a high recovery rate of 256% (Table 3). These results imply either existing contamination of these samples, an issue in the extraction process, or a problem with the mass spectrometry assay. Additionally, Glyphosate had recovery rates of 2.41% in water samples and 2,105% in muck samples. However, in Glyphosate's case this may be due to the difficulty that this chemical can present to detection, making it incompatible with the general LC-MS protocol run in this study (Ferrer et al., 2013; Hao et al., 2011). For this reason, Glyphosate readings from this protocol are not reliable and were excluded from the study. All of the muck standards did show recovery rates above the 1.26% maximum seen in the water standards, with Sucralose reaching over 90% recovery (Table 3). The water sample and muck sample standards for the recovery runs were at different concentrations from one another due to insufficient volumes of each standard at each individual concentration and insufficient chemicals to make more standards for all contaminants. Instead, all water samples were run with 1 mg/ml standards, except for Diazepam which was not available above 100 µg/ml, and all muck samples were run with 1 µg/ml standards.

Table 3: Recovery of EOC standards after either the water or muck extraction process. Original concentrations were 1000 µg/ml for water samples, except for Diazepam which was at 100 µg/ml. original concentration for the muck samples was 1 µg/ml. ND=Not Detected

Contaminant	Water Recovery (µg/ml)	Water Recovery (%)	Muck Recovery (µg/ml)	Muck Recovery (%)
Diazepam	0.238	0.238	0.069	6.89
Sulisobenzone	4.61	0.461	12.3	1230
Oxybenzone	ND	ND	ND	ND
Acetaminophen	7.62	0.762	ND	ND
Atrazine	ND	ND	0.43	43
Chloramphenicol	9.55	0.955	2.56	256
Ibuprofen	12.58	1.26	ND	ND
Sucralose	7.3	0.73	0.905	90.5

Detection of EOCs in Samples

Based on the calibration curves developed, concentrations for each contaminant of interest were detected in water samples (Table 4). In addition to Oxybenzone and Atrazine, which were not recovered from samples, Acetaminophen and Sucralose were not found in any samples and Ibuprofen was only found in 2 (Table 4). The total concentration of EOCs in each site ranged from about 6,1851 ng/ml at site 51 to about 30,460 ng/ml at site M42 (Figure 16). Diazepam and Chloramphenicol were the only pharmaceuticals detected consistently throughout the study, with Chloramphenicol typically being found in much higher concentrations, averaging over 7,500 ng/ml per site, while Diazepam averaged just under 260 ng/ml. (Table 4). The two most abundant contaminants were the sunscreen and ED Sulisobenzone and the antibiotic Chloramphenicol (Figure 17).

After site M42 the next highest concentration of EOCs were found at site M7. Site M7 had the highest concentration of Chloramphenicol in the study, with over 19,000 ng/ml of the antibiotic present in the water. Site M42 had the highest concentration of the other dominant water EOC, Sulisobenzone, with a concentration of more than 15,500 ng/ml. These two sites are both located in fairly open areas away from the shoreline or urbanized areas (Figure 18).

As for the muck samples, there was a significant Sucralose spike of nearly 25 ng/ml at sample site M21, the highest concentration of any individual EOC outside of Chloramphenicol in the muck samples (Table 5; Figure 17). At the same time, Sucralose was only detected in two sample sites (Table 5). Additionally, Acetaminophen was not detected at all and despite having the highest average concentration of any individual EOC in muck samples Chloramphenicol was detected in only 16 samples (Table 5). The total EOC load in the muck samples ranged from 2.15 ng/ml at site M16 to 448.3 ng/ml at site M31 (Figure 16). The majority of the EOC load in sample M31, and several other sites including M68, the site with the second highest concentration of EOCs, is carried by Chloramphenicol (Figure 17; Table 5). Unlike the water samples with the highest

EOC concentrations both samples M31 and M68 are near urbanized shorelines, with M68 being inside of a canal (Figure 19). The EOC with the next highest average concentration in the muck samples was Sulisobenzone, though Sucralose was present at a relatively high concentration at site M21 (Figure 17).

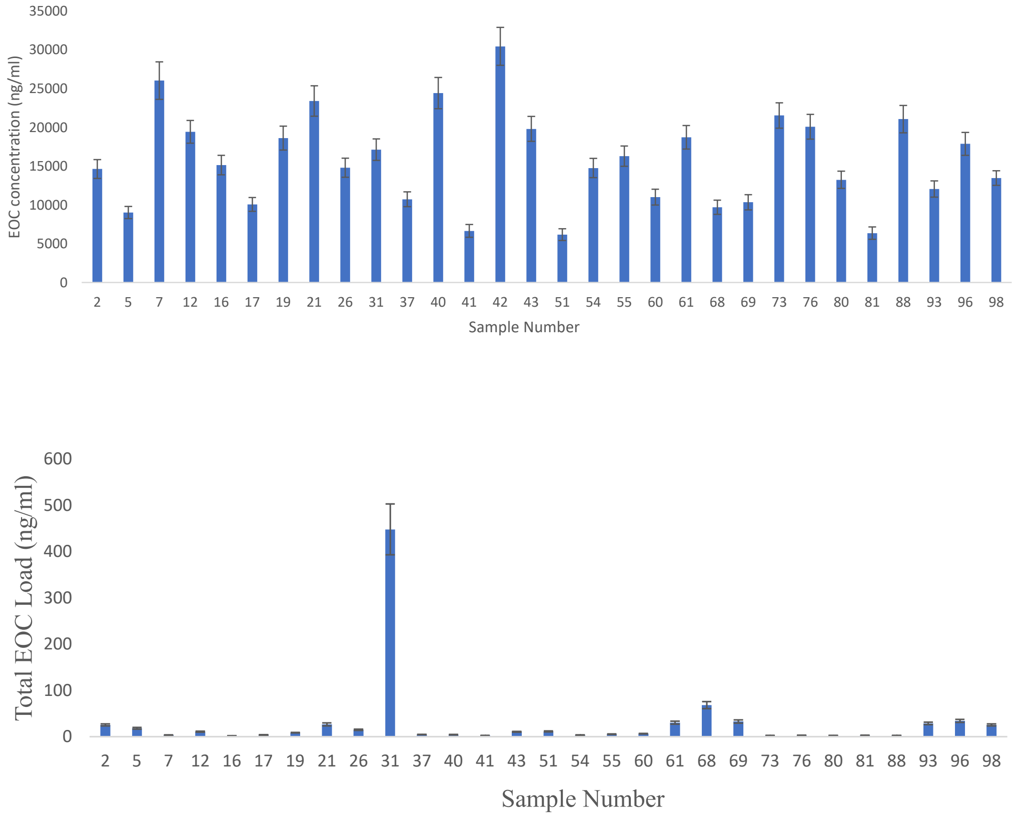


Figure 16: Total number of counts of all eight EOCs measured at each study site in Sykes Creek. The top graph represents data from water samples, and the bottom graph represents data from muck samples. Error bars represent the standard error of three LC-MS runs.

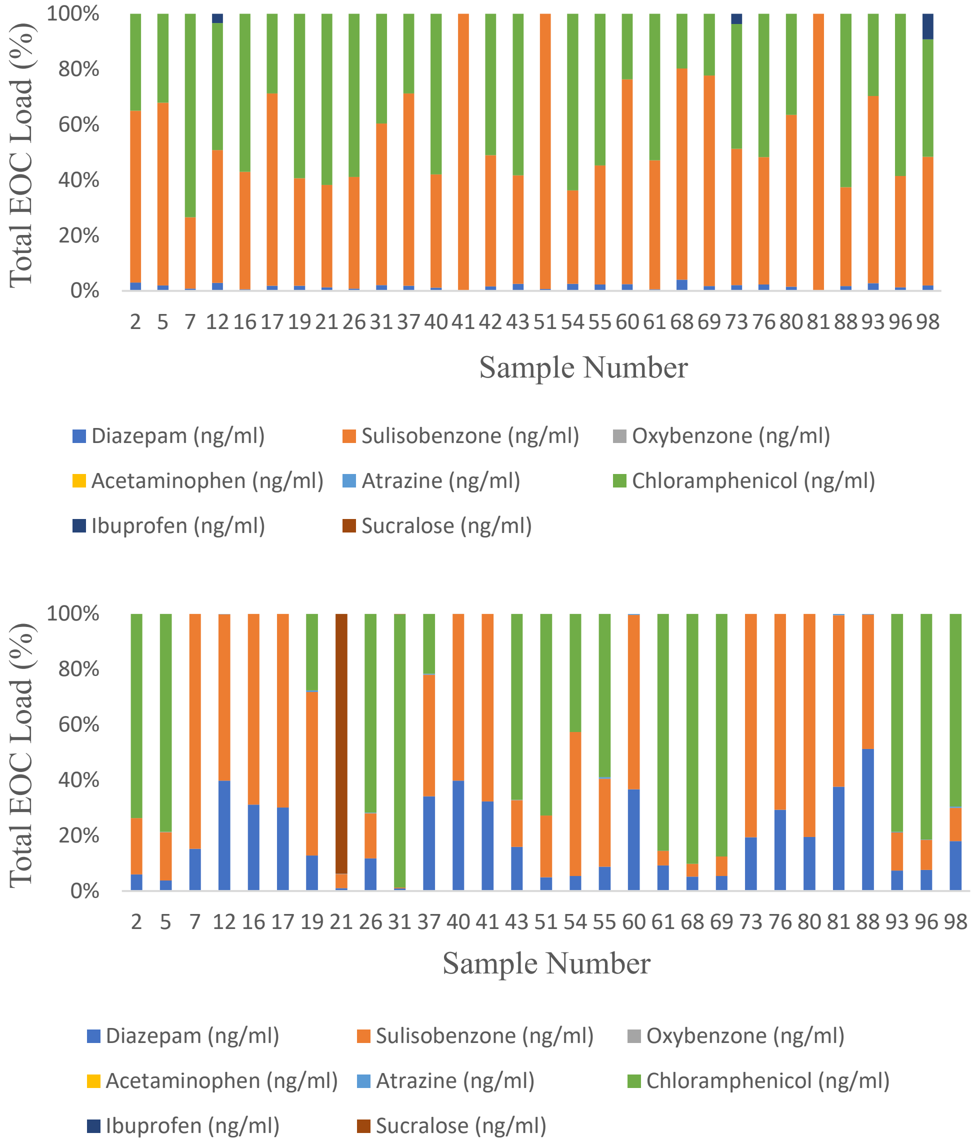
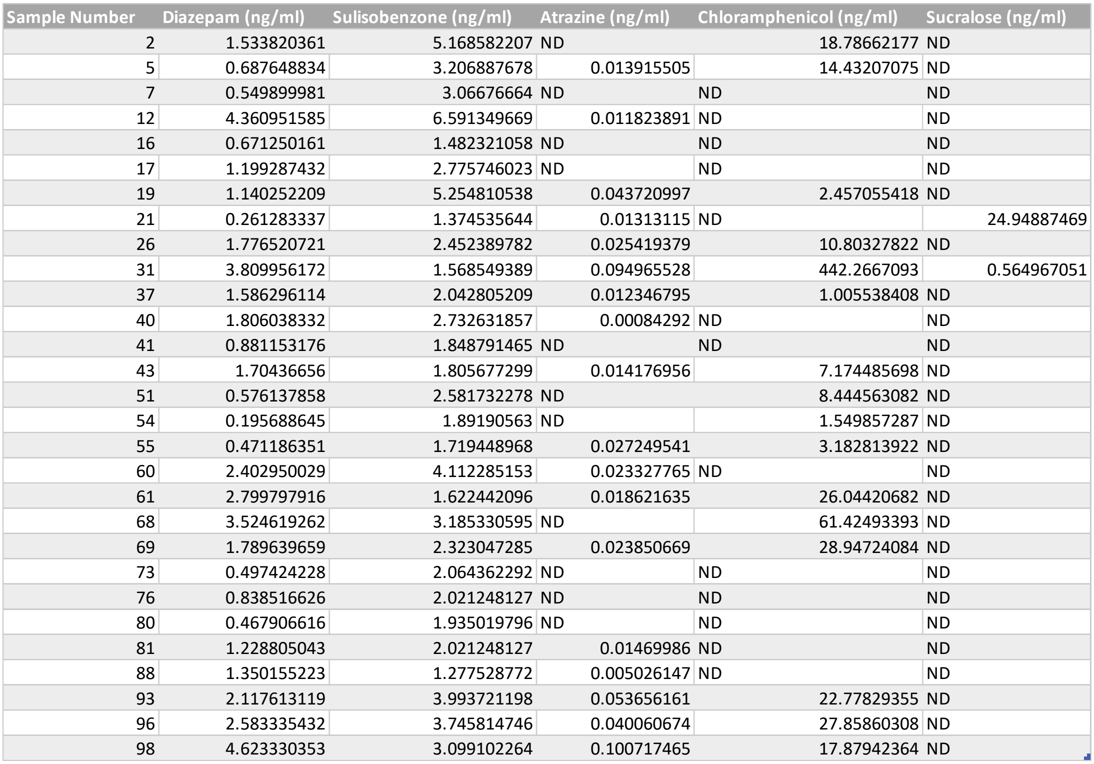


Figure 17: Relative abundance of EOCs in the water at each site. Stacked bar graph showing total contaminant load at each site and the amount of that total made up of each individual EOC. The top graph represents data from water samples, and the bottom graph represents data from muck samples. Sulisobenzone and Chloramphenicol dominate each water sample, and most muck samples, though Diazepam and Sucralose have a significant presence in several of the muck samples.

Table 4: Concentrations of EOCs measured in water samples taken from the Sykes Creek area of the IRL. EOCs which were not detected in any sites have been removed from the table. ND=Not Detected

Sample Number	Diazepam (ng/ml)	Sulisobenzone (ng/ml)	Chloramphenicol (ng/ml)	Ibuprofen (ng/ml)
2	444.7450485	9073.988611	5133.269385	ND
5	171.2971764	5968.085279	2895.957304	ND
7	195.8131925	6715.802748	19140.7885	ND
12	561.667587	9304.055524	8926.97248	643.0770233
16	86.43404362	6428.219106	8635.149165	ND
17	186.3839555	7003.386389	2895.957304	ND
19	333.4800523	7233.453303	11067.01012	ND
21	278.7904778	8671.371512	14471.61546	ND
26	114.7217545	5968.085279	8732.423603	ND
31	361.7677632	9994.256264	6786.934837	ND
37	190.1556503	7463.520216	3090.50618	ND
40	271.2470883	9994.256264	14179.79215	ND
41	7.228453062	6658.286019	ND	ND
42	495.6629282	14423.04435	15541.63429	ND
43	508.8638599	7751.103858	11553.38231	ND
51	44.94540095	6140.635464	ND	ND
54	369.3111528	4990.300897	9413.344672	ND
55	369.3111528	7003.386389	8926.97248	ND
60	261.8178513	8153.720957	2604.133989	ND
61	105.2925176	8728.88824	9899.716863	ND
68	393.8271689	7406.003488	1923.21292	ND
69	180.7264133	7866.137315	2312.310674	ND
73	433.4299642	10626.94028	9705.167987	793.8570991
76	461.7176751	9246.538796	10386.08905	ND
80	199.5848873	8211.237685	4841.44607	ND
81	ND	6370.702377	ND	ND
88	352.3385262	7521.036945	13207.04777	ND
93	327.8225101	8153.720957	3576.878372	ND
96	233.5301404	7175.936575	10483.36349	ND
98	259.9320039	6255.668921	5716.916015	ND

Table 5: Concentrations in muck of EOCs of interest measured in 30 sites from the Sykes Creek area of the IRL. EOCs which were not detected in any sites have been removed. ND=Not Detected



Sample Number	Diazepam (ng/ml)	Sulisobenzone (ng/ml)	Atrazine (ng/ml)	Chloramphenicol (ng/ml)	Sucralose (ng/ml)
2	1.533820361	5.168582207	ND	18.78662177	ND
5	0.687648834	3.206887678	0.013915505	14.43207075	ND
7	0.549899981	3.06676664	ND	ND	ND
12	4.360951585	6.591349669	0.011823891	ND	ND
16	0.671250161	1.482321058	ND	ND	ND
17	1.199287432	2.775746023	ND	ND	ND
19	1.140252209	5.254810538	0.043720997	2.457055418	ND
21	0.261283337	1.374535644	0.01313115	ND	24.94887469
26	1.776520721	2.452389782	0.025419379	10.80327822	ND
31	3.809956172	1.568549389	0.094965528	442.2667093	0.564967051
37	1.586296114	2.042805209	0.012346795	1.005538408	ND
40	1.806038332	2.732631857	0.00084292	ND	ND
41	0.881153176	1.848791465	ND	ND	ND
43	1.70436656	1.805677299	0.014176956	7.174485698	ND
51	0.576137858	2.581732278	ND	8.444563082	ND
54	0.195688645	1.89190563	ND	1.549857287	ND
55	0.471186351	1.719448968	0.027249541	3.182813922	ND
60	2.402950029	4.112285153	0.023327765	ND	ND
61	2.799797916	1.622442096	0.018621635	26.04420682	ND
68	3.524619262	3.185330595	ND	61.42493393	ND
69	1.789639659	2.323047285	0.023850669	28.94724084	ND
73	0.497424228	2.064362292	ND	ND	ND
76	0.838516626	2.021248127	ND	ND	ND
80	0.467906616	1.935019796	ND	ND	ND
81	1.228805043	2.021248127	0.01469986	ND	ND
88	1.350155223	1.277528772	0.005026147	ND	ND
93	2.117613119	3.993721198	0.053656161	22.77829355	ND
96	2.583335432	3.745814746	0.040060674	27.85860308	ND
98	4.623330353	3.099102264	0.100717465	17.87942364	ND

Discussion

The EOCs selected for this study cover a range of organic contaminants commonly found in waterways around the world (Lapworth et al., 2012; Serra-Compte et al., 2018; Voigt et al., 2020). They include the herbicide Atrazine, sunscreens such as Sulisobenzone and Oxybenzone, the antibiotic Chloramphenicol, Diazepam, the anxiety medication, pain relievers Acetaminophen and Ibuprofen, and the artificial sweetener Sucralose. These contaminants typically show up in waters and sediments in the environment at concentrations ranging from ng/L (ppb) to µg/L(ppm) (Lapworth et al., 2012; Voigt et al., 2020). For example, Oxybenzone has been found in waters off the US Virgin Islands at concentrations ranging from 75-1400 ng/L, well within the range where it can cause death in corals, determined to be between 8 and 3100 ng/L depending upon conditions and species of coral (DiNardo & Downs, 2018). Atrazine has been found in Great Lakes sediments at concentrations of up to 1.7 ng/g and in Chinese lakes up to 171 ng/g with negative impacts in aquatic life appearing at concentrations as low as 20 ng/L once it is in the water (Qu et al., 2017). Many of the EOCs examined in this study do not fall into the ranges that were found in previous studies. One of the potential reasons for this is the poor recovery rates obtained in this study, especially in the water samples which only reached above 1% recovery for a single EOC. Additionally, out of the eight EOCs and two sample types two were not recovered at all from water samples and three were not recovered at all from muck samples, with Oxybenzone not being recovered from either sample type. The technique was used in order to make the most of a limited amount of samples, but these poor recovery rates suggest that the general, “one-size-fits-all”, extraction and quantification technique used here is far from ideal.

The Atrazine levels found throughout the study site in the muck seems to be just below the range of Atrazine levels seen elsewhere in the world, which has been reported to range from 20-200 ng/L with an average concentration of 18.54 ng/L (Table 5) (Shenoy, 2012). Many of the samples with higher Atrazine concentrations, such as the muck samples for sites M31 and M98 are also near residential housing and may therefore be caused by homeowners using the chemical to kill weeds on their property and having excess Atrazine runoff into the water.

The amounts of both Sulisobenzone and Chloramphenicol identified in the study area are incredibly high when compared with concentrations found in other water bodies in previous studies. Sulisobenzone in water samples averaged 7,883 µg/L in this study (Table 4) but only reached a maximum concentration of just 4.8 µg/L in previous studies (Beel et al., 2013). Similarly, Chloramphenicol had a maximum concentration in previous studies of 15.6 µg/L (Marson et al., 2021), while in this study it averaged 7,534 µg/L in water samples (Table 5). As for the muck samples, Sulisobenzone averaged only 2.72 µg/L and Chloramphenicol averaged 23.97 µg/L, both significantly lower than in the water (Table 5). It is possible that this large discrepancy in concentrations is due at least in part to the nature of the Sykes Creek area. Both of these chemicals have potential half-lives of over one year and with the restricted flow of the Sykes Creek area chemicals that enter the environment there will remain for a long period of time and can build up to high concentrations if the chemicals are continuously added to the system (Semones et al., 2017; Xiao et al., 2021). Additionally, Sulisobenzone would be expected to be found in water more than in sediments due to its high water solubility of 250,000 mg/L, though this result is unexpected for Chloramphenicol, which has a much lower water solubility of 2500 mg/L (Jithan et al., 2008; Semones et al., 2017). However, when looking at just the muck EOC data Chloramphenicol is found in significantly higher concentrations than Sulisobenzone, which would be expected based upon their water solubilities.

The range of Benzodiazepine, including Diazepam, concentrations found in waterways previously stretches from as low as 0.14 ng/L up to 840 µg/L (Chen et al., 2021). In this study, the average concentrations ranged from 1.64 µg/L in the muck samples up to 273.4 µg/L in the water samples (Table 4; Table 5). Diazepam has also been shown to have a Bioconcentration Factor (BCF) of up to 927, meaning that it has been found in organisms at concentrations up to 927 times higher than in the surrounding environments (Chen et al., 2021). With the Diazepam concentrations found in the water in this study being well above the 12 µg/L shown to cause negative impacts in fish, it is likely that organisms in the Sykes Creek area are at risk of negative effects resulting from the bioaccumulation of Diazepam from the environment around them (Chen et al., 2021). Like many of the EOCs in this study, Diazepam is found in statistically significantly higher concentrations in the water samples than in the muck samples.

Ibuprofen was found in only two samples total in this study, the water samples for sites M12 and M73. The average concentration of these two sites was 718.5 μg/L (Table 4). Neither of these sites are especially near to any urbanized areas. Previous studies had found Ibuprofen levels in untreated wastewater in Europe of up to 143 μg/L, though it was found in concentrations of only several μg/L in surface waters (Moro et al., 2021). It is possible that these two sites have significantly more Ibuprofen than other surface waters due to the nature of Sykes Creek as a restricted waterway allowing concentrations of contaminants to build up beyond levels seen in freely flowing waterways. However, the low water solubility of Ibuprofen, which is only 21 mg/L, makes it unlikely that Ibuprofen would stay in the water column for an extended period of time. Like Diazepam previously, Ibuprofen is known to bioconcentrate and bioaccumulate in organisms (Moro et al., 2021; Serra-Compte et al., 2018). Substances that bioaccumulate like Ibuprofen or Diazepam can get sequestered in aquatic organisms and are then brought into the sediments by those organisms after they die and begin to decompose, though this may be in different areas than where the contaminant was picked up depending upon movement, predation, fishing, etc.

The average Sucralose concentration in the muck samples was 0.88 μg/L, though the average of the only two sites where Sucralose was detected, sites M21 and M31, was over 12 μg/ml (Table 5). Previously Sucralose concentrations of up to 119 µg/L have been recorded in aquatic environments, which is well above the levels found in Sykes Creek (Alves et al., 2021). Due to the fact that Sucralose is freely soluble in water, it was expected to be present in water samples more than in muck samples, however it was not detected in water samples at all. With only two sites having detectable levels of Sucralose in the muck samples it may be possible that Sucralose is only present in pockets throughout the study site and that there was none in the water samples tested during this study. It is not readily apparent why site M21 has such a high concentration of Sucralose, but there is a triangular feature in the shoreline nearby that appears to be man-made. Behind this feature is a residential community; it is possible that this feature is associated with wastewater and/or runoff from this community and that is the source of the Sucralose.

Figure 18: Locations of two sites with the highest concentrations of EOCs in the water samples. Google Earth images zoomed in on site M42 (top) which contained the most EOCs and site M7 (bottom) which contained the second most. Site M7 is located in the center of the southern portion of Sykes Creek away from any shoreline.

Figure 19: Locations of two sites with levels of individual EOCs significantly above those found in other muck samples. Google Earth images zoomed in on site M68 (top) which contained the second highest concentration of EOCs in muck samples and site M31 (bottom) which contained the highest concentration. Chloramphenicol is the most common EOC in both sites.

Chapter 3: Modeling

Introduction

Sampling and testing benthic sediments across an entire estuary or nearshore area for contaminants is both expensive and time consuming. Creating a predictive model to track these contaminants can save both time and money, while still providing important information about where these pollutants may be present in order to focus efforts to identify and quantify them. Using mass spectrometry for EOCs may allow for the initial identification of a variety of contaminants in a study site that are required to construct a model. When combined with data on other parameters of the study site, the mass spectrometry data could be used to potentially create a model that may be able to predict which contaminants are present elsewhere based on the similarity of those other locations to the site used without necessarily requiring extensive sampling and analysis. The study site used for this project is nearly ideal due to the heavily urbanized area around Sykes Creek, where the samples were taken, also being adjacent to the Ulumay Wildlife Sanctuary which allows for a comparison between heavily urbanized shorelines and relatively natural shorelines. In addition, the site has never been dredged and any muck or other sediment deposits located within the site have not been significantly disturbed.

Modeling is a common method for visualizing and assisting in the analysis of multi-variate datasets. Models organize data into a mathematical equation that can be used to make predictions about what is occurring in other locations that are similar to the site used to create the model in question. However, much of the modeling done on aquatic environments focuses on wildlife or the water column with less effort being put forth to study the sediment even in highly polluted environments (Heuner et al., 2016; Zimmer-Faust, Brown, & Manderson, 2018). Modeling efforts in estuarine environments have seen little attention paid to the sediments (Barile, 2018; Buzzelli et al., 2012). Models previously made of the IRL typically focused on algae or seagrass. Barile (2018) used data on macroalgae growth to trace sewage pollution in the lagoon. Buzzelli et al. (2012) used data following hurricanes passages in 2004 and 2005 to model responses of seagrass to varying water quality. Additionally, several models have been created to determine the behavior of the wind and the tides within the lagoon (Bilskie et al., 2019; Colvin, et al., 2018).

Tracking hydrocarbons in sediments can be used to create a model capable of differentiating their sources (Venturini et al., 2015). For example, Venturini et al., (2015) were able to separate sample sites into those which had aliphatic hydrocarbons from natural sources and those which came from human petroleum activities. Modeling can also be used to answer temporal questions such as how long it takes metal pollutants in sediments to lose enough bioavailability to be considered safe (Huang et al., 2019). This study measured the rate at which available metals transferred to unavailable forms in relation to depth. That data was then fed into a model specifically for the metals arsenic and cadmium and found that arsenic inputs would become safe after 15 years, while cadmium required 47 years.

This study aims to use modeling to improve our understanding of the muck in the IRL and potentially other restricted lagoons. Using Generalized Linear Models (GLMs) we seek to use the data collected previously by ORCA in conjunction with the data collected in this study to determine if it is possible to predict the presence of EOCs in muck samples. If the model is able to accomplish this, it will allow researchers and policymakers to find these potentially dangerous contaminates without spending the time and expense required for examining entire muck deposits.

Methods

Generalized Linear Model

Data previously gathered on nutrient and metal levels in muck and water from the Sykes Creek region of the IRL by ORCA were combined with data on organic contaminants and anthropogenic bacteria collected in this study into a single CSV file for use in R (R Core team, 2021) (Tables 9&10). The data were then used to create a series of GLMs to search for relationships among the variables. The models used all parameters measured in the samples; after these initial models were created the least impactful variable from each model was removed. This process was repeated until each model reached its strongest state as indicated by its lowest Akaike Information Criterion (AIC) value. It was then noted which parameters showed significant p-values in the final models.

Testing Toxicity Units (TU)

Linear regressions were used in an effort to determine whether Toxicity Units (TU), the unitless measure from 1 to 100 used for the toxicity assay and determined by the relative loss of luminescence in a sample that has been spiked with bioluminescent bacteria compared to a nontoxic sample, was related to any of the other variables collected by ORCA. The same process was carried out using the total load of EOCs measured in this study in both the water and the muck.

Results

Modeling EOCs

To begin, a GLM was created for the concentration of each EOC in all water samples across the study site using water depth, concentrations of nitrate, nitrite, ammonia, ammonium, and phosphorus in the water. Each model was then refined as stated in the methods section, with the least impactful variable was removed for each iteration of the model (Table 6). This process was repeated with the muck parameters: total nitrogen, ammonia, total phosphorus, total sulfur, copper, lead, mercury, iron, and total organic carbon, as well as also including the toxicity value assigned to each sample (Table 6). Each model was then checked for any significant correlation between these variables and the concentration of the EOC in the water samples. This process was repeated with the EOCs quantified from the muck samples (Table 6). The parameters still included in the final version of each model are shown in Table 7 and Table 8, with variables that had a significant P-value shown in bold.

Table 6: Simplified summaries of GLMs showing Sulisobenzone concentration in water samples used as the response variable and modeled against parameters modeled by parameters measured in the water of each sample site (top) and by parameters measured in the sediment present at each sample site (bottom). The summaries show the version of each model with the lowest AIC value. TKN=Total Kjeldahl Nitrogen, TOC=Total Organic Carbon, TU=Toxicity Units

Coefficients	Estimate	Pr(>|t|)
Water Depth	280	0.11
Ammonia	9.2E4	2.3E-5
Total_Phosphorus	2.3E4	0.29
	AIC=522.59	

Coefficients	Estimate	Pr(>|t|)
TKN	0.787	0.807
Ammonia_Muck	82.8	0.147
Total_Phosphorus_Muck	-3.21	0.792
Total_Sulfur	-0.230	0.849
Copper	-53.3	0.650
Lead	251	0.684
Mercury	1.99E5	0.223
Iron	-0.455	0.650
TOC	-429	0.238
TU	19.6	0.418
	AIC=552.65	

Table 7: Table showing the ORCA parameters included in the final, best model for each EOC isolated from muck samples. Parameters written in bold had significant relationships with EOC concentrations. Dashes indicate where models could not be made due to insufficient EOC data. TKN=Total Kjeldahl Nitrogen, TOC=Total Organic Carbon, TU=Toxicity Units

EOC	**Water Parameter Model**	**Muck Parameter Model**
Diazepam	Water Depth, Ammonia	TKN, Ammonia, Phosphorus, Sulfur, Copper, Lead, Iron, TU
Sulisobenzone	Nitrate	TKN, Ammonia, Phosphorus, Sulfur, Copper, Iron, TU
Oxybenzone	-	-
Acetaminophen	-	-
Atrazine	-	-
Chloramphenicol	**Water Depth**, Nitrate	TKN, Ammonia, Phosphorus, **Sulfur**, Copper, **Mercury**, Lead, Iron, TOC, TU
Ibuprofen	-	-
Sucralose	Water Depth	TU

Table 8: Table showing the ORCA parameters included in the final, best model for each EOC isolated from water samples. Parameters written in bold had significant relationships with EOC concentrations. Dashes indicate where models could not be made due to insufficient EOC data. TKN=Total Kjeldahl Nitrogen, TOC=Total Organic Carbon, TU=Toxicity Units

EOC (Sample)	Water Parameter Model	Muck Parameter Model
Diazepam	Ammonia	TKN, Ammonia, Phosphorus, Sulfur, Copper, Lead, Mercury, Iron, TOC, TU
Sulisobenzone	Water Depth, **Ammonia**, Phosphorus	TKN, Ammonia, Phosphorus, Sulfur, Copper, Lead, Mercury, Iron, TOC, TU
Oxybenzone	-	-
Acetaminophen	-	-
Atrazine	Water Depth	TKN, **Ammonia**, Phosphorus, Sulfur, Iron, TU
Chloramphenicol	Nitrate, Ammonia	TKN, Ammonia, Phosphorus, Sulfur, Copper, Lead, Mercury, Iron, TOC, TU
Ibuprofen	Water Depth	Iron
Sucralose	**-**	**-**

The modeling data shown above indicates that only two of the EOC samples collected in this study can be potentially predicted using the water data collected by ORCA, with those two parameters being water depth and Ammonia (Table 7; Table 8). In addition, two total samples, one from water samples and one from muck samples, had significant predictors in the models for data that ORCA retrieved from the muck (Table 7; Table 8). Those two EOCs, Chloramphenicol from the water and Atrazine from the muck, had three total potential predictors; for Atrazine it was Ammonia from the muck, while for Chloramphenicol it was both Sulfur and Mercury (Table 7; Table 8).

Toxicity Data and Other Parameters from ORCA

The data provided by ORCA for the water samples taken at each site included water depth as well as the concentrations of: Ammonia, Ammonium, Nitrite, Nitrate, and Phosphorus. The data on the muck samples included muck depth at most sites as well as Nitrogen, Phosphorus, Ammonia, Sulfur, Copper, Lead, Mercury, Iron, and Organic Carbon for each sample. These data can be used to determine if there are any parameters here that correlate specifically to toxicity in the muck samples. The water data collected by ORCA showed no Nitrite or Nitrate in any of the selected sites. Ammonia and Ammonium only appeared in the water of site 42, a moderate toxicity site which contained muck with 69 Toxicity Units. Only phosphorus was detected in all water samples but does not appear to have any immediate correlation with TU, based upon the linear regression using the phosphorus and TU data. However, there was a great deal of data from the muck samples tested at each site. A linear regression run using the parameters gathered by ORCA from the muck found that Phosphorus, Sulfur, and the metals do not significantly contribute to toxicity, but Total Kjeldahl Nitrogen (TKN), Ammonia, and Total Organic Carbon (TOC) all showed a significant correlation to TU.

TU does not appear to have any significant relationship with the total EOC contamination of the water at any given site; however, Diazepam and Atrazine from muck samples do correlate to toxicity (Figure 22). This indicates that if a prediction about EOC pollution in general is to be made, it must use other information instead of, or in addition to, toxicity alone. The correlation does not seem to be reversable as TU was not a potential predictor for either Diazepam or Atrazine (Table 7; Table 8).

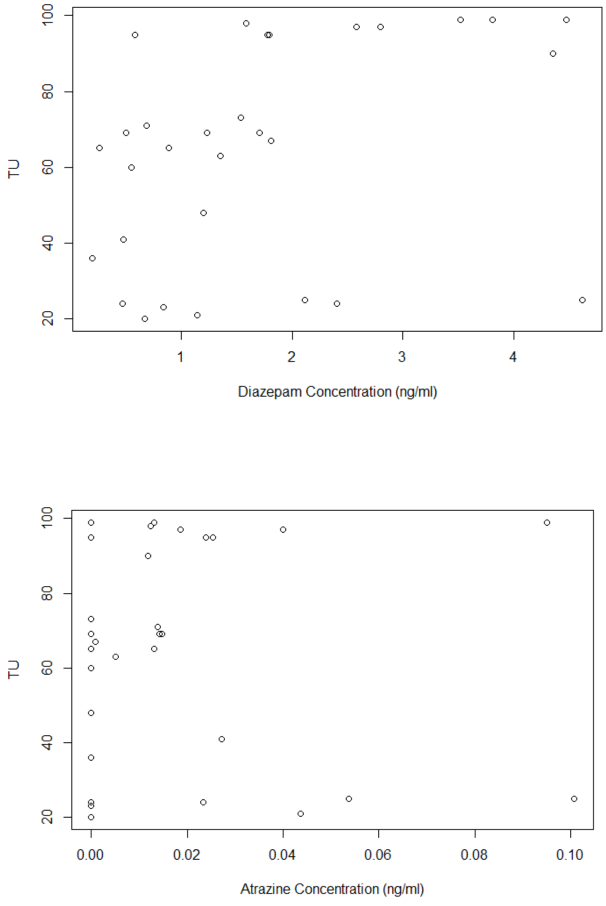


Figure 22: Image from RStudio showing the relationship between Diazepam from muck samples and Toxicity Units (Top) and the relationship between Atrazine from muck samples to Toxicity Units (Bottom)

Discussion

Based on the data collected and the modeling studies performed here, I conclude that EOCs overall are not a significant contributor to sample toxicity (toxicity units - TU). Many of the other pollutants that ORCA collected data on are also not significant contributors to TU, though they seem to have a stronger correlation overall than EOCs. However, three of the pollutants measured from the muck, TKN, Ammonia, and TOC, were shown to contribute significantly to toxicity in the sediment. It is also possible that TU may be impacted by EOCs or pollutants that were not included in this study in addition to the three identified here. TU itself was found not to significantly correlate to any of the water sample EOCs included in this study but did have a significant relationship with Diazepam and Atrazine from the muck samples. This seems to indicate that TU values are not a good general predictor of the EOCs in this study and that the majority of them do not correlate to this value.

The results from the GLMs created with the data from this study show that EOC presence can occasionally be predicted based upon other parameters that can be more easily measured in the environment. The models found two samples that could be predicted by a parameter measured in the water of the sample sites: one being Ammonia and the other being water depth. A possible explanation for this is that water depth can essentially indicate a basin where things can potentially ‘get stuck’. This is the reason many muck deposits form in areas of deeper water, which also makes it more likely that pollutants associated with the muck, or released from it into the water column, may be found in deeper water (Foster et al., 2018). Ammonia was also found to be a potential predictor of Atrazine from muck samples, Ammonia can occur naturally as organisms decompose, but can also be introduced into the environment by fertilizer runoff, a significant contributor to muck formation and deposition (Van Damme et al., 2018). Another parameter found to be significantly correlated to an EOC is sulfur. The main source of sulfur in aquatic systems is sulfur dioxide produced by bacteria through anaerobic respiration and is commonly associated with wastewater and sewage (Taheriyoun et al., 2019). However, there is also a high degree of anaerobic respiration associated with muck due to the low oxygen levels within and around muck deposits, which may also be a significant source of sulfur found in the study samples (Foster et al., 2018). The other parameter significantly correlated to an EOC is Mercury, which can be produced during mining activities or the combustion of fossil fuels and can travel long distances through the atmosphere before being absorbed into the soil or water (US EPA, 2014). The ability to link most of the parameters that correlate significantly with the EOCs in this study to muck deposits provides supporting evidence for the conclusion that the muck is sequestering these contaminants, and potentially acting as a source and releasing them into the water column. This conclusion is potentially compromised however by the concentrations of EOCs found in the water relative to those found in the muck in this study.

Chapter 4: Summary/Conclusion

Quantification of EOCs by LC-MS

This study used a general LC-MS procedure to identify all EOCs of interest at once from each sample. This method met with mixed success and was unable to identify Oxybenzone in either water and muck samples, Atrazine in water samples, or Acetaminophen or Ibuprofen in muck samples. The average total load of EOCs in water samples was 15,781 ng/ml, while muck sites averaged 29.22 ng/ml: about 540 times less than the water sample average, with water samples having most of their highest EOC loads in sites away from the shoreline, like samples M42 and M7, and muck samples having their highest EOC loads in sites that were near the shoreline or inside of canals, such as samples M68 and M31. This represents a very significant difference between the EOC content in the water and in the muck for which several possible explanations exist.

The first possible that some of the EOCs measured in the water had been in the muck and released into the water column by some disturbance before or during sampling. However, the low concentration of EOCs in the muck mean that this is an unlikely explanation. There is also the potential impact of Humic Acids, a type of organic matter created from decomposed organisms which can be found in muck and other sediments (Zhuan & Wang, 2020). Humic acids have been shown to have a variety of impacts on the rate of decomposition of organic contaminants (Koumaki et al., 2015; Zhuan & Wang, 2020). Humic Acids have been shown to both increase and decrease the degradation rates of multiple EOCs in various experiments (Koumaki et al., 2015; Rodríguez-López et al., 2021; Zhuan & Wang, 2020). This dichotomy seems to stem from the balance of Humic Acids acting as a photosensitizer, a substance that increases the sensitivity of another substance to photodegradation, and acting as a screen that blocks light from reaching another compound, although in some cases even if Humic Acids absorb light instead of an EOC they can still release additional radicals and speed up the degradation of other substances (Koumaki et al., 2015; Rodríguez-López et al., 2021; Zhuan & Wang, 2020). It is difficult to know the impact of Humic Acids on the EOCs in the muck of Sykes Creek without having specifically tested for it, but it is possible that these substances may be increasing the degradation of EOCs in the muck and contributing to the significantly lower concentrations of muck EOCs when compared to the water. Finally, the recovery rates measured for the water samples were much lower than those for the muck, which makes more room for error in their calculated concentrations than in the muck samples. Despite these potential confounding factors, the EOC data offers no support for the idea that muck acts as a reservoir for EOCs as I had hypothesized and that it is the water that instead holds significantly more of these contaminants.

Pharmaceuticals

The pharmaceutical EOCs showcased a wide range of concentrations in both the water and the muck samples, with those that were detected ranging from 7.23 ng/ml in Diazepam up to 19,140 ng/ml in Chloramphenicol in the water, with a much smaller range of 0.0008 ng/ml in Atrazine to 442.3 ng/ml in Chloramphenicol. In both water and muck samples Chloramphenicol had the highest single concentration, though Sulisobenzone had a slightly higher average concentration in the water samples. Chloramphenicol was also the only pharmaceutical to be found in the Sykes Creek area above levels reported elsewhere. This could be due to the Sykes Creek area, and the IRL at large, being low-flow, restricted waterways allowing chemicals with relatively long half-lives, like Chloramphenicol, to build up to higher levels than in higher flow water bodies (Xiao et al., 2021).

Sunscreens

Of the two sunscreens examined in this study, both potential EDs, only Sulisobenzone could be recovered from the extraction procedure used in this study. Sulisobenzone was also the only EOC in the study to be found in a higher average concentration than Chloramphenicol in the water samples. As a UV blocker commonly used in sunscreen, Sulisobenzone typically enters the environment directly when washed off of skin after application and so is unique in this study as being the only EOC which is often never subjected to wastewater treatment

(DiNardo & Downs, 2018; Narla & Lim, 2020; Semones et al., 2017). This may help to explain why Sulisobenzone is present at such high concentrations in the study site. Sulisobenzone was also expected to be found in water more often than many other EOCs in the study due to its high water solubility of 250,000 mg/L, and was one of the few EOCs in the study found where it was expected to be (Semones et al., 2017).

Herbicide

The herbicide Atrazine made up a relatively small portion of the total concentration of EOCs in the study site, being the smallest contributor in muck samples, and not being recovered from extraction in the water samples. When Atrazine was found the locations with the highest concentrations were along urbanized shorelines or within canals. Much of the shoreline in the study area is urbanized, with a large portion of that area being residential and containing canals connecting to the open waters of the creek. These enclosed canals surrounded by housing may be receiving runoff herbicide from homeowners attempting to kill weeds in their lawns. Though each lawn is small, the large number of houses can add up to a significant amount of herbicide.

Artificial Sweetener

The artificial sweetener Sucralose, also known as Splenda®, was not detected in water samples, and only showed up in two muck samples. Sucralose averaged a concentration of 0.88 ng/ml in the muck samples. Due to its high water solubility, it was expected to be found in water samples more often and at higher concentrations than in the muck. The highest concentration of Sucralose in a single sample was seen in a muck sample at site M21, which contained nearly fifty times as much Sucralose as the only other site where it appeared. This site is located just offshore of a triangular feature in the shoreline that appears manmade and could possibly be related to wastewater outflow from the nearby community, or from the industrial complex to the north. If this is the case and has been for an extended period of time it is possible that Sucralose from the wastewater has begun to be integrated into the water associated with muck over time. Muck is mostly water by weight, and it is possible that Sucralose may have become stuck in water that makes up the muck sample from site M21.

Predictive Models for EOCs

The Generalized linear models created as part of this study indicate that several of the parameters measured by ORCA are potentially capable of predicting whether at least one EOC is present in a given site. The only water parameters which ORCA measured that had significant correlations with any EOCs were water depth, which correlated with Chloramphenicol water samples, and Ammonia, which correlated with Sulisobenzone muck samples. None of the other water parameters measured by ORCA showed any significant relationship with any of the EOCs, though as stated above most of the water parameters were found to not be present in the water samples tested and so may still potentially be predictors of EOCs.

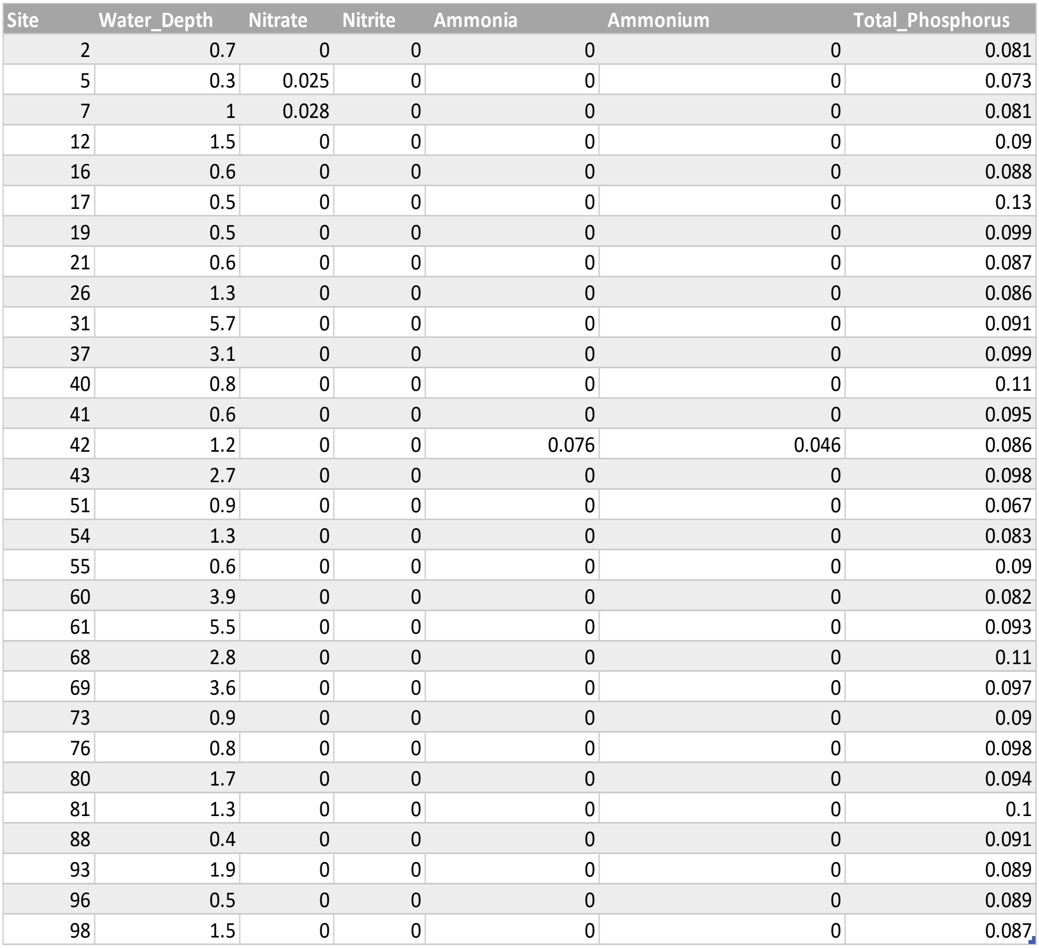
As for the parameters measured from the sediment, there were not only more of them, but they were all present in every sample with the exception of Mercury, which was present in just under half of the samples. This made them more likely to have the potential to correlate to EOC presence, though there were still only three potential predictors in this category, with one of them being a repeat. Ammonia was measured in both the water and the muck of the samples and correlated to an EOC in each, though Ammonia from the muck correlated with Atrazine rather than with Chloramphenicol again. The other two parameters were Sulfur and Mercury which both showed as potential predictors for Chloramphenicol from the water. Toxicity Units, the value ORCA used to determine the overall toxicity of a site, had a significant correlation with none of the EOCs. It is also worth noting that this study covered eight of the most common EOCs found in waters around the world, but that is far from the total number of EOCs that have been found in the environment, and some of these parameters may be correlated with other EOCs that were outside the scope of this study. Likewise, the parameters tested by ORCA are all commonly studied in water and sediments, but they are not all of the parameters that are collected from the environment.

As for Toxicity Units itself, it was found that three of the parameters measured by ORCA had a significant correlation with Toxicity Units. These three consisted of Ammonia and Phosphorus, which are common in fertilizers, one of the major contributors to muck, especially in the Indian River Lagoon (Foster et al., 2018; Wigand et al., 2014). The third significant muck parameter is organic Carbon, which is often found in high amounts in muck deposits (Fox & Trefry, 2018). Diazepam and Atrazine from muck samples were also found to contribute significantly to TU in the muck, though TU is not a significant predictor of either of these EOCs. It is possible that parameters which were not measured by ORCA may also contribute to TU. The fact that Toxicity Units is dependent upon the measurement of bioluminescence given off by bacteria in the sample may also come into play depending upon how susceptible the bacteria are to each factor present within the sample and what conditions may cause them to stop bioluminescing.

Appendix A

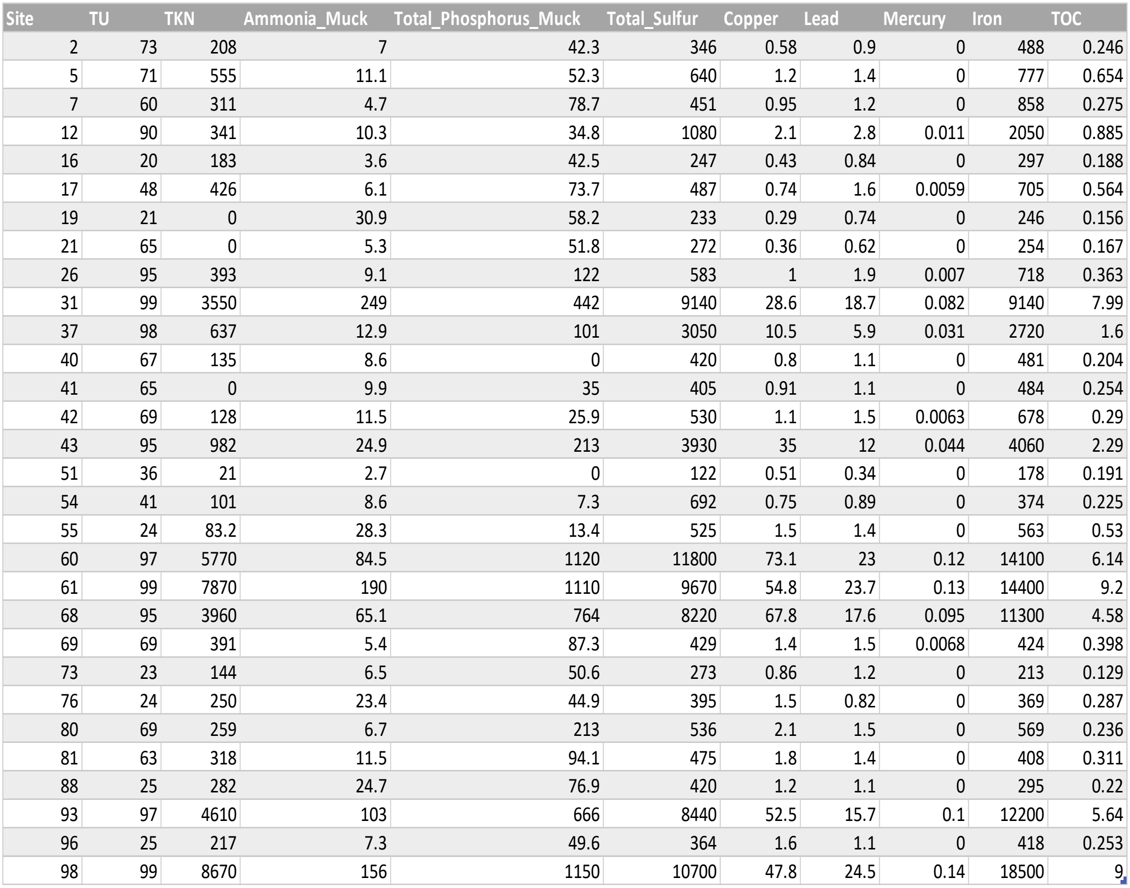
ORCA data

Table 9: Data on water and muck depth and nutrient levels in water collected at sample sites in Sykes Creek by ORCA



Site	Water_Depth	Nitrate	Nitrite	Ammonia	Ammonium	Total_Phosphorus
2	0.7	0	0	0	0	0.081
5	0.3	0.025	0	0	0	0.073
7	1	0.028	0	0	0	0.081
12	1.5	0	0	0	0	0.09
16	0.6	0	0	0	0	0.088
17	0.5	0	0	0	0	0.13
19	0.5	0	0	0	0	0.099
21	0.6	0	0	0	0	0.087
26	1.3	0	0	0	0	0.086
31	5.7	0	0	0	0	0.091
37	3.1	0	0	0	0	0.099
40	0.8	0	0	0	0	0.11
41	0.6	0	0	0	0	0.095
42	1.2	0	0	0.076	0.046	0.086
43	2.7	0	0	0	0	0.098
51	0.9	0	0	0	0	0.067
54	1.3	0	0	0	0	0.083
55	0.6	0	0	0	0	0.09
60	3.9	0	0	0	0	0.082
61	5.5	0	0	0	0	0.093
68	2.8	0	0	0	0	0.11
69	3.6	0	0	0	0	0.097
73	0.9	0	0	0	0	0.09
76	0.8	0	0	0	0	0.098
80	1.7	0	0	0	0	0.094
81	1.3	0	0	0	0	0.1
88	0.4	0	0	0	0	0.091
93	1.9	0	0	0	0	0.089
96	0.5	0	0	0	0	0.089
98	1.5	0	0	0	0	0.087

Table 10: Data on nutrient and metal levels present in muck collected at sample sites in Sykes Creek by ORCA



Site	TU	TKN	Ammonia_Muck	Total_Phosphorus_Muck	Total_Sulfur	Copper	Lead	Mercury	Iron	TOC
2	73	208	7	42.3	346	0.58	0.9	0	488	0.246
5	71	555	11.1	52.3	640	1.2	1.4	0	777	0.654
7	60	311	4.7	78.7	451	0.95	1.2	0	858	0.275
12	90	341	10.3	34.8	1080	2.1	2.8	0.011	2050	0.885
16	20	183	3.6	42.5	247	0.43	0.84	0	297	0.188
17	48	426	6.1	73.7	487	0.74	1.6	0.0059	705	0.564
19	21	0	30.9	58.2	233	0.29	0.74	0	246	0.156
21	65	0	5.3	51.8	272	0.36	0.62	0	254	0.167
26	95	393	9.1	122	583	1	1.9	0.007	718	0.363
31	99	3550	249	442	9140	28.6	18.7	0.082	9140	7.99
37	98	637	12.9	101	3050	10.5	5.9	0.031	2720	1.6
40	67	135	8.6	0	420	0.8	1.1	0	481	0.204
41	65	0	9.9	35	405	0.91	1.1	0	484	0.254
42	69	128	11.5	25.9	530	1.1	1.5	0.0063	678	0.29
43	95	982	24.9	213	3930	35	12	0.044	4060	2.29
51	36	21	2.7	0	122	0.51	0.34	0	178	0.191
54	41	101	8.6	7.3	692	0.75	0.89	0	374	0.225
55	24	83.2	28.3	13.4	525	1.5	1.4	0	563	0.53
60	97	5770	84.5	1120	11800	73.1	23	0.12	14100	6.14
61	99	7870	190	1110	9670	54.8	23.7	0.13	14400	9.2
68	95	3960	65.1	764	8220	67.8	17.6	0.095	11300	4.58
69	69	391	5.4	87.3	429	1.4	1.5	0.0068	424	0.398
73	23	144	6.5	50.6	273	0.86	1.2	0	213	0.129
76	24	250	23.4	44.9	395	1.5	0.82	0	369	0.287
80	69	259	6.7	213	536	2.1	1.5	0	569	0.236
81	63	318	11.5	94.1	475	1.8	1.4	0	408	0.311
88	25	282	24.7	76.9	420	1.2	1.1	0	295	0.22
93	97	4610	103	666	8440	52.5	15.7	0.1	12200	5.64
96	25	217	7.3	49.6	364	1.6	1.1	0	418	0.253
98	99	8670	156	1150	10700	47.8	24.5	0.14	18500	9

References

Alves, P. da C. C., Rodrigues-Silva, C., Ribeiro, A. R., & Rath, S. (2021). Removal of low-calorie sweeteners at five Brazilian wastewater treatment plants and their occurrence in surface water. *Journal of Environmental Management*, *289*, 112561. https://doi.org/10.1016/j.jenvman.2021.112561

Beel, R., Lütke Eversloh, C., & Ternes, T. A. (2013). Biotransformation of the UV-Filter Sulisobenzone: Challenges for the Identification of Transformation Products. *Environmental Science & Technology*, *47*(13), 6819–6828. https://doi.org/10.1021/es400451w

Bilskie, M. V., Bacopoulos, P., & Hagen, S. C. (2019). Astronomic tides and nonlinear tidal dispersion for a tropical coastal estuary with engineered features (causeways): Indian River lagoon system. *Estuarine, Coastal and Shelf Science*, *216*, 54–70. https://doi.org/10.1016/j.ecss.2017.11.009

Buzzelli, C., Robbins, R., Doering, P., Chen, Z., Sun, D., Wan, Y., Welch, B., & Schwarzschild, A. (2012). Monitoring and Modeling of Syringodium filiforme (Manatee Grass) in Southern Indian River Lagoon. *Estuaries and Coasts*, *35*(6), 1401–1415. https://doi.org/10.1007/s12237-012-9533-8

Cantwell, M., Perron, M., Sullivan, J., Katz, D., Burgess, R., & King, J. (2014). Assessing organic contaminant fluxes from contaminated sediments following dam removal in an urbanized river. *Environmental Monitoring & Assessment*, *186*(8), 4841–4855.

Chen, K., Wu, M., Chen, C., Xu, H., Wu, X., & Qiu, X. (2021). Impacts of chronic exposure to sublethal diazepam on behavioral traits of female and male zebrafish (Danio rerio). *Ecotoxicology and Environmental Safety*, *208*, 111747. https://doi.org/10.1016/j.ecoenv.2020.111747

Colvin, J., Lazarus, S., Splitt, M., Weaver, R., & Taeb, P. (2018). Wind driven setup in east central Florida's Indian River Lagoon: Forcings and parameterizations. *Estuarine, Coastal and Shelf Science*, *213*, 40–48. https://doi.org/10.1016/j.ecss.2018.08.004

de Araujo, F. G., Bauerfeldt, G. F., Marques, M., & Martins, E. M. (2019). Development and Validation of an Analytical Method for the Detection and Quantification of Bromazepam, Clonazepam and Diazepam by UPLC-MS/MS in Surface Water. *Bulletin of Environmental Contamination and Toxicology*, *103*(2), 362–366. https://doi.org/10.1007/s00128-019-02631-z

Dellapenna, T. M., Fielder, B., Noll, C. J., & Savarese, M. (2015). Geological Responses to Urbanization of the Naples Bay Estuarine System, Southwestern Florida, USA. *Estuaries and Coasts; Port Republic*, *38*(1), 81–96. http://dx.doi.org.portal.lib.fit.edu/10.1007/s12237-013-9704-2

DiNardo, J. C., & Downs, C. A. (2018). Dermatological and environmental toxicological impact of the sunscreen ingredient oxybenzone/benzophenone-3. *Journal of Cosmetic Dermatology*, *17*(1), 15–19. https://doi.org/10.1111/jocd.12449

Ferrer, I., Zweigenbaum, J. A., & Thurman, E. M. (2013). Analytical methodologies for the detection of sucralose in water. *Analytical Chemistry*, *85*(20), 9581–9587. https://doi.org/10.1021/ac4016984

Foster, G., Riegl, B.M., Foster, K. A., & Morris, L.J. (2018). Acoustic Detection and Mapping of Muck Deposits in the Indian River Lagoon, Florida. *Journal of Coastal Research*, 34(4), 856-. Academic OneFile

Fox, A.L., & Trefry, J.H. (2018, August). *Environmental Dredging to Remove Fine-Grained, Organic-Rich Sediments and Reduce Inputs of Nitrogen and Phosphorus to a Subtropical Estuary*. https://doi.org/info:doi/10.4031/MTSJ.52.4.3

Downs, C. A., Kramarsky-winter, E., Segal, R., Fauth, J., Knutson, S., Bronstein, O., Ciner, F. R., Jeger, R., Lichtenfeld, Y., Woodley, C. M., Pennington, P., Cadenas, K., Kushmaro, A., & Loya, Y. (2016). Toxicopathological Effects of the Sunscreen UV Filter, Oxybenzone (Benzophenone-3), on Coral Planulae and Cultured Primary Cells and Its Environmental Contamination in Hawaii and the U.S. Virgin Islands. *Archives of Environmental Contamination and Toxicology; New York*, *70*(2), 265–288. http://dx.doi.org.portal.lib.fit.edu/10.1007/s00244-015-0227-7

Gasnier, C., Dumont, C., Benachour, N., Clair, E., Chagnon, M.-C., & Séralini, G.-E. (2009). Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. *Toxicology*, *262*(3), 184–191. https://doi.org/10.1016/j.tox.2009.06.006

Gomez, E., Bachelot, M., Boillot, C., Munaron, D., Chiron, S., Casellas, C., & Fenet, H. (2012). Bioconcentration of two pharmaceuticals (benzodiazepines) and two personal care products (UV filters) in marine mussels (mytilus galloprovincialis) under controlled laboratory conditions. *Environmental Science and Pollution Research International*, *19*(7).

Hao, C., Morse, D., Morra, F., Zhao, X., Yang, P., & Nunn, B. (2011). Direct aqueous determination of glyphosate and related compounds by liquid chromatography/tandem mass spectrometry using reversed-phase and weak anion-exchange mixed-mode column. *Journal of Chromatography. A*, *1218*(33), 5638–5643. https://doi.org/10.1016/j.chroma.2011.06.070

Hashem, Md. A., Nur-A-Tomal, Md. S., Mondal, N. R., & Rahman, Md. A. (2017). Hair burning and liming in tanneries is a source of pollution by arsenic, lead, zinc, manganese and iron. *Environmental Chemistry Letters*, *15*(3), 501–506. https://doi.org/10.1007/s10311-017-0634-2

Heuner, M., Weber, A., Schröder, U., Kleinschmit, B., & Schröder, B. (2016). Facilitating political decisions using species distribution models to assess restoration measures in heavily modified estuaries. *Marine Pollution Bulletin*, *110*(1), 250–260. https://doi.org/10.1016/j.marpolbul.2016.06.056

Huang, B., Guo, Z., Xiao, X., Zeng, P., & Peng, C. (2019). Changes in chemical fractions and ecological risk prediction of heavy metals in estuarine sediments of Chunfeng Lake estuary, China. *Marine Pollution Bulletin*, *138*, 575–583. https://doi.org/10.1016/j.marpolbul.2018.12.015

Huang, S. S. Y., Benskin, J. P., Veldhoen, N., Chandramouli, B., Butler, H., Helbing, C. C., & Cosgrove, J. R. (2017). A multi-omic approach to elucidate low-dose effects of xenobiotics in zebrafish (Danio rerio) larvae. *Aquatic Toxicology*, *182*, 102–112. https://doi.org/10.1016/j.aquatox.2016.11.016

Jithan, A. V., Mohan, C. K., & Vimaladevi, M. (2008). Development and Evaluation of a Chloramphenicol Hypertonic Ophthalmic Solution. *Indian Journal of Pharmaceutical Sciences*, *70*(1), 66–70. https://doi.org/10.4103/0250-474X.40334

Johannesson, K. H., Chevis, D. A., Burdige, D. J., Cable, J. E., Martin, J. B., & Roy, M. (2011). Submarine groundwater discharge is an important net source of light and middle REEs to coastal waters of the Indian River Lagoon, Florida, USA. *Geochimica et Cosmochimica Acta*, *75*(3), 825–843. https://doi.org/10.1016/j.gca.2010.11.005

Joyner, J., Wanless, D., Sinigalliano, C. D., & Lipp, E. K. (2014). Use of Quantitative Real-Time PCR for Direct Detection of Serratia marcescens in Marine and Other Aquatic Environments. *Applied and Environmental Microbiology*, *80*(5), 1679–1683. https://doi.org/10.1128/AEM.02755-13

Kataoka, C., Sugiyama, T., Kitagawa, H., Takeshima, A., Kagami, Y., Tatsuta, H., & Kashiwada, S. (2019). Temperature-dependent toxicity of acetaminophen in Japanese medaka larvae. *Environmental Pollution*, *254*, 113092. https://doi.org/10.1016/j.envpol.2019.113092

Kjelland, M. E., Woodley, C. M., Swannack, T. M., & Smith, D. L. (2015). A review of the potential effects of suspended sediment on fishes: potential dredging-related physiological, behavioral, and transgenerational implications. *Environment Systems and Decisions*, *35*(3), 334–350. https://doi.org/10.1007/s10669-015-9557-2

Koumaki, E., Mamais, D., Noutsopoulos, C., Nika, M.-C., Bletsou, A. A., Thomaidis, N. S., Eftaxias, A., & Stratogianni, G. (2015). Degradation of emerging contaminants from water under natural sunlight: The effect of season, pH, humic acids and nitrate and identification of photodegradation by-products. *Chemosphere*, *138*, 675–681. https://doi.org/10.1016/j.chemosphere.2015.07.033

Lapointe, B. E., Herren, L. W., Debortoli, D. D., & Vogel, M. A. (2015). Evidence of sewage-driven eutrophication and harmful algal blooms in Florida's Indian River Lagoon. *Harmful Algae*, *43*, 82–102. https://doi.org/10.1016/j.hal.2015.01.004

Lapworth, D. J., Baran, N., Stuart, M. E., & Ward, R. S. (2012). Emerging organic contaminants in groundwater: A review of sources, fate and occurrence. *Environmental Pollution*, *163*, 287–303. https://doi.org/10.1016/j.envpol.2011.12.034

Li, J., Ye, Q., & Gan, J. (2015). Influence of organic amendment on fate of acetaminophen and sulfamethoxazole in soil. *Environmental Pollution*, *206*, 543–550.

Liu, Q., Li, Y., Song, P., Zhou, F., Qiao, Y., Dong, J., Yue, J., Yang, J., & Shi, M. (2020). Diversity of viruses infecting sweet potato in Beijing based on small RNA deep sequencing and PCR or RT-PCR detection. *Journal of General Plant Pathology*, *86*(4), 283–289.

Lynch, K. M., Fair, P. A., Houde, M., Muir, D. C. G., Kannan, K., Bossart, G. D., Bartell, S. M., & Gribble, M. O. (2019). Temporal Trends in Per- and Polyfluoroalkyl Substances in Bottlenose Dolphins (*Tursiops truncatus*) of Indian River Lagoon, Florida and Charleston, South Carolina. *Environmental Science & Technology*, *53*(24), 14194–14203. https://doi.org/10.1021/acs.est.9b04585

Marson, E. O., Paniagua, C. E. S., Costa-Serge, N. M., Sousa, R. M. F., Silva, G. D., Becker, R. W., Sirtori, C., Starling, M. C. V. M., Carvalho, S. R., & Trovó, A. G. (2021). Chemical and toxicological evaluation along with unprecedented transformation products during photolysis and heterogeneous photocatalysis of chloramphenicol in different aqueous matrices. *Environmental Science and Pollution Research*, *28*(19), 23582–23594. https://doi.org/10.1007/s11356-020-09756-3

Moro, I., Matozzo, V., Moschin, E., Trentin, R., & Dalla Vecchia, F. (2021). Morpho-physiological responses by *Chlamydomonas reinhardtii* to different concentrations of ibuprofen. *Chemistry and Ecology*, *37*(4), 352–368. https://doi.org/10.1080/02757540.2021.1886279

Nandi, B., Nandy, R. K., Mukhopadhyay, S., Nair, G. B., Shimada, T., & Ghose, A. C. (2000). Rapid Method for Species-Specific Identification of Vibrio cholerae Using Primers Targeted to the Gene of Outer Membrane Protein OmpW. *Journal of Clinical Microbiology*, *38*(11), 4145–4151.

Narla, S., & Lim, H. W. (2020). Sunscreen: FDA regulation, and environmental and health impact. *Photochemical & Photobiological Sciences*, *19*(1), 66–70. https://doi.org/10.1039/C9PP00366E

Neumann, B., Vafeidis, A. T., Zimmermann, J., & Nicholls, R. J. (2015). Future coastal population growth and exposure to sea-level rise and coastal flooding - a global assessment. *PLoS ONE*, *10*(3). Gale Academic OneFile. http://link.gale.com/apps/doc/A428932960/AONE?u=melb26933&sid=zotero&xid=f0fa7703

Omar, T. F. T., Aris, A. Z., Yusoff, F. Md., & Mustafa, S. (2018). Occurrence, distribution, and sources of emerging organic contaminants in tropical coastal sediments of anthropogenically impacted Klang River estuary, Malaysia. *Marine Pollution Bulletin*, *131*, 284–293. https://doi.org/10.1016/j.marpolbul.2018.04.019

O’Mullan, G. D., Juhl, A. R., Reichert, R., Schneider, E., & Martinez, N. (2019). Patterns of sediment-associated fecal indicator bacteria in an urban estuary: Benthic-pelagic coupling and implications for shoreline water quality.

Science of The Total Environment, *656*, 1168–1177. https://doi.org/10.1016/j.scitotenv.2018.11.405

Palmer, P. M., Wilson, L. R., O'Keefe, P., Sheridan, R., King, T., & Chen, C.-Y. (2008). Sources of pharmaceutical pollution in the New York City Watershed. *Science of The Total Environment*, *394*(1), 90–102. https://doi.org/10.1016/j.scitotenv.2008.01.011

Parolini, M., Magni, S., Traversi, I., Villa, S., Finizio, A., & Binelli, A. (2015). Environmentally relevant concentrations of galaxolide (HHCB) and tonalide (AHTN) induced oxidative and genetic damage in Dreissena polymorpha. *Journal of Hazardous Materials*, *285*, 1–10. https://doi.org/10.1016/j.jhazmat.2014.11.037

Peeler, K. A., Opsahl, S. P., & Chanton, J. P. (2006). Tracking Anthropogenic Inputs Using Caffeine, Indicator Bacteria, and Nutrients in Rural Freshwater and Urban Marine Systems. *Environmental Science & Technology*, *40*(24), 7616–7622. https://doi.org/10.1021/es061213c

Perkins, T. L., Clements, K., Baas, J. H., Jago, C. F., Jones, D. L., Malham, S. K., & McDonald, J. E. (2014). Sediment Composition Influences Spatial Variation in the Abundance of Human Pathogen Indicator Bacteria within an Estuarine Environment. *PLoS ONE*, *9*(11), e112951. https://doi.org/10.1371/journal.pone.0112951

Qu, M., Li, H., Li, N., Liu, G., Zhao, J., Hua, Y., & Zhu, D. (2017). Distribution of atrazine and its phytoremediation by submerged macrophytes in lake sediments. *Chemosphere*, *168*, 1515–1522. https://doi.org/10.1016/j.chemosphere.2016.11.164

Rodríguez-López, L., Cela-Dablanca, R., Núñez-Delgado, A., Álvarez-Rodríguez, E., Fernández-Calviño, D., & Arias-Estévez, M. (2021). Photodegradation of Ciprofloxacin, Clarithromycin and Trimethoprim: Influence of pH and Humic Acids. *Molecules*, *26*(11), 3080. https://doi.org/10.3390/molecules26113080

Rudershausen, P. J., Hightower, J. E., Buckel, J. A., O'Donnell, M. J., Dubreuil, T., & Letcher, B. H. (2019). Survival and Density of a Dominant Fish Species Across a Gradient of Urbanization in North Carolina Tidal Creeks. *Estuaries and Coasts*, *42*(6), 1632–1653. https://doi.org/10.1007/s12237-019-00575-5

Schaefer, A. M., Goldstein, J. D., Reif, J. S., Fair, P. A., & Bossart, G. D. (2009). Antibiotic-Resistant Organisms Cultured from Atlantic Bottlenose Dolphins (Tursiops truncatus) Inhabiting Estuarine Waters of Charleston, SC and Indian River Lagoon, FL. *EcoHealth; New York*, *6*(1), 33–41. http://dx.doi.org.portal.lib.fit.edu/10.1007/s10393-009-0221-5

Seitz, R., Lipcius, R., Olmstead, N., Seebo, M., & Lambert, D. (2006). Influence of shallow-water habitats and shoreline development on abundance, biomass, and diversity of benthic prey and predators in Chesapeake Bay. *Marine Ecology Progress Series*, *326*, 11–27. https://doi.org/10.3354/meps326011

Semones, M. C., Sharpless, C. M., MacKay, A. A., & Chin, Y.-P. (2017). Photodegradation of UV filters oxybenzone and sulisobenzone in wastewater effluent and by dissolved organic matter. *Applied Geochemistry*, *83*, 150–157. https://doi.org/10.1016/j.apgeochem.2017.02.008

Serra-Compte, A., Corcoll, N., Huerta, B., Rodríguez-Mozaz, S., Sabater, S., Barceló, D., & Álvarez-Muñoz, D. (2018). Fluvial biofilms exposed to desiccation and pharmaceutical pollution: New insights using metabolomics. *Science of The Total Environment*, *618*, 1382–1388. https://doi.org/10.1016/j.scitotenv.2017.09.258

Shenoy, K. (2012). Environmentally Realistic Exposure to the Herbicide Atrazine Alters Some Sexually Selected Traits in Male Guppies. *PLoS ONE*, *7*(2), e30611. https://doi.org/10.1371/journal.pone.0030611

Sorinolu, A. J., Tyagi, N., Kumar, A., & Munir, M. (2020). Antibiotic resistance development and human health risks during wastewater reuse and biosolids application in agriculture. *Chemosphere*, 129032. https://doi.org/10.1016/j.chemosphere.2020.129032

Sung, H.-H., Chiu, Y.-W., Wang, S.-Y., Chen, C.-M., & Huan, D.-J. (2014). Acute toxicity of mixture of acetaminophen and ibuprofen to Green Neon Shrimp, Neocaridina denticulate. *Environmental Toxicology and Pharmacology*, *38*(1), 8–13.

Taheriyoun, M., Salehiziri, M., & Parand, S. (2019). Biofiltration performance and kinetic study of hydrogen sulfide removal from a real source. *Journal of*

Environmental Health Science and Engineering, *17*(2), 645–656. https://doi.org/10.1007/s40201-019-00378-7

Tollefsen, K., Nizzetto, L., & Huggett, D. (2012). Presence, fate and effects of the intense sweetener sucralose in the aquatic environment. *Science of The Total Environment*, *438*, 510–516. https://doi.org/10.1016/j.scitotenv.2012.08.060

Toptan, T., Hoehl, S., Westhaus, S., Bojkova, D., Berger, A., Rotter, B., Hoffmeier, K., Cinatl Jr., J., Ciesek, S., & Widera, M. (2020). Optimized qRT-PCR Approach for the Detection of Intra-and Extra-Cellular SARS-CoV-2 RNAs. *International Journal of Molecular Sciences*, *21*(12), 1–11.

Van Damme, M., Clarisse, L., Whitburn, S., Hadji-Lazaro, J., Hurtmans, D., Clerbaux, C., & Coheur, P.-F. (2018). Industrial and agricultural ammonia point sources exposed. *Nature*, *564*(7734), 99–103. https://doi.org/10.1038/s41586-018-0747-1

Venturini, N., Bícego, M. C., Taniguchi, S., Sasaki, S. T., García-Rodríguez, F., Brugnoli, E., & Muniz, P. (2015). A multi-molecular marker assessment of organic pollution in shore sediments from the Río de la Plata Estuary, SW Atlantic. *Marine Pollution Bulletin*, *91*(2), 461–475. https://doi.org/10.1016/j.marpolbul.2014.06.056

Voigt, A. M., Zacharias, N., Timm, C., Wasser, F., Sib, E., Skutlarek, D., Parcina, M., Schmithausen, R. M., Schwartz, T., Hembach, N., Tiehm, A., Stange, C., Engelhart, S., Bierbaum, G., Kistemann, T., Exner, M., Faerber, H. A.,

& Schreiber, C. (2020). Association between antibiotic residues, antibiotic resistant bacteria and antibiotic resistance genes in anthropogenic wastewater – An evaluation of clinical influences. *Chemosphere*, *241*, 125032. https://doi.org/10.1016/j.chemosphere.2019.125032

Wang, R., Li, H., Peng, X., Zhang, G., & Zhang, L. (2019). New ecological dam for sediment and overlying water pollution treatment based on microbial fuel cell principle. *Environmental Science and Pollution Research*, *26*(18), 18615–18623.

Wang, R. X., Wang, A., & Wang, J. Y. (2014). Antibiotic resistance monitoring in heterotrophic bacteria from anthropogenic-polluted seawater and the intestines of oyster Crassostrea hongkongensis. *Ecotoxicology and Environmental Safety*, *109*, 27–31. https://doi.org/10.1016/j.ecoenv.2014.07.028

Wigand, C., Roman, C. T., Davey, E., Stolt, M., Johnson, R., Hanson, A., Watson, E. B., Moran, S. B., Cahoon, D. R., Lynch, J. C., & Rafferty, P. (2014). Below the disappearing marshes of an urban estuary: historic nitrogen trends and soil structure. *Ecological Applications*, *24*(4), 633–649. https://doi.org/10.1890/13-0594.1

Xiao, L., Li, J., Lichtfouse, E., Li, Z., Wang, Q., & Liu, F. (2021). Augmentation of chloramphenicol degradation by Geobacter-based biocatalysis and electric field. *Journal of Hazardous Materials*, *410*, 124977. https://doi.org/10.1016/j.jhazmat.2020.124977

Yuxuan, Z., Peiyong, G., Yanmei, W., Xiaoyan, Z., Meixian, W., Simin, Y., Yinshi, S., Jun, D., & Haitao, S. (2019). Evaluation of the subtle effects and oxidative stress response of chloramphenicol, thiamphenicol, and florfenicol in Daphnia magna. *Environmental Toxicology and Chemistry*, *38*(3), 575–584. https://doi.org/10.1002/etc.4344

Zeng, J., & Han, G. (2020). Preliminary copper isotope study on particulate matter in Zhujiang River, southwest China: Application for source identification. *Ecotoxicology and Environmental Safety*, *198*, 110663. https://doi.org/10.1016/j.ecoenv.2020.110663

Zhuan, R., & Wang, J. (2020). Degradation of diclofenac in aqueous solution by ionizing radiation in the presence of humic acid. *Separation and Purification Technology*, *234*, 116079. https://doi.org/10.1016/j.seppur.2019.116079

Zimmer-Faust, A. G., Brown, C. A., & Manderson, A. (2018). Statistical models of fecal coliform levels in Pacific Northwest estuaries for improved shellfish harvest area closure decision making. *Marine Pollution Bulletin*, *137*, 360–369. https://doi.org/10.1016/j.marpolbul.2018.09.028