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Mercury Cycling in Sulfur Rich Sediment From The Brunswick Estuary

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by

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(Under the direction of major professor Franciscan Cubas)

ABSTRACT

Mercury is potentially toxic to the environment. Mercury is absorbed into anaerobic sediments of surface waters, which may be converted to methylmercury, a toxic form of mercury that bio-accumulates in aquatic biota. Sources of mercury in the environment vary, but the production of methylmercury is common in sulfur-rich sediments containing mercury. In such environments, sulfur reducing bacteria (SRB) produce methylmercury as a by-product. The metabolic process uses energy from the reduction of sulfate to sulfide. This study focuses on determining the methylmercury production and release potential from sulfur-rich sediments extracted from different areas of the Brunswick Estuary. Previous studies note considerable levels of mercury in the Brunswick Estuary due to a local super fund site. Water and sediment samples were collected from six different sites to feed microcosms. The design measures the potential of the sediments to produce methylmercury. Microcosms were operated under anaerobic conditions to determine if sediments produced methylmercury under extreme conditions (e.g. low dissolved oxygen, low oxidation-reduction potential, and highly productive environment). This may seasonally exist in different zones of the estuary. Results revealed that sediments have the potential to reduce sulfate under anaerobic conditions. In the microcosms, sulfate concentrations rapidly decreased from values as high as 290 mg/L to practically 0 mg/L. This suggests that sediments provide an adequate environment to support SRB activity, which may result in methylmercury production. Further, results revealed that the production potential of methylmercury varies across different zones of the estuary. Precise methylmercury concentrations collected from the different sites are currently being evaluated. Due to the environmental conditions that prevail in the estuary, its proximity to a mercury super fund site, and its accessibility for fishing activities, it is crucial to further assess the methylmercury formation in this area.

INDEX WORDS: Mercury Cycling, Methyl mercury, Sulfur reducing bacteria

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DEDICATION

To my wife, whom I love very much, thank you for standing by me for the last five years.

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To Dr. Cubas: Thank you for all the support you have given me in the last year. The mentorship you have given me has helped develop me professionally.

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CHAPTER 1:
INTRODUCTION

1.1 Overview

Mercury can be a very hazardous substance to the environment and humans. It can be found in surface waters where it can be consumed by aquatic life and then consumed by humans. Fish and other aquatic biota may consume the organic form of mercury, called methyl mercury (Chemical Formula CH_3Hg), which is highly toxic at low concentrations (Clarkson et al. 2003). Mercury can cause problems with the central nervous system, kidneys, and liver. It can also disturb the immune system in humans (Maqbool et al. 2016). Other known problems that mercury (methylmercury) can cause to humans are tremors, negatively effects fetal development impaired vision and hearing, paralysis, insomnia and emotional instability and possible death (Clarkson et al. 2003). Multiple studies were conducted on the negative effects of methyl mercury. A study conducted in 2012 revealed that low level dosages of methyl mercury negatively affect fetal development (Karagas et al. 2012). Then a study done in 2007 showed that methyl mercury adversely impacts the central nervous system (Crespo-López et al. 2007). The effect of methyl mercury has on the central nervous system is seen in a study done on the Amazonian populations. The study related the consumption of methyl mercury to nervous system dysfunction within the Amazonian populace (Lebel et al. 1996).

Mercury can enter the ecosystem through two types of sources: natural or human promoted. Naturally, mercury enters the ecosystem through plate tectonics, like volcanoes (Ferrara et al. 2000). These sources of mercury usually do not cause contamination because the amounts of mercury released are usually low (Hartung and Bertram 1972). Mercury from anthropogenic activities comes from many industrial processes that discharge high concentrations of mercury to the environment. Mercury from these activities is usually emitted

or discharge from a manufacturing plant. From the atmosphere, mercury can reach the environment through dry or (Lindberg 1992) wet deposition (Iverfeldt 1992). Most of the mercury comes from anthropogenic sources (Zhang and Wong 2007). When mercury is discharged into the environment it is usually transported to the sediments of surface waters. There, it can be absorbed into the sediments and stay inert under certain environmental conditions that will promote the absorption of mercury to the sediments. Generally, oxidized environments rich in oxygen or nitrate will promote the absorption of mercury to sediments.

In sediments, mercury attaches mostly to sulfide creating cinnabar, a common form of mercury found in the environment. From the sediment-water interface, mercury can be absorbed by plants, methylated, or reenter the atmosphere through volatilization. The soil can also be eroded and mercury may enter the aquatic systems. When mercury enters an aquatic environment, it enters has an oxidized species. Mercury is known to combine to with sulfur (in water) producing mercury sulfate ($Hg(SO_4)$) or mercury sulfide (HgS), but it can also bond with other elements. It can bind with chlorine, creating mercury (II) chloride (HgCl), or BiSulfide (Hg(HS)). Mercury moves through the aquatic environment until it settles to the sediment-water interface. Once at the bottom of the aquatic environment it can undergo changes. If there is a large amount of Dissolved Oxygen (DO), mercury will bond with the sediment, most likely creating mercury sulfide. If the environment is anaerobic, mercury is released from the soil in the sediment water interface, by diffusion. Most of the time mercury gets pushed around the aquatic system or is returned to the atmosphere by volatilization.

If the environment is productive and contains large amount of nutrients, especially sulfate, then methylation of mercury may occur. Mercury is converted to methylmercury in anaerobic and reduced environments. This type of environment promotes the reduction of iron III

to iron II or the reduction of sulfate to sulfide. These reduction processes are typically microorganism catalyzed. The sequence of reductions reaction at 20 degrees C is: oxygen (reduction≈ 500-300mV), nitrogen (reduction≈ 300-200mV), manganese (reduction≈200-100mV), Iron (reduction≈100-0mV) and sulfate (reduction≈0- -150mV). Once the reductions process reaches sulfate, the sulfur reducing bacteria (SRB) consume the sulfate. This produces sulfide (most likely hydrogen sulfide), but some of the mercury sulfate is consumed by the SRB. The mercury sulfate could enter the SRB through four pathways: Mer based transport, passive diffusion, facilitated diffusion and active diffusion. All ways are possible and can lead to methylation. The byproduct would be methylmercury. Sulfur reducing bacteria carry specific genes called "hcg a" and "hcg b" cluster. It is believed that these genes are required to produce methyl mercury (Hsu-Kim et al. 2013). The SRB either releases the methyl mercury into the environment, where biota can breathe it through their gills, or are consumed. Methyl mercury then bioaccumulates up the trophic sphere until human consumption (Park and Curtis 1997).

This study focuses on the Brunswick Estuary in southeast Georgia. Industrial activities discharged pollutions between 1950-1980. Before the 1970s manufacturing plants dumped contaminants at water sites causing a high contamination level. This occurred because there were no governmental regulations preventing these actions until the 1970's. This is the case for the Brunswick Estuary. This is documented by USGS in a study of GA rails (Odom 1975). Today, this area in Brunswick, Georgia is now considered a superfund site due to all of the contamination from the industry. There is suspicion that the environment has the potential to produce methyl mercury. If the suspicions are correct, it could be causing serious damage to the local animal and human populations.

1.2 Problem statement

There is historical evidence of mercury contamination in the Brunswick Estuary (Odom 1975). Currently there are fish advisories that are in place but these advisories are limited in quantity. Fishing is very common for the local population. Due to bioaccumulation through the trophic level, the amount of methyl mercury could be harmful to the public. However, this is dependent on the amount of sulfate in the Brunswick Estuary. ***If*** sulfur concentrations are high, ***then*** the amount of methyl mercury to total mercury increases significantly, causing possible harm to the public.

1.3 Objective of Research

This research will contribute to the knowledge of:

- Relationship between methyl mercury and sulfur.
- Role of Oxidation-Reduction Potential (ORP) in reducing sulfate.
- Conducting research to find mercury contaminated areas.

The main objective of the thesis is to determine if methyl mercury can be produced in the Brunswick Estuary. Sediments are taken from the surrounding area and place in a reduced environment to produce methyl mercury. The potential is measured by deriving the amount of sulfate reduced and then, comparing it to the amount of methyl mercury from the total mercury. The experiment also provides key indicators that could be used to identify possible methyl mercury sites.

3.3 Criteria for Success

The success of the experiment is dependent on the correlation between reduced sulfate and methyl mercury to total mercury. All studies, both current and previous, show a relationship

of R^2 no less than 0.5 (Shao et al. 2012, Pollman and Donald 2014, Johnson et al. 2016). The relationship would also show that an increase in methyl mercury must correlate to a decrease in sulfate. This indicates that the consumption of sulfate is necessary to produce methyl mercury.

Any values lower than 0.01 ng L^{-1} of mercury and methyl mercury are deemed irrelevant because it does not show to have a large enough of an effect on on bioaccumulation concentrations. Mercury and methyl mercury will be measured to 0.01 ng L^{-1} to provide accurate calculation for experiment. The Environmental Protection Agency states that the drinking water standard is a maximum daily consumption of 2.0 µg L^{-1} day^{-1} of inorganic mercury. The minimum amount of mercury of 0.01 ng L^{-1} can be hazardous because methyl mercury can still exceed the maximum daily consumption. It can be done though bioaccumulation of methyl mercury. It can reach maximum allowable level set by EPA, which is acute 1.8 µg L^{-1} similar to the amount derived by Ouédraogo in 2013.

CHAPTER 2:
LITERATURE REVIEW

2.1 Sources of Mercury

Before mercury settles within a sulfur rich wetland, it must be discharged or emitted from a source. Mercury can be retrieved via two sources: it is found abundant in nature or it can be manmade. The abundance of natural sources of mercury plays an important role in mercury cycling. Mercury cycles are a natural part of the ecosystem. Mercury can be naturally derived from the environment through: mantle materials (such as: volcanoes and tectonic plates), wild fires, and reemission. How mercury will enter the environment is dependent upon the source of which it originated.

Regardless of wet or dry deposition, when mercury is emitted into the atmosphere it is accumulated within the soil of the surrounding environment. Mercury found in the earth's mantle enters the ecosystem via volcanoes. The mantle originates as molten lava, which is rich in mercury. When the molten lava is discharged from a volcano, it forms a layer atop the earth's surface and hardens creating mercury-rich igneous rock. The ash emitted from the volcano also distributes mercury throughout the soil, water, and atmosphere. Throughout the ocean, mercury is also abundant in supply due to underwater vents (or underwater volcanos) located throughout the ocean floor. These vents emit molten lava, from the earth's mantle, which harden to contribute to the mercury-rich sediment of the ocean soil. The process of "sea floor spreading" also contributes to the emission of mercury from the mantle into the ocean. Tectonic plates moving in opposite directions, or divergent plates, create gaps in which molten lava escapes the mantle and hardens on the ocean floor, adding sediment to the already mercury-rich environment. There is no natural regulation as to when the environment has reached a toxic level

of mercury production, so a buildup of mercury could occur and contaminations could develop (Bank 2012).

Since the soil contains mercury, the surrounding and implanted vegetation absorbs its mercury from the soil. The level of mercury contamination within said vegetation is location dependent. For example, an area that is at an increased risk for forest fire, has an increased risk of mercury contamination in the air, as well as in the soil. In a forest fire, the mercury within the vegetation is burned and released into the surrounding air creating toxic levels of mercury in the atmosphere. When the trees decay, the mercury is seeped into the soil. The effects of a forest fire can redistribute the mercury contamination throughout both air and land environments (Schroeder and Munthe 1998).

Mercury naturally found on the earth's surface accounts for only a small percentage of mercury production. Most of the mercury is derived from its reemission into the atmosphere. A study performed in 2013 measured the amount of mercury in the global environment. The study concluded that 70% of mercury in the atmosphere is derived from reemission (United Nations Environmental Program 2013).

Mercury contamination is not typically caused by the anthropogenic sources of mercury in the environment. In fact, naturally produced mercury further pollutes an already contaminated area. The greatest risk of contamination is a result of anthropogenic (or manmade) sources of mercury. There are a multitude of said sources responsible for contamination, such as: goldmining, metal production, cement production, waste incineration, Chlor-Alkali industry and dental amalgam filling creation. Today, the source responsible for the majority of mercury production is the combustion of fossil fuels, for power and heating. When there are toxic levels

of mercury, derived from a multitude of sources and focused on a given area, contamination occurs.

Combustible fuels comprise 45% of the world's production of mercury. Of the total amount of mercury generated globally, coal produces 40% of the total mercury emission from combustible fuels (United Nations Environmental Program 2013). In the United States, coal generates 13-26% of mercury emission. The concentration of mercury in coal averages 0.17ppb, based on a 7,000 samples selection (Tewalt et al. 2001).

Goldmining is the second leading cause of global mercury contamination. Miners use mercury to help recover gold that was deposited deep within the earth. To ensure efficient and effective gold retrieval, mercury is used in all methods of hydraulic, drift, and dredging for maximum mining effectiveness (Alpers et al. 2005). All methods of retrieval discard the excess mercury-rich waste into rivers, lakes, and aquafers. The retrieval process also emits a percentage mercury into the atmosphere as a waste product (Alpers et al. 2005).

Production of metals is the third leading cause of mercury contamination. Non-ferrous metal production emits 27 tons of mercury per year. Mercury assists in the production of lead, copper and zinc. Zinc is the leader in non-ferrous metal mercury emission as it emits 19 tons yr^{-1} of mercury. Copper emits six tons yr^{-1} of mercury and lead emits two tons yr^{-1} of mercury (United Nations Environmental Program 2013).

Mercury is used in all types of cement production. Coal, which contains mercury, is used to produce heat to eliminate carbon dioxide (CO_2) from the raw material. During the production, increased levels of mercury is discharged as a byproduct or emitted and could cause contamination (Mlakar 2010).

When anthropogenic waste products, containing mercury, are disposed and incinerated, these products release various levels of mercury into the atmosphere (United Nations Environmental Program 2013). The level of mercury emitted is dependent on the type and process in which the product is discarded. Products, such as oil-based products, contain higher levels of mercury. When oil-based products are disposed, they are incinerated and release mercury in to the atmosphere as a waste byproduct, which can lead to higher levels of mercury toxicity.

Chlor-Alkali plants are used to produce chlorine. Prior to the 1970's, the majority of chlor-alkali plants used a mercury-cell processing method in the production of chlorine. When mercury was used in this process, plants emitted mercury into the water and air. Mercury was discharged into the local aquatic system via runoff, seepages and intentional disposal of brine. Mercury would also contaminate the atmosphere through the emission of brine. Although today another cleaner and more efficient method of processing is used in chlorine production, up until the 1970's it was a common and major form of environmental mercury contamination (Maserti and Ferrara 1991).

Dental amalgam fillings are comprised of liquid mercury and metal alloy used to fill cavities caused by tooth decay. In the production of these fillings, mercury is released into the atmosphere as a byproduct of the process. Although it is a trace amount in comparison to other anthropogenic sources, it still plays a role in mercury contamination (Mackert 1991). The use of dental amalgam fillings is a contributing factor to the mercury contamination problem the world faces today.

It is evident that the vast majority of the global mercury contamination is due to anthropogenic sources. Although naturally producing sources of mercury only contribute to the

already present contamination, both anthropogenic and naturally derived sources contribute to the mercury cycling process. The mercury cycling process is the movement of mercury from the earth's surface, where it begins as a byproduct of a manmade or naturally derived source, through the various ecological spheres (atmosphere, pedosphere/lithosphere, hydrosphere, and biosphere).

2.2 Mercury Cycling

The production of manmade mercury has changed over time. Prior to the 1970's, chlor-alkali plants were the leading producers in mercury derived from an anthropogenic source (Schroeder and Munthe 1998). Today, chlor-alkali plants are noted as the smallest producers due to technological advancements and modern revisions to government regulations (United Nations Environmental Program). Coal is now considered the largest producer of mercury in the atmosphere. The total amount of mercury, derived from an anthropic source, ranges from 910-6,200 ton yr^{-1}, with a median of 3,560 tons yr^{-1} (United Nations Environmental Program 2013).

The mercury cycle typically begins when mercury is emitted into the air as a byproduct, of anthropogenic or naturally derived sources. When this mercury is released, it collects in the atmosphere and commences the cycling process. Mercury in the atmosphere has a natural background of 1.3-1.7ng m^{-3} (in the Northern Hemisphere) and 1.1-1.3 ng m^{-3} (in the Southern Hemisphere) (Driscoll 2013). Knowing the natural background of each hemisphere is critical in the understanding of the mercury cycling process. Estimated mercury emission, derived in a study from 1970-1982, measured mercury from natural sources ranging in value from 2,500-30,000 tons yr^{-1}, on a global scale (Lindqvist 1984). A later study, in 1991, revealed an average global flux of 6 gkm^{-2} yr^{-1} (or 0.7ng $m^{-2}h^{-1}$) (Lindqvist et al. 1991). More recent reports note 70% of emission come from natural sources (United Nation Environment Program 2013). The

majority of the 70% comes from reemission of mercury back into the atmosphere. The other 30% comes from anthropogenic sources, which have increased over time (United Nations Environmental Program 2013).

The transportation of mercury in and through the atmosphere is considered a global phenomenon. The movement is dependent on the physical and chemical composition of mercury. It has been discovered, that mercury in the atmosphere can be found in three forms: none-oxidized mercury (Hg^0), oxidized mercury (Hg II), and other species of mercury (noted as: Hg(p)). Non-oxidized mercury comprises approximately 95% of mercury in the atmosphere. This form is the most abundant due to its ability to travel large distances, ≈10,000 miles, prior to being deposited into the water and soil. Oxidized mercury comprises ≤ 3% of mercury in the atmosphere and can travel only a 10-100 miles prior to being deposited. Other species of mercury makes up only scant amounts in the atmospheres and will deposit in the immediate vicinity once emitted (Schroeder and Munthe 1998). The length of travel of each form of mercury is variable to the season and location in which the movement occurs.

The total gaseous state of mercury differs due to location and season, as well. If atmospheric mercury is located near its emission source, there will be an influx of gaseous mercury. If the atmospheric mercury is a great distance from its emission source, there will be a decrease in the amount of gaseous mercury found (Keeler et al.., 1995). A colder, wintery season can be attributed to the increase the total gaseous mercury. A study performed on the Nordic networks showed the total gaseous mercury concentration to be higher during winter months and snowy areas (Lindqvist et al. 1991). Although, another study performed in the rural areas surrounding the Great Lakes region in Vermont, where the winter season is heavily felt, found that the total gaseous mercury concentration was not significantly affected by the cold weather

(Burke et al.. 1995). It can be concluded that the interaction between mercury and the atmosphere is influenced by the seasons but can also be inversely influenced by the location, regardless of the season.

Atmospheric transformation and reaction occurs at the source of emission, but can occasionally occur at a great distance from the originating source. There are five gaseous reactions that dictate the state of mercury that are found in the atmosphere, as seen in Table 1 below. All reactions listed are commonly occurring in the atmosphere and are able to travel at great distance from the originating source.

The most commonly sources of mercury in the atmosphere is $HgCl_2$. Cl is not very common in the atmosphere, but comes from the same sources of mercury. Then it reacts with it in the atmosphere creating $HgCl_2$ had seen in table 1. Little is known how Hg react to form oxidized mercury in the atmosphere, but non-oxidized mercury could be oxidized to form oxidized mercury. The type of mercury in atmosphere dictates how accumulates on land or in water.

Table 1: Reaction and Rates of Mercury Found in Atmosphere

Number	Reactions	Rate (cm^3/moles-sec)	References
1	Hg^0+O_3-Hg(p)	3.0E-20	(Hall 1995)
2	Hg^0+HCl-$HgCl_2$	1.0E-19	(Hall, Bloom and Munthe 1995)
3	$Hg^0+H_2O_2$-Hg(p)	8.5E-19	(Sommar et al. 1998)
4	Hg^0+Cl_2-$HgCl_2$	4.0E-18	(Sommar et al. 2003)
5	Hg^0+OH-Hg(p)	8.7E-14	(Sommar et al. 2001)

Deposition can begin in the following three areas: in the atmosphere, at the air/soil interface, or at the air/water interface. Mercury can be deposited via a dry deposition or a wet deposition. Wet deposition occurs in the form of acid rain. Air that contains an increased concentration of oxidized mercury or other species of mercury (at approximately 100pg m^{-3}) will absorb mercury through wet deposition. The absorption is dependent upon the oxidation factor of Hg^0 in the atmosphere (Schroeder and Munthe 1998). Dry deposition occurs in the form of gas. If the environment is dry, such as forest areas, the dry deposition of mercury can become potentially problematic because the vegetation will absorb any amount of mercury from the air.

Although the atmosphere plays a vital role in the deposition of gaseous mercury, it also plays an important role in the volatilization at the interfaces of air/soil (to include vegetation) and air/water. Soils containing a high amount of mercury are considered "Hg pools." These "Hg pools" are a large source of reemission and volatilization. Just like deposition, volatilization is season and location specific (Lindqvist et al. 1991). During warm, summer months there is an increase in the occurrence of mercury volatilization. This is due to the increase in precipitation during this season, at the air/soil interface. There is a direct relationship between the amount of precipitation to the occurrence of volatilization. During the cold, winter season there is a decrease in the amount of precipitation causing this time of the year to be low in volatilization (Schroeder et al. 1998).

Volatilization is highly dependent on the location and the type of soil. The background emission rates of mercury in the atmosphere range from 1-10 ng m^{-2} h^{-1}. Soil high in sledge and waste content has a daily flux of methyl mercury emission values, from 12-24 pg $m^{-2}h^{-1}$ and approximately 100 ng $m^{-2}h^{-1}$ (Lindberg 1992). For example, the Boreal forest areas in Sweden

had emission values of 5 ng $m^{-2}h^{-1}$ (Schroeder et al. 1989) whereas, in eastern Tennessee, during summer and fall months, experience fluxes of 30ng $m^{-2}h^{-1}$ (Kim, Lindberg and Meyers 1995).

The vast majority of volatilization comes from the soil. As much as 25% of the mercury found in soil, leaches down to the sea horizon and bedrock. The background of mercury within the sea horizon and bedrock varies from 455 to 20,000 ppb g^{-1} (location dependent on concentration). While there are a variety of types of mercury located within the sediment, the majority of mercury found in the bedrock is in the form of cinnabar (chemical formula: HgS). Cinnabar encompasses 86% of the total mercury found in bedrock (United Nations Environmental Program 2013). Mercury that does not undergo volatilization is either stored in the bedrock or transported to a watershed. Mercury enters the watershed primarily through the atmosphere. It can also enter the watershed via runoff and soil erosion, as a secondary form of pollution. This can lead to the contamination of rivers, lakes, and streams. When an aquafer is already contaminated with mercury, in-flowing rivers from the aquafer can spread tertiary mercury contamination.

The majority of the mercury found in water is in the form of oxidized mercury (chemical formula: Hg^2). If mercury were to enter the surface water in a non-oxidized state (chemical formula: Hg^0), it would convert to oxidized mercury to become adaptable to the water environment, as seen on Table 2. Oxidized mercury encompasses 98% of mercury found in surface water (Weber 1993). The abundance of oxidized mercury is due to the chemical reactions noted in Table 2. Reactions 3 and 6 depict the very slow interaction of non-oxidized mercury in a water environment. The remaining reactions depict the efficiency that oxidized mercury has within a water environment. In water, mercury can bind to a myriad of elements. The most common of these are: $HgSO_4$, HgHS, HgHs, Hg-dissolved organic matter, $HgCl_2$, $Hg(HS)_2$ and

HgS (Hsu-Kim et al. 2013). For a more detailed and comprehensive list of other possible compounds of mercury in water, please reference the journal of Hsu-Kim, 2013, page 2447.

Table 2: Reaction and Rates of Mercury Found in Water

Number	Reactions	Rate (moles-sec)	References
1	$Hg^0+O_3—Hg^2$	4.7E+7	(Munthe 1992)
2	$Hg^0+OH—Hg^2$	2.0E+9	(Lin and Pehkonent 1997)
3	$Hg^2+OH—Hg^0$	≈0	(Anderson 2003)
4	$Hg^0+HOCl—Hg^2$	2.1E+6	(Pehkonen and Lin 1998)
5	$Hg^0+OCl^{-1}—Hg^2$	2.0E+6	(Lin and Pehkonen (1998)
6	$Hg^2+H—Hg^0$	6.0E-7	(Bullock and Brehme 2002)

To this day, little research has been performed on the movement of mercury through an aquatic environment. The transportation of mercury through a watershed is due to, but not limited to, turnovers, tidal forces, and/or weather. In the presence of water, mercury undergoes a phase of equilibrium. During this phase, mercury can volatilize into the atmosphere, be dispersed into the water, and/or transported through the watershed (Hsu-Kim et al. 2013). Due to the density of mercury, in compounded form or singularly, it will percolate to the bottom of the aquatic environment. Mercury that is in the water singularly, will form a compound before, or at the time, it reaches the bottom of the aquatic floor. In study performed by the United States Geological Survey, mercury in sediment at the bottom of bodies of water would increase in depth until it reached 10 cm, where it would start decreasing. Mercury is imbedded deeper into the sediment over time due to the addition of new sediment pushed upon it by the moving water

body (Colman 1999). Once in the sediment, mercury can methylate by sulfur reducing bacteria or iron reducing bacteria. Both types of micro bacteria contain a two-gene cluster called “hcg a” and “hcg b” (Hsu-Kim et al. 2013). It is these two-gene clusters that the reaction results in the production of methyl mercury.

2.3 Dissolved Oxygen

Dissolved oxygen plays an important role in aquatic environments. Dissolved Oxygen (DO) is the amount of free oxygen in an aquatic environment and is measured in mg/L (Wetzel 2012). Dissolved oxygen takes the form of O_2 and is not compounded with another element. Dissolved oxygen is the most essential element in the aquatics system, next to actual water. The amount of DO in the water effects fish, plankton and microorganisms. Too much or too little DO can have a serious impact on the biota (Wetzel 2012).

There is three processes in which DO enters the aquatics system: diffusion, photosynthesis, and rivers (moving water). Oxygen in the air enters the water via diffusion. Oxygen moves form a higher energy state (in the air) to a lower energy state (in the water). Plants that are submerged or emergent in the water consume CO_2. The consumption of carbon dioxide reacts with water to create methane and DO ($CO_2 + H_2O \rightarrow (CH_2O) + O_2$). Dissolved oxygen also enters the aquatic system through rivers that usually contain high amount of DO (Wetzel 2012).

Dissolved oxygen saturation in water is dependent on the equilibrium of DO between water and air. The equilibrium is the percentage of air in the area, which is equivalent to the percentage of DO in the water. Water slowly absorbs gasses, like DO, into the water until equilibrium is reached. Equilibrium is easily facilitated in shallow water versus water great in depth. Respirations of organism, microorganism and decomposition through microorganisms

prevent DO from reaching 100% equilibrium, in deep waters. Some water bodies do not experience equilibrium because they are to shallow in depth. The decrease of DO occurs below an invisible boundary, known as the thermocline: an area that separates the metalimnion from the hypolimnion. Below the thermocline, there is a steady decrease in temperature, which decreases the maximum amount of DO (Wetzel 2012).

Temperature and solubility of DO have an inverse relationship. As temperature rises, the DO saturation decreases. Inversely, in cooler temperatures, DO saturation increases. An increase in depth causes a decrease in temperature, increasing saturation potential. While it is expected that saturation will increase with depth that is not the case for every type of water body. Since DO is derived from the atmosphere it is more difficult for DO to reach greater depths. Organisms and microorganisms will decrease DO through consumption and respiration. This reduces the amount of DO in deeper waters since DO cannot be replaced due to depth (Wetzel 2012).

Salinity decreases DO solubility equilibrium by 20% lower than fresh water. Saltwater intrusion can cause a decrease in DO within a coastal water body. The introduction of saltwater to fresh water bodies will decrease the overall amount of DO soluble equilibrium (Sánchez et al. 2007).

Altitude affects the DO solubility equilibrium through air pressure. As air pressure increases, the DO solubility equilibrium also increases. Water bodies at lower altitudes have a higher DO solubility equilibrium due to the atmospheric pressure. With every increase in altitude by ten meters, there is a 10% loss of DO, which equates to smaller amounts of DO in water bodies (Sánchez et al. 2007).

Oversaturation is also possible in aquatic environments because of the biota in the ecosystem. Supersaturation occurs when DO concentrations in the water exceed the equilibrium in the air. Aeration in a water body is usually due to photosynthesis in the environment. This occurs during plant respiration when the plant consumes in carbon dioxide and releases oxygen ($6CO_2+H_2O+\text{light energy}\rightarrow C_6H_{12}O_6+6O_2$). Supersaturation is most probable during the day when the plants can receive the most amount of sunlight. However, if the plants are not close enough to the surface the plant will not be able to receive the maximum amount of sunlight (Sánchez et al. 2007).

In rivers, DO is usually high in concentration. This is due to the amount of runoff from precipitations, tributaries and the movement in the water. The runoff from precipitation introduces fresh sources of DO through tributaries or higher elevated landmasses. The river receives more DO than it consumes providing a high concentration of DO. However rivers or tributaries that are fed by springs, have a naturally lower concentration of DO, which lowers the overall concentration of DO in the water body (Sánchez et al. 2007).

The limnology of a lake is different than that of a river. The amount of DO is different in lakes because of stratification and low flow. Dissolved oxygen in lakes changes due to depth and stratification. In the upper layers of the lake, known as the epilimnion, DO is high. Dissolved oxygen typically begins to decline when passing into the metalimnion (middle layer), passing the thermocline boundary. Frequent changes of the metalimnion can cause higher or lower concentration of DO. The hypolimnion, the lowest layer of the lake, contains 40% less concentrations then the upper two layers. In some lakes, the hypolimnion becomes anaerobic when DO is unable to reach (Sánchez et al. 2007).

Estuary stratification is different from the rivers and lakes because of the combining of salt water and fresh water. Salinity values change during high and low tides. This causes a vertical stratification due to the push and pull relationship of salt water and fresh water. There is a inverse relationship between the distance towards land and the concentration of DO. The further inland, the greater the DO concentration whereas, measurements taken in open sea, decreases concentrations of DO (Sánchez et al. 2007).

The changes of DO have a varying affect on different types of aquatic life. Higher trophic level organisms are more susceptible to change in DO. Higher trophic organisms typically require a higher amount of DO. When DO decreases, it could have a negative effect on higher trophic organisms (possibly death). Where lower trophic organism, like crawfish, can survive in lower concentration of DO (Sánchez et al. 2007).

The greatest threat to organisms, in water, is turnovers. Turnovers are typically associated with lakes. Lakes typically turnover twice a year: in the winter and in the spring. However, between those times, the hypolimnion can become anaerobic or anoxic, which can kill many types of organisms. In colder regions where ice covers a water body, the DO cannot reach the bottom of the water body due to the ice. During the summers, DO is removed from the bottom of water bodies due to decomposition and consumption of DO (Sánchez et al. 2007).

2.4 Phosphorus Cycle

Phosphorus plays a unique role in cells. Phosphorus functions in energy storage and makes up the cellular wall. The most important role is energy storage. In storing energy, phosphorus take the form of Adenosine Tri-Phosphate (ATP or $C_{10}H_{16}N_5O_{13}P_3$). When an enzyme needs the energy, it loses it endmost phosphate group for energy to create proteins. The loss of the phosphate group releases a large amount of energy usually in the form of HPO_4. The

chain is now adenosine diphosphate. If the cell requires more energy, it loses another phosphate group creating adenosine monophosphate. When cells are not using energy, it continues to build up the ATP storage. Phosphorus is also found in the cellular wall. The walls are comprised of lipids that contain phosphorus. The presence of phosphorus can strengthen the cellular wall allowing for protection (Wetzel 2012).

Phosphorus takes three forms: orthophosphate (PO_4), metaphosphate (or polyphosphate) and organically bound phosphate. Each compound contains phosphorous in a different chemical arrangement. The majority of phosphorus is found in rock. While phosphorus does not enter the atmosphere, precipitation such as rain, erodes the rock. The rock either moves to soil or water. Phosphorus has a tendency to combine with the soil and can be absorbed by plants in an inorganic form. The plant can be eaten and phosphorus then changes to an organic form. Once in the soil, phosphorus mineralizes to its inorganic form through bacteria. Phosphorus can enter the water through run off from fertilizer, mining, and natural processes. Phosphorus is not easily soluble and likes to bind with soil. However, phosphate can be available if the soil is stirred up or the environment is anaerobic and/or reduced (Wetzel 2012).

2.5 Nitrogen Cycle

Nitrogen cycle is very important to life and the ecosystem. There are four processes in the nitrogen cycle: nitrogen fixation, decay, nitrification, and denitrification. Microorganisms play an important part in the transformation of nitrogen. Since these processes are meditated by micro-bacteria the process occurs quickly. So the rate of the process is controlled by environmental factors, such as temperature, moisture and resources (Wetzel 2012).

In the atmosphere nitrogen is in its inert state, N_2. There are three ways for nitrogen to enter soil or water from the atmosphere. Atmospheric fixation is the most rare, which accounts

for 5-8% of nitrogen leaving the atmosphere. This method needs lightning to break the bonds of nitrogen gas. Once broken, nitrogen combines with oxygen. The compound dissolves in rainwater to form nitrates and becomes part of the soil or water. The industrial method uses heat and nitrogen gas to form fertilizer, usually in the form of ammonium nitrate. The most common method of nitrogen fixation is biological. The microorganisms convert the nitrogen gas into ammonia, $N_2 + 8\ H^+ + 8\ e^- \rightarrow 2\ NH_3 + H_2$.

Nitrogen can also be derived from the decay of deceased organisms, feces or urine. The process of decay is also known has purification where fungi or bacteria breakdown the organic nitrogen. The organic nitrogen is then convert into ammonia. In the soil the ammonia forms NH_4. In water the form of ammonia depends on the pH. If the pH is high then the ammonia is NH_4. If the pH is low then ammonia forms NH_3 (Wetzel 2012).

Once in the form of ammonia nitrification occurs. Nitrification is the oxidation of ammonia to nitrate. The first step in the process is oxidizing the ammonia to nitrite (NO_2-), $2\ NH_4^+ + 3\ O_2 \rightarrow 2\ NO_2^- + 2\ H_2O + 4\ H^+$ or $NH_3 + O_2 \rightarrow NO_2^- + 3H^+ + 2e^-$. This happens through the microorganism called Nitrosamines. The second step in the process is converting the nitrite into nitrate (NO_3-), $NO_2^- + O_2 \rightarrow 2\ NO_3^-$. This portion of the process occurs through Nitrobacteria or Nitrospina. Once in the form of nitrate, plants or other organism usually consume the nitrate for nourishment. Once nitrogen is in the form of nitrite or nitrate bacteria start conducting denitrification. During the process these organism convert the two compounds into nitrogen gas and releases it back into the atmosphere (Wetzel 2012).

2.6 Sulfur Cycle

Sulfur is the 6th most commonly found element on earth. Sulfur is necessary for plants and animal in the synthesis of amino acids as cysteine, methionine and proteins. The sulfur is

available to organism through the process of the sulfur cycle. Sulfur cycles through four zones: atmosphere, lithosphere, hydrosphere and biosphere (Kutney 2007).

Sulfur enters the atmosphere through volcanoes, volatilization, plankton, or manmade sources. When sulfur enter the atmosphere has hydrogen sulfide (H_2S). The hydrogen sulfide reacts with oxygen to create sulfur dioxide (SO_2), $2H2S+3O2 \rightarrow 2H2O+2SO2$. Volcanos can also emit sulfur as sulfur dioxide. Volatilization comes from anaerobic decay on the earth surface. When volatilized sulfur enter the atmosphere has hydrogen sulfide. It then reacts with oxygen to create sulfur dioxide. Plankton located on the surface of water emit sulfur has dimethyl sulfide ($(CH_2)_3S$). Once in the atmosphere, dimethyl sulfide reacts with oxygen and creates sulfur dioxide (SO_2), $2(CH_3)_2S + 9O_2 \rightarrow 2SO_2 + 4CO_2 + 6\ H_2O$. Manmade sources are found in industry and emit sulfur dioxide. Once sulfur dioxide is in the atmosphere it reacts with O_2 and creates sulfur trioxide (SO_3), $2SO_2+O_2 \rightarrow 2SO_3$. Sulfur trioxide can react with tiny water droplets and fall to the surface. It could also react with ammonia to form ammonium sulfate ($(NH_4)_2SO_4$). The sulfur is then carried by wind until it falls to the ground or water through wet or acid deposition (Kutney 2007).

Once in the soil sulfur takes the form of a sulfate and can bond with other elements in the soil. In a more dry setting, sulfur bonds with hydrogen to form hydrogen sulfide or mercury to create mercury sulfide. If this occurs the sulfide can be stuck in the soil or lithosphere for a while. If it stays in the form of sulfide it could reenter the atmosphere through volcanos or other physical mechanism. If the soil is moist enough the sulfur can bond to another element has sulfate. Sulfate can bond with mercury creating mercury sulfate. If the environment is right the sulfate could be reduced by SRB and take the form of Sulfide. It could also be taken up by plants, has a salt. Plants could then release the sulfur into the atmosphere. Animals could also

consume the plant, but the sulfur would leave the animal a fecal matter. Then it decays and starts the process all over again (Kutney 2007).

Sulfur can also enter a water system through deposition. In a water environment, sulfur usually takes the form of sulfate. Sulfate can attached to many different elements and compounds. It can be taken up by plankton or plants and volatilize into the atmosphere. It can also be consumed by another biota. If the environment is reduced and anaerobic the sulfate can be consumed by bacteria and transformed into sulfide. In its sulfide state, it can transform back to sulfate or descend into the sediments (Kutney 2007).

2.7 Sulfur Reducing Bacteria vs. Iron Reducing Bacteria

For mercury to methylate, it enters the cells of sulfur reducing bacteria or iron reducing bacteria through four possible pathways. Not all pathways end in methylation. These pathways are marked with gram-negative micro bacteria. Pathway 1 is Mer based transport. Lipophilic oxidized species are able to pass through the outer layer of the cell and into the periplasm. Mer P transports the mercury, through the inner membrane; to Mer T. Pathway 1 is the only pathway that does not lead to methylation. Pathway 2 is the passive diffusion of lipophilic mercury. Passive diffusion allows mercury to pass through both cell membrane layers. Pathway 3 is facilitated diffusion. By way of the transmembrane protein channel, mercury is pass through a neutrally charge compound into the cell. Pathway 4 is active transport. Oxidized mercury is forced through a protein channel located on each cell membrane (Hsu-Kim et al. 2013).

The elements present in the sediment dictate the amount of sulfur reducing bacteria and iron reducing bacteria located within the sediment. If there is an increase in the amount of iron, in the sediments, and a decrease in the amount of sulfur, then there will be a large population of iron reducing bacteria. If there is an increase in the amount of sulfur in the sediment and a

decrease in the amount of iron found, then there will be a large population of sulfur reducing bacteria. If there is an increased amount of both iron and sulfur found in the sediment, then there will be large amount of both micro-bacteria.

Iron reducing bacteria is not as readily found in the environment. A study performed in 2015, used oxidized iron ($FeCl_3$, $Fe(OH)_3$, and $C_6H_3FeO_7$), oxidized mercury and iron reducing bacteria to produce methyl mercury. The experiment took sediment, which contained iron reducing bacteria, and introduced oxidized iron and oxidized mercury compounds. The introduction of both would reduce the amount of iron and create methyl mercury. The study found that lower concentrations of oxidized iron produced more methyl mercury than higher concentrations of oxidized iron (Si et al. 2015). It could also be deduced that the iron reducing bacteria reversed the process when increased amounts of oxidized iron were introduced. The study found iron reducing bacteria can produce methyl mercury but deduced that using sulfur reducing bacteria yields a more productive outcome and is far more abundant in nature.

There is presently more knowledge on using sulfur reducing bacteria, which is more efficient in the process of methylation, than iron reducing bacteria. Given the extensive studies performed on sulfur reducing bacteria, the entire process of methylation is more widely understood using this type of micro-bacteria. An example of said study is one performed in 2012, which compared varying levels of sulfate (no sulfate used, low, and high) with inorganic mercury to understand the relationship between concentrations of sulfate and methyl mercury. The study found the reactor with no sulfate produced the least amount of methyl mercury. The reactor with low amounts of mercury produced at least 10 ng L^{-1} more than the reactor with no sulfate. The reactor with the highest amount of sulfate produces the most amount of methyl mercury, by at least a 100 ng $^{L-1}$ (Shao 2012).

2.8 Water Environment and Mercury

Mercury can be found in rivers, lakes/reservoirs, wetlands and other aquatics environments. Mercury does not respond the same in different aquatic environment. There has been different studies on production of methyl mercury in relationship with river, lakes/ponds/reservoirs, and wetlands (Wetzel 2012)..

Rivers are usually considered to have low amounts of mercury and methyl mercury because the movement of the water. SRBs live in an anaerobic environment and rivers don't usually provide that environment. Rivers have moving waters that constantly introduces oxygen because of the speed of the moving water. However, in some cases the water moves slower than usually because of the environment. This means less oxygen is being introduced into the environment and can cause the accumulation of mercury and methyl mercury. (Paller et al. 2004).

Lakes, ponds and reservoirs are conducive for SRB. Lakes are broken of into three stratification: epilimnion, metalimnion and hypolimnion. The epilimnion and metalimnion are the top two portion of a lakes, ponds and reservoirs but also have the most oxygen. The bottom part of a lake, pond and reservoirs is the hypolimnion. Since water moves slower and there is little oxygen it consider and anaerobic environment. The anaerobic environment in lakes, ponds and reservoirs is very conducive for SRB. Mercury also falls to the bottom of these aquatics environment where the SRB can produce methyl mercury. Since there is very little movement the amount of mercury and methyl mercury can build up over time. That's why lakes ponds and reservoirs are consider to be the best environment for production of methyl mercury (Wetzel 2001).

Wetlands are unique situation because they are made up of plants and very slow moving water. Water may even reseed over time and expose sediment surfaces. In most cases wetlands are usually basin or/and catchments. The rivers and lakes usually discharge all the nutrients, metal and contaminants into the wetland. Wetlands are unusually anaerobic environment where SRB thrive. Wetland can easily form reduced and anaerobic environments. This is way many believe that wetlands produce methyl mercury. A study done by the USGS (Eagles-Smith et al. 2012) found that wetlands are a good environment to produce methyl mercury. However, a study done in 2008 (Hall et al. 2008) showed that the sediments contain very little methyl mercury in comparison with the local lakes and river. Another study found that many biota, especially biota found in wetlands, accumulate the methyl mercury in their biomass removing it from the sediment (Wetzel 2012).

CHAPTER 3:
METHODOLOGY

3.1 Overview of Methodology

The samples were collected from Brunswick GA estuary. A total of six samples were taken. Sample site 1 and 2 were taken from Middle Turtle River and Academy creek at coordinates 31 09 .899N 81 27 .911W and 31 09 .929N 81 30 .355W. Sample Site three was next to 17 in Terry Creek at coordinates 31 09 .183N 81 31 .030W. The 4th sample site taken from a lake located near Mellow Marsh Park at coordinates 31 09 .117N 81 28 .347W. The 5th sample site taken from a stream called Clubb Creek at coordinates 31 08 .849N 81 27 .548W. The control samples were taken from Buffalo Creek. The location is off of 405 at coordinates 31 15 .594N 81 35 .399W.

The research objective is to contribute to the knowledge of production of methyl mercury by sulfur reducing bacteria in sulfur-rich sediments. The site selected was derived from the research gathered regarding Brunswick, Georgia. From 1970 to 1971, a study performed by Georgia Geological Survey, found that the fish in the Brunswick watershed were contaminated with copious amounts of mercury. The historical data showed Allied Chemical, a chlorine production company located in Brunswick, Georgia, was responsible for discharging 1.36-4.55 L of brine per day. The brine was a byproduct of mercury cell processing from chlor-alkali plants. The study also revealed an increasing concentration of methyl mercury in fish. The two highest concentrations of methyl mercury were found on Turtle River where GA-17 and GA-301 cross over the river (Odom 1975). The Georgia Geological Survey also performed a study on the sediment in Brunswick, Georgia. The sediment samples retrieved revealed increasing amounts of mercury at depths of 30.5cm (Zeller and Finger 1971). A more recent study performed by the Georgia Department of Natural Resources, in 2015, retrieved samples of aquatic life in the

Brunswick Estuary, Turtle River, Back River and their respective tributaries. Based off the study findings, fish advisories have since been created warning the local population of increasing mercury levels in the fish (Georgia Department of Natural Resources 2015). The mercury found at these sites is believed to be from the superfund site. At adjacent sites, sulfur-rich sediment has also been found adjacent to the superfund site (United States Department of Health and Human Services 2014).

Sampling Sites:

Six sampling locations were chosen to analyze the methylmercury production potential in the sediments from the Brunswick Estuary, which based on research conducted by the Georgia Natural Resources and the United States Department of Health and Human Services, have been historically contaminated with mercury (United States Department of Health and Human Services 2014). To test the methyl mercury potential, the samples have to be spatial and environmentally different from each other. Three different aquatic environments (wetlands, lakes and streams) were tested for microcosm studies in the lab at Georgia Southern University. They were chosen to give an idea of the potential in each sample site to produce methyl mercury

All sediment samples were collected using a dredge that holds about 250cm^3 of sediment. The samples were then stored in mason jars holding 250cm^3 of soil. The samples were stored, in their original collection jars, at the Environmental Lab at Georgia Southern University in a 39ºF refrigerator during the construction of the reactors. The samples were categorized in the following fashion: Sample Site and Reactor 1 – Middle Turtle River (wetland area); Sample Site and Reactor 2 – Academy Creek (wetland area); Sample Site and Reactor 3 – Terry Creek (wetland area); Sample Site and Reactor 4 – Mellow Lake (lake); Sample Site and Reactor 5 -

Clubb Creek (stream); and Sample Site Reactor 6 - Buffalo Creek (stream). Sample Site 6 will act as the Control Site for this experiment (Figure 1).

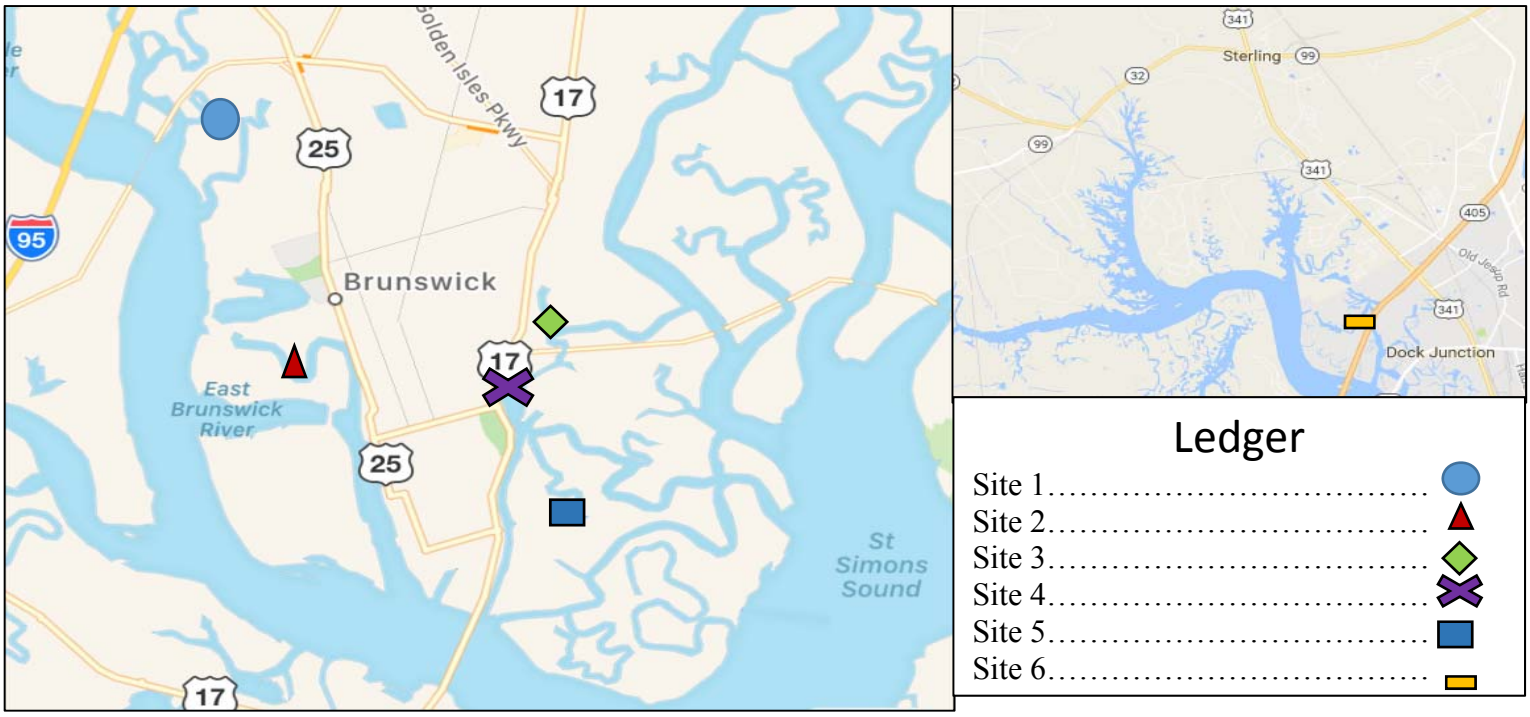


Figure 1: Samples site selected (Google Maps)

Microcosm Setup:

Lab-scaled reactors were used to test the methylmercury production potential from the sediment collected in each site. The reactors emulated an environment that allowed sulfur-reducing bacteria to thrive and to produce methyl mercury. Six 2000mL flasks were used to create the reactors. Each sample site was designated its own reactor. The sediment from each sample site was measured to 600ml, and added to the 2000mL Flask. The reactor required 1400mL of DI water. Nutrients and metals (iron, manganese, and mercury) concentrations in the water used to seed the reactors were low enough to considerably affect the results for this study (see results section). Four small glass tubes were installed in the top of each reactor, through the

rubber stoppers, and where used to collect water samples and to purge the reactors. The first tube inserted through the stopper was a large, glass tube. This tube was connected to a nitrogen tank with a valve to introduce nitrogen into the environment with the purpose of preventing oxygen intrusion into the reactors. The valve connected to the reactor is then connected to a six-piece splitter that will distribute nitrogen to each reactor. The splitter is connected to a main regulatory valve, which controls the flow of nitrogen. The second tube acts as an exhaust allowing the nitrogen to flow out of the flask. The third tube inserted was used for sample retrieval. The fourth tube was used to reintroduce water to each reactor, after samples have been retrieved for analysis, to maintain a constant volume of 2000 mL within the flasks (figure 2). When water was reintroduced, it was controlled by a valve, which regulated the flow of water by a slow drip over a 24-hour period. The valve is connected to water, from the corresponding sample site that is gravity fed to the reactor. Before retrieving samples from the reactors on November 14, 2016, oxygen was added until water values reached 8 mg/L (figure 3 page 38). Samples from each reactor were taken weekly from November 14, 2016 through December 19, 2016. A day of

Figure 2: The figure shows of the setup used in the experiment.

sample consisted of 75-350 ml removal of water from the reactors (the sample removed was split into two 75 mL vials). A day of sample consisted of 75-350 ml removal of water from the reactors (the sample removed 50 mL of water were taken for iron and manganese analyses from each reactor and placed in a 50 mL plastic vile. An average of 40 Drops of .1 moles of nitric acid were added to the iron and manganese samples to decrease pH to 2. 200 mL of water for mercury and methyl mercury analysis were taken (see table A2 in appendixes for dates samples were taken) and placed in and 250 mL amber bottle. An average of 20 drops of 1mole of hydrochloric acid were added to mercury and methyl mercury sample to lower pH to 2. Then 25 mL of nutrients samples were taken in a 50 mL plastic vile (See table A1 in appendixes for dates sampled) for dates taken). All sample vials were placed in the refrigerator set at 39 ºF. After every sampling run, nitrogen was immediately circulated through the flasks to purge out any oxygen that was introduced when sampling. Iron and manganese samples were tested in the Georgia Southern Chemistry Lab. Mercury and methyl mercury samples were sent to the Environmental Engineering Department at UC Merced. Nutrient (ammonia, nitrate, orthophosphate, and sulfate) and total organic carbon samples were tested in the Civil Engineer and Construction Management Department at George Southern University. Metals, nutrients, and organic carbon samples were analyzed following standard methods (American Public Health Association, 2014). Mercury and methylmercury samples were analyzed following the most current EPA method (Method 1630).

3.2 Operational Controls

In this study, all reactors have to be kept sealed to environment to promote the development of anaerobic conditions (figure 3). For this reason, decreasing oxygen intrusion during the sampling runs in all reactors was a necessary step. The implementation of operational

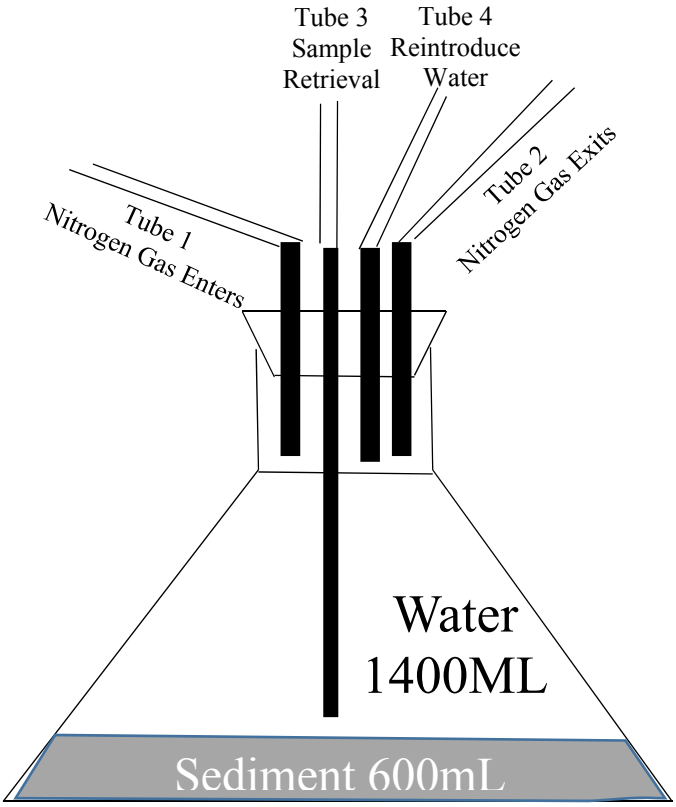


Figure 3: The figure shows how each individual reactor was setup.

controls that were traceable during the experiments was developed. The goal of the controls was to: limit the introduction of oxygen, prevent the contamination of samples, and preserve the samples during storage and shipment.

To limit the oxygen exposure, the reactors sampling and exhaust tubes remained clamped and sealed until use. When sampling, it was possible that minimal amounts of oxygen entered the reactors, thereby potentially contaminating the samples. For instance, using a probe to measure DO brought some oxygen into the headspace of the reactors. To rapidly remove the oxygen from

the headspace, each reactor was purged with nitrogen gas for approximately 2 minutes and then they were immediately sealed to the atmosphere once again. To prevent contamination, multiple preventative steps were taken. All sample vials were labeled and numbered to prevent cross-contamination from another sample. Each reactor received a designed syringe to prevent contamination between reactors. Each syringe was rinsed thoroughly before and after using DI water. Due to the wet environment, allege growth was potentially problematic so, tin viol was used to cover each reactor to prevent bacterial growth. Setting a schedule to collect samples from the reactors was very important in this experiment. If samples were taken at the incorrect interval, it would negatively affect data collection and results. Adherence to maintaining a clean environment to prevent contamination was critical amongst all individuals working on the reactors.

Sediment samples were taken from each site with van veen, that was rinsed with tap water before gather each sample (figure 4). The samples were taken with glove and stored in 500mL mason jars. The jars were immediately placed in a cooler, on ice, once samples were taken. All sample were stored in a refrigerated cooled to 39 ºF. Mercury samples were stored in amber bottle to prevent light from making contact with the sediment, this prevented the light from breaking the CH_3 bond with Hg^{+2}. Iron and Manganese sample received, on average 40 drops of nitric acid to preserve metals, this lowered the ph down to two (Clesceri et al. 2005). Mercury samples received 20 drop of hydrochloric acid to preserve metal, this lowered the pH

down to two (Clesceri et al. 2005). Samples were ship overnight in a cooler packed with stuffing to prevent stuffing.

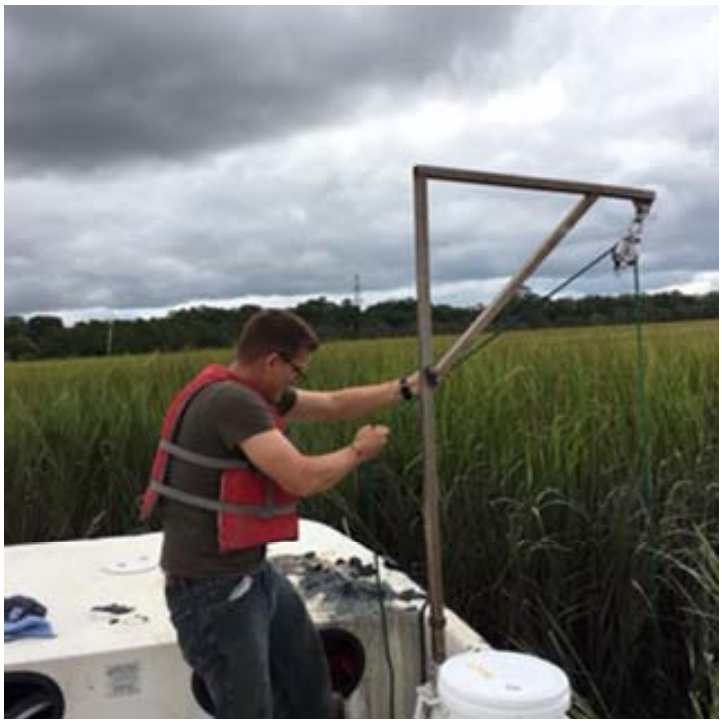


Figure 4: Picture of samples being collect from Middle Turtle River.

CHAPTER 4:
RESULTS AND ANALYSIS

Laboratory experiments were performed to measure the methylmercury production and release potential from sediments collected at different locations in the Brunswick Estuary area adjacent to a mercury super fund site. To test for sediment mercury release potential, sediments were incubated in laboratory-scaled reactors subject to ideal conditions for methylmercury formation and subsequent release. Reactors were initially left open to the atmosphere for two days to allow oxygen penetration to the upper sediments and the water column (day 0). Equilibrium between the two interfaces was also achieved during this time. After day 0, reactors were sealed to the atmosphere to encourage the development of anaerobic conditions for the duration of the experiment. DO concentrations rapidly decreased to values lower than 1 mg/L during the first six days of the experiment (Figure 5). After the second week of the experiment, DO concentrations decreased to very low values and stayed that way until the culmination of the study.

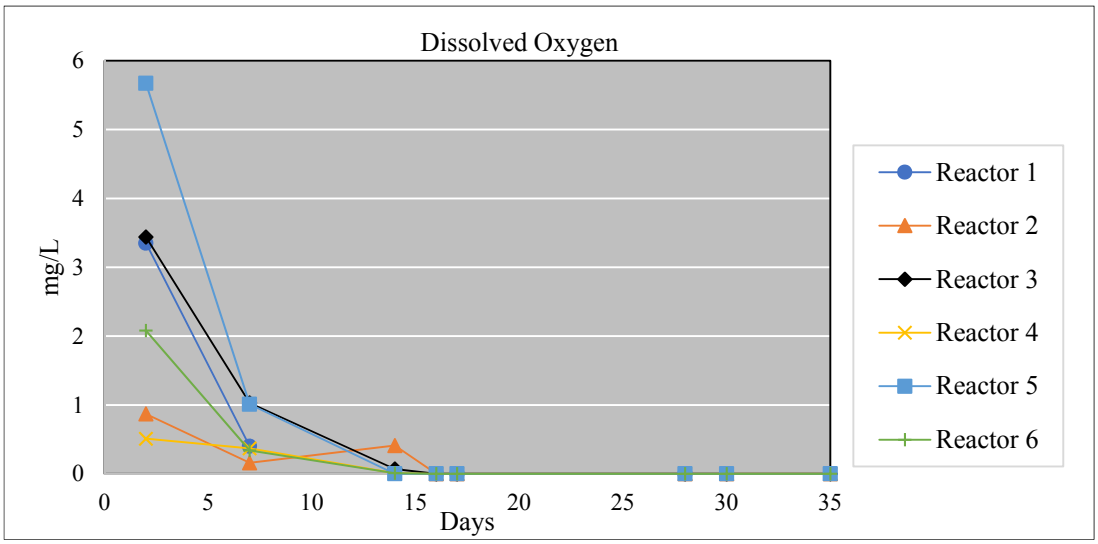


Figure 5: Dissolved oxygen concentrations in all reactors in the experiment.

Oxidation reduction potential values were measured to attest for reducing conditions in each reactor and to confirm that the resulting environment would sustain the reduction of sulfate to sulfide. The ORP values, amongst all reactors, declined since the initiation of the study and decreased to values lower than 0 mv at week three (Figure 6). The ORP fell due to the reductions of oxidized elements of oxygen, nitrogen, manganese, iron and sulfate. When other elements are reduced the lower the potential becomes. After weeks two and three, after the majority of sulfate was reduced to sulfide, ORP values slowly increased probably due to oxygen intrusion towards the end of the study. R1 falls until day 14 when it reaches below zero, then starts to rise. R2 decreases in week one, but increases in week three. The lowest ORP value in R2 occurred in the middle of week three. R3, R5 and R6 saw a decline from the beginning of the experiment until week three when they hit their lowest point, R5 had the lowest ORP values. R4 took the longest to bottom out at the end of week three (day 17). The pH values, in all reactors, decreased until week three where it began to gradually increase until the end of the study (Figure 7). R6 had the steadiest decrease and lowest pH values out of all the reactors. R1 one had the highest amounts of pH and stayed the highest throughout the experiment. R2-5 was close in pH through the

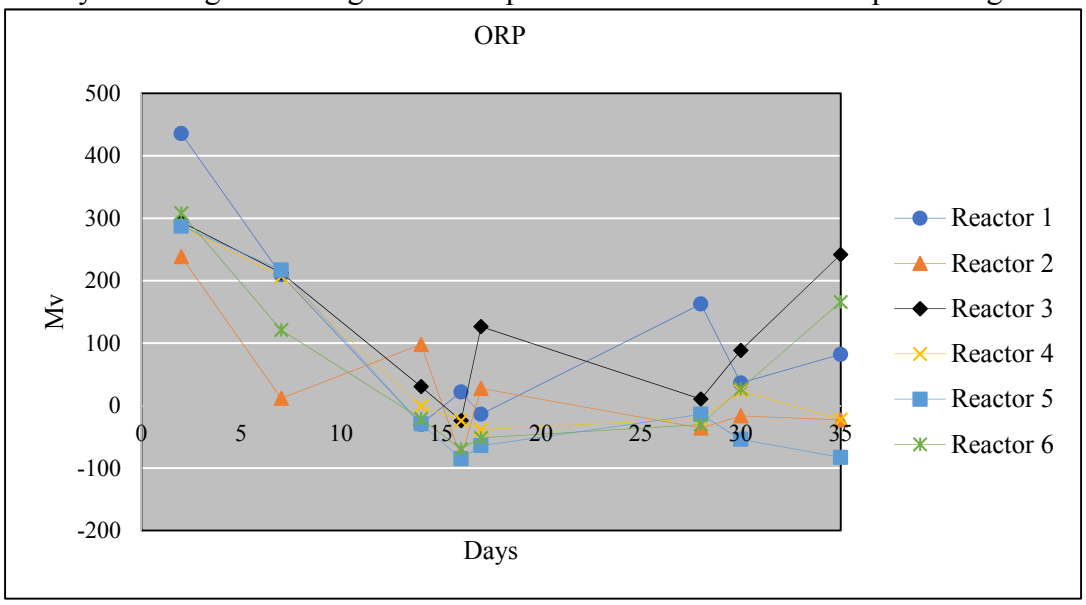


Figure 6: ORP values in each reactor corrected for standard conditions.

experiment, which deceased until week three. In week four R2-5 increased until the end of the experiment.

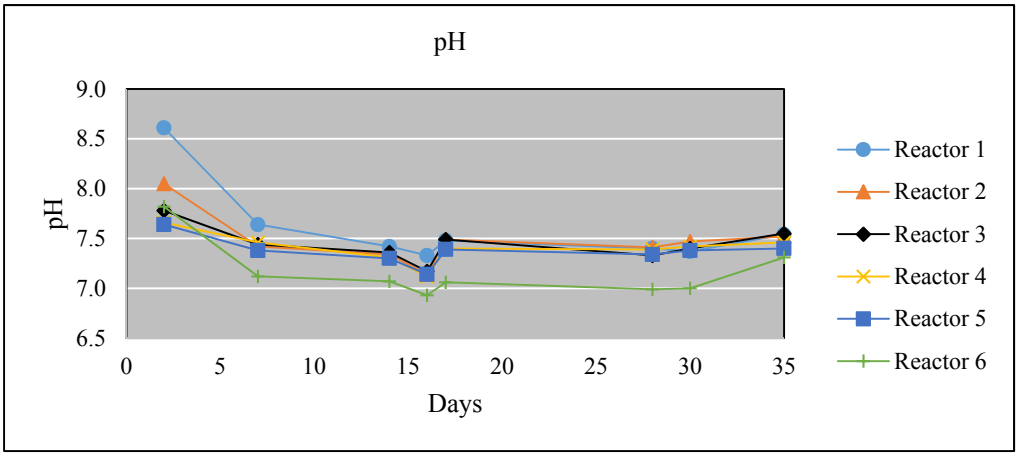


Figure 7: pH values within each reactor throughout the experiment.

Sulfate was measured to determine if the amount of available sulfur would limit the proliferation of sulfur reducing bacteria (SRB). In addition, sulfate concentrations were used as an indicator showing that SRBs were actively using sulfur to oxidize the available organic matter under reduced conditions. Sulfate concentrations were high in all reactors. Initially, sulfate concentrations in the water column ranged from 60-175 mg/L, except for R4 which was 175 mg/L, and increased to values higher than 250 mg/L after day 10. As expected, sulfate values decreased towards the end of the study, under anaerobic conditions (Figure 5&6). Initially, during the first two weeks of the study, sulfate concentrations increased in all reactors while oxygen was still present in the water column. It is possible that sulfate increased due to sulfate diffusing from the rich sulfur sediments used in the experiment, and due to the oxidation of reduced sulfur (sulfide) within the sediments and in the water column. Sulfate began to decrease at day 15 immediately after the development of anaerobic conditions and the establishment of a reduced environment in each reactor. All reactors reduced the most amount of sulfide from day 15 to 24. R5 and R6 reduced the highest amount of Sulfate (130 and 133 mg/L respectively) out

of all the reactors. R2 and R3 reduced similar amount of sulfate (122 and 123 mg/L respectively), which was lower than the amount reduced in R5 and R6. R1 reduced the second lowest amount of sulfate (≈ 115 mg/L) and R4 reduced the lowest amount (≈ 82 mg/L). After Day 28, oxygen introduced into the reactors caused some of the sulfide to convert to sulfate, causing an increase in the sulfate concentration towards the end of the experiment.

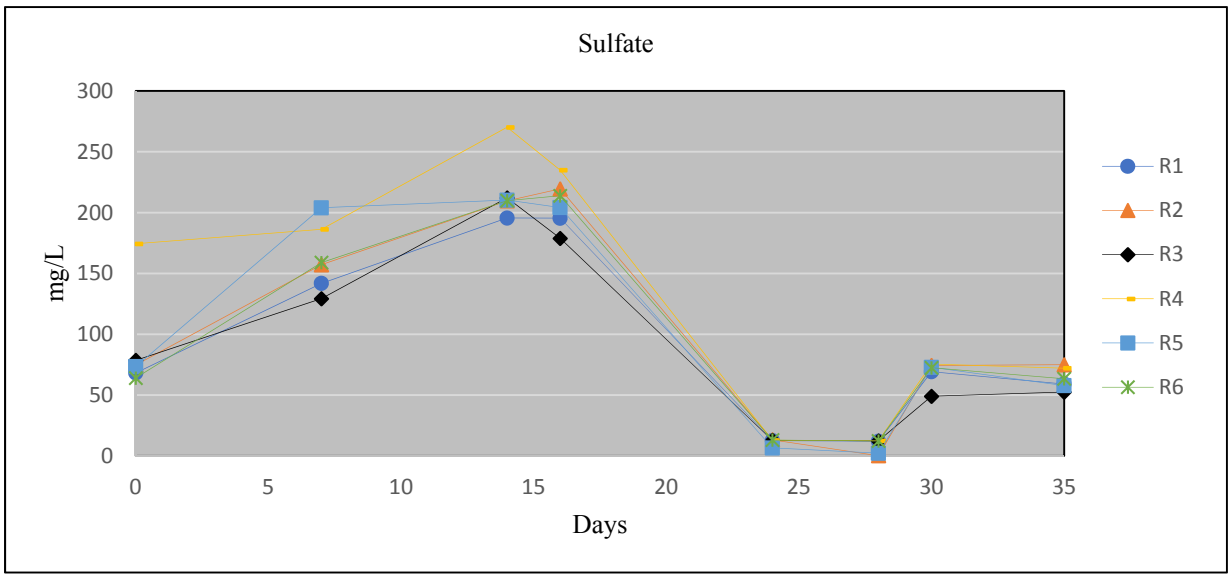
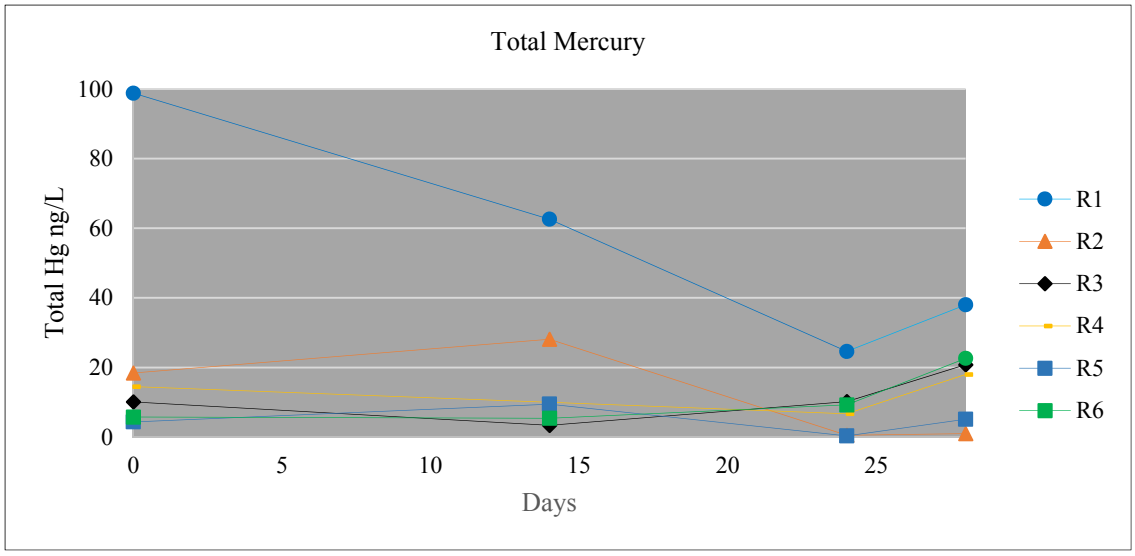


Figure 8: Sulfate concentrations in each reactor throughout the experiment.

Total mercury was measured in each reactor to verify if the sediments contained mercury after being exposed to a mercury discharge forty-five years earlier. In addition, total mercury and methylmercury concentrations were measured to determine if microorganism within the sediments could use the available mercury (Hg^{2+}) and produce methylmercury as a byproduct of their metabolic processes. Mercury cycling followed a similar trend in all reactors. Mercury concentrations at the beginning of the study ranged from 5-20 ng/L in all reactors with the exception of reactor 1, which had an initial mercury concentration of almost 100 ng/L (Figure 9).

The high concentration of mercury may be an indication of the high amount of mercury contained in the sediments.

In reactor 1, mercury decreased from 100 to 60 ng/L when the system was still aerobic. It is possible that some of the mercury was suspended from the sediments at the beginning of the experiment and it was gradually precipitated and sorbed back to the sediments during the first 15 days. An opposite trend was observed in reactor 2, where mercury concentrations increased during the first 15 days and then suddenly decreased towards the end of the experiment. It is possible that in reactor 2 mercury continue to diffuse from the sediments to the water column during the aerobic period in this reactor. In reactors 3-6, total mercury concentrations did not vary (a lot) during the first 15 days of the experiment. A total mercury decrease of 5 ng/L was observed in reactors 3 and 4 during the aerobic part of the experiments, while total mercury concentrations slightly increased in reactor 5 and stayed constant in reactor 6. Mercury concentrations changed mainly either by precipitation or diffusion processes that happened in the presence of oxygen in each reactors. It is possible that some methylation is already occurring deeper in the sediments, consuming some of the available mercury in solution. Total mercury concentrations, however, decrease in the anaerobic portion of the experiment and slightly increases toward the end of the experiment in all reactors.



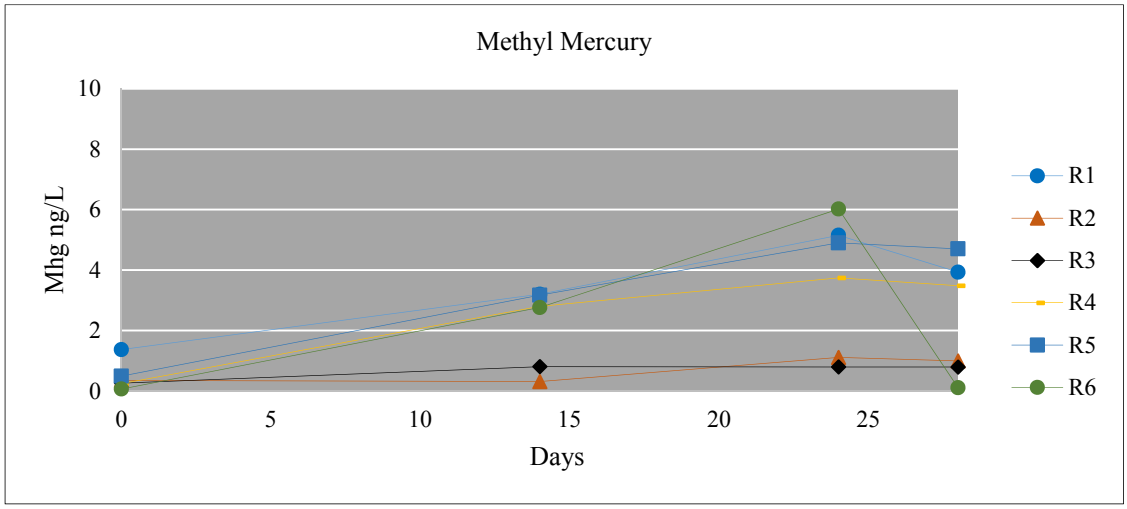


Figure 9: The top graph shows total mercury excluding methyl mercury. The second graph shows how much methyl mercury is present.

Methyl mercury was produced in all reactors and its accumulation varied depending on the initial amount of mercury and on the sulfate consumption rate. In reactors 4, 5, and 6 methylmercury concentrations increased from values lower than 0.5 ng/L to values higher than 5 ng/L during the first 25 days of the experiment (Figure 9). In the same manner, methylmercury concentrations in reactor 1 increased from 1.37 to 5.15 ng/L in the same time period. Results

suggest that methylmercury production occurred at a lower rate during the aerobic period and increased during the anaerobic period in these reactors. In reactors 2 and 3, methylmercury production was lower than in the other reactors. Maximum methylmercury concentrations measured were 0.8 and 1.1 ng/L in R3 and R2 respectively, R3 having the lowest maximum. Methylmercury accumulation in these two reactors occurred during the anaerobic period in each reactor. Examination of R4 and R5 measured a maximum of 3.75 ng/L and 4.9 ng/L. The highest accumulation of methylmercury transpired during the anaerobic period in R4 and R5. The two highest amount of methylmercury were found in R5 and R6 (5.15ng/L and 6.02ng/L). Methylmercury accumulation in the last two reactors happened during the anaerobic period in each reactor. During the end of the experiment the amount of Methylmercury decreased because of the introduction of oxygen.

Figures 10 and 11, describe the development of Iron and Manganese over a 30-day period. Iron was measured to test for the activity of iron reducing bacteria. A high concentration of soluble iron in the samples would indicate high rates of iron reduction in the sediments. Dissolved iron concentrations were low (< 1 mg/L) in reactors 2, 4, 5, and 6 throughout the duration of the experiment. Sediment iron release was observed only in reactors 1 and 3 after the development of anaerobic conditions. Reactor 1 and 3 had the largest amount of iron in the water column with a maximum above 2.7 mg/L. Iron release followed the same pattern in both reactors 1 and 3, reaching their maximum values around day 16, which coincides with the development of anaerobic conditions in the sediment-water interface. Results suggest that low iron concentrations were the result of low iron concentrations in the sediments and subsequent iron reducing bacteria activity.

The amount of manganese accumulated in the water column was less than iron. Reactors 1 and 3 had the largest amount of manganese. They peaked on day 16 and degreased until the completion of the experiment. Reactor 4 had the third highest amount of manganese and spiked

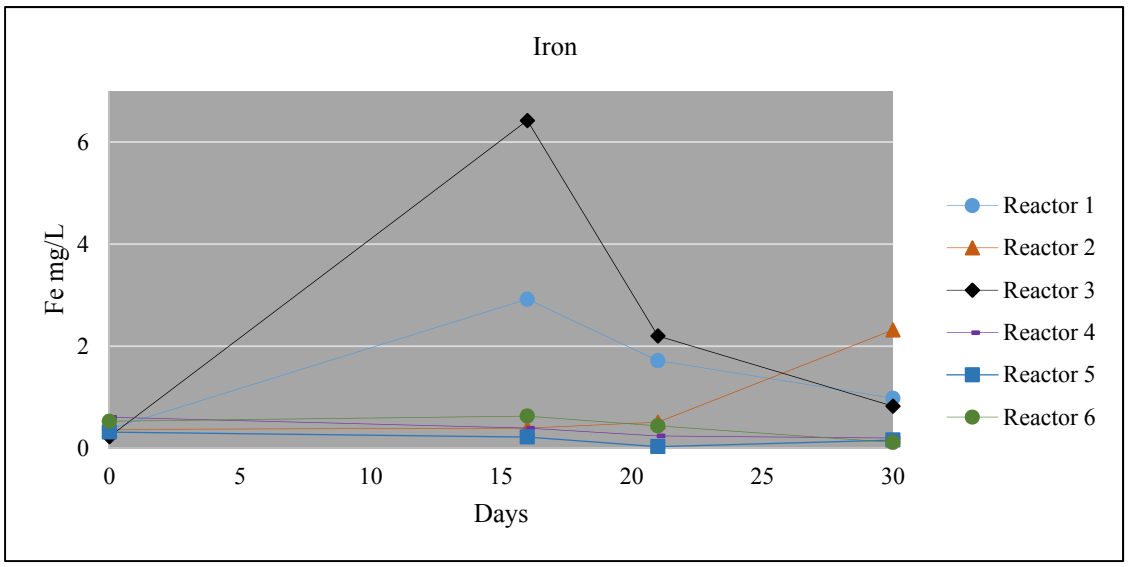


Figure 10: Dissolved iron concentrations throughout the experiment (mg/L).

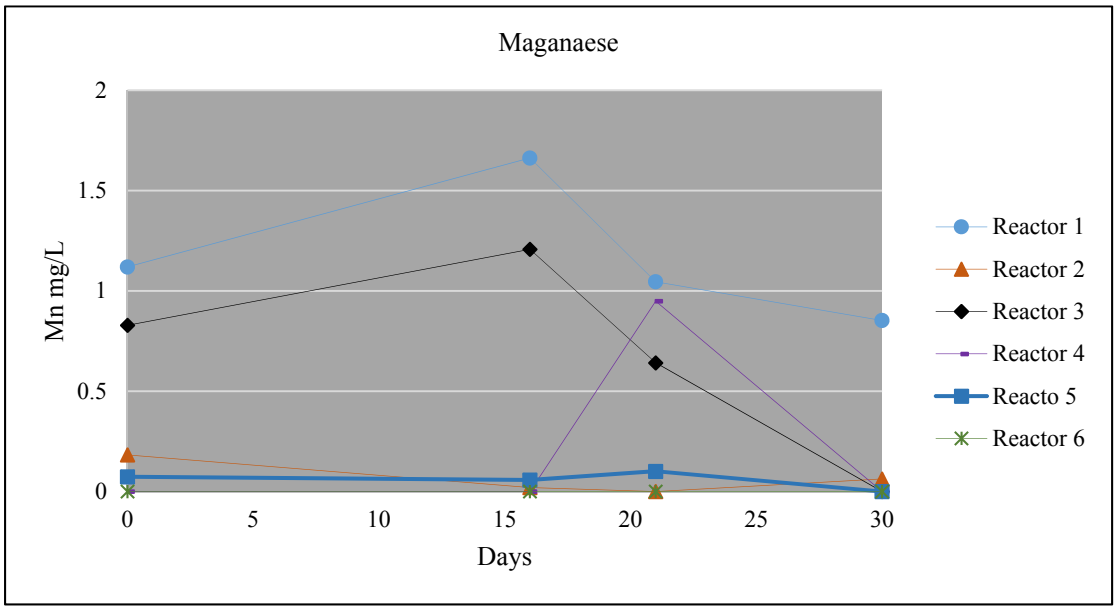


Figure 11: Dissolved Manganese concentrations throughout the experiment (mg/L).

on day 21 and decreases until the end of the experiment. Reactor 2, 5 and 6 had the least amount of manganese in the experiment.

To determine the contribution of sulfate to the total mercury methylation, a comparison of the total methylmercury produced in each reactor was done. To effectively compare methylmercury concentrations, a ratio (in mass units) between the methylmercury and mercury was first defined. The mercury ratio is expressed in percentage and it reflects the total amount of methylmercury produced based on the reactor's mercury concentration. The ratio was defined to decrease the effect of having a variable initial mercury concentration in some of the reactors. The mercury ratio was plotted against the change in sulfate concentration measured between each day data was collected (Figure 12). Data shows that as more sulfate is consumed in the reactors, the higher the amount of methylmercury produced related to the amount of mercury available. The figure reveals that when greater amounts of sulfate are consumed the higher the percentage of MHg (week 4) to THg (week 4).

Figure 12 Graph 1 show correlation between reduction of sulfate (ΔS) to mercury ration of R^2<.23. Graph 1 also depicts a direct relationship among ΔS and mercury ration. The lower amount of sulfate reduced the lesser amount of methyl mercury produced. The reactors that consumed less the 200mg/L had less significant mercury ration, less than 30 MeHg/THg in ng/L. However, reactor that measured ΔS>200 mg/L had mercury ration above 50 MeHg/THg in ng/L. R2 had the highest mercury ration of 209 MeHg/THg in ng/L, but only had the third highest sulfate consumption, 206mg/L. In Graph 2 R2 is remove and now had an correlation of R^2>.77, which is a much higher correlation then graph 1. Reactor 6 has the highest mercury ration with a

delat suflate of 202mg/L. Then R4 has the second highest mercury ration with a delta Sulfate of 222mg/L

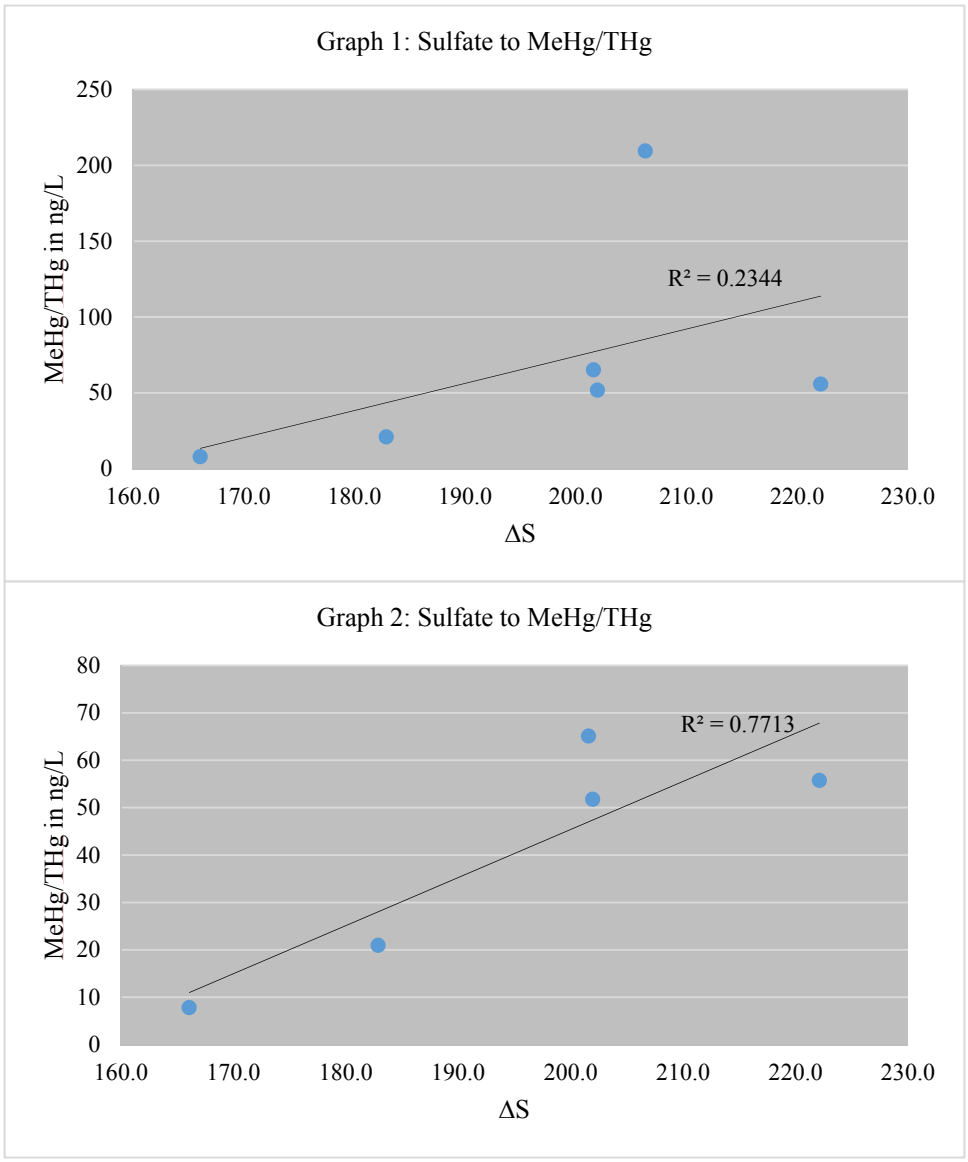


Figure 12: This figure compares the changes in sulfate with MeHg(Week4)/THg(Week 4). The first graph includes reactors 1-6. The second graph only excludes reactor 2.

Figure 13 shows the amounts of: ammonia (NH_3), Nitrate (NO_3-) and phosphate (PO_4-). The concentration of ammonia had varying patterns and results amongst the reactors. Reactors 1-4 exhibited the most similar patterns of concentration throughout the experiment. At the commencement of the experiment, Reactors 1-4 had an increased concentration and would display two large spikes prior to conclusion. Reactors 1 and 2 noted a gradual decrease in concentration prior to the culmination of the study whereas Reactors 3 and 4 concluded with a gradual increase in concentration. Both Reactors 5 and 6 began with low concentrations of ammonia. Reactor 5 was the only reactor that began the experiment with a decrease in ammonia concentration and a subsequent decrease in concentration by the completion. Rector 6 began with an increase in concentration and an increase by completion with only one spike noted throughout.

Within all six reactors the experiment commences with low concentrations of Nitrate. Each reactor behaved differently. Reactor 1 showed a spike at beginning of experiment (approximately day 7) and another spike in concentration approximately at day 16 where the concentration decreased until conclusion. Reactor 2 spiked in concentration at approximately week 3 of the experiment where the Nitrate decreased until a secondary spike was noted towards the end of the experiment. Reactor 3 decreased in concentration at the beginning, hitting a low plateau until an increase is noted in week 4. Following this increase, another decrease occurs at week 5 followed by a spike in week 6 where the concentration increased until the culmination. Reactor 4 began with high concentrations of Nitrate and a spike noted in the first week, which decreased concentrations until week 5. Towards the culmination, a subsequent spike at week 6 increases the concentrations until the end of the experiment. Reactor 5 showed a gradual increase

in concentration until week 3 where there was a gradual decrease in concentration until end of experiment. Reactor 6 showed a gradual increase in concentration until week 3 where concentrations decreased until week 5. Concentrations increased again at week 6 leading to the culmination.

The concentration of phosphate behaves identically in all six reactors, regardless of the varying amounts. At the commencement of the experiment, in each reactor, an increase the

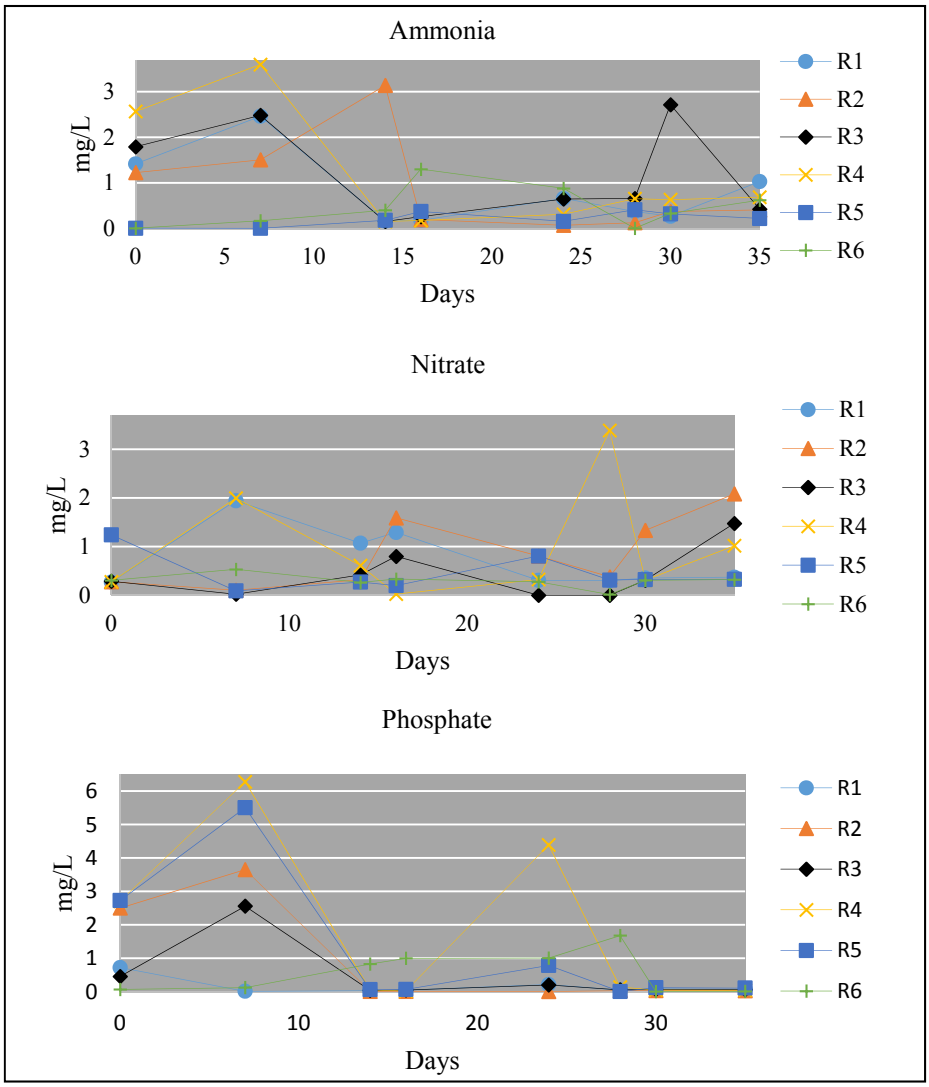


Figure 13: Shows the amount of NH_3, NO_3 and PO_4 in mg/L in each reactor.

concentration of phosphate is noted. The increase in concentration continues until week 3 where the concentration then decreases until the conclusion of the study. The concentration of sulfate also behaved identically amongst all six reactors but in varied levels of concentration. There was a significant increase in concentration noted until approximately week 3 where a drastic drop in levels occurred. Following this decrease was a gradual increase in concentration until the culmination of the study.

Table 4 shows the maximum, minimum and mean of the Total Amount of Organic Carbon (TOC). The two highest maximum TOC are R4-6 with 38.37 and 31.63 mg/L. The lowest two maximums are R2 and R3 with 20.85 and 21.73 mg/L. In the middle are R1 and R5 with 25.44 and 22.97. The lowest Min is R3 with 0 mg/L and the second lowest in R1 with 10.22mg/L. The majority of the time maximums were found in week 3 or week 4 of the experiment. The highest minimum displayed is R2 and R4 with 11.68 and 14.85 mg/L. The highest mean is seen in R4, R5 and R6 with 20.61, 18.58 and 18.46 mg/L. The lowest mean were R2 and R3 with 17.80 and 15.93 mg/L. R1 is in the middle with 19.80 mg/L

Table 3: Total Organic Carbon (TOC) Max, Min and Mean (mg/L)

	Max	Min	Mean
R1	25.44	10.22	19.80
R2	20.85	11.68	17.37
R3	21.73	0.00	15.93
R4	38.37	14.85	20.61
R5	22.97	11.13	18.58
R6	31.63	11.30	18.46

CHAPTER 5:
DISCUSSION

Methylmercury production potential was tested by measuring methylmercury concentrations and the productivity of the sediments collected from each site in laboratory-scaled experiments. The microcosm study performed presented a snap shot of the potential for production and accumulation of methylmercury in sediments from the Brunswick Estuary. There are many different factors that may cause the reactors to produce different methylmercury concentrations and in this study, three different conditions exacerbating methylmercury production have been identified. These conditions include: a productive environment (high concentrations of organic matter and nutrients) to support anaerobic conditions needed to promote the reduction of sulfur species; high concentrations of sulfate to sustain the continuous reduction of sulfate to sulfide and promote sulfur reducing bacteria proliferation; and enough mercury in the sediments that will act as substrate for methylmercury production.

Microcosms were operated to sustain anaerobic conditions using solely the sediments that were collected from each site to seed the reactors. Different parameters such as DO, pH, and ORP levels were used as indicators of the existing environmental conditions in the reactors. In all reactors DO was depleted to nearly to 0 mg/L by the middle of week 3 (day 18). The DO was quickly depleted as microorganism use it to oxidize organic matter and other reduced constituents. The ability to consume oxygen so quickly demonstrated the productivity of the reactors. Low DO concentrations throughout the experiment shows that all reactors, resembling the Brunswick Estuary, had enough organic matter available to sustain anaerobic conditions for the duration of the experiment. The low DO concentrations setup an adequate environment for the production and accumulation of methylmercury. It is possible that during sampling and refilling of the reactors (see methods), minor amounts of oxygen were introduced to each reactor.

However, DO readings didn't show any increase in any reactor because microorganisms within the reactors rapidly consumed any available oxygen.

ORP was used as an indicator for the development of reduced environments and was also measured because the ORP plays an important role in the productions and accumulation of methylmercury in sediments. Low ORP conditions, indicative of a reduced environment, promote the reduction of sulfate to sulfide as well as the growth and proliferation of SRB. In the sediments, SRB can assimilate sulfate compounds containing mercury (mercury (II)- sulfate) resulting in the formation of methylmercury as a byproduct (Parks et al. 2013). Once low ORP conditions are met and sustained, methylmercury production will occur as long as there is enough mercury and sulfate in the sediments. The ORP measures the oxidation reduction potential in a system. In this study, we see that some reactors had very high ORP values reflecting a highly oxidized environment, which is typically found in aerobic environments (high oxygen concentrations). Once oxygen is consumed, ORP values decreased in all reactors. Decreasing ORP values reflected a change from an oxidized to a reduced environment following the electron potential tower. The sequence of reductions reaction in the reactors and their associated electro-potential values was as follows: oxygen reduction (≈ 500-300mV), nitrate reduction (≈ 300-200 mV), manganese reduction (≈200-100mV), iron reduction (≈100-0mV), and sulfate reduction (≈0- -150mV). Temperature plays an important role in ORP and pH (Stumm 2012).

In this study, ORP in all reactors fell below zero at least once during the experiment. The majority of DO is depleted in R1, R3, R4 and R5 when ORP values are around 300 as expected. R2 and R4 probably reduced the majority of DO when they were exposed to the air in the lab, because the flask was left open prior to the start of the experiment. In R1, R4, and R5, nitrate

was consumed after DO was depleted (end of week 1) delaying the decrease in ORP in these reactors. R2 and R6 didn't start reducing nitrate until week 3 (around day 15-18). The late reduction probably due to a slower release of nitrogen from the sediments or maybe due to lower productivity at the beginning of the experiments. During Days 10 and 24, ORP values in R1-R6 fell between 100 and -100mV where manganese (Mn_4O_2), iron (Fe^{3+}), and sulfate (SO_4^{-2}) were reduced to manganese (Mn^2), Fe^2, and Hydrogen Sulfide (S^{-2} usually in the form of H_2S). The reduction of all these species suggests that there was an adequate environment to promote the production of methylmercury. The ORP values gradually increased towards the end of the experiment probably due to oxygen intrusion into the reactors.

The pH varies daily and between each reactor. R4, R5 and R6 were relatively the most acidic and R1, R2 and R3 were the least. Methylmercury was typically produced in an environment where pH was slightly below 7.0 due to the susceptibility of the SRB to variable pH (Pak and Bartha 1998). The reduction of oxygen, nitrogen, manganese, iron and sulfate caused a pH decrease in all reactors as seen between days 15-20. The pH became more basic at the end of the experiment because it lost the ability to produce compounds lowering the pH such as H_2S due to the possible intrusion of oxygen. This is very evident after day 20 in when the pH starts increasing towards the end (Figure 7).

It was found in this study that sulfate and total mercury were the most important factors controlling the production and release of methylmercury in the sediments from the Brunswick Estuary. The history of the site showed that large amount of mercury contamination was being introduced into the environment in the 60's and 70's. 1.36-4.55 liters of mercury were being discharged into the Brunswick estuary every day. The Brunswick area contains three superfund sites due to this historical dumping of mercury (EPA 2017). It is highly possible that most of the

mercury discharged from these sites ended up in the sediments. Soluble mercury in surface waters is typically absorbed into sediments in aerobic and anoxic environments (reference). This is why the sediments analyzed contained high levels of mercury that sustained methylmercury production under anaerobic conditions. The initial concentration of total mercury measured in all reactors was above 7 ng/L. In some reactors, total mercury concentrations increased two weeks after the beginning of the experiment because it took longer for mercury to diffuse from the sediments. Regardless of retarded diffusion mercury was present in all reactors.

Sulfate is also a key parameter because it is the reduction of mercury-sulfate mediated and catalyzed by SRB that results in the production of methylmercury ($CH_3+Hg(SO_4)\rightarrow CH_3Hg$). The amount sulfate in the reactors was extremely high compared to other aquatic systems at the beginning of the experiments. The average aquatic system sulfate concentration usually doesn't exceed 50mg/L (Orem 2017). In this study, all reactors exceeded levels above 150mg/L, which shows an abundance of sulfate in the sediments. The lowest concentrations of sulfate observed in all reactors coincided with lower ORP measured in periods of anoxia during the experiment. Although sulfide was not measured in the experiments, a pungent hydrogen sulfide odor was sensed during the weeks when sulfate concentrations were lower. The sulfur reducing bacteria primarily operate in ORP value below 0 mV, which were observed during weeks 3 and 4 in all reactors. During these periods high amounts of sulfate were reduced to sulfide. Corresponding with the sulfate reduction, a major increase in methylmercury occurred in week 4 in each reactor except in R3, were the increase was in week 3.

During the experiment, the amount of total mercury in R1-4 and R6 decreases until the week 3 or 4. Mercury in R5 increased in week three then decreased in week 4. Additionally, methylmercury increased in R1-2 and R4-6 during week 4 and 5. Methylmercury in R3 stopped

to increase in week three. The loss of total mercury in the reactors was due to the methylation of mercury. The amount of methylmercury produced in each reactor tripled the original levels throughout the experiment. During weeks 3 and 4 sulfate began to be reduced through SRB, which coincided with the decrease in mercury and the increase of methylmercury concentrations. In all reactors, the reduction of sulfate to sulfide directly relates to the methylation of mercury.

To determine and compare the influence of sulfate reduction with methylmercury production a ratio between the methylmercury and total mercury concentrations was developed (methylmercury/total mercury) and was expressed as a percentage. The ratio shows the amount of methylmercury produced based on the initial and available mercury. With a higher mercury ratio, there is a higher potential for producing methyl mercury in each reactor. Comparing the mercury ratio with the reduction of sulfate (delta sulfate) shows a solid relationship between the methylmercury produced and the amount of sulfate consumed, which can be considered to fuel the process that resulted in mercury methylation (Figure 9 and 12). A closer look at these figures reveals that when sulfate (sulfate consumption) decreases, so does the mercury ration, and as the delta sulfate concentration increases the higher the methylmercury production normalized by the initial amount of available mercury. A different trend, however, was observed in reactor 2.

R4, R5 and R6 produce the highest amount methyl mercury to total mercury because the amount of sulfate reduced in those reactors was higher compared to the other reactors. Conversely, R1 and R3 had the lowest mercury ratio (Figure 9) because less methylmercury was produced due to lower sulfate reduction. The second graph in Figure 9 also shows a high correlation between mercury and methylmercury to sulfate reduced, $R^2 > 0.7$. The R^2 value of 0.7 suggests a good correlation of the mercury and sulfate data. This correlation provides evidence that the high sulfate reduction is most one of the most important factors in the

production of methylmercury in this study. This can be seen in R4-6 where higher consumption of sulfate (delta sulfate) lead to a higher mercury ratio. Most studies that compare data sulfate to methyl mercury usually get values less than 0.7, showing better results from other study of .5 (Shao et al. 2012, Pollman and Donald 2014, Johnson et al. 2016).

R4 had the highest reduction rate of sulfate and it was slightly lower than R5 and R6. This is due to the length of time the reactors ORP values stayed below 0 mV. R5 and R6 consumed large amounts of sulfate to have a high methylmercury production rate. They also stayed in an environment, with low ORP and pH, long enough for the methylmercury to accumulate. R4 was not able to sustain that environment long enough allowing a lower methylmercury production. It is also possible that the amount of nitrate present increased the ORP level for some time to decrease the methylation in this reactor. R2 probably had the potential to produce a higher amount of methyl mercury, but because higher ORP and pH may occurred in this reactor, methylmercury production was lower. R2 did have the right ORP values for SRB to reduce the sulfate to produce methyl mercury. However, R2 did not sustain that environment long enough for methylmercury to accumulate. A further analysis revealed that if R2 is treated as an outlier, then it is possible to obtain a better correlation between the sulfates consumed and the methylmercury produced (Figure 9 graph 2).

It is possible that the amount of iron in the reactor also played a small role in the production of methylmercury. The small concentration of iron in the reactor could have methylated some mercury through Iron Reduce Bacteria (IRB). These bacteria Reduce iron instead of sulfate. In this study, however, the amount of iron reduced is insignificant to the amount of sulfate reduced. This can be seen by comparing the amount sulfate reduced (no less

than 160mg/L) to amount of Iron (no more than 6mg/L) between days 14-24. Therefore, results suggest that most of the methylation was done by SRBs.

Nutrients play a significant role in the productivity of aquatic systems. Usually, in the presence of considerable amounts of nutrients, there is a highly productive environment. The reactors used for this study were not the exception. High nutrients, mainly nitrogen species such as ammonia and nitrate along with orthophosphate were measured in all reactors. R4 has the highest amount of nutrients and it is the reactors that had the highest reduction of sulfate. This mean that R4 was the most productive out of all the reactors. Sediments used in R4 sample were taken from a lake. Lakes receives their nutrient from runoff and moving water. The flow rate of the water enters the lake slows significantly. This low flow rate prevent erosion of nutrient from the lake. Similarly, R1-3 had very high amount of nutrient because their samples were taken from wetlands. Wetlands collect large amount of nutrients from rivers and streams. The large amount of vegetation slows the flow of water and catches the nutrients. Conversely, it is possible that R5 and R6 had lower amount of nutrient than the other reactors because their sediments came from streams. Steams are constantly moving and have much higher flow then lakes or wetlands. This prevents the nutrient from settling and building up.

The amounts of nitrogen and phosphate are higher in reactor R1-4 than they are in R5 and R6. It is clear that in R1-6 the nitrification process is occurring because of the increase in nitrate concentrations. In those same reactors, we see an increase of nitrate because nitrifying bacteria take the nitrite and consume it to produce nitrate, which can be observed in the beginning of the experiment and in the middle of the experiment in R1, R3 and R4. In R2, R5 and R6 this process occurs later in the experiment probably due to less nitrogen being released from the sediments. Nitrate decline can be due to numerous factor. The most likely possible is that the nitrate is

convert to N_2 gas through microbial denitrification. (Payne 1998). When the denitrification process occurs, the ORP levels increase retarding or preventing the reduction of sulfate to sulfide. Therefore, the production of methylmercury decreased in those reactors having high concentrations of nitrate that would promote the denitrification process.

Phosphate was also measured as an indicator of productivity in each reactor. When transitioning from an aerobic to an anaerobic environment, phosphates are released as iron III is reduced to iron II, thereby releasing any iron bound phosphorus (Panswad 2003). This process was observed in all reactors as high concentrations of both phosphates and dissolved iron were measured in the reactors. Nitrogen and phosphorus species were not limiting bacterial growth in this experiment. The presence of nitrogen and phosphorus were significantly increasing the productivity in all reactors, thereby promoting highly reduced conditions and the continuous reduction of sulfate to sulfide. Measuring TOC was also important in showing the productivity in each reactor. All reactors had large amount of organic carbon, which mean that they all showed potential to sustain reduced conditions during the anaerobic environment.

All rectors had potential to produce methylmercury, but each reactor had different potential. Reactor 6 showed the highest potential because of its mercury ration. However, some sites (where the samples were taken) are less likely to produce condition to methylate mercury. Sites that have high productivity and slow moving water, like lakes and wetland, are more likely to create condition for methylation of mercury. Site with a higher flow are less likely to create those conditions. The sites need to be in the right location with high amount of nutrients and potential to methylate mercury.

R6 produced the most amount of methyl mercury followed by R1, R5, R4, R2 and R3. When looking at the reactor ability to produce mercury R6, R5 and R4 high the highest chances

because the methylmercury to total mercury ratio was higher and the sulfate consumption was higher as well. However, these condition were induced in the lab and may not reflect the ability of each sampling site to achieve these conditions all year round. Reactor 4 has the highest chance out of all the other sites because its flow regime resembles more to that of a lentic water body such as a lake. Lakes are able to reach an anaerobic environment like the reactors faster because the lack of DO penetration to the sediments during periods of thermal stratification, which generates the release of nutrients from the sediments (Wetzel 2001). DO is usually easily introduced into aquatic system in running waters. Generally the higher the flow of water the more DO there is in a system. In a lake there is usually a very low flow and lower flow of oxygen to the hypolimnion, especially during the warmest months of the year.

R1-3 sites have the second highest chance to produce and environment supportive for production and accumulating of methyl mercury. R1-3 were seeded with sediments collected from a wetland environment, which have large amount of nutrients similar to a lake, but can have high seasonal amounts of DO depending on the depth of the system. Wetlands have a large amount of emergent plants use a process of photosynthesis. This process produces O_2 increases the DO in the water (chemical process $6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$). Dissolved oxygen fluctuates on a daily basis, but the environment is so productive because the large amount of nutrient that it creates an anaerobic environment. Anaerobic productive environment can produce and accumulate large amount of methyl mercury. Streams are less like to create anaerobic environment because the amount of DO constantly introduced into the system from running water. Moving water usually has high amount of DO and would prevent R6 and R5 from becoming anaerobic enough to even produce and accumulate methyl mercury. R6 and R5 are the least likely to have high amount of methyl mercury naturally.

The high methylmercury production potential found in all reactors suggests that all sites from which the sediment were collected from have a high chance of having high concentrations of methylmercury above the sediment-water interface. Local biota could consume the methyl mercy and work it way up the food chain. Bioaccumulation would then occur increase the concentration of methyl mercury. The bioaccumulation factor could be in the thousands (Gobas 2001) breaching EPA standards of 1.8 mg/L. This would be considerable hazard to local fishermen and population of the Brunswick area. Sample of biota and water should be taken to evaluate the levels of methyl mercury. However, due to the high risk that mercury poses to the environment, it is pertinent to continuously monitor the conditions that prevail in each sampling site to assure that methylmercury is not being produced in the estuary.

CHAPTER 6:
CONCLUSION AND RECOMMENDATIONS

All reactors in the experiment showed the ability to produce methylmercury. Reactors that consumed over 200mg/L of sulfate produced a higher amount of methylmercury as described by the high methylmercury to mercury ratio. The strong relationship between sulfate depletion and methylmercury production confirms that the most important factors controlling the production of methylmercury are the initial mercury concentration and the amount of sulfate present to fuel the microbial reduction of sulfur to sulfide in anaerobic environments. Without one of these factors the production of methylmercury would be low in the Brunswick area.

This does not mean however, that the potential to produce methylmercury is always high in the actual sampling sites. In the estuary, water flow and temperature may affect the environment that exists in the water-sediment interface. Seasonally, cooler temperature and a water movement may, including tides, may promote oxygen intrusion to the bottom waters creating an oxidized environment above the sediments which will inhibit the methylmercury production. However, results suggest that if conditions are adequate (anaerobic environments) methylmercury production will inevitable and the resulting methylmercury may be consumed by aquatic biota living in the estuary. Spatially, environment such as streams, where water is constantly moving, may have a lower potential for mercury production despite the sediments contain mercury. This is due to the flow of water brining a constant amount of oxygen into the environment. On the other hand, wetlands and lake environments may enhance methylmercury production because of their low moving water and higher productivity in these sites.

In the Brunswick Estuary, it may be hard to track the conditions that would enhance methylmercury production. Therefore, by knowing how water flows, how oxygen and nutrient

cycle in different areas and environment is important to estimate if methylmercury will be produced in the Estuary. Results show that mercury can still be found in the sediments from areas around the superfund site locations in the Brunswick Estuary even fifty years after the mercury discharge was stopped. Despite the constant water movement generated by tides, mercury still persists in the sediment. Furthermore, it is possible, as shown in this study, that tidal flows had transported mercury upstream the superfund sites. Sediments from R6, which were collected upstream the estuary, contained high mercury concentrations as shown in the results. Therefore, it is very important to develop a comprehensive monitoring strategy to measure the parameters that control the environment conditions in the estuary and to monitor mercury and methylmercury concentrations at least during the summer months.

REFERENCES

Alpers, Charles N., Michael P. Hunerlach, Jason T. May, and Roger L. Hothem. "Mercury contamination from historical gold mining in California." (2005).

American Public Health Association, American Water Works Association, Water Pollution Control Federation, and Water Environment Federation. *Standard methods for the examination of water and wastewater*. Vol. 2. American Public Health Association., 2014.

Andersson, Maria E., Katarina Gårdfeldt, IngvarWängberg, and Dan Strömberg. "Determination of Henry's law constant for elemental mercury." *Chemosphere* 73, no. 4 (2008): 587-592.

Bank, Michael S., ed. *Mercury in the environment: pattern and process*. Univ of California Press, 2012.

Bullock, O. Russell, and Katherine A. Brehme. "Atmospheric mercury simulation using the CMAQ model: formulation description and analysis of wet deposition results." *Atmospheric Environment* 36, no. 13 (2002): 2135-2146.

Burke, Janet, Marion Hoyer, Gerald Keeler, and Timothy Scherbatskoy. "Wet deposition of mercury and ambient mercury concentrations at a site in the Lake Champlain Basin." *Water, Air, & Soil Pollution* 80, no. 1 (1995): 353-362.

Colman, John A., Marcus C. Waldron, Robert F. Breault, and Robert M. Lent. "Distribution and transport of total mercury and methylmercury in mercury-contaminated sediments in reservoirs and wetlands of the Sudbury River, east-central Massachusetts." *US Geol. Surv. Water-Resour. Invest. Rep* (1999): 99-4060.

Crespo-López, María Elena, Andrea Lima De Sa, Anderson Manoel Herculano, Rommel Rodríguez Burbano, and José Luiz Martins do Nascimento. "Methylmercury genotoxicity: a novel effect in human cell lines of the central nervous system." *Environment international* 33, no. 2 (2007): 141-146.

Driscoll, Charles T., Robert P. Mason, Hing Man Chan, Daniel J. Jacob, and Nicola Pirrone. "Mercury as a global pollutant: sources, pathways, and effects." Environmental science & technology 47, no. 10 (2013): 4967-4983.

Eagles-Smith, Collin A., Joshua T. Ackerman, Julie Yee, and Terrence L. Adelsbach. "Mercury demethylation in water bird livers: dose–response thresholds and differences among species." *Environmental Toxicology and Chemistry* 28, no. 3 (2009): 568-577.

Ferrara, R., B. Mazzolai, E. Lanzillotta, E. Nucaro, and N. Pirrone. "Volcanoes as emission sources of atmospheric mercury in the Mediterranean basin." *Science of the Total Environment* 259, no. 1 (2000): 115-121.

Gobas, F. A. P. C. "Assessing bioaccumulation factors of persistent organic pollutants in aquatic food-chains." *Persistent organic pollutants: environmental behaviour and pathways of human exposure. Norwell, Massachusetts: Kluwer* (2001): 145-165.

Hall, B. "The gas phase oxidation of elemental mercury by ozone." *Water, Air, & Soil Pollution* 80, no. 1 (1995): 301-315.

Hall, B., N. S. Bloom, and J. Munthe. "An experimental study of two potential methylation agents of mercury in the atmosphere: CH3I and DMS." *Water, Air, and Soil Pollution* 80, no. 1-4 (1995): 337-341.

Hartung, Rolf, and Bertram D. Dinman. "Environmental mercury contamination." (1972).

Hintelmann, Holger, R. Douglas Evans, and Janice Y. Villeneuve. "Measurement of mercury methylation in sediments by using enriched stable mercury isotopes combined with methylmercury determination by gas chromatography–inductively coupled plasma mass spectrometry." *Journal of Analytical Atomic Spectrometry* 10, no. 9 (1995): 619-624.

Hsu-Kim, Heileen, Katarzyna H. Kucharzyk, Tong Zhang, and Marc A. Deshusses. "Mechanisms regulating mercury bioavailability for methylating microorganisms in the aquatic environment: a critical review." *Environmental science & technology* 47, no. 6 (2013): 2441-2456.

Iverfeldt, Åke. "Occurrence and turnover of atmospheric mercury over the Nordic countries." *Water Air & Soil Pollution* 56, no. 1 (1991): 251-265.

Johnson, Nathan W., Carl PJ Mitchell, Daniel R. Engstrom, Logan T. Bailey, Jill K. Coleman Wasik, and Michael E. Berndt. "Methylmercury production in a chronically sulfate-impacted sub-boreal wetland." *Environmental Science: Processes & Impacts* 18, no. 6 (2016): 725-734.

Karagas, Margaret R., Anna L. Choi, Emily Oken, Milena Horvat, Rita Schoeny, Elizabeth Kamai, Whitney Cowell, Philippe Grandjean, and Susan Korrick. "Evidence on the human health effects of low-level methylmercury exposure." *Environmental health perspectives* 120, no. 6 (2012): 799.

Keeler, G., G. Glinsorn, and N. Pirrone. "Particulate mercury in the atmosphere: its significance, transport, transformation and sources." *Water, Air, and Soil Pollution* 80, no. 1-4 (1995): 159-168.

Kim, Ki-Hyun, Steven E. Lindberg, and Tilden P. Meyers. "Micrometeorological measurements of mercury vapor fluxes over background forest soils in eastern Tennessee." *Atmospheric Environment* 29, no. 2 (1995): 267-282.

Kutney, Gerald. *Sulfur: history, technology, applications & industry*. ChemTec Publishing, 2007.

Lebel, Jean, Donna Mergler, Marc Lucotte, Marucia Amorim, Julie Dolbec, Daniela Miranda, Guilherme Arantes, Isabelle Rheault, and Pierre Pichet. "Evidence of early nervous system dysfunction in Amazonian populations exposed to low-levels of methylmercury." *Neurotoxicology* 17, no. 1 (1996): 157-168.

Lin, Che-jen, and Simo O. Pehkonen. "Aqueous free radical chemistry of mercury in the presence of iron oxides and ambient aerosol." *Atmospheric Environment* 31, no. 24 (1997): 4125-4137.

Lin, Che-Jen, and Simo O. Pehkonen. "The chemistry of atmospheric mercury: a review." *Atmospheric Environment* 33, no. 13 (1999): 2067-2079.

Lindberg, S. E., J. G. Owens, and W. Stratton. *Application of throughfall methods to estimate dry deposition of mercury*. No. CONF-9206380--1. Oak Ridge National Lab., TN (United States), 1992.

Lindqvist, Oliver, A. Jernelöv, K. Johansson, and H. Rodhe. Mercury in the Swedish environment. Global and local sources [emissions, natural waters, transformations, distribution, deposition]. SNV, 1984.

Lindqvist, Oliver, Kjell Johansson, Lage Bringmark, Birgitta Timm, Mats Aastrup, Arne Andersson, Gunnar Hovsenius, Lars Håkanson, Åke Iverfeldt, and Markus Meili. "Mercury in the Swedish environment—recent research on causes, consequences and corrective methods." *Water, Air, & Soil Pollution* 55, no. 1 (1991): xi-261.

Mackert, John Rodway. "Dental amalgam and mercury." The Journal of the American Dental Association 122, no. 9 (1991): 54-61.

Maqbool, Faheem, Haji Bahadar, Kamal Niaz, Maryam Baeeri, Mahban Rahimifard, Mona Navaei-Nigjeh, Seyedeh Farnaz Ghasemi-Niri, and Mohammad Abdollahi. "Effects of methyl mercury on the activity and gene expression of mouse Langerhans islets and glucose metabolism." *Food and Chemical Toxicology* 93 (2016): 119-128.

Maserti, B. E., and R. Ferrara. "Mercury in plants, soil and atmosphere near a chlor-alkali complex." *Water Air & Soil Pollution* 56, no. 1 (1991): 15-20.

Mlakar, Tanja Ljubič, Milena Horvat, Tomaž Vuk, Andrej Stergaršek, Jože Kotnik, Janja Tratnik, and Vesna Fajon. "Mercury species, mass flows and processes in a cement plant." *Fuel* 89, no. 8 (2010): 1936-1945.

Munthe, John. "The aqueous oxidation of elemental mercury by ozone." *Atmospheric Environment. Part A. General Topics* 26, no. 8 (1992): 1461-1468.

Odom, Ron R. "Mercury contamination in Georgia rails." In *Proc. Ann. Conf. Southeast. Assoc. Game & Fish Comm*, (1975): 649-658

Orem, William H. "Sulfate as a Contaminant in Freshwater Ecosystems: Sources, Impacts and Mitigation." USGS Conference. Accessed Feb. & March, 2017. http://www.bibme.org/chicago/website-citation/new. Online Presentation

Ouédraogo, Ousséni, and Marc Amyot. "Mercury, arsenic and selenium concentrations in water and fish from sub-Saharan semi-arid freshwater reservoirs (Burkina Faso)." *Science of the total environment* 444 (2013): 243-254.

Pak, K-R., and R. Bartha. "Mercury methylation and demethylation in anoxic lake sediments and by strictly anaerobic bacteria." *Applied and Environmental Microbiology* 64, no. 3 (1998): 1013-1017.

Panswad, Thongchai, Apiradee Doungchai, and Jin Anotai. "Temperature effect on microbial community of enhanced biological phosphorus removal system." *Water Research* 37, no. 2 (2003): 409-415.

Park, J-G., and L. R. Curtis. "Mercury distribution in sediments and bioaccumulation by fish in two Oregon reservoirs: point-source and nonpoint-source impacted systems." *Archives of Environmental Contamination and Toxicology* 33, no. 4 (1997): 423-429.

Parks, Jerry M., Alexander Johs, Mircea Podar, Romain Bridou, Richard A. Hurt, Steven D. Smith, Stephen J. Tomanicek et al. "The genetic basis for bacterial mercury methylation." *Science* 339, no. 6125 (2013): 1332-1335.

Payne, William Jackson. *Denitrification*. John Wiley & Sons Inc., 1981.

Pehkonen, Simo O., and Che-Jen Lin. "Aqueous photochemistry of mercury with organic acids." *Journal of the Air & Waste Management Association* 48, no. 2 (1998): 144-150.

Pollman, Curtis D., and Donald M. Axelrad. "Mercury bioaccumulation and bioaccumulation factors for Everglades mosquitofish as related to sulfate: a re-analysis of Julian II (2013)." *Bulletin of environmental contamination and toxicology* 93, no. 5 (2014): 509-516.

Sánchez, Enrique, Manuel F. Colmenarejo, Juan Vicente, Angel Rubio, María G. García, Lissette Travieso, and Rafael Borja. "Use of the water quality index and dissolved oxygen deficit as simple indicators of watersheds pollution." *Ecological Indicators* 7, no. 2 (2007): 315-328.

Schroeder, W. H., J. Munthe, and O. Lindqvist. "Cycling of mercury between water, air, and soil compartments of the environment." *Water, Air, and Soil Pollution* 48, no. 3-4 (1989): 337-347.

Schroeder, William H., and John Munthe. "Atmospheric mercury—an overview." *Atmospheric Environment* 32, no. 5 (1998): 809-822.

Search Superfund Site Information." EPA. December 28, 2016. Accessed June 01, 2017. https://cumulis.epa.gov/supercpad/cursites/csitinfo.cfm?id=0401634

Shao, Dingding, Yuan Kang, Shengchun Wu, and Ming H. Wong. "Effects of sulfate reducing bacteria and sulfate concentrations on mercury methylation in freshwater sediments." *Science of the Total Environment* 424 (2012): 331-336.

Si, Youbin, Yan Zou, Xiaohong Liu, Xiongyuan Si, and Jingdong Mao. "Mercury methylation coupled to iron reduction by dissimilatory iron-reducing bacteria." *Chemosphere* 122 (2015): 206-212.

Sommar, Jonas, Katarina Gårdfeldt, Dan Strömberg, and Xinbin Feng. "A kinetic study of the gas-phase reaction between the hydroxyl radical and atomic mercury." *Atmospheric*

Environment 35, no. 17 (2001): 3049-3054.Clarkson, Thomas W., Laszlo Magos, and Gary J. Myers. "The toxicology of mercury—current exposures and clinical manifestations." *New England Journal of Medicine* 349, no. 18 (2003): 1731-1737.

Sommar, Jonas, Katarina Gårdfeldt, Dan Strömberg, and Xinbin Feng. "A kinetic study of the gas-phase reaction between the hydroxyl radical and atomic mercury." *Atmospheric Environment* 35, no. 17 (2001): 3049-3054.

Stumm, Werner, and James J. Morgan. *Aquatic chemistry: chemical equilibria and rates in natural waters*. Vol. 126. John Wiley & Sons, 2012.

Tewalt, Susan J., Linda J. Bragg, and Robert B. Finkelman. *Mercury in US coal; abundance, distribution, and modes of occurrence*. No. 095-01. 2001.

United Nations Environmental Program. "Global Mercury Assessment 2013: Sources, Emissions, Releases and Environmental Transport." (2013).

United States Department of Health and Human Services. "LCP CHEMICALS SUPERFUND SITE." (2014): 1-223

USEP, A. "Method 1630: Methyl mercury in water by distillation, aqueous ethylation, purge and trap, and cold vapor atomic fluorescence spectrometry." *Draft. USEP A, Office of Water* (1998).

Weber, James H. "Review of possible paths for abiotic methylation of mercury (II) in the aquatic environment." *Chemosphere* 26, no. 11 (1993): 2063-2077.

Wetzel, Robert G. *Limnology: lake and river ecosystems*. Gulf Professional Publishing, 2001.

Zhang, L., and M. H. Wong. "Environmental mercury contamination in China: sources and impacts." *Environment international* 33, no. 1 (2007): 108-121.