EFFECTS OF OXYGEN AND NITRATE ON MERCURY CYCLING IN THE PROFUNDAL ZONE OF LAKES AND RESERVOIRS

By

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Abstract

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Methylmercury, a neurotoxin, has been identified as a serious public health concern. To date, effective remediation techniques for preventing and remediating methylmercury contamination have remained elusive, mainly due to the lack of knowledge in regard to how methylmercury is generated and degraded in the aquatic environment. This dissertation evaluated methylmercury accumulation in both replicate experimental chambers containing undisturbed sediment-water interface samples from mercury-contaminated lakes in northern California, and a two-year field study evaluating spatial and temporal patterns of nitrate, oxygen and methylmercury during summer stratification in Occoquan Reservoir, Virginia. The primary objective of this research was to access how oxidants, including nitrate, affect the cycling of mercury in the profundal zone of lakes and reservoirs. Results demonstrated that methylmercury accumulation in both experimental chamber water and field sampling was lower under aerobic and anoxic (no oxygen but nitrate present) conditions versus anaerobic conditions. In Guadalupe Reservoir, California, MeHg efflux measured in replicate bench-scale chambers averaged 5.5 ng/m²/d under aerobic conditions and 22 ng/m²/d under anaerobic conditions. In Almaden Lake, California, MeHg
efflux was -2.3 ng/m$^2$/d (uptake) under aerobic conditions and 11 ng/m$^2$·d under anaerobic conditions. Like oxygen, nitrate repressed MeHg efflux from sediments from Almaden Lake to < 0.2 ng/m$^2$/d. In Occoquan Reservoir, Virginia, MeHg was < 0.1 ng/L in a tributary enriched with nitrate from treated wastewater effluent compared to 0.04-1.1 ng/L in a low nitrate reference tributary. MeHg accumulation correlated with elevated iron and manganese concentrations. Nitrate addition to the reservoir likely kept downstream bottom waters from going highly reduced, thereby reducing MeHg accumulation in bottom waters. This dissertation also describes a fellowship performed with the World Bank, as part of the WSU NPSIRE IGERT program, evaluating environmental and economic policy in the water/wastewater sector in Cartagena, Colombia. The investigation found that Cartagena's water supply coverage has increased from 73% to 99.9% and sewer coverage from 61% to 90%. However, a lack of transparent environmental policy regarding public participation has limited the effectiveness of achieving meaningful benefits without negatively impacting the public.
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Dedication

This dissertation is dedicated to the memory of my beloved father, Succes Joseph, a man full of love and compassion. You will always be profoundly appreciated for your unflinching commitment to my career pursuit!

“To accomplish great things, you must not only act, but also dream; not only plan, but also believe.”
CHAPTER 1
INTRODUCTION

1.1 Overview of Dissertation

This dissertation examines the use of nitrate as a resource to control mercury cycling in lakes and reservoirs. The dissertation is divided into five chapters. The Introduction herein provides an outline and rationale for chapters 2, 3, 4 and 5 and an introduction to the Nitrogen Systems: Policy-oriented Integrated Research and Education (NSPIRE) and the Bullitt Foundation Environmental Fellowship programs that helped to fund my research.

Chapter 2 is a discussion of the nitrogen cycle and the linkages between the nitrogen cycle and the mercury cycle. Chapter 2 also includes a discussion of case studies where nitrate has been used to enhance surface water quality. Chapter 3 is a policy paper based on my NSPIRE internship with the World Bank and describes the implementation of a water and sanitation project to enhance access to services and improve coastal water quality in Cartagena, Colombia. Chapters 4 presents a chamber study that evaluated oxygen and nitrate control on mercury fluxes from mercury-contaminated reservoir sediments from San Jose, CA. Chapter 5 presents results of field work at Occoquan Reservoir, VA that focused on understanding the spatial and temporal patterns of nitrate and mercury in the water column of a reservoir that is loaded high levels of nitrate.
1.2. Outline and Rationale for Dissertation Chapters

1.2.1. Chapter 2: Overview of Nitrogen Cycling in Aquatic Ecosystems

Chapter 2 is a review of major processes and factors controlling nitrogen transport and transformations in aquatic ecosystems. This chapter first describes the main processes controlling nitrogen cycling in aquatic environment. The chapter emphasizes heterotrophic denitrification, with organic carbon as an electron donor; autotrophic denitrification using reduced sulfur; dissimilatory nitrate reduction to ammonium; and anoxic oxidation of ammonium. This chapter also reviews environmental factors that affect denitrification rates such as nitrate concentration, nutrient availability, microbial acclimation, pH, temperature, and presence of toxic compounds. This chapter also discusses denitrification process that influence air quality. For example, incomplete denitrification in aquatic ecosystems may enhance the biogenic production of the potent greenhouse gas nitrous oxide (N\textsubscript{2}O). Furthermore, this chapter summarizes the linkages between the nitrogen cycle and the mercury cycle, illustrating that nitrogen, in form of nitrate, can affect mercury biogeochemistry in low-oxygen environments. Finally, this chapter acknowledges that significant accumulation of nitrate in aquatic ecosystems can be a major pollution source for humans and wildlife. However, a number of case studies are presented that illustrate how managed nitrate addition can be used to enhance surface water quality.

1.2.2. Chapter 3: Policy Study in Cartagena, Colombia

Chapter 3 describes the history and development of environmental policies in Cartagena, Colombia, where I worked for the World Bank as part of my NSPIRE fellowship. The chapter focuses on the Cartagena Water Supply, Sewerage and Environmental Management
Project, which was part of the World Bank Water and Sanitation program. The objectives of the project were to: 1) improve the water and sewage services of the city of Cartagena and the sanitary conditions of Cartagena’s poorest population; 2) facilitate the environmental cleanup of surrounding water bodies, including Cartagena Bay, Caribbean beaches, and Cienaga de la Virgen Lagoon; and 3) improve the sustainability of water quality. The project had a number of interrelated components including construction of water supply and sewerage system, the construction of a submarine outfall for discharging treated effluent to the Caribbean Sea near Punta Canoa, Cartagena, and the implementation of environmental and social policies to promote project success.

Unexpectedly, the proposed submarine outfall off the coast of Punta Canoa turned out to be especially contentious. Local citizens argued that the outfall would negatively affect the coastal zone's fisheries, which was the primary source of food and income for the local people. They believed that the project's untreated wastewater would be discharged into the sea, contaminate marine life, and have serious and permanent impacts on people’s health and livelihoods. This belief was of particular concern for the indigenous people of Punta Canoa, whose lives are inextricably linked to the health of the Caribbean Sea.

This chapter focuses on the main efforts to improve water quality in Cartagena, mechanisms for overcoming impediments, and key points for success. This chapter also evaluates two fundamental policy aspects of the Cartagena effort, the lack of a water management program and efforts to empower local citizens’ participation to enhance project outcomes and best environmental management practices. Finally, this chapter discusses the difficulties that any environmental policy faces when technical drivers take precedence over
social and cultural concerns of local stakeholders. Consequently, policy lessons from this project are also revealed.

1.2.3. Chapter 4: Chamber Study at Almaden Lake and Guadalupe Reservoir, California

Chapter 4 of this dissertation is a summary of a sediment-water-interface chamber study that was conducted in Almaden Lake and Guadalupe Reservoir, two mercury-impacted lakes managed by the Santa Clara Valley Water District near San Jose, California. The study evaluated the sediment release of methylmercury (HgCH$_3^+$) under aerobic conditions, anaerobic conditions, anoxic conditions with nitrate addition, and anaerobic conditions with sodium aluminate addition.

The hypothesis of this study was that anaerobic chambers would accumulate more methylmercury than aerobic chambers or anoxic chambers with nitrate. This is because anaerobic conditions, in which both oxygen and nitrate are depleted, tend to promote methylmercury production in sediments through a number of potential mechanisms. For example, anaerobic microorganisms such as sulfate-reducing bacteria (SRB) have been implicated in methylmercury production (Benoit et al., 2003). Anoxic conditions also promote the dissolution of iron and manganese oxides in surficial sediments, which may release sorbed methylmercury upon reduction (Mathews et al., 2013). Only a handful of studies have documented nitrate suppression of methylmercury accumulation in lake bottom waters, including one by the consulting firm CH2M Hill, which added nitrate to a number of small lakes in Minnesota (Austin, 2011), and a large-scale nitrate addition pilot project in Onondaga Lake, New York (Matthews et al., 2013).
An additional hypothesis was that addition of aluminum, which fosters the formation of an aluminum hydroxide floc at the sediment-water interface, would prevent the release of methylmercury from anaerobic sediment to overlaying water by sorbing effluxing methylmercury. Aluminum addition as sodium aluminate and/or aluminum sulfate is a common lake management additive to enhance the sorption capacity of surfacial sediments. Aluminum additions are generally used to control sediment phosphorus release (DeGasperi et al., 1993). Little research has been conducted on the effects of this common lake additive on mercury cycling.

1.2.4. Chapter 5: Field Study at Occoquan Reservoir, Virginia

Chapter 5 presents the results of a two-year field study that evaluated the spatial and temporal patterns of nitrate and mercury during the summer stratification period in Occoquan Reservoir, VA. The reservoir receives large inputs of nitrate from tertiary treated wastewater that is discharged into the upper part of the reservoir (Randall and Grizzard, 1995). This nitrate tends to flow with cool water into the profundal zone of the lake and impact biogeochemical processes in the bottom of the lake. The hypothesis was that methylmercury would only be observed in substantial levels in bottom waters if oxygen and nitrate were not present at the sediment-water interface, that is, when or where water conditions shifted from anoxic (no oxygen but some nitrate) to anaerobic (no oxygen or nitrate). The rationale for this hypothesis was that the production of methylmercury, as noted above in sub-section 1.2.3, is usually associated with anaerobic conditions that enhance the activity of SRB and the dissolution of mercury-rich metal oxides in surfacial sediments.
Only a limited number of field studies have documented interactions between nitrogen and mercury cycling in large, human-impacted reservoir ecosystems. A comparable evaluation of methylmercury accumulation was performed in Lake Onondaga in Syracuse, NY, a mercury-polluted lake that receives 20% of its annual inflow from the Syracuse Metropolitan wastewater treatment plant (WWTP). Todorova et al. (2009) reported that the presence of nitrate from wastewater effluent alleviated anaerobic conditions in bottom waters and repressed methylmercury accumulation. Nitrate is currently being added to the profundal zone of the Onondaga Lake in an effort to further inhibit methylmercury release from sediments (Matthews et al., 2013).

1.3. Funding Sources

This dissertation was funded in part by a two-year fellowship with the Nitrogen Systems: Policy-oriented Integrated Research and Education (NSPIRE) program at Washington State University and a two-year Bullitt Foundation Environmental Fellowship. NSPIRE was funded by the Integrative Graduate Education and Research Traineeship (IGERT) program. IGERT is part of the National Science Foundation's flagship interdisciplinary training program, educating U.S. Ph.D. scientists and engineers by building on the foundations of their disciplinary knowledge with interdisciplinary training. Nitrogen pollution is one of world's most widespread, costly, and challenging environmental problems. NSPIRE focuses on creating a new generation of scientists with broad and rigorous training in nitrogen cycling and who can integrate nitrogen cycle science for effective communication with public policy makers. This program prepares graduate students for leadership roles in science and engineering to help solve nitrogen cycle problems. In addition,
diversity among the students contributes to their preparation to solve large and complex research problems of significant scientific and societal importance at national and international levels.

In 2012, I received a Bullitt Foundation Environmental Fellowship. This program focuses on educating environmentally knowledgeable graduate students from a community under-represented in the environmental movement. Students awarded the fellowship must also have demonstrated an exceptional capacity for leadership and scholarship. This fellowship embraces the idea of stewardship, supporting projects that touch the future and promote a healthy, sustainable environment. The Foundation supports a variety of tactics to advance its mission. Grantees tend to be creative in devising projects and campaigns to build understanding, advance policy innovations, and garner the political power needed to affect change. These tactics generally include the following: developing a diverse pipeline of future environmental and sustainability leaders; ensuring compliance with environmental laws and policies; and convening environmental, scientific, business, and political leaders to clarify differences and seek common ground.
1.4 References


CHAPTER 2

OVERVIEW OF NITROGEN CYCLING IN AQUATIC ECOSYSTEMS

2.1. Introduction

Nitrogen (N) cycling in aquatic ecosystems is complex. Figure 1 of N cycling in a surface-flow wetland illustrates this complexity. The figure includes the following key components of N cycling in aquatic ecosystems: 1) ecological compartments (sediment, water, air, biota); 2) ecological niches (aerobic, anoxic, anaerobic); 3) N species [organic N, ammonium (NH$_4^+$), ammonia (NH$_3$), nitrite ions (NO$_2^-$), nitrate ions (NO$_3^-$), nitrogen gas (N$_2$), nitrous oxide (N$_2$O)]; 4) N transformations (assimilation, ammonification, nitrification, denitrification, anammox, fixation); and 5) N transfer processes (leaching, volatilization, diffusion). This overview will focus on exploring three key N transformations of significance in aquatic ecosystems: nitrification, denitrification, and anaerobic ammonium oxidation (anammox). Microorganisms, particularly bacteria, play a major role in most N transformations. Bacteria obtain metabolic energy by redox processes, for example, using nitrate, nitrite, and soluble N oxides as terminal respiratory oxidants under oxygen-limiting conditions during denitrification (Burging et al., 2011). Thus, this discussion will also focus on bacteria and their capacity to transform N compounds in aquatic ecosystems. Finally, this discussion will examine the linkages between the N cycles and mercury (Hg) cycles and present case studies illustrating how nitrate can be used as a resource to improve water quality.
Figure 2-1. The nitrogen cycle in a surface flow wetland. Figure from Kadlec and Knight (1996). Treatment Wetlands. First Edition, CRC Press, Boca Raton, Florida.

2.2. Nitrification

Nitrification, the microbial oxidation of ammonia to nitrate, is a key transformation in the N cycle. In aquatic environments, decomposing microorganisms, such as bacteria and fungi, facilitate ammonification, the breakdown of organic N to ammonia. Next, during the process of nitrification, ammonium is oxidized to nitrite and then to nitrate. Thus, nitrifying microorganisms such as nitrosomonas and nitrobacter within aquatic ecosystems reduce total
ammonia concentrations through nitrification according to the following overall reactions (Chin, 2006):

$$4 \text{NH}_4^+ + 6 \text{O}_2 \rightarrow 4 \text{NO}_2^- + 8 \text{H}^+ + 4 \text{H}_2\text{O}$$

$$4 \text{NO}_2^- + 2 \text{O}_2 \rightarrow 4 \text{NO}_3^-$$

$$\text{NH}_4^+ + 2 \text{O}_2 \rightarrow \text{NO}_3^- + 2 \text{H}^+ + \text{H}_2\text{O}$$

Nitrification requires the presence of oxygen; therefore, nitrification can only happen in aerobic environments such as surface layers of soils and sediments in micro-oxic zones (Barnard et al., 2005). Because nitrification is central to the accumulation and ultimate loss of nitrate (via denitrification), factors regulating nitrification are critical to eutrophication and elevated nitrate concentrations in fresh waters. Nitrification converts excessive ammonia to nitrate, which can then be lost from the aquatic environment via denitrification (see Section 2.3); therefore, this process can play a potentially important role in protecting surface waters from eutrophication (Hagopian and Riley, 1998). However, some important human health consequences must be considered. If nitrate assimilation does not occur at a rate at least equal to nitrate production, the aquatic environment may accumulate nitrate. Excessive nitrate in drinking water is linked to several illnesses in humans including methemoglobinemia and the internal production of carcinogenic nitrosamines (Vitousek et al., 1997; Carpenter et al., 1998).

2.2.1. Ammonia Oxidation

Ammonia oxidation, a reaction mediated by both ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA), is the first and rate-limiting step of nitrification (Falkowski
et al., 2008). AOB are thought to be largely responsible for the oxidation of ammonia to nitrite in natural environments. However, Erguder et al. (2009) summarized the current knowledge on environmental conditions influencing the amount and presence of AOA and identified several site-related properties and environmental factors. These properties and factors included ammonium, organic carbon, temperature, salinity, oxygen, pH, sulfide, phosphate, and some metal contents. The two key variables that appear to control AOA versus AOB dominance of ammonia oxidation include temperature and pH.

Temperature exhibited different effects on the abundance and diversity of AOA and AOB. Higher temperature tends to select for AOA because elevated temperature enhances the decomposition of extracellular DNA or dead cells in sediment (Wu et al., 2013), which may promote higher AOA abundance. Temperature variations could lead to the fluctuations of other environmental factors such as organic carbon, oxygen availability, and ammonia. These additional factors might also contribute to community shifts of ammonia oxidizers and lead to significant variations in AOA and AOB. For example, elevated temperature could increase the decomposition rate of organic matter and release more organic molecules, which could also affect AOB (Park and Noguera, 2004). Racz et al. (2010) reported that organic carbons such as peptone and glucose could affect the abundance and diversity of AOB in a mixed culture with the heterotroph community. Elevated temperature could also increase the decomposition rates of organic matter and decrease the dissolved oxygen availability due to heterotrophic bacteria, which have higher affinities for dissolved oxygen than the nitrifiers (Starry et al., 2005). Elevated decomposition rates of organic matter would also increase the ammonia availability, which is the substrate for nitrification (Jantti et al., 2011). Consequently, variations due to
elevated temperature could change the abundances and community compositions of AOA and AOB in the lake sediments and, in turn, affect the N cycling process.

Besides temperature, low pH environments may favor AOA because lower pH could drive the conversion from ammonia to ammonium, decreasing substrate availability and increasing energy stress that may select for AOA over AOB as the dominating ammonia oxidizer (Valentine, 2007). As a result, AOA communities more easily adapt to low pH environments compared to their AOB counterparts. This speculation was recently confirmed by cultivation and characterization of an obligate, acidophilic, thaumarchaeal ammonia oxidizer from a nitrifying acidic soil (Lehtovirta-Morley et al., 2011).

Both AOA and AOB have ammonia mono-oxygenase, one of the key enzymes responsible for the transformation of ammonia to hydroxylamine (NH$_2$OH) (Jin et al., 2010). Hydroxylamine then undergoes a redox reaction with oxygen to produce nitrite and energy. This reaction generates a small amount of energy relative to many other types of metabolism; as a result, nitrosofiers (nitrifying bacteria) are notoriously slow growers. Additionally, aerobic ammonia oxidizers are autotrophs, fixing carbon dioxide to produce organic carbon much like photosynthetic organisms, but using ammonia as the energy source instead of light.

Conditions for ammonia oxidizers are best at the interface between oxic environments with adequate oxygen concentrations and anoxic environments with high ammonium concentrations (Voytek and Ward, 1995). This part of the N cycle is especially sensitive to pH. Acidification forces a proton onto ammonia, producing ammonium and lowering concentrations of ammonia, the actual substrate used by ammonia oxidizers. Lower ammonia concentrations may result in slower rates of ammonia oxidation (Kirchman, 2012). Despite limited research, it is well-known that AOB and AOA play a vital role in linking the input of fixed N, through N-
fixation and remineralization, to potential loss processes, including denitrification and anammox (Bouskill et al., 2011).

2.2.2. Nitrite Oxidation

Nitrite oxidation is the second step of the nitrification process, during which nitrite is quickly oxidized to nitrate by a separate group of prokaryotes, known as nitrite-oxidizing bacteria. Some of the genera involved in nitrite oxidation include *Nitrospira, Nitrobacter, Nitrococcus, and Nitrospina* (Koenneke et al., 2005). Although nitrite rarely builds up in the environment, the nitrite oxidation reaction is an essential component of nitrification. Similar to ammonia oxidizers, the energy generated from the oxidation of nitrite to nitrate is small, and thus growth rates are low (Zehr et al., 2003). In fact, ammonia- and nitrite-oxidizers must oxidize many molecules of ammonia or nitrite in order to fix a single molecule of carbon dioxide (Bernhard, 2012). For complete nitrification, both ammonia oxidation and nitrite oxidation must occur.

2.3. Denitrification

2.3.1. Conventional Denitrification

Denitrification is the process that converts nitrate to N gas, thus removing bioavailable N and returning it to the atmosphere. Dinitrogen gas is the ultimate end product of denitrification, but other intermediate gaseous form, including nitric oxide and nitrous oxide, a potent greenhouse gas (see subsection 2.3.3). Unlike nitrification, denitrification is an anaerobic process, occurring mostly in soils and sediments and anoxic zones in lakes and oceans. Similar to
N fixation, denitrification is carried out by a diverse group of prokaryotes, but recent evidence suggests that some eukaryotes are also capable of denitrification (Risgaard-Petersen et al., 2006). Some denitrifying bacteria include species in the genera Bacillus, Paracoccus, and Pseudomonas. Denitrifiers are chemoorganotrophs and thus must also be supplied with some form of organic carbon. The overall energy reactions are (Metcalf and Eddy, 2003):

\[
\begin{align*}
6 \text{NO}_3^- + 2 \text{CH}_3\text{OH} & \rightarrow 6 \text{NO}_2^- + 2 \text{CO}_2 + 4 \text{H}_2\text{O} \\
6 \text{NO}_2^- + 3 \text{CH}_3\text{OH} & \rightarrow 3 \text{N}_2 + 3\text{CO}_2 + 3 \text{H}_2\text{O} + 6\text{OH}^- \\
6 \text{NO}_3^- + 5 \text{CH}_3\text{OH} & \rightarrow 5 \text{CO}_2 + 3 \text{N}_2 + 7 \text{H}_2\text{O} + 6 \text{OH}^- 
\end{align*}
\]

Denitrification is an important microbial process with beneficial consequences for water quality (Burgin et al., 2012). The importance of denitrification in the N cycle in marine and freshwater ecosystems has been the focus of an increasing number of studies in recent decades (Seitzinger et al., 1993). Every year, nearly 50 million tonnes of reactive N enter marine ecosystem via rivers, resulting in eutrophication of coastal ecosystems (Galloway et al., 2004). Denitrification is the major pathway of fixed N loss from aquatic systems. Thus, the process is a critical component of the global N budget and a balancing mechanism for removing anthropogenic N along the terrestrial-freshwater-marine continuum (Galloway et al., 2004; Seitzinger et al., 2006). Additionally, denitrification in wastewater treatment plays a beneficial role by removing nitrate from wastewater effluent, thereby reducing the chances that the treated discharge will cause undesirable environmental consequences (e.g., algal blooms). However, the denitrification process is a major concern in agricultural areas. Significant losses from some surface-applied N sources can occur, leading to limited plant growth (Bernhard, 2012).
In lakes, denitrification occurs in low-oxygen bottom waters, where rates generally range from 0.2 to 1.9 µmol N/L/d (Seitzinger, 1988). In lakes, denitrification rates are greater in the sediments compared to the water column. In the absence of oxygen, denitrifiers respire nitrate sequentially to nitrite, nitric oxide, and nitrous oxides, and finally to dinitrogen gas, with organic carbon used as the electron donor (Devol, 2008). In consuming organic matter, nitrate acts as the terminal electron acceptor for the oxidation of organic matter under anaerobic conditions. In aquatic sediments, nitrate is usually converted to dinitrogen gas, with a variable but small fraction escaping as nitrous oxide (Burgin et al., 2007). During this process, microorganisms regenerate inorganic nutrients such as carbon dioxide, ammonium, and phosphate, which are necessary to sustain continued primary production. However, this process is controlled by a variety of environmental parameters and their interactions, including substrate availability, temperature, and/or oxygen concentration. Why one or the other parameter dominates is under debate (Deutsch et al., 2010; Seitzinger, 1988). The specific issue of nitrous oxide production during denitrification is discussed in greater detail in subsection 2.3.3.

An alternative to heterotrophic, biological denitrification is autotrophic, reduced sulfur-driven denitrification. Common autotrophic denitrifiers, *Thiobacillus denitrificans* and *Thiomicropira denitrificans*, can use various reduced sulfur compounds as electron donors (Campos et al., 2008; Sengupta et al., 2007; Krishnakumar and Manilal, 1999). Sulfide, particularly, can fuel the activity of some autotrophic denitrifiers. Also, sulfide inhibition can reduce denitrification rates and stimulate nitrous oxide production because nitrous oxide reductase, the enzyme catalyzing the reduction of nitrous oxide to dinitrogen, is sensitive to sulfide (Brunet and Garcia-Gil, 1996; Joye, 2002; Porubsky et al., 2009). The overall reaction for sulfur-driven denitrification is (Soares, 2002):
\[ 5S^0 + 6NO_3^- + 2H_2O \rightarrow 5SO_4^{2-} + 3N_2^- + 4H^- \]

Hydrogen sulfide may play an important role in regulating N-cycling processes and has the ability to repress N transformations, such as nitrification and heterotrophic denitrification. However, in lakes, where nitrate and reduced sulfur compounds occur, chemolithoautotrophs can use reduced sulfur as an energy source and nitrate as the electron acceptor to produce N gas, thus enhancing denitrification rates (Burgin et al., 2012).

### 2.3.2. Dissimilatory Reduction of Nitrate to Ammonia

Fermentative dissimilatory nitrate reduction to ammonia (DNRA) is a process by which nitrate reduction can produce ammonium via a bacterial-mediated heterotrophic process in anaerobic environments. This process is different from the assimilatory process in that ammonia is not directly incorporated into biomass. Fermentative DNRA couples organic matter oxidation to nitrate reduction by fermentation reactions (Tiedje, 1988). The reaction has been found in anoxic sediments with bacteria of the *Thioploca* and *Thiomargarita* species. Both types of bacteria are able to concentrate nitrates within their own cells for the subsequent oxidation of sulphur-containing compounds in reduced form. In this way, the bacteria are able to reduce nitrate to ammonium passing through nitrite as an intermediary compound. This reaction, although still needing clarification, would potentially supply nitrite and ammonium to the anammox reaction in anoxic sediments (Burgin et al., 2007; Francis et al., 2007). This process has been identified as an important sink for nitrate in organic-rich marine and freshwater sediments (Gardner et al., 2006). The overall reaction is (Schumacher et al, 1992):
\[
\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NH}_4^+
\]

DNRA represents a potential positive feedback to ecosystems because it converts nitrate to ammonia, a biologically available form of N that can be taken up by plants (Scott et al., 2008). However, ammonia generated by DNRA could also fuel nitrification. Thus, DNRA may be an important pathway for nitrate removal in aquatic sediments, particularly in sediments containing labile carbon, reduced sulfur, and reduced iron (Burgin et al., 2007). Additionally, in highly reducing environments, sulfides rather than organic substrates can serve as electron donors for sulfur-driven lithoautotrophic DNRA (Gardner et al., 2006). Sulfide concentrations play a critical role in determining the dominant pathway of nitrate reduction. At low sulfide concentrations, nitrate is reduced mainly via denitrification; however, at higher sulfide concentrations, DNRA becomes the dominant pathway (Senga et al., 2006; Brunet and Garcia-Gil, 1996). As noted by Brunet et al. (1996), DNRA accounted for up to 30% of the total nitrate reduction in freshwater sediments amended with calcium nitrate.

2.3.3. Nitrous Oxide Production during Denitrification

Nitrous oxide is a denitrification byproduct of concern. In all systems, the formation of nitrous oxide during denitrification results in tropospheric warming and stratospheric ozone depletion (Davidson and Seitzinger, 2006). Nitrous oxide is produced during several microbiological processes, including nitrification, denitrification, and dissimilatory nitrate reduction to ammonium (De Wilde and De Bie, 2000). However, denitrification and chemolithotrophic nitrification appear to be the main biological sources of nitrous oxide.
emission in natural systems (Bonin et al., 2002). Recently, attention has been directed to redox processes in the N cycle that operate in tandem, stressing the importance of considering total nitrous oxide production rates from nitrification and denitrification (Meyer et al., 2008; Davidson and Seitzinger, 2006). In addition, nitrous oxide production is reliant on a number of environmental factors such as inorganic N concentrations, sediment redox potential, and organic carbon availability (Oliveira Fernandes et al., 2010). Therefore, assessment of environmental parameters and their interrelationships with net nitrous oxide production in sediments is crucial for determining the key parameters governing nitrous oxide formation.

In many aquatic systems, most of the nitrous oxide produced during denitrification is subsequently reduced to dinitrogen gas. However, the ratio of nitrous oxide to dinitrogen gas is variable and remains uncertain because a comprehensive understanding of denitrification rates and controlling factors across ecosystems is lacking (Davidson and Seitzinger, 2006; McCutchan et al., 2003). Some key factors include dissolved oxygen concentration, pH, temperature, nitrate concentrations, and carbon availability (de Klein et al., 2001, 2010). These factors influence denitrifier populations and their enzyme activities, and hence, affect the production of nitrous oxide relative to dinitrogen gas.

Because denitrification is an anaerobic reaction, oxygen availability is one of the most important factors inhibiting this process in aquatic environments. The amount of nitrous oxide produced via denitrification is a function of the overall denitrification rate, which is often controlled by oxygen concentration (Mulholland et al., 2002). While denitrification is inhibited in the presence of oxygen, a potential source of nitrous oxide under low-oxygen conditions is nitrification by ammonium-oxidizing bacteria. On a global basis, well-oxygenated flowing waters are a much smaller source of nitrous oxide than aquatic ecosystems such as estuaries or
freshwater lakes that exhibit periods of hypoxia or fluctuate between oxic and anoxic conditions (Beaulieu et al., 2014).

In addition to oxygen concentrations, pH and temperature can affect nitrous oxide production. For example, at lower pH levels, enhancement of abiotic transformations of nitrate to nitrite may contribute to an increased proportion of nitrous oxide production (Koskinen and Keeney, 1982). Water temperature can cause temporal fluctuations in denitrification rates (Ryden, 1983). Because the activation energy of nitrous oxide reduction is higher than that of nitrous oxide production (Holtan-Hartwig et al., 2002), more nitrous oxide is produced at low temperatures, resulting in increased production of nitrous oxide relative to dinitrogen gas (Keeney et al., 1979; Avalakki et al., 1995).

The ratio of nitrous oxide to dinitrogen gas can also be influenced by biotic and abiotic transformations of nitrogenous compounds that occur during denitrification. For example, presence of suitable electron donors and denitrifying bacteria, possessing metabolic enzymes capable of enhancing nitrous oxide production rates, can affect reaction rates and redox potential, which facilitate nitrous oxide accumulation. As a result, nitrous oxide production is a complex process that cannot be easily related to denitrification fluxes (Barnard et al., 2005). Meyer et al. (2008) argued that the nitrous oxide produced by denitrification in deeper sediment layers is consumed during its diffusion toward the sediment-water interface in nutrient-enriched mangrove sediments.

In terrestrial systems, lower nitrate concentrations usually result in lower nitrous oxide to dinitrogen gas ratios (Zaman et al., 2007). As nitrate concentration decreases, nitrous oxide acts as a major electron acceptor, thereby decreasing nitrous oxide production (Swerts et al., 1996; Dendooven et al., 1997). Increasing carbon availability tends to decrease the ratio of nitrous
oxide to dinitrogen gas (Dendooven et al., 1998). Senbayram et al. (2009), for example, observed low nitrous oxide emissions following a large input of labile carbon in soils. This observation was possibly due to microbial immobilization of some of the added N (Tiedje, 1988) or dissimilatory nitrate reduction to ammonium (DNRA). Under anaerobic conditions, nitrate concentration may control the denitrification product ratio of nitrous oxide to dinitrogen gas, whereas labile carbon concentration may control the denitrification rate (Tiedje, 1988).

2.4. Anaerobic Ammonium Oxidation

Anammox (anaerobic ammonium oxidation) is the oxidation of ammonium coupled with the reduction of nitrite under anaerobic conditions. This process is considered more thermodynamically favorable than aerobic ammonium oxidation (Hu et al., 2011). Anammox is an important pathway for N gas production in anaerobic marine sediments (Dalsgaard et al., 2005). Anammox has been identified as an alternative microbial pathway of N gas production and, from a biogeochemical perspective, can be considered a denitrifying process (Devol, 2008). Anammox reduces nitrite to nitric oxide, which then reacts in a one-to-one stoichiometry with ammonium to form hydrazine, and finally dinitrogen gas (Kartal et al., 2011). The overall reaction is (Dalsgaard et al., 2005):

\[
\text{NH}_4^+ + \text{NO}_2^- \rightarrow \text{N}_2 + 2 \text{H}_2\text{O}
\]

Although aerobic ammonia oxidation and anammox both act on ammonia, each process involves different organisms. Anammox is mediated only by bacteria from the *Planctomycetes*
phylum; aerobic ammonia oxidation is carried out by both bacteria and archaea (Kirchman, 2012). The first described anammox bacterium was *Brocadia* anammoxidans (Dalsgaard et al., 2005) Anammox bacteria oxidize ammonia by using nitrite as the electron acceptor to produce gaseous N. Anammox bacteria were first discovered in anoxic bioreactors of wastewater treatment plants, but have since been found in a variety of aquatic systems, including low-oxygen zones of the ocean, coastal and estuarine sediments, mangroves, and freshwater lakes. The rate of anammox appears to be regulated by the availability of reduced substrates, nitrite concentrations, and sediment organic content. However, in marine sediments, the reduction of nitrate, and thus the production of nitrite, is fast enough to allow the anaerobic ammonium oxidation (Dalsgaard et al., 2005).

In marine environments, temperature plays an important role in the anammox process. Anammox is microbially catalyzed and therefore dependent on temperature. Ammonium oxidizers have a temperature optimum from 12° to 15° C, whereas denitrifying bacteria have a much higher temperature optimum between 25° and 30° C (Saad et al., 1993). The lower temperature optimum of the anammox bacteria enables them to outcompete denitrifies in cold environments.

In addition, organic matter content in sediments is a key factor for the anammox process. The more organic matter mineralized, the more nitrite produced, and the higher the anaerobic ammonium oxidation rate. Normally, organic matter loading of sediments decreases with increasing water depth because of the long settling time of particles. The longer it takes for organic matter to sink toward the ground, the higher the possibility of it being mineralized before arrival. Thus, we can expect decreasing absolute anammox rates with increasing water depth (Saad et al., 1993; Dalsgaard and Thamdrup, 2002; Dalsgaard et al., 2005; Schubert et al., 2006).
The importance of anammox in N-cycling processes across a range of aquatic ecosystems is being increasingly recognized. In some areas of the ocean, anammox may be responsible for a significant loss of N (Kuypers et al., 2005). However, Ward et al. (2009) argue that denitrification, rather than anammox, is responsible for most N loss in other areas. Regardless of whether anammox or denitrification is responsible for most N loss in the ocean, clearly, anammox represents an important process in the global N cycle (Bernhard, 2012).

In wastewater treatment systems, the anammox process has several advantages. For example, oxygen addition can be reduced by 60%. In turn, the energy required to perform the oxygenation process can also be reduced. Anammox bacteria do not require organic carbon and produce very little biomass, reducing the amount of sludge disposal (Fux et al., 2002; Egli et al., 2001). In addition, greenhouse gas emissions are enormously reduced using the anammox process. The overall carbon dioxide emission of a wastewater treatment plant can be reduced with 95%, because the anammox process itself consumes carbon dioxide. Furthermore, the production of nitrous oxide, which easily forms as a by-product of conventional denitrification, is eliminated using the anammox process (Van Loosdrecht et al., 2004; Van Dongen et al., 2001). Overall, the anammox process provides a sustainable alternative for nitrification.

2.5. Impacts of Nitrate on Mercury Cycling

Hg contamination in the water column and sediments of aquatic ecosystems raises concerns related to biological exposure, including potential health effects in humans and wildlife caused by consumption of contaminated fish (Benoit et al., 2003). On a global scale, rates of atmospheric Hg deposition have increased three-fold as a result of human post-industrial
activities. An estimated 5-20% of deposited Hg is delivered to aquatic ecosystems where it may accumulate in aquatic biota (Swain et al., 2007). In the U.S., three-fourths of states currently have blanket fish consumption advisories due to elevated Hg in fish tissue. Nearly half of the total U.S. lake acres and over a third of U.S. river miles are subject to Hg-related advisories (USEPA, 2009). Hg cycling in aquatic ecosystems is greatly influenced by metabolic activity, including primary production, decomposition processes, and sulfate reduction. Of primary concern is the production of methylmercury (MeHg) which is a neurotoxin that strongly bioaccumulates in aquatic food webs. MeHg represents more than 95% of total Hg in fish biomass (Tchounwou et al., 2003).

The accumulation of MeHg in the anaerobic bottom waters of lakes is a recognized phenomenon (Watras, 2009). Hg typically enters a lake in the inorganic form via aerial deposition and watershed runoff. Inorganic Hg can complex with dissolved organic carbon (DOC), and co-precipitate with settling particles such as metal oxides of iron (Fe) and manganese (Mn) (Watras, 2009; Chadwick et al., 2006). MeHg is produced in anaerobic environments by SRB, which use sulfate as the terminal electron acceptor during the oxidation of organic matter (Benoit et al., 2003). Sulfate reduction proceeds in the absence of energetically favorable electron acceptors such as oxygen, nitrate, and oxidized manganese and iron. MeHg can also enter the water column from the sediment as it is released from metal oxide complexes that become reduced after the onset of anoxia (Merritt and Amribahman, 2008; Chadwick et al., 2006).

Due to its potential effect on redox conditions at the sediment-water interface, increased nitrate concentration should reduce MeHg efflux from sediments by both of the mechanisms described above: MeHg production by SRB and MeHg release from reduced metal oxides. SRB
activity in lake sediments and related internal production of MeHg may be inhibited by maintaining adequate concentrations of alternate, energetically favorable electron acceptors, such as oxygen and/or nitrate (Stephan et al., 1988). As detailed by Golterman (2001), oxidation-reduction potential at the sediment-water interface is particularly critical in regulating the flux of reduced and oxidized substances into and out of profundal sediments. Depressed oxidation-reduction potential at the sediment-water interface has a profound impact on metal cycling, as reduced Fe (II) and Mn (II) tend to accumulate in anaerobic waters (Bryant et al., 2011). The presence of nitrate at the sediment-water interface may poise redox potential in surficial sediments and repress metal oxide reduction and associated efflux of MeHg sorbed to metal oxides.

Recent studies confirm nitrate’s ability to repress MeHg accumulation in lakes (Mathews et al., 2013; Todorova et al., 2009). For example, Matthews et al. (2013) conducted a two-year pilot study of nitrate addition to Onondaga Lake, a Hg-contaminated lake in New York. Results showed that maximum concentrations of MeHg and phosphate in bottom waters decreased by 94% and 95%, respectively. The research analysis suggested that increased sorption to Fe and Mn oxyhydroxides in surficial sediments was the likely regulating mechanism. Clearly more study is needed to assess the potential for oxidants such as nitrate to inhibit MeHg efflux from lake sediment, as well as the mechanisms by which oxidants repress MeHg efflux.

2.6. Case Studies of Managed Nitrate Addition in Lakes and Reservoirs for Water Quality Improvement

2.6.1. Nitrate as a Pollutant of Concern
Nitrate is recognized as a critical pollutant in surface and ground waters. The most remarkable global impacts include the leaching of nitrate to groundwater, the eutrophication of surface waters and resultant marine “dead zones,” atmospheric deposition that acidifies ecosystems, and the emission of N oxides (NOx) that deplete stratospheric ozone (Keeney and Hatfield, 2007; Beever et al., 2007; Foley et al., 2005). These widespread environmental changes can also threaten human health (Galloway et al., 2008; Guillette and Edwards, 2005; Galloway et al., 2004; Vitousek et al., 1997; Fan and Steinberg, 1996; Jordan and Weller, 1996). In surface waters, nitrate can stimulate algal productivity leading to eutrophication and associated water quality problems and impairment of aquatic biota.

Whereas phosphorus (P) is a critical limiting nutrient in aquatic ecosystems (Schindler, 2012), N limitation or co-limitation of N and P in freshwater lakes is more common than generally recognized (Elser et al., 2007). Therefore, nitrate is a potentially significant driver of eutrophication in many freshwater systems. N-limited coastal waters are especially vulnerable to eutrophication and the development of hypoxic conditions (Howarth and Marino, 2006). Nitrate pollution from agricultural activities is responsible for the hypoxic dead zone at the mouth of the Mississippi River in the Gulf of Mexico (Mitsch et al., 2001).

Nitrate contamination also limits the use of groundwater resources for human consumption. A recent study concluded that nitrate contamination of drinking water wells, resulting from agricultural activities, poses a health risk to hundreds of thousands of people in California's Central Valley (Lund, 2012). When present in large quantities, nitrate in drinking water is a potential health hazard to infants due to methemoglobinema, also known as blue baby syndrome (Knobeloch et al., 2000). In addition, scientific evidence suggests that ingested nitrates may result in mutagenicity, teratogenicity, and birth defects (Camargo and Alonso, 2006).
Unsuitably high nitrate levels may also affect pregnant women and adults with hereditary cytochrome b5 reductase deficiency. In addition, nitrate and nitrite ingestion in humans has been linked to goitrogenic (anti-thyroid) actions on the thyroid, fatigue and reduced cognitive functioning due to chronic hypoxia, maternal reproductive complications including spontaneous abortion, and a variety of carcinogenic outcomes deriving from N-nitrosamines formed via gastric nitrate conversion in the presence of amines (Ward et al., 2005).

The concentration of nitrate fluctuates with the rate of nitrification and denitrification processes. Nitrification requires the presence of oxygen; as a result, nitrification occurs only in oxygen-rich environments and the surface layers of soils and sediments. However, the process of nitrification has some important consequences. For example, ammonium ions are positively charged and therefore stick to negatively charged clay particles and soil organic matter. The positive charge prevents ammonium N from being leached out of the soil by rainfall. In contrast, the negatively charged nitrate ion is not held by soil particles and can be washed out of the soil, leading to decreased soil fertility and nitrate enrichment of downstream surface and groundwater (Ravishankara et al., 2009; Compton et al., 2011; Harrison, 2003).

During denitrification, oxidized forms of N such as nitrate and nitrite are converted to dinitrogen and, to a lesser extent, nitrous oxide gas. However, nitric oxide and nitrous oxide are gases that have environmental impacts. Both gases are known as contributors to the atmospheric ozone destruction. For example, nitric oxide contributes to smog which is known to cause respiratory illnesses like asthma in both children and adults, and nitrous oxide is an important greenhouse gas that is contributing to global climate change. The Intergovernmental Panel on Climate Change (IPCC) estimates that the microbial conversion of agriculturally derived N to nitrous oxide in soils and aquatic ecosystems is the largest source of anthropogenic nitrous oxide.
to the atmosphere (Forster et al., 2007). Therefore, microbial denitrification is a large source of nitrous oxide emissions in terrestrial and aquatic ecosystems.

The proportion of denitrified nitrate converted to nitrous oxide, rather than dinitrogen gas, partially controls how much nitrous oxide is produced via denitrification (Codispoti, 2010). Although nitrous oxide emission rates have been reported for streams and rivers (Cole and Caraco, 2001; Beaulieu et al., 2008), the nitrous oxide yield has been studied mostly in lentic freshwater and marine ecosystems, where it generally ranges between 0.1 and 1.0%. However, yields as high as 6% have been observed (Seitzinger and Kroeze, 1998). These nitrous oxide yields are low compared with observations in soils (0–100%) (Schlesinger, 2009), which may be a result of the relatively lower oxygen availability in the sediments of lakes and estuaries. Few studies provide information on the nitrous oxide yield in streams and rivers because of the difficulty of measuring gas production in these systems. Yet, we know that N enrichment increases nitrous oxide production by stimulating nitrification and denitrification. Overall, nitrous oxide is a byproduct of both of these microbially mediated transformations (Seitzinger et al., 2006; Forster et al., 2007; Compton et al., 2011).

2.6.2. Nitrate as a Resource to Improve Water Quality

While nitrate is a pollutant, it can also have beneficial effects on water quality. A number of studies have documented that nitrate at the sediment-water interface can inhibit the release of problematic compounds such as P, reduced iron and manganese, and sulfide. P can exacerbate eutrophication, reduced iron and manganese can complicate potable water treatment, and sulfide is extremely toxic to aquatic biota (Beutel et al., 2008; Wauer et al., 2005; Jonas et al., 2003; DeGasperi et al., 1993). Some studies have documented that phytoplankton communities shift
away from harmful N-fixing cyanobacteria as N to P ratios increase in surface waters due to changes in nutrient loading (Schindler, 2012; Effler et al., 2008; Levich, 1996).

Managed nitrate addition to anoxic sediment has been used in lakes and reservoirs to oxidize organic sediment and repress internal P loading (Søndergaard et al., 2000a; Foy, 1986; Ripl, 1976). Recent examples of managed nitrate addition to repress MeHg production in profundal lake sediments also exist (Austin et al., 2011; Nolan et al., 2012). Thus, in some cases, nitrate may have a beneficial role to play in managing the quality of water resources.

The following case studies revisit the concept of managed nitrate addition to lakes and reservoirs for water quality improvement. Specifically, we focus on the potential for reuse of nitrate-rich treated wastewater to improve water quality in raw water reservoirs used as a drinking water sources. This concept is not especially novel. Ripl and Lindmark (1978) first highlighted the potential to use nitrate in treated wastewaters to improve profundal water and sediment quality over 30 years ago. A renewed focus on the topic is merited as water quality and availability continues to be stressed by growing populations and climate change.

In a recent review of N discharges to European freshwater ecosystems, Petzoldt and Uhlmann (2006) asked, "Is there a need for nitrate elimination in all wastewater treatment plants?" The authors noted that nitrate could be used as an ecotechnological strategy to limit the release of redox-sensitive P from anaerobic sediment, thereby improving water quality in P-limited aquatic ecosystems. They further argued that, rather than being mandated in all cases, nitrate reductions in discharge to surface waters should be evaluated on an ecosystem-by-ecosystem basis. The reuse of nitrate in wastewater to improve the quality of raw drinking water sources is compelling because it enhances the sustainability of both treatment processes. Since the added nitrate is potentially converted to harmless dinitrogen gas via biological denitrification,
negative impacts related to eutrophication could be minimal if nitrate addition is properly managed. In addition, nitrate addition to freshwater bodies in which P loading is low should not result in substantial enhancement of productivity.

2.6.3. *Sequential Treatment of Sediment with Nitrate*

Rilp (1976) was one of the first researchers to propose the in-lake addition of nitrate to manage internal P loading. The sediment treatment scheme consisted of a sequence of three chemical additions. First, ferric chloride is added. The formation of iron oxides and resulting acidic conditions in sediment have the dual effect of enhancing sediment P binding potential while promoting hydrogen sulfide volatilization, which enhances subsequent nitrate treatment since sulfide is a potential sink for nitrate via abiotic reduction. Second, calcium hydroxide is added to neutralize the pH of the sediment, a precondition for effective microbial denitrification. Third, calcium nitrate is added to oxidize organic matter in sediment via microbial denitrification. Treated sediment should have a higher P retention capacity and lower oxygen demand, resulting in lower rates of internal P loading. In 1975, sediment treatment was evaluated in Lake Lillesjön (surface area = 4.2 ha; maximum depth = 4.2 m; mean hydraulic retention time = 0.3 yr), a eutrophic lake in Sweden that received sewage loading before wastewater inflow was diverted in 1971. During the treatment, water column nitrate concentrations peaked to 7 mg-N/L but declined to zero after two months. After the treatment sediment oxygen demand decreased by 50% and water column P dropped from 0.2-0.4 mg-P/L in the previous year to < 0.1 mg-P/L.

Informed by the early work of Ripl (1976), Wauer at al. (2005) recently evaluated the use of a newly developed compound, consisting of a matrix of iron oxide imbedded with calcium and nitrate ions, to limit P release from lake sediment. The compound was added to replicate
experimental enclosures (diameter = 10 m; depth = 8.5 m) in eutrophic Dagowsee (surface area = 0.3 km$^2$; volume = 1.5 x 10$^6$ m$^3$; maximum depth = 9.5 m) located 100 km north of Berlin. Three months after treatment, phosphate in bottom waters in untreated enclosures was 706 µg-P/L compared to 16 µg-P/L in treated enclosures. P efflux from sediment was 4-10 mg-P/m$^2$/d in untreated enclosures and negligible in treated enclosures. The repression of P efflux from treated sediment was still observed 1 year after the sediment treatment.

2.6.4. Quarry Lakes Experiment, Habo, Sweden

Swedish researchers were also the first to propose the use of nitrate-rich effluent from wastewater to improve water quality in lakes (Ripl et al., 1979). They suggested that nitrate addition could have a number of positive water quality impacts, including a shift away from N-fixing cyanobacteria, lower rates of P release from sediment, and better oxygen conditions as organic matter in sediment is oxidized by nitrate-consuming bacteria. During the summer and fall of 1978, Ripl et al. (1979) performed multiple low level additions of sodium nitrate to three quarry ponds near Habo, Sweden. Two ponds were treated (surface area = 0.25 and 0.72 ha, maximum depth = 5.5 m and 2.8 m) and another pond was used as a control (surface area = 1.6 ha, maximum depth = 4.5 m). Nitrate levels in water in the treated ponds ranged from 1-6 mg-N/L. Nitrate addition resulted in a shift from a cyanobacteria dominated system to a green algae dominated system with a more robust large-bodied zooplankton population with relatively lower levels of N fixation, primary production and chlorophyll.
2.6.5. Lake Lyng, Denmark

A number of in-lake restoration measures have been implemented in Danish lakes over the past two decades to improve water quality including manipulation of fisheries, macrophyte implantation, dredging, and aeration (Søndergaard et al., 2000b). In one case, nitrate was added to oxidize sediment and inhibit internal P release. Lake Lyng (surface area = 10 ha; maximum depth = 7.6 m) is a eutrophic lake with a history of sewage loading from a nearby municipality (Søndergaard et al., 2000a). Summertime surface waters were highly turbid (Secchi depth < 2 m) and commonly dominated by small colony-forming cyanobacteria. Bottom waters were anoxic and accumulated P up to a concentration of 3 mg-P/L. In the 1950s sewage inputs were diverted but the water quality in the lake remained degraded due to high rates of sediment P release. In the summers of 1995 and 1996, 500-600 kg of N as calcium nitrate was added to bottom waters, a relatively low dose compared to other lake treatments. Nitrate addition yielded a 50% drop in summertime P levels. Nitrate enhanced the co-precipitation of iron and phosphate in the bottom waters of the lake, with the resulting iron-phosphate precipitate settling to the sediment, resulting in less P available to stimulate phytoplankton growth in the water column. Nitrate also indirectly enhanced iron-phosphate precipitation by inhibiting the formation of sulfides. Sulfides can sequester iron through the precipitation of iron sulfide, thereby lowering the amount of iron available to bind with P.

2.6.6. Schlei Fjord, Germany

Researchers have also evaluated the effect of nitrate addition on P cycling in estuaries and coastal waters (Petzoldt and Uhlmann, 2006; McAuliffe et al., 1998; Feibicke, 1997). In one European study, Feibicke (1997) evaluated the impact of nitrate addition to estuary sediment on
water quality in the Schlei, a eutrophic fjord in northern Germany. During summer, highly reduced conditions in sediment lead to sulfide formation. Sulfide abiotically reduces P-containing iron oxides in sediment, resulting in P efflux from estuary sediment ranging from 1-30 mg-P/m²/d. Released P exacerbated eutrophic conditions by further stimulating phytoplankton productivity. Nitrate addition, which would favor biological denitrification over sulfate reduction, was hypothesized to impeded sulfide production and lower sediment efflux of P. A nitrate solution was injected into sediment in a 3.5 ha enclosure in the summer of 1994. As expected, nitrate addition resulted in higher levels of sulfate and dissolved iron and lower levels of phosphate in sediment pore water. Total P and nitrate in water overlaying sediment was 0.4 mg-P/L and < 1 mg-N/L in untreated enclosures and 0.1 mg-P/L and 4-10 mg-N/L in treated enclosures. A drop in phytoplankton biomass in treated enclosures, comparable to that for total P, was also observed. Phytoplankton composition also shifted from cyanobacteria to diatoms and other non-blue-green algal species in the treated enclosures. An estimated 1% of added nitrate remained in the sediment after 100 days.

2.6.7. Occoquan Reservoir, USA.

The Occoquan Reservoir is a large, run-of-the-river reservoir (surface area = 6.23 km²; volume = 31.4 x 10⁶ m³; maximum depth = 19.8 m; mean hydraulic retention time = 20 d) near Manassas, Virginia (Randall and Grizzard, 1995). The reservoir, which serves as the water source for 2 million people, has a history of excessive nutrient loading from wastewater discharges and storm runoff. In the late 1960s, massive cyanobacteria blooms led to frequent taste and odor problems, depletion of oxygen in bottom waters, and complications and increased costs to treat reservoir water for potable use. To address water quality concerns, regional
authorities evaluated a range of wastewater management options including a moratorium on new sewage connections and the export of wastewater from the basin. Seeing the value in utilizing wastewater as a resource, managers ultimately developed the "Occoquan Policy" – a farsighted program of indirect reuse via tertiary wastewater treatment.

The Upper Occoquan Service Authority discharges $360 \times 10^6 \text{ m}^3/\text{yr}$ of highly treated, low nutrient wastewater to one of the two main tributaries of the reservoir. During the summer, treatment is modified so that discharged effluent is rich in nitrate. Much of the added nitrate flows with the colder stream water to the profundal zone of the reservoir. Typical nitrate levels are $>10 \text{ mg-N/L}$ in discharge and $<2 \text{ mg/L}$ in bottom waters near the reservoir outlet 26 km downstream (Cubas et al., 2014). The Fairfax County Water Authority withdraws water near the outlet of the reservoir and treats it for potable use. Maintaining elevated nitrate levels in effluent discharge to the reservoir has multiple benefits: decreasing tertiary treatment costs by avoiding the denitrification step during the summer, improving water quality by reducing internal loading of P and other reduced compounds from sediment, and increasing water supply.

2.6.8. Lake Tegel, Germany

Lake Tegel (volume $= 23.2 \times 10^6 \text{ m}^3$; maximum depth $= 16 \text{ m}$; mean hydraulic retention time $= 0.2 \text{ yr}$) is a eutrophic lake in northwestern Berlin. The lake, which serves as a water supply reservoir, has a long history of cultural eutrophication and has been impacted by polluted urban runoff, sewer overflows, and sewage discharges. The lake provides a unique case study in which reductions in external P and N loading were implemented sequentially, resulting in an interim period when the lake was relatively rich in nitrate (Schauser et al., 2006).
To improve water quality, a P elimination plant was constructed to remove P in surface inflow in the 1980s. Then in the 1990s, to meet European Union nutrient discharge requirements, regional wastewater treatment plants implemented N removal. Nitrification, the conversion of ammonia to nitrate, began in 1992 and denitrification, the further conversion of nitrate to dinitrogen gas, began in 1997. Nitrate rich conditions repressed P release from profundal sediment. Bottom water P concentrations in 1994-1996 were < 0.2 mg-P/L, while prior to and after this period P concentrations were 0.5-1.5 mg-P/L. 1994-1996 also yielded the three lowest consecutive years of P accumulation in the hypolimnion (< 2 mg-P/m²/d). Accumulation rates after 1996 were 4-8 mg-P/m²/d. Even in years before and after nitrification, the onset of P accumulation in bottom waters correlated with depletion of nitrate in bottom waters. Detailed evaluation of sediment biogeochemistry showed that sediment in Lake Tegel was saturated with P relative to iron. While nitrate inhibited release of P bound to iron oxides, sediment still released mineralized P since sediment had low capacity to retain P. These results indicate that the ratio of P to iron in lake sediment is an important parameter in predicting the effectiveness of nitrate in limiting P release from sediment.

2.6.9. Lake Onondaga, USA

Lake Onondaga (volume = 131 x 10⁶ m³; maximum depth = 19.5 m; mean hydraulic retention time = 0.25 yr) is located in Syracuse, New York. Since the 1920s, the urban lake has received effluent from the Metropolitan Syracuse Wastewater Treatment Plant, which now accounts for 20% of annual lake inflow. The input of treated wastewater has had profound impacts on water quality, with elevated ammonia and P levels resulting in high phytoplankton productivity and low dissolved oxygen levels (Effler et al., 2010; Matthews and Effler, 2006).
One historical industrial pollutant, Hg, is extremely elevated in lake sediment and waters (Todorova et al., 2009). In 2004, the wastewater treatment plant underwent a $130 million treatment upgrade to improve effluent quality. Treatment enhancements included conversion of ammonia to less toxic nitrate via biological nitrification, removal of P via chemical precipitation, and a new ultraviolet disinfection system. A 80% decrease in P loading to the lake has lowered primary production in surface waters (Effler and O’Donnell, 2010), and nitrification has resulted in lower ammonia levels and increased dissolved oxygen in lake bottom waters (Effler et al., 2010). An unanticipated effect of the shift from ammonia to nitrate in wastewater effluent was a 50% drop in MeHg in bottom waters. MeHg, a potent neurotoxin that bioaccumulates in aquatic food webs, is produced by anaerobic bacteria in the profundal zone of lakes. As detailed in Todorova et al. (2009), the presence of nitrate in bottom waters alleviated anaerobic conditions and repressed MeHg accumulation. The New York State Department of Environmental Conservation is implementing a $400 million remediation cleanup effort focused on managing Hg contamination in Lake Onondaga, including a 3-year pilot test to assess the benefits of nitrate addition to impede MeHg accumulation in bottom waters (Nolan et al., 2012).
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CHAPTER 3
ENVIRONMENTAL AND ECONOMIC POLICY STUDY IN CARTAGENA, COLOMBIA: WATER SUPPLY AND ENVIRONMENTAL MANAGEMENT PROJECT

3.1. Background

For decades, water pollution and lack of sanitation have been serious concerns for the citizens of Cartagena, Columbia. Cartagena was the historic city where the “1983 Cartagena Convention for the Protection of the Caribbean” was signed. The primary aim of this agreement was to protect the coastal marine environment and the public heath of the Caribbean's citizens through improved wastewater management. However, many water bodies near urban areas are still highly polluted (ACUACAR, 1995, 2009b). Pollution emanating from domestic and industrial wastewater has contaminated marine waters and the highly productive estuaries and bays that provide critical ecological connections to marine environments. Inadequate wastewater management has also polluted urban beaches, threatening public health and undermining tourism.

Cartagena is a city of almost 900,000 people on the northern coast of Colombia. The city serves as the capital of Bolívar, one of the Columbia's departments (states), and is an economic hub and popular tourist destination. Prior to 1995, Cartagena's water and wastewater services were extremely unreliable; less than 70 percent of households had water connections and less than 55 percent had sewage service (World Bank, 1999, 2009). A large portion of the population, particularly those with lower incomes, obtained water from private vendors. The other portion, with connections to the city's water system, frequently experienced water pressure so low that
service was practically nonexistent. The pre-existing system operated with substantial financial losses and had insufficient funding for maintenance or improvements (UNEP, 2010).

Considerable political pressure from the citizens of Cartagena led to the creation of a Public-Private Partnership (PPP) to repair and operate the water and sewage systems. In 1994, under the leadership of the mayor, the city's municipal council approved the creation of an “empresa mixta” (mixed enterprise), which combined the resources of the public works department and a private Spanish water firm (World Bank, 2007, 2008). Together, these two entities operate under the name Aguas de Cartagena, known as ACUACAR.

Despite ACUACAR's significant progress in improving the efficiency and quality of water and sanitation services, Cartagena still faced many challenges, including: (i) insufficient water supply coverage, especially in poor neighborhoods located in the vicinity of the Cienaga de la Virgen; (ii) insufficient sewerage services, especially in the poorest areas around the Cienaga and in zones adjacent to the Cartagena Bay where open sewage canals on streets was common; (iii) inadequate domestic wastewater management system with untreated wastewater discharging to the Cartagena Bay, the Cienaga, and the inner-city water courses; and (iv) minimally treated industrial wastewater discharging from the Mamonal zone to the Cartagena Bay. Together, these conditions generated sanitation, environmental, and public health problems that reduced the quality of life for residents and posed serious restrictions for sustainable economic development, especially the city's tourism sector (World Bank, 2005).

To address these significant water and sanitation challenges, the District of Cartagena and ACUACAR developed a water and wastewater master plan and implementation strategy. This strategy primarily relied on a project called "The Cartagena Water Supply, Sewerage, and Environmental Management Project (“the Project”)", financed by the World Bank. The Project's
goals were to: (i) expand water supply coverage and production capacity; (ii) expand sewerage networks and improve the sewerage system in the Cienaga de la Virgen basin; and (iii) enhance wastewater management in Cartagena. The wastewater management plan used a conveyance system that transported the city’s wastewater to a preliminary treatment plant and subsequently discharged the treated wastewater through a submarine outfall into the Caribbean Sea.

This chapter summarizes Cartagena’s experience and environmental policy issues associated with implementing a water and wastewater management program. Specifically, this chapter discusses the main efforts to improve water quality in Cartagena, the mechanisms for overcoming impediments, and the key factors for achieving success. The political and environmental empowerment of Cartagena is analyzed relative to implementing an effective water and sanitation management program. Finally, this chapter examines the key environmental policy lessons revealed through the Cartagena project experience.

A portion of this chapter is based on the publication, *Restoring the Coastal Environment of Cartagena, Columbia*, authored by Ricardi Duvil (Consultant, World Bank) and Greg Browder (Lead Water and Sanitation Specialist, World Bank), in collaboration with the World Bank, the Colombian Ministry of Environment and Sustainable Development, the Cartagena Water Utility (ACUACAR), and the Global Partnership for the Oceans (GPO) (Box 1). Also I would like to thank Dr. Steven Stehr, associate professor of politics, philosophy and public affairs at Washington State University for helpful discussions and reviews of this chapter.
Box 1: Global Partnership for the Oceans

The Global Partnership for Oceans is a new and powerful approach to restoring ocean health. It mobilizes finance and knowledge to activate proven solutions at an unprecedented scale for the benefit of communities, countries and global well-being. The GPO is a growing alliance of over 140 governments, international organizations, civil society groups, and private sector interests committed to addressing the threats to the health, productivity and resilience of the ocean. The Partnership aims to tackle documented problems of overfishing, pollution, and habitat loss. Together these problems are contributing to the depletion of a natural resource bank that provides nutrition, livelihoods and vital ecosystem services.

3.2. Project Objectives

The objectives of the Project were to: (i) improve the water and sewerage services of Cartagena and the sanitary conditions of the city's poorest population by expanding water and sewerage coverage, particularly in the poor neighborhoods; (ii) facilitate the environmental cleanup of water bodies surrounding the city (Cartagena Bay, Caribbean beaches, and Cienaga de la Virgen estuary) (Figure 1) by providing adequate collection, treatment, and disposal of the city's wastewater; and (iii) improve the sustainability of water and sewerage services in Cartagena by leveraging Bank support to shore up the private sector participation (PSP) model, pioneered by ACUACAR, against the prospect of political interference.

A related objective of the city was to decrease the amount of non-revenue-water (NRW) caused by leaks in the existing pipes. The city believed that 60 percent of the water was being
lost in this way and wished to reduce the loss to no more than 25 percent. Reducing NRW losses would increase water pressure, water supply, and daily duration of water availability. In addition, lowering NRW would improve the city's long-term financial situation because a larger portion of water treatment and distribution costs could be recovered from consumers.

Another objective of ACUACAR was to transform the water and sewerage system into a financially self-sustaining operation. This objective was to be achieved through increased efficiency in the management of water supply and sewage services, paired with an increase in the tariff collection ratio. The tariff structure incorporates a system of cross-subsidies based on the 1991 National Constitution. The structure divides customers into six categories, with the top two categories subsidizing the lowest three and the remaining one paying cost-recovery tariffs (World Bank, 2006). The municipal provider had over one thousand employees, but water service was intermittent and tariff collection was less than 50 percent. Prior to the creation of ACUACAR, the financial working ratio (the cash operating costs/cash collections) for the municipal-owned provider was about 152 percent. The company was losing money and providing inadequate service to customers. ACUACAR aimed to improve management, to improve all-around service, and to eliminate the operating losses.
Figure 3-1. Map of project area.
3.3. **Cartagena: A Dynamic Cultural and Industrial City**

The District of Cartagena has experienced rapid growth with the population doubling over the last two decades. Due to its history and spectacular natural scenery, Cartagena is Colombia’s largest tourist area, with an annual influx of around 1 million visitors. In 1984, UNESCO declared the key landmarks of Cartagena as a World Cultural Heritage Site. Cartagena also has a thriving industrial sector with important petrochemical, beverage, and seafood processing industries. In 2012, the Port of Cartagena was Colombia's main container port and one of the busiest ports for transportation of grain in South America.

The socio-economic structure of Cartagena is complex and the city suffers from high levels of poverty. Thirty-five percent of the population is classified as extremely poor, or “Strata 1” in the Colombian system, implying that they cannot meet their basic needs. Another 30% falls under the “Strata 2” classification, implying that their basic needs are not met in a satisfactory manner (Deloitte, 2009). Households classified as Strata 1 or 2 pay subsidized rates for utility services, such as water and sanitation. A significant percentage of the population is composed of recently arrived residents from rural areas seeking better economic opportunities and fleeing drug trafficking violence in the countryside.

Cartagena is surrounded by water on all sides: the Caribbean Sea to the north, Cartagena Bay to the west, and a lagoon known as the Ciénaga de la Virgen to the east (Figure 1). Recognizing the importance of the marine environment for Cartagena, and its standing as a leading city in the region, the Convention for the Protection and Development of the Marine Environment in the Wider Caribbean Region, known as The Cartagena Convention, was signed in 1983 in Cartagena (Box 2).
The Convention for the Protection and Development of the Marine Environment in the Wider Caribbean Region (WCR) is a comprehensive, umbrella agreement for the protection and development of the marine environment. This regional environmental convention provides the legal framework for cooperative regional and national actions. The Convention was adopted in Cartagena, Colombia on 24 March 1983 and entered into force on 11 October 1986, for the legal implementation of the Action Plan for the Caribbean Environment Programme. The Cartagena Convention has been ratified by 25 national governments in the Wider Caribbean Region. The Convention is supplemented by three Protocols which entered into force with the following dates: i) Combating Oil Spills (1986); ii) Protected Areas and Wildlife (2000); and iii) Pollution from Land-Based Sources (2010).

3.4 Confronting Cartagena's Coastal Environmental Crisis

By the mid-1990s, rapid population growth, unplanned urban development, and poor wastewater management had severely deteriorated Cartagena’s rich coastal resources and generated a public health crisis. Less than half of the households had connections to a wastewater disposal system and all wastewater was untreated. Untreated wastewater flowed into coastal waters carrying organic waste and nutrients that destroyed rich fisheries in the Bay and the Lagoon. Disease-causing bacteria and parasites from untreated wastewater flowed through streets and into water courses, amplifying public health risks, particularly for the numerous low-
income and vulnerable communities living around the Lagoon. Cartagena’s famous beaches were frequently closed due to microbiological contamination. In addition, industries further contributed to the contamination of Cartagena’s environment by discharging wastewater with impunity into the nearest water body.

The environmental crisis confronting Cartagena in the mid-1990s was undermining the city’s long-term sustainability by threatening its world-class tourism industry, posing public health risks, and lowering the quality of life. Furthermore, the rich ecosystems in the Bay, the Lagoon, and along the coast—mangrove forests, highly productive and diverse fisheries, seagrasses and coral reefs—were in ecological decline. The prospects for recovery appeared bleak in the absence of a comprehensive and effective water pollution control program.

3.5 Resetting the Institutional Framework for Water Supply and Management

Prior to 1995, Cartagena’s water and sanitation service suffered from an inefficient water utility that was owned and controlled by the municipal government. Due to low tariffs and inefficient operations, the lack of cost recovery on behalf of the water utility contributed to financial problems. Ultimately, these problems resulted in inadequate service delivery to its users. Major portions of the population, particularly those with lower incomes, were forced to rely on water provided by private vendors. Even those with connections to the city water system frequently experienced such low water pressure that their service was essentially nonexistent. Unable to recover costs due to its inefficiency, the city’s system operated with substantial financial losses and had insufficient funding for maintenance or improvement of the system.
The situation in Cartagena was not an isolated case in Colombia during this time period. As a result, in 1994, the Colombian government passed the Public Services Law that expanded the role of specialized operators in public water utilities and created national tariff and service regulatory agencies. To address its environmental and socio-economic problems, the District of Cartagena was one of the first municipalities in Colombia to introduce private participation in the water and sanitation sector.

In 1995, the Cartagena District Council and the City Mayor liquidated the municipal utility and created a “mixed-capital” company for the operation of the water and wastewater system, called ACUACAR. A private company from Barcelona, Spain (AGBAR) was selected as the specialized operator and owns 46% of ACUACAR’s shares. Cartagena District retained 50% of the shares, with the remaining 4% owned by private shareholders. Although this model is commonly used in Spain, this was the first time such an arrangement had been tried in Latin America (BID, 2006).

The ACUACAR contract included various performance targets to improve the quality of service and maintenance, reduce leakages in the distribution system, and improve the collection rate. The District originally retained primary responsibility for financing future capital investments, while ACUACAR was responsible for implementing the capital works program. However, the contract has evolved over time and ACUACAR now self-fines a significant share of capital works through its tariff revenue.
3.6 Developing New Environmental Policy and Management Institutions

In 1993, a new National Environmental Law created the Ministry of Environment and formulated a framework for environmental management. The law also established autonomous regional environmental authorities, which are responsible for implementing national environmental policies at the local level. The regional environmental authorities in Colombia are governed by a combination of national, regional, and local government representatives and stakeholders from the business community, non-governmental groups, and indigenous peoples. The regional environmental authorities have a steady funding stream, based mainly on a 15% environmental property tax surcharge, to implement projects and cover operating costs. However, these funds were often insufficient because the coastal regional environmental authorities' jurisdiction now includes marine environments, which are an added responsibility and contribute to higher operating costs.

The environmental authority for the region surrounding Cartagena is called CARDIQUE. This institution is responsible for issuing environmental licenses and setting standards for water quality and municipal and industrial discharge. The larger cities of Columbia, including Cartagena, also have their own municipal agencies that are responsible for environmental management and entitled to one-half of the city's environmental property tax surcharge.

3.7 Financing and Implementing a Phased Infrastructure Development Program

In the late 1990s, the financing needs for upgrading Cartagena’s water supply system, and essentially constructing a new wastewater collection, treatment, and disposal system were
daunting. The upgrade would require over $150 million for wastewater infrastructure alone. Fortunately, the Colombian national government and the District of Cartagena were committed to providing most of the required financing. International development institutions, such as the World Bank and the Inter-American Development Bank (IDB), also provided long-term low-interest loans, coupled with technical assistance, to help Cartagena achieve its environmental objectives (World Bank, 2004).

Tackling Cartagena’s severe coastal environmental degradation required a long-term and incrementally phased approach, grouped into the following components:

1) **Improving Water Supply Service**: ACUACAR’s highest priority during the period from 1995 to 2000 was to improve water service to customers by rehabilitating and upgrading the existing water supply system (Hazen and Sawyer, 2001). Improvements included increasing the treated water supply, stabilizing water pressure, improving the electrical supply system, and improving commercial practices such as billing, collection, and customer service. These interventions allowed ACUACAR to enhance its financial stability and cost recovery and to secure the confidence of its customers and the Cartagena District government, thus paving the way for large-scale wastewater investments.

2) **Improving Drainage in High-Value Economic Areas**: Prior to 2000, key tourism and commercial areas in Cartagena were subject to frequent flooding and sewer overflows into the streets and surrounding beaches. For example, the low-lying Bocagrande zone of popular, high-end tourist beaches and hotels is particularly vulnerable. With the help of an IDB loan of
US$40.5 million, ACUACAR improved the wastewater system draining into Cartagena Bay and essentially eliminated sewage discharges in key tourism areas.

3) Improving Water Circulation in the Ciénaga de la Virgen: While ACUACAR focused on water supply and high-priority drainage issues in the late 1990s and early 2000s, the District of Cartagena and CARDIQUE developed an innovative project to improve water quality in the Lagoon (Ciénaga de la Virgen). The Lagoon originally had numerous outlets to the sea and its brackish waters supported a diverse and highly productive aquatic ecosystem. However, the construction of a road along the coastline in the late 1980s sealed off most of the natural outlets, thus severely curtailing tidal circulation in the estuary. By 2000, the Lagoon was receiving around 60% of Cartagena’s untreated wastewater. The combination of high pollution loads with poor water circulation had created an environmental disaster. The District of Cartagena and CARDIQUE, with the financial and technical support of the Netherlands' government, constructed a stabilized tidal inlet entrance known as “La Bocana Project” in the early 2000s. The project consists of an enlarged canal entrance to the Lagoon, self-actuating tidal gates that increase flow into the Lagoon, and a long seawall within the estuary that routes the tidal flows and helps flush pollution out of the Lagoon.

4) Collecting, Treating, and Disposing of Cartagena’s Wastewater: By the early 2000s, ACUACAR, with the support of a US$85 million loan from the World Bank, was poised to fully implement its wastewater master plan. The plan consisted of the following components (Figure 2):
• Convey the wastewater from the Cartagena Bay drainage area to the new central pump station;

• Complete the wastewater collection system in the Laguna drainage area, particularly in the poor, low-lying squatter neighborhood along the Lagoon, and convey the wastewater to the new central pump station;

• Pump all of Cartagena’s collected wastewater from the central pump station to a new treatment plant located 19 kilometers to the north; and

• Treat the wastewater at a new wastewater treatment plant, and then discharge the effluent through a 4.3-kilometer long, 2-meter diameter, submarine outfall into the Caribbean Sea at a depth of approximately 20 meters.

A submarine outfall (Box 3), coupled with preliminary wastewater treatment was the selected alternative due to its relatively low costs and the adequate natural conditions close to Cartagena’s coast. The preliminary treatment plant was designed to remove floatable material such as oils and plastic bags, as well as sand and grit particles. The submarine outfall technology efficiently reduces organic material and suspended solids, producing up to a 99% reduction in the concentration of these key pollutants outside the designated mixing zone around the outfall diffusers. Extensive modeling was done with international experts to confirm the feasibility of a submarine outfall and the plan was approved by CARDIQUE. The treatment plant layout also allowed for upgrading the treatment process in the future, if so required.
A marine outfall is a pipeline or tunnel that discharges wastewater under the sea’s surface. For municipal wastewater, effluent is often discharged after only preliminary treatment, with the intent of using the assimilative capacity of the sea for further treatment (McCarthy, 2006). The siting and design of a submarine outfall through sophisticated modeling and oceanographic studies is critical to ensuring that the receiving seawater has sufficient dilution capacity. High quality construction is required to avoid leakage in the outfall pipe or tunnel, and continuous monitoring under independent regulatory supervision is indispensable to confirm that the outfall performs as planned. Submarine outfalls, combined with preliminary treatment tend to be less expensive than advanced wastewater treatment plants, using the natural assimilative capacity of the sea instead of energy-intensive treatment processes in a plant. The costs of preliminary treatment are about one tenth that of secondary treatment and preliminary treatment also requires much less land than advanced wastewater treatment. Preliminary treatment, however, typically does not remove nutrients such as nitrogen or phosphorous which are a concern in many marine environments. Submarine outfalls are common throughout the world and probably number in the thousands. More than 200 large outfalls have been listed in a single international database maintained by the Institute for Hydromechanics at Karlsruhe University for the International Association of Hydraulic Engineering and Research (IAHR) / International Water Association (IWA) Committee on Marine Outfall Systems.
3.8 Social Impact Concerns Raised by the Local Non-Governmental Organization

The selection of a submarine outfall option, combined with preliminary treatment, was controversial (Neotropicos, 2002). The local non-governmental organization (LNGO), on its behalf and on the behalf of 424 residents of the areas, was opposed to the project. As a result the LNGO filled a number of claims to the World Health Organization (WHO), the United Nations Environmental Programme (UNEP) and the Colombian government. These claims include:

- The outfall would affect fishing village near the start of the 4-km long marine outfall pipe.
- Local property developers feared odor and seawater quality problems would affect real estate value.
- The Bank failed to identify affected communities as indigenous and failed to safeguard their livelihood and reliance on fishing and farming as a subsistence living.
- The Project would have serious impacts on the communities' culture and way of life, as well as the impact of pollution on their health.
- The Bank failed to perform proper supervision and insufficiently scrutinized the economic investment and the environmental risk evaluations for the proposed submarine outfall system.
- The Project would place undue fiscal strain on the city of Cartagena and would violate Colombia’s international obligations under the 1983 Cartagena Convention on Land-Based Sources of Pollution and the related 1999 Aruba Protocol.
As a result, the marine outfall approach was contested in the Colombian environmental regulatory system. In 2002, the Ministry of Environment ratified the environmental license originally approved by CARDIQUE. Opponents of the outfall option also appealed to the World Bank’s Inspection Panel in 2004. After a lengthy and extensive review, the Panel endorsed the outfall alternative as an acceptable solution.

3.9 Addressing Concerns Raised by the LNGO

To address the LNGO’s concerns, the World Bank Panel conducted a Social Impact Assessment (SIA) and an Environmental Impact Assessment (EIS) in the Punta Canoa areas. The Bank found that the environmental impact and social impact assessment demonstrated the project would not have significant adverse effects on the environment and the affected population. The investigation concluded that the project would achieve significant public benefits with a high rate of return, without significant environmental or social risks, and in a fiscally sustainable manner. However, the Bank agreed that the project failed to address possible long-term environmental and health effects on the coastal and marine environment as a result of multiple outfalls and increased volumes of sewage and organic wastes. The Bank acknowledged that this issue needed to be addressed in future. In addition, the bank agreed to support a program to strengthen fishing activities and requested ACUACAR work with the communities to identify specific activities for optimized fishing opportunities in Punta Canoa. Finally, the Bank included the affected communities in the project decision-making process and ensured they would share in project benefits, rather than compensating them for the project’s existence. Later, the Bank and
ACUACAR compensated for the potential impacts, for example, a new school and community center were built in the north zone area.

3.10 Implementations Metrics and Summary of ACUACAR’s Achievements

With the Bank's financial support, Cartagena water and sanitation services improved significantly. Current customers saw an increase in the reliability of water and water pressure. Existing pipelines and water meters were evaluated and repairs were made. A large percentage of the loss of water was corrected, which increased water pressure to customers. Service was extended into poor areas of the city and management improvements were identified and implemented. Even before new pipelines were constructed, water trucks were sent into poor areas to supply those areas with less expensive water.

Another aspect of Colombia’s Law No. 142 was that it required a survey of all residential buildings, including location, form, state, and quality of housing. This information was used by local governments to classify all residences on a six-level scale. Residences assigned a classification of 1-3 were considered poor, residences categorized as 4 were middle-class, and residences assigned a 5 or 6 were deemed upper class. Using this information, ACUACAR created a new tariff system that included a cross-subsidy to benefit poor customers. Residences were evaluated and classified per the six-category scale. For customers with high incomes, tariff rates were set higher than the actual cost of service. This extra revenue then went to subsidize service to poor residents, whose rates were set below the cost of service.

The new tariffs were intended to generate enough funds to cover operational and investment costs. ACUACAR proposed the tariff rates and the Drinking Water Regulation
Commission reviewed and approved them before rates were assigned to customers. Residents living in level 4 houses (middle-class) were assigned a tariff equivalent to the actual cost of service. Lower rates were given to people in levels 1-3 (lower income households), while higher rates were given to people living in levels 5-6 (the upper income households). This practice of cross subsidization was used to cover the deficit in tariff revenues that would have resulted from a general subsidy to poor households (ACUACAR, 2009c, 2009e).

Efficiency within the company and in provision of services was another objective of ACUACAR. To increase efficiency within the organization, ACUACAR only hired back about 400 of the former 1,500 unionized municipal employees (those let go received severance pay based on years of service). This move improved productivity from about 5.5 employees per one thousand accounts in 1995 to 3.4 employees per one thousand accounts in 2005.

To extend service, about 35,000 new connections were made, almost exclusively in poor neighborhoods. Many poor citizens were skeptical and suspicious of ACUACAR because they were not used to dealing with a water company. In response, the company hired social workers and community relations specialists to explain to residents how water and sewage service works. Local unskilled laborers were also hired to complete the construction of the new pipelines for the new connections. These actions, coupled with visible service expansion, improved ACUACAR’s relationship with the community.

Another achievement was the decrease in the level of NRW. Originally, losses were estimated at about 40 percent of system volume, leading to an objective of lowering NRW to 25 percent (a 15 percent net reduction) over a period of ten years. However, reevaluation determined that the NRW was actually closer to 60 percent. Nonetheless, with the improvements initiated by ACUACAR, the NRW was reduced to about 40 percent by 1999, a net reduction of
20 percent in only four years. In addition, water coverage went from less than 70 percent in the early 1990s to close to 99 percent in 2005. The hours of water service increased from about 17 hours a day to 24. Sewage service availability increased from 55 percent of households in the early 1990s to over 75 percent in 2005. ACUACAR has added an additional 260 km of pipeline, bringing the water and sewage network up to 700 km in total, an increase of 48 percent (World Bank, 2009; ACUACAR, 2009a, 2009d).

Equally important was the objective of increasing the collection fee ratio from less than 50 percent to 95 percent. Many households lacked a water meter. In other households, meters were frequently broken. This situation made tariff collection difficult for the municipal-owned provider and contributed to the negative cash flows. Within the first few months of their operations, ACUACAR replaced or added water meters to existing connections and added water meters to all new connections. By 2005, over 99 percent of connected households had water meters. The improved billing and tariff collection has improved the ratio to just over 90 percent in 2005. As a result, the financial working ratio dropped to less than 70 percent, from the 152 percent historical figure. ACUACAR also experienced an increase in the ratio of operating revenue/operating costs to about 120 percent in 2005. Increases in efficiency, coupled with a better collection record, gave ACUACAR positive cash flows.

As a result of improvements in utility management, moderate increases in tariffs, and infrastructure investments, ACUACAR has been able to significantly improve the quality of water and sanitation services and achieve sustainability. Potable water, which meets the national quality standards, is provided on a continuous basis to all households—even to the poorest neighborhoods. Based on its tariff revenues, ACUACAR is able to cover all operating and maintenance costs, and help contribute to infrastructure investments. ACUACAR’s mixed capital
model has become institutionalized in Cartagena, and the combination of local political control of the company combined with professional private sector management has performed remarkably well (Table 1).

Table 3.1. Implementation Metrics Water Supply and Wastewater Treatment.

<table>
<thead>
<tr>
<th>Year</th>
<th>Water Supply Coverage</th>
<th>Water Supply Customers</th>
<th>Length Water Network</th>
<th>Continuity of Water Service</th>
<th>Unaccounted for Water</th>
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<tr>
<td>1995</td>
<td>73%</td>
<td>92,572</td>
<td>700 kms</td>
<td>14 hours</td>
<td>45%</td>
</tr>
<tr>
<td>2012</td>
<td>99.9%</td>
<td>225,815</td>
<td>1,480 kms</td>
<td>24 hours</td>
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<table>
<thead>
<tr>
<th>Year</th>
<th>Sewerage Coverage</th>
<th>Sewerage Customers</th>
<th>Length Sewage Network</th>
<th>Wastewater Treated</th>
<th>Revenues COP-Million</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>61%</td>
<td>77,553</td>
<td>500 kms</td>
<td>0%</td>
<td>25,592</td>
</tr>
<tr>
<td>2012</td>
<td>89%</td>
<td>200,150</td>
<td>1,062 kms</td>
<td>&gt;90%</td>
<td>165,889</td>
</tr>
</tbody>
</table>

Source: Table based on ACUACAR water data information system.

3.11 ACUACAR’s Methods for Overcoming Impediments

Prior to the decision in 1994 to create the public-private partnership ACUACAR, the unionized water service workers expressed strong opposition to the project because of potential job loss. Many of these workers had been hired based on their relationship to the mayor and managers of the municipal-owned provider, rather than on job-related qualifications. Under ACUACAR’s management, many of these employees lost their jobs, but were provided severance pay based on years of service (Gómez Torres, 2003).

ACUACAR also faced the immediate challenge of many complaints from consumers based on their experiences with the previous service provider. Citizens voicing their opinions
about poor service helped bring about the creation of ACUACAR, but once the company took over the operations and management, some of the vocal criticism continued. Only through continuous efforts at improving services and public outreach was ACUACAR ultimately able to turn around public opinion about Cartagena’s water and sewage services.

Another problem ACUACAR faced was that providing services to poor areas would take time, because of the need to construct new pipelines. Many poor people had been purchasing their water from independent vendors at a higher price than that paid by households with municipal connections. Once ACUACAR was operational, the PPP began trucking water to customers in these poorer neighborhoods. Even though these customers would have to wait for a connection, they saw an immediate increase in service and were willing to support ACUACAR during construction.

ACUACAR worked hard to earn and keep the support of the community, which is a necessary step in the success of a PPP. The majority of the new connections were created in poor areas where residents were not used to dealing with a water company. Many residents were confused about the construction process and suspicious of, even hostile towards, the workers. Community relations specialists and social workers were hired by ACUACAR to overcome this obstacle. Public meetings were held explaining how water and sewage service worked and ACUACAR’s methods. The social workers also helped set up payment schedules for customers with irregular incomes. To further show dedication to the project and the poor areas, ACUACAR hired local unskilled laborers to construct the pipelines and other necessary parts of the network.
3.12 Commissioning of Cartagena’s Wastewater System

Recognizing the importance of this achievement, President Juan Manuel Santos of Colombia inaugurated the wastewater treatment system on March 20, 2013; his translated comments are presented below:

“This is an extremely important project, a project that has great significance for the development of the country, Cartagena, and the thousands of people which in one way or another will benefit from no longer having a contaminated Bay or a contaminated Ciénaga de la Virgen. With this project, Cartagena becomes the first city in the country to have a comprehensive sanitation program with 100% wastewater treatment. This is the project that you have been waiting for 20 years, and after 20 years of much effort and challenges, finally today it is a reality.

3.13 Coastal Environmental Improvements

In addition to improved water services, the completion of the wastewater system has generated environmental improvements that were hardly imaginable two decades ago. Although there is less than one year of extensive water quality monitoring to date after the commissioning of the new wastewater treatment system, the results are evident. Initial opposition to the preliminary treatment with the marine outfall solution has dissipated and there is a consensus that the approach is working well.

Figure 2 provides a summary of key water quality indicators “before” and “after” the commissioning of the wastewater treatment system in mid-2013. This data was provided as a
The final data set by ACUACAR based on their monitoring data collection. Figure 2 also compares the water quality data with the regulatory requirements contained in ACUACAR’s environmental license. For example, Figure 2 illustrates the total coliforms along the beaches of Cartagena. “Before” reflects data analysis completed prior to the start of the wastewater treatment system, and the average concentration was 920 MPN/100 mL and was calculated based on a historical data analysis performed by ACUACAR. Data were collected between 2005 and 2012 from different regions along the beaches, with 13 sampling stations from Castillo Grande to Punta Canoa. However, there was high variability in the data set. The standard deviation was 672 MPN/100 mL (sample size = 55) and the peak value was > 34,000 MPN/100 mL. Data indicated that beaches were unsafe at certain times. “After” reflects data analysis completed after the start of the wastewater treatment system, and the average concentration is 31 MPN/100 mL and was calculated based on a monthly monitoring system that utilized 11 sampling stations to collect data from April 2013 through August 2013. All water quality sampling and monitoring was also completed by ACUACAR. As a result, the beaches are suitable for swimming, fishing and recreational activities. As indicated in Figure 2, both average values before and after the project are below the standard value (1,000 MPN/100 mL) for primary contact, but the total coliform concentration shows a 97% decrease from 2005 to 2013.

Furthermore, Figure 2 shows Cienaga de la Virgen water quality improvements, the average concentration of total coliforms before was 8,667 MPN/100 mL and the average concentration “after” was 885 MPN/100 mL. The average value before the projects (8,667 MPN/100 mL) was calculated based on a historical data analysis performed by Hazen and Sawyer (2001). Data were collected in the 1990s and 2000s from different regions of the lagoon, and a peak value of > 20,000 MPN/100 mL was recorded close to the point of domestic
wastewater discharge. The average value after the projects (885 MPN/100 mL) was calculated based on a monthly monitoring system that utilized 7 sampling stations to collect data from January 2013 through July 2013. This water quality sampling and monitoring was completed by ACUACAR. As indicated in this graphical representation, the average value in 2013 (885 MPN/100 mL) is below the standard value (1,000 MPN/100 mL) for primary contact. In this data set, the highest concentrations were observed close to the urban areas. Monitoring indicated a 90% decrease in total coliform concentration from the 1990s to 2013.

In addition, Figure 2 shows the average concentration of total nitrogen, total phosphorus and dissolved oxygen in the lagoon before (25 mg/L, 15 mg/L, and 1.7 mg/L) and after (0.4 mg/L, 0.2 mg/L, and 5.54 mg/L) the outfall project. Poor water quality before the project was the result of human activity, which greatly increased nitrogen and phosphorus pollution. These nutrients trigger the growth of algae that block light to seagrass and reduce dissolved oxygen concentration through the decay of sea grass and algae. This in turn can result in fish kills. The average values before the projects were calculated based on a historical data analysis completed by Hazen and Sawyer (2001) as well as Grupo Ambiental de Cartagena, which utilized 5 sampling stations (as highlighted in the 2008 water quality survey). The average value after the project was calculated based on a monthly monitoring system that utilized 7 sampling stations to collect data from January 2013 through July 2013. This water quality sampling and monitoring was completed by ACUACAR. The results of 2013 show that Cienaga de La Virgen meets the water quality standards for a healthy lagoon with an overall decrease of 98% in total nitrogen, 97% in total phosphorus, and an increase of 69% in dissolved oxygen.

Finally, Figure 2 illustrates the total coliform concentration in Cartagena Bay. The average concentration before was 6,200 MPN/100 mL and the average concentration after was
500 MPN/100 mL. The average value before the completion of the projects (6,200 MPN/100 mL) was calculated based on a historical data analysis performed by ACUACAR. Data were collected in the 1990s and 2000s from different regions of the bay, and a peak value > 24,000 MPN/100 mL was found close to the industrial discharges. This peak value was detected during high rain intensity; prior studies by Kress and Gifford (1984) have confirmed that high rainfall intensity has an effect on peak coliform levels. The average after value (500 MPN/100 mL) was calculated based on a monthly monitoring system that utilized 11 sampling stations to collect data from January 2013 through July 2013. This water quality sampling and monitoring was also completed by ACUACAR using the surface water sampling rules as guidelines. As indicated in this graphical representation, the average value in 2013 (500 MPN/100 mL) is below the standard value (1,000 MPN/100 mL) for primary contact. The highest concentrations were observed at the surface waters of the Bay because coliforms are largely retained near the surface and the Bay received continuous fecal contamination from a variety of sources, including industrial wastewater and urban runoff with historically high coliform levels. The data shows a 92% decrease in total coliform concentration from the 1990s to 2013.

In addition, the level of dissolved oxygen concentration in the Cartagena Bay before was 0.5 mg/L and the after average concentration was 6.2 mg/L. In the Bay area, the dissolved oxygen concentration varies from region to region, but in Colombia environmental regulations, healthy water quality requires that the dissolved oxygen concentration should fall between 6 to 8 mg/L (IDEAM, 2000). In this data analysis, the average value before the project (0.6 mg/L) was calculated based on an historical data analysis performed by the consulting firm Hazen and Sawyer (2001) in 1998 and was used in the 1999 Environmental Impact Assessment for Wastewater Management in Cartagena. The results indicate that dissolved oxygen concentration
was significantly influenced by pollution discharge from domestic and industrial waste.

Furthermore, the average value after the project (6.2 mg/L) was calculated based on a monthly monitoring system that utilized 8 sampling stations to collect data from January 2013 through July 2013 in varied weather conditions. During this sampling event, the Bay maintained an average temperature of 26.5 °C and pH of 8.5. This data analysis was reported by ACUACAR in September 2013. As indicated in this figure, the average value in 2013 (6.2 mg/L) was between the standard value (6-8 mg/L) for healthy water quality. The lowest concentrations were observed at the bottom of the Bay because of high level of organic matters. The data shows a 90% increase in dissolved oxygen concentration from the 1990s to 2013.

Since the wastewater treatment system was only commissioned in May 2013, the results are preliminary, but encouraging. ACUACAR and CARDIQUE have a comprehensive and continuous water quality monitoring program. The impacts can be qualitatively described as follows:

**Pollution Free Coastal Beaches:** Cartagena’s Caribbean beaches are essentially free of contamination from sewage and the “red-flag” days of beach closures are now history. Coliforms are a broad class of bacteria found in our environment, including the feces of human and other warm-blooded animals. The presence of coliform bacteria in water indicates a possible presence of harmful, disease-causing organisms associated with human waste. The Colombian standard for safe bathing is 1000 MPN/100 mL and was frequently exceeded in the past. Total coliform concentrations have declined dramatically after the wastewater treatment system was commissioned (Figure 2).
**Significant Improvements in Cartagena Bay Water Quality:** The wastewater generated in the western part of the city, accounting for approximately 35% of the total pollution load, is now conveyed to the wastewater treatment plant and disposed through the submarine outfall. Pollution levels in the Bay, particularly alongside Cartagena in the “Inner Bay,” are now significantly reduced. There are still challenges, however, in achieving a full Bay clean-up including contaminated stormwater run-off, industrial pollution, and pollution from the “Canal del Dique” in the southern part of the Bay which discharges untreated effluent from numerous small municipalities.

**Restoration of the Ciénaga de la Virgen:** The removal of wastewater discharges into the Lagoon, coupled with improved water circulation produced by the La Bocana project, has transformed the estuary. All key parameters, including coliforms, dissolved oxygen, biochemical oxygen demand, and suspended solids are now within regulatory standards and odor problems have been eliminated. Cartagena residents can now enjoy the Lagoon through boating and fishing activities, and CARDIQUE is planning to transform the Lagoon and its surrounding area into a protected ecological park.

**Water Quality around the Submarine Outfall:** ACUACAR, under the regulatory supervision of CARDIQUE, has undertaken extensive water quality monitoring around the outfall discharge area. The monitoring program follows international standards and indicates that outside of the prescribed mixing zone there is no discernible impact on the seawater quality. Outside of the 500 meter mixing zone around the outfall diffuser, biochemical oxygen demand and suspended solid concentrations are equal to ambient seawater quality levels, and total coliform levels are less than 5 MPN/100 mL—a low level and considered suitable for human contact.
Preliminary and Indicative Water Quality Monitoring Results in Cartagena

Figure 3-2. Preliminary Water Quality Monitoring in Cartagena.
3.14 Future Challenges in Coastal Environmental Restoration

Cartagena has passed through the first and perhaps most important stage: the collection, treatment, and safe disposal of its wastewater. International experience has shown, however, that this first phase must be complemented with additional initiatives to protect the coastal environment.

- Ensuring all households are connected to the sewerage system and that sewer overflows are eliminated;
- Achieving full compliance with industrial discharge standards;
- Reducing stormwater related pollution;
- Dealing with non-traditional pollutants including nutrients, heavy metals, pesticides, and pharmaceutical waste;
- Monitoring water quality in a continuous and extensive basis to assess progress in pollution control and help determine the need to upgrade the level of wastewater treatment;
- Managing land-use, particular with respect to protecting riparian areas along beaches, estuaries, and rivers; and
- Managing fisheries and natural resource exploitation to ensure sustainable use of resources.

In addition, the environmental condition of the Canal del Dique with heavy metals contamination, which generates delta formations in the Bay of Cartagena, has raised serious concerns. Current sedimentation along Canal del Dique has prevented inland water transport, which is vital for the economic development of Cartagena. Besides, the canal provides multiple
environmental functions such as source of water and nutrients for the neighboring wetlands, natural control of salt intrusion, and vital source of drinking water for the different settlements. Thus, the canal will still require an intense review and planning to protect water quality in the Bay (Menahem and Roberts, 2002).

Furthermore, Cartagena Bay is known to have problems involving sedimentation and eutrophication. Urban planning and management will be required to organize settlements along the canal. For example, in the absence adequate planning, the Cerro de la Popa could suffer from landslides. La Mojana, a sub-region near the canal, could undergo an accelerated process of environmental degradation including the desiccation of wetlands, alteration of the natural hydrological regime, severe deforestation, critical habitat alterations, and remarkable loss of biodiversity. Finally, agricultural and fishing production could be affected by negative effects of illegal mining practices, which are responsible for the sedimentation and contamination with mercury and other highly toxic heavy metals.

3.15 Analysis of the Cartagena Water Supply and Environmental Management Project

Overall, the Cartagena Water Supply, Sewage, and Environmental Management Project was successful. Service was extended into poorer neighborhoods and the existing service was greatly improved. The system has continuous water pressure and leakage has been reduced. Residents are happy with the service and vocal in supporting ACUACAR. Each time that a new mayor is elected, residents want to make sure the new mayor does not do anything to change the service.
Several of the keys to success for a PPP are evident in the case of ACUACAR. The initial investment by the private sector to organize ACUACAR required an income stream for repayment of this investment. Not only did the Spanish water firm receive dividends for being a shareholder in ACUACAR, but it also received a percentage of the profits. This arrangement gave the Spanish firm two reasons to operate all aspects of its business efficiently, including operations and maintenance, infrastructure investments, long-term debt management, and collected tariffs.

ACUACAR also sought out and obtained the support of the community, which made success possible. Hiring social workers, community relations specialists, and local unskilled laborers for the construction of pipelines helped show customers that the company was different from the previous municipal provider.

3.16 Environmental Policy Issues Revealed in this Project

Although the District of Colombia has extensive environmental regulations, they are inadequate for a number of reasons. First, in many cases, urgently needed regulation simply does not exist. Second, some regulations are incomplete and lack critical details. For example, the lack of regulations regarding the scope and applicability of environmental and social impact analysis has made the use of public participation virtually incoherent. Third, some regulations are overly prescriptive and potentially inappropriate for local economic and social circumstances. These inadequacies in Colombia’s regulations lead to many problems. They contribute to poor coordination between the Ministry of Environment, ACUACAR, and CARDIQUE, causing difficulties for carrying out one of their basic functions—implementing regulations established at the national level. The inadequacies also make it difficult for other institutions to
perform their assigned roles. For example, lack of regulation—from constitutional precepts to specific information standards—limits advancement of the Colombian System of Environmental Information. As previously noted, incomplete licensing and permitting regulations lead to inconsistent requirements and enforcement across the water and wastewater sector and create opportunities for corruption.

In addition, environmental management capacity varies markedly across the water/wastewater sector (Galán Santos, 1998). This marked variability in regulatory capacity is a significant problem that has far-reaching consequences. For example, environmental regulations are stringently enforced in some areas and virtually ignored in others. Also, locally generated funds are efficiently collected and invested in some areas but are scarce and inefficiently invested in others. At both the national and the regional levels, regulatory capture and corruption are significant problems within the water/wastewater sector.

At the national level, private-sector interests have far more influence on environmental policymaking than the organizations responsible for representing civil society—Non-governmental organizations (NGOs). Private-Sector interest groups have a strong influence on decision-making. Members of boards of directors with strong ties to the private sector include not only two dedicated private-sector representatives, but often mayors and even NGO representatives. The latter members sometimes represent spurious local organizations set up by, or closely tied to, industry. Private-sector influence aside, water/wastewater project decision-making is often unduly influenced by political considerations. For example, environmental investments such as water distribution systems are sometimes spatially targeted to maximize political payoffs instead of environmental benefits.
Furthermore, inadequate data on environmental quality and institutional performance is a critical contributor to the water/wastewater failings sector. For example, environmental laboratories, measuring stations, documentation centers, and basic cartography services are inadequate. In addition, lack of priority-setting across environmental subsectors and programs contributes to imbalances in budgetary priorities. Budgetary allocations are apparently driven more by institutional history than by environmental needs (Gómez Torres, 2003). For example, a recent audit of the Ministry of Environment found that rural environmental issues accounted for three-quarters of the ministry’s investment budget, even though more than 70% of Colombia’s population is urban.

Finally, inadequate mechanisms for public participation have been an issue throughout Colombia water/wastewater regulatory systems. Water/wastewater projects at the local level, particularly in rural areas, confront problems from both lack of security and leadership. Thus, Colombia environmental regulatory systems currently lack a consistent system of prior notification of the government’s intention to take many major actions, such as promulgation of major regulations. A systematic provision for public comment is also lacking.

3.17 Summary of Key Lessons in the Cartagena Project

Cartagena’s experience can be summarized into the following key points:

1) An efficient and sustainable water utility is crucial for effective wastewater management—which is fundamental for coastal cities. The creation of ACUACAR in 1995 was a key component of Cartagena’s success.
2) Partnerships at the local, national, and international levels can facilitate and expedite environmental improvements. The Colombian national government formulated the policy framework for private participation in the water sector and enhanced environmental management, as well as providing significant financial support for Cartagena’s infrastructure. Strong partnerships at the local level between CARDIQUE, the District of Cartagena, and ACUACAR were indispensable to program continuity and coherence. International institutions, such as the World Bank, provided financial support and technical assistance.

3) Efficient environmental regulatory policies are very important to address water and sanitation problems. Recently, a key role of the Colombian government in the emerging field of water sustainability management was the development of effective and efficient environmental policies. Although Cartagena water and sanitation services have improved significantly, such new policies have helped the Ministry of environment, CARDIQUE, and ACUACAR to better understand the causes of environmental problems.

4) Public relations, community outreach and building consensus among local stakeholders is critical to planning and implementing wastewater programs. There is usually no obvious “best technical solution,” rather in consultation with all stakeholders and taking into account all dimensions a “preferred alternative” often emerges. The challenge is then to implement this approach in an expeditious manner to avoid further environmental degradation.
5) Comprehensive wastewater management is long-term process and can take a decade or longer to implement. In Cartagena, it took around five years (1995-2000) to ensure the proper policy, institutional planning and financial arrangements were in place before the construction could commence, and then over ten years (2000-2013) before the wastewater treatment system could be fully constructed and commissioned.

6) Long-term, incrementally phased and prioritized programs are necessary for water pollution control and environmental restoration. With the commissioning of the wastewater management system, Cartagena has completed the first phase of its long-term program to restore the coastal environment.

7) Submarine outfalls, combined with preliminary treatment, can be an appropriate solution for protecting coastal areas such as beaches, bays, and estuaries, while providing flexibility for future upgrades as necessary and when affordable. The feasibility of an outfall approach depends, of course, on the capacity of the receiving water body to assimilate the discharges and must be accompanied by extensive environmental, engineering, and social studies.
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Chapter 4

Effects of Oxygen, Nitrate and Aluminum Hydroxide on Methylmercury Efflux from Mine-Contaminated Profundal Lake Sediments

ABSTRACT

Experimental and field-based studies have evaluated remedial actions to mitigate mercury (Hg) bioaccumulation in lake ecosystems. This chapter discusses results of chamber studies evaluating the effects of oxygen, nitrate and sodium aluminate addition on methylmercury (MeHg) efflux from profundal reservoir sediments from Guadalupe Reservoirs and Almaden Lake near San Jose, CA. These reservoirs' sediments were highly contaminated as a result of historical Hg mining in their watershed (Guadalupe total Hg ~3 mg/kg dry wt.; Almaden total Hg 6-20 mg/kg dry wt.).

Aerobic conditions in the overlying water column corresponded with a decrease in MeHg efflux. In Guadalupe Reservoir, MeHg efflux was $5.5 \pm 2.2$ ng/m$^2$/d (average plus/minus standard deviation, n = 4) under aerobic conditions and $22 \pm 11$ ng/m$^2$/d under anaerobic conditions. In Almaden Lake, MeHg efflux was $-2.3 \pm 0.8$ ng/m$^2$/d (loss from water column) under aerobic conditions and $11 \pm 4$ ng/m$^2$.d under anaerobic conditions. MeHg efflux corresponded with the release of other redox-sensitive compounds at the sediment-water interface including iron, manganese, ammonia and phosphate. Results suggest that the dissolution of reduced metal oxides in surficial sediments may be an important source of MeHg efflux from sediments.

Furthermore, additional chamber experiments were conducted in Almaden Lake examining the effects of nitrate and sodium aluminate on MeHg accumulation. Like oxygen, nitrate repressed
MeHg efflux to less than 0.2 ng/m$^2$/d by maintaining a higher redox condition at the sediment-water interface. However, sodium aluminate, a common lake management additive that enhances the sorption capacity of surficial sediments mainly for phosphorus control, did not appear to impede MeHg efflux from profundal sediment.

4.1. Introduction

Methylmercury (MeHg), a known neurotoxin, is one of the most widespread waterborne contaminants in the U.S. (USGS, 2001a; UNEP, 2002; USEPA, 2004) and poses major public health concerns (WHO, 2009). During the 20th century, mercury (Hg) concentrations in lake sediments across Europe, Asia, and North America have increased three-fold, with the highest contamination in lower-latitude lakes near major emission sources (UNEP, 2008). In the U.S, Hg pollution has contaminated 18 million acres of lakes, estuaries, and wetlands, and 1.4 million river miles (NRDC, 2014). Exposure to toxic levels of MeHg affects more than 630,000 newborns each year, interfering with brain and nervous system development (Babal, 2005). Studies have established a link between children exposed to MeHg and developmental deficits in language, memory, cognitive thinking, and visual spatial skills (Babal, 2005; Bose-O’Reilly et al., 2010).

Hg found in aquatic systems originates from both natural and anthropogenic sources. Natural sources include volcano eruptions, geologic Hg deposits, and volatilization from the ocean. The primary anthropogenic sources include coal combustion, chlorine alkali processing, gold mining operations, and metal processing (Bose-O’Reilly et al., 2010). In addition, atmospheric deposition of Hg from fossil fuel combustion and other sources associated with
atmospheric particulate matter has led to increased concentrations in freshwater systems and biota, even in remote areas free from direct anthropogenic influences (Cohen et al., 2004; Rada, et al., 1989). Once Hg is discharged from paper mills, coal power plants, and mining operations and settles into bottom waters, the potential for ionic Hg, Hg(II), to be methylated increases at the sediment-water-interface under anoxic conditions (Wang et al., 2004). Primarily taken up by pelagic phytoplankton, MeHg then accumulates through aquatic food webs and can cause a range of toxic and reproductive effects in fish and wildlife (Gilmour et al., 1998). The strong reactivity of MeHg with sulphhydryl groups of proteins is responsible for its high degree of bioaccumulation (Beckvar et al., 1996). Phytoplankton can concentrate dissolved MeHg in the water column approximately 100,000 times, making this a critical step in the bioaccumulation process (Watras et al., 1994). Klasing and Brodberg (2008) summarized human exposure and toxicity of MeHg. The primary route of MeHg exposure in humans in the U.S. is fish consumption. In turn, human consumption of contaminated fish and wildlife poses significant health risks (USEPA, 2009).

MeHg forms via the methylation of dissolved inorganic Hg by anaerobic bacteria in reduced lake sediments and bottom waters (Benoit et al., 2003). Specifically, sulfate-reducing bacteria (SRB) become active using sulfate as the terminal electron acceptor during organic matter oxidation, which leads not only to MeHg production, but also hydrogen sulfide (Slotton et al., 1995). This production of MeHg represents an important pathway for bioaccumulation of MeHg in the food chain due to the availability of organic matter and the sulfate-rich environment in lakes (Todorova et al., 2009). Furthermore, hydrogen sulfide is extremely toxic to aquatic biota (Reiffenstein et al., 1992) and can degrade the water quality of lakes and reservoirs so that potable water use becomes problematic (Levine et al., 2004).
Another pathway leading to MeHg production and accumulation, under anaerobic conditions, is the release of highly reduced compounds such as iron (Fe) and manganese (Mn) from lake sediments to overlaying waters (Chadwick et al., 2006). These redox-sensitive metal oxides undergo reductive dissolution in the methylation zone. This dissolution, in turn, promotes the release of previously sorbed or complexed MeHg and Hg(II). This additional source of Hg(II) in water bodies adds to the background Hg concentrations, deposited from the atmosphere and watershed runoff, further enhancing the potential for MeHg production (Chadwick et al., 2006; Regnell et al., 2001).

As noted earlier, human exposure to MeHg is mainly through consumption of contaminated fish and shellfish (Al-Saleh, 2009). As a result, MeHg consumption has caused serious health problems in humans and wildlife worldwide. For example, Japanese women who consumed MeHg-contaminated fish (50 µg/g on average), from Minamata Bay, during pregnancy gave birth to children who developed severe neurological defects, including cerebral palsy, microcephaly, blindness, and seizures (Tsubaki, 1977; Harada, 1995; Goto, 2000). In China, in the Guizhou Province near heavy coal-based industries, surface water samples contained total Hg (THg) concentrations up to 12,000 ng/L (Horvat et al., 2003; Zhang et al., 2010), which bioaccumulated in the aquatic food chains leading to serious health concerns (Cheng et al., 2009). Similar results and effects were reported in Philippines (Gray et al., 2003), and India (Sharma, 2003). In Canada, MeHg contamination in the Arctic ecosystem has been linked to both autism and Alzheimer disease in humans (El-Hayek, 2007).

In the U.S., freshwater fish contaminated with MeHg has affected the livelihood and health of citizens in California, New York, Wisconsin, and Minnesota (Genuis, 2009; Hightower, 2009; Krabbenhoft, 2004; WDNR, 1999). For example, in Northern California, several studies
have reported an increasing trend in cases of Hg exposure that negatively impact fetus development (EHHA, 2003; CDPH, 1987). The National Research Council, in its 2000 report on the toxicological effects of MeHg, pointed out that the population at highest risk is the offspring of women who consume large amounts of fish and seafood (NRC, 2000). The report went on to estimate that more than 60,000 children are born each year at risk for adverse neurodevelopmental effects due to in utero exposure to MeHg.

Owing to in-situ production of MeHg in anaerobic zones of lakes and reservoirs, mechanisms regulating MeHg formation, accumulation, and biomagnification are poorly understood (Eckley et al., 2005). Despite much literature on the topic, the production and transformation mechanisms of MeHg in aquatic environment are still somewhat unclear (USEPA, 2006; Ullrich et al., 2001). Knowledge is also lacking about the many chemicals (sulfides and dissolved organic carbon) and biological processes (bacterial activity levels) that control Hg methylation and bioaccumulation in aquatic environment, particularly at the sediment-water-interface (de Wit et al., 2012; Ullrich et al., 2001). A better understanding of the environmental factors affecting the formation of MeHg in lakes is also needed (Winfrey et al., 2009; Langston et al., 1998).

To address MeHg contamination issues, USEPA has increased fish consumption advisories for MeHg, which now account for more than three-quarters of all fish consumption advisories in the U.S. These new advisories illustrate the severity of the problem (USEPA 2010). In addition, the Food and Drug Administration (FDA) released a new advisory warning the public about eating Hg-contaminated fish (FDA, 2004). From 2006 to 2008, the number of lake acres under advisory increased by 18%, and the number of river miles increased by 52%. In
2008, all 50 U.S. states issued fish consumption advisories, warning citizens to limit how often they eat certain types of fish because of known Hg contamination (NRDC, 2014).

Growing concerns about MeHg accumulation related to anoxic profundal sediment in lakes and reservoirs in the United States have stimulated interest in remediation alternatives for the inhibition of MeHg production in aquatic environments. USEPA has proposed to strengthen efforts to control Hg (USEPA, 2010). Federal and State agencies are looking for management strategies and remedial techniques for these polluted sediments that protect the public adequately. For example, the State of California Water Board has initiated a statewide control program for Hg that will focus first on Hg in California’s reservoirs. Fish containing potentially harmful amounts of Hg are found in numerous reservoirs across the state. The program’s objective is to reduce concentrations of inorganic Hg in reservoir sediment to minimize MeHg production and its subsequent bioaccumulation in fish. Potential source controls include remediation of historic gold and Hg mines upstream of reservoirs. In addition, reservoir management practices and management of fish species are being implemented which are important tools in addressing Hg impairments (CWB, 2013). Moreover, the California Water Board has determined that total maximum daily load (TMDL) is the primary regulatory vehicle for achieving water quality goals. As a result, a number of TMDL programs are being implemented. For example, a single TMDL for the Guadalupe River Watershed near San Jose, California, that considers all Hg sources in the watershed has been established. This program includes all tributaries of the Guadalupe River upstream of tidal influence (CWB, 2013).

The objective of the research in this study was to provide a more comprehensive understanding in MeHg accumulation at the sediment-water-interface by analyzing three different lake remediation alternatives. This study presents a clear and logical approach to
evaluate how Hg efflux from sediment behaves under: (1) aerobic versus anaerobic conditions, (2) anoxic conditions with nitrate addition, and (3) anaerobic conditions with sodium aluminate addition. In contrast to many studies that have focused on anaerobic sediment release of phosphorus (Nurnberg, 1988; Golterman, 2001; Schauser et al., 2006), this study analyzed replicate experimental chamber incubations that quantify the effects of oxygen, nitrate, and aluminum hydroxide on MeHg efflux from contaminated profundal sediments. A few recent studies have correlated the presence of oxygen and nitrate with suppression of MeHg accumulation in bottom water (Matthews et al., 2013; Auer et al., 2013; Austin, 2011; Todorova et al., 2009). For our study, it was hypothesized that nitrate and oxygen would repress MeHg accumulation by inhibiting the activity of SRB and/or repressing the release of MeHg from Fe and Mn oxides in surficial sediments while maintaining an adequate concentration of the oxygen and nitrate in the hypolimnion (Todorova et al., 2009; Benoit et al., 2003). Further, it was hypothesized that aluminum hydroxide floc unlike Fe would not be reduced, but rather would act as a potential sorption sink for MeHg, even under anaerobic conditions (DeGaspari et al., 1993). A unique focus of this paper was the evaluation of correlation between aluminum hydroxide and MeHg efflux at the sediment-water-interface using undisturbed sediment cores. This allowed for a conclusive evaluation of the ultimate fate Hg methylation in replicate experimental profundal sediments under anaerobic conditions.
4.2. Materials and Methods

4.2.1. Study Sites

Almaden Lake and Guadalupe Reservoir, part of the Guadalupe River Watershed (Fig. 4-1), suffer the impacts of Hg mining, particularly during large runoff events when Hg-laden sediments from mine wastes are discharged into the surface waters and accumulate downstream (Tetra Tech, 2005). Both water bodies are highly productive, with abundant phytoplankton. Almaden Lake (maximum depth of 12.5 m; surface area of 3.2 ha) is a eutrophic quarry pond located in an urban park in San Jose, California. Guadalupe Reservoir (mean depth of 12 m; maximum depth of 17; surface area of 30 ha; storage capacity of 4,212,000 m³) is a eutrophic reservoir located east of San Jose. For decades, the Guadalupe Watershed has experienced cultural eutrophication and Hg contamination. Historical Hg mining in the region fed the demand of gold rush miners who used Hg to separate gold from crushed rock (SFBRWQCB, 2008; Tetra Tech, 2006). The watershed, managed by the Santa Clara Valley Water District (SCWVD), is used as a source for drinking water for Santa Clara County's 1.8 million residents. The water is captured and then percolated into the regional groundwater basin for later withdrawal for potable use.

The low dissolved oxygen concentrations in bottom waters during the summer and fall dry season create conditions that enhance MeHg production, as demonstrated by the sampling results from Almaden Lake and Guadalupe Reservoirs. As a result of these conditions and processes, the water bodies facilitate the production and downstream export of MeHg, the form of Hg that most readily bioaccumulates. Average total MeHg concentrations in deep water discharges measured during a 2004 study were 12.8 ng/L in Almaden Lake and 23.6 ng/L in
Guadalupe Reservoir (SFBRWQCB, 2008; Tetra Tech, 2005). Water quality profiles collected in 2011 show that MeHg accumulation is associated with anaerobic/anoxic conditions. Field monitoring of temperature and DO showed the reservoirs were thermally stratified and that DO in bottom water was depressed (DO < 1 mg/L) (Fig. 4-2). Data collected by the SCVWD also show a tight linkage between dissolved oxygen, nitrate and MeHg in bottom waters (Fig. 4-3). Low dissolved oxygen concentrations correlated with an increase in MeHg efflux and depletion of nitrate concentrations during anaerobic conditions also associated with an increase in MeHg efflux.

4.2.2. Collection of Sediment Cores

This study included two sampling events. Sediment-water interface samples were collected using a 15 x 15 cm Wilco standard Ekman dredge and cylindrical 1.8-L polycarbonate chambers. The dredge was brought to the surface with minimal disturbance. The collected sediment was sub-sampled out of the dredge and placed into the chambers. Chambers have a diameter of 9.5 cm, a height of 14 cm and a surface water of 71 cm² (Beutel et al., 2007, 2006; Beutel, 2003). The sediment-water interface samples were 4-10 cm thick cores.

During the first sampling event on November 16, 2012, replicate sediment-water interface chambers were collected from Almaden Lake (5 chambers) and Guadalupe Reservoir (4 chambers). For this experiment, quadruplicate chambers were incubated under aerobic followed by anaerobic conditions. For the second event on May 3, 2013, sixteen profundal sediment-water interface samples were collected at the deepest station in Almaden Lake. In this experiment, quadruplicate sediment chambers were incubated under four conditions: anaerobic control, oxygen addition (air), nitrate addition (calcium nitrate), and aluminum floc (sodium aluminate).
4.2.3 Sediment Incubations

The nine chambers from the first sampling event were stored in an incubator at 14 °C. Chambers were incubated over two phases: an aerobic phase for 10 days by bubbling with air, and an anaerobic phase for 18 days by bubbling with nitrogen gas. Chamber water was tracked over time for MeHg, THg, nitrate, ammonia, phosphate, sulfate, Fe and Mn. One chamber from Almaden Lake was monitored every few days during the anaerobic phase for a limited number of parameters (Fe, Mn, MeHg) to develop a more complete time series of accumulation of redox-sensitive compounds in chamber water.

The sixteen chambers from the second event were stored in an incubator at 14 °C. Quadruplicate chambers were incubated as an anaerobic control (bubbled with nitrogen gas) and three treatments: oxic (bubbled with air), anoxic (bubbled with nitrogen gas and enriched with nitrate), and aluminum hydroxide floc (bubbled with nitrogen gas and enriched with sodium aluminate). For nitrate chambers, 4.215g of calcium nitrate (CaNO$_3$) were diluted with 250 mL of lake water and chambers were spiked with a mean of 20 mg N/L of nitrate. For sodium aluminate (NaAlO$_2$) chambers, a primary solution was made with 12.5 g NaAlO$_2$ which were diluted with 250 mL of lake water and neutralized to circumneutral pH with 2N HCl. Chambers were then spiked with 12 mL of NaAlO$_2$ solution resulting in an areal Al dose of 30 g/m$^2$, which is a typical dose range (10 to 40g/ m$^2$) used in lake applications (DeGasperi et al., 1993; Cooke et al., 1993; Welch and Cooke, 1999). Chambers were incubated for 24 days.
4.2.4. Water Quality Analyses

THg samples were collected from the chambers in clear 250 ml glass bottles and preserved with 1% bromine monochloride solution; MeHg samples were collected in 250 ml amber glass bottles to reduce photodegradation and preserved with 0.5 % HCl. Trace metals (Fe and Mn) and nutrients were collected in 60 ml pre-wash plastic bottles. Metals were preserved with 0.5% nitric acid and nutrients were filtered through 0.45 micrometer filters and frozen for analysis. Each sample was drawn from chambers with clean syringes and Teflon tubing. All Hg sampling protocol followed USEPA methods 1630 and 1631(USEPA, 2001, 2002). MeHg and THg samples were analyzed using the Brooks Rand MERX-M Auto Analyzer with EPA method 1630 modular analytical technique, which includes sample distillation, ethylation, purge and trap, thermal desorption, gas chromatography separation, pyrolyzation, and subsequent detection via a Tekran 2500 CVAFS (USEPA, 2001). Standard quality control procedures for MeHg included duplicates (< 25% relative percent difference), matrix spikes (77-125% recovery), and method blanks. Method detection limits were 0.2 ng/L for THg and 0.02 ng/L for MeHg. Nutrient samples were analyzed for soluble reactive phosphorus (SRP), nitrate, and ammonia on a Lachat QuikChem 8500 nutrient auto-analyzer using standard colorimetric method (APHA, 1998). Nutrient detection limits were 0.01 mg/L. Samples for Fe and Mn analyses measured using inductively coupled plasma mass spectrometry (APHA, 1998). Method detection limits were 50 µg/L for Fe and 0.1 µg/L for Mn. Sulfate samples were analyzed using ion chromatography with EPA method 300 (USEPA, 1993). Due to the high concentration of sulfate, the matrix spike/matrix spike duplicate (MS/MSD) were reported above the calibration range. The associated laboratory control sample (LCS) met acceptance criteria. Method detection limit was 20 µg/L.
4.2.5. **Sediment Quality Analyses**

Sediment was analyzed for a number of standard characteristics. Subsamples were placed in weighed crucibles and weighed. Weight loss was measured after heating at 100 °C overnight to remove water, at 550 °C for four hours to remove organic matter, and at 1000 °C for two hours to remove carbonates. Weight losses associated with water and carbon dioxide evolutions were quantified by recording sample weights before and after controlled heating (ignition at 550 and 1000 °C) (Heiri et al., 2001; Santisteban et al., 2004). From these data the percentages by dry weight of sand, silt, and clay were calculated for each sample and classified according to the nomenclature of Shepard (1954). Sediment cores were analyzed using the ultrasensitive DMA-80 analyzer to determine THg following EPA method 7473 (USEPA, 2007). Sediments were also analyzed for total nitrogen (N), total carbon, total phosphorus and total sulfur (S), iron, and Mn using inductively coupled plasma mass spectrometry (ICP-MS) (APHA, 1998).

4.3. **Results**

4.3.1. **Sediment Quality**

In Almaden Lake, sediment cores were collected from 4 different stations (depth = 12.5 m, 10.5 m, 8.5 m and 8 m). Sediment compositions were reported with clay (16-24%), silt (32-39%), sand (35-53%), loss on ignition (83-88%), total carbon (2.4%), total nitrogen (0.27), total Hg (7-35 mg/kg dw), total Fe (51 mg/g dw), total phosphorus (0.84 mg/g dw) and total sulfur (2.3 mg/g dw) (Table 1). Guadalupe sediment cores were collected from a single station at depth 15 m. Sediment compositions included clay (38%), silt (30%), sand (32%), loss on ignition
(91%), total carbon (5.9%), total nitrogen (5.9%), total Hg (6 mg/kg dw), total iron (40 mg/g dw), total phosphorus (1 mg/g dw), and total Sulfur (7 mg/g dw) (Table 4-1).

4.3.2 Study 1: Total Mercury, Sulfate and Methylmercury Effluxes under Aerobic and Anaerobic Conditions

In Guadalupe Reservoir, aerobic conditions yielded high rates of THg efflux (119 ± 110 ng/m²/d) and sulfate efflux (66.9 ± 21.7 ng/m²/d). But in Almaden Lake, aerobic conditions substantially repressed THg efflux (-207 ± 98 ng/m²/d) and lowered sulfate efflux (-90.3 ± 53.1 ng/m²/d) (Table 4-2; Fig. 4-4). Note negative values indicate loss from the water column. Data sets were statically significantly different (two-tailed paired t-test, p < 0.05).

Under anaerobic conditions, Guadalupe showed THg efflux (45.9 ± 74.4 ng/m²/d) slightly lower than levels Almaden (51 ± 35 ng/m²/d), but sulfate efflux (-167 ± 17.6 ng/m²/d) in Almaden was substantially lower than levels in Guadalupe (-56.3 ± 37.3 ng/m²/d) (Table 4-2; Fig. 4-4). In Almaden Lake, aerobic conditions decreased MeHg efflux (-2.4 ± 0.8 ng/m²/d) but Guadalupe showed positive MeHg efflux (5.5 ± 2.2 ng/m²/d) at the sediment-water interface. Under anaerobic conditions, Almaden MeHg efflux (11 ± 4 ng/m²/d) was slightly lower than Guadalupe MeHg efflux (22 ± 11 ng/m²/d) (Table 4-2; Fig. 4-4). Data sets were nearly significantly different (two-tailed paired t-test, p = 0.07). The ratio of MeHg to THg in Almaden was 0.04 ± 0.2 in aerobic chamber water but increased to 0.17 ± 0.06 in anaerobic chambers. In Guadalupe Reservoir, the ratio of MeHg to THg was 0.09 ± 0.01 in aerobic chamber water but increased to 0.25 ± 0.07 in anaerobic chambers.
4.3.3 Study 1: Iron and Manganese in Experimental Chambers

In Almaden Lake, both Fe (-2.2 ± 0.9 mg/m²/d) and Mn (-1.1 ± 0.9 mg/m²/d) efflux rates were negative under aerobic conditions. However, in Guadalupe, only Mn (-4.2 ± 0.5 mg/m²/d) efflux showed a negative efflux while Fe (2.5 ± 0.5 mg/m²/d) showed a positive efflux under aerobic conditions (Table 4-2; Fig 4-4). During the anaerobic phase, Fe (6.7 ± 5.6 mg/m²/d; 4.1 ± 2.3 mg/m²/d) and Mn (17.7± 6.7 mg/m²/d; 20.6 ± 7.0 mg/m²/d) efflux increased at both sites, with substantial increases in Mn. During all incubation phases, Fe and Mn showed a strong correlation revealing the common cycling mechanisms shared by these two metals at the sediment-water interface.

4.3.4 Study 1: Cumulative Mass of Methylmercury, Manganese and Iron in Anaerobic Phase

One chamber from Almaden Lake was monitored every few days for a period of 17 days under anaerobic conditions. A limited number of parameters (Fe, Mn, MeHg) were evaluated to develop a more complete time series of accumulation of redox-sensitive compounds in chamber water. Results showed at day 0, mass of MeHg was 0.41 ng while Fe and Mn were 0.05 and 0.01 mg respectively. At day 3, mass of MeHg slightly increased to 0.98 ng but mass of Fe showed no change (0.05 mg) while Mn increased to 0.77 mg. At day 8, mass of MeHg substantially increased to 4.79 ng while Fe and Mn increased to 2.81 and 1.35 mg (Fig. 4-5). At day 12, mass of MeHg slightly increased to 5.62 ng while Fe and Mn continued to increase to 3.05 and 1.89 mg respectively. At day 17, mass of MeHg showed an increase from 5.62 to 6.03 ng but Mn dropped to 3 mg while mass of Fe increased slowly from 1.89 to 1.92 mg (Fig. 4-5).
4.3.5 Study 2: Efflux Rates for Methylmercury, Manganese, Iron and Ammonia

Four alternative treatments were evaluated: anaerobic, aerobic, anoxic with nitrate addition, and anaerobic with sodium aluminate addition. In the anaerobic control, high level of MeHg and ammonia efflux were observed at 10.8 ng/m²/d and 3.2 mg/m²/d, respectively, but Fe efflux decreased negatively to -0.56 mg/m²/d while Mn efflux was 1.7 mg/m²/d. Data sets were statically significantly different (two-tailed paired t-test, p < 0.05). In aerobic control, all parameters showed a decrease in efflux rates. MeHg and ammonia efflux rate dropped to 0.5 ng/m²/d and 1.6 mg/m²/d while Mn and Fe decreased to -1.05 and -1.95 mg/m²/d respectively (Fig. 4-6). In anoxic with nitrate treatment, MeHg and Fe efflux continued to decrease with efflux rates of 0.2 ng/m²/d and -0.32 mg/m²/d, respectively, while ammonia and Mn were 3.2 and 1.4 mg/m²/d, respectively. In sodium aluminate/anaerobic treatment, MeHg efflux substantially increased to 10.5 ng/m²/d and ammonia also increased to 3.7 mg/m²/d. Fe and Mn effluxes were 0.2 and 1.0 mg/m²/d (Fig. 4-6).

4.4. Discussion

4.4.1. Comparison of Release Rates of Methylmercury Efflux

In our experimental study, MeHg efflux ranged from -2.3 to 22 ng/m²/d which was similar to MeHg efflux rates found in other Hg-contaminated freshwater lakes and reservoirs (Table 2). Kuwabara et al. (2003) measured MeHg efflux rates of 1-3 ng/m²/d from aerobic profundal sediments from Camp Far West Reservoir, a mesotrophic reservoir impacted by historic Hg mining activity in the Sierra Nevada Mountains of Northern California. Kuwabara et al. (2002) in collaboration with USGS examined sediment-water core incubations in Lahontan...
Reservoir, NV, a mining impacted reservoir, to determine the benthic flux from the sediment into overlaying water. MeHg efflux rates ranged from -3.9 to 27.4 ng/m$^2$/d. Our rates were generally near or higher than levels measured in uncontaminated systems, though there are few studies of non-contaminated systems. Efflux rates of MeHg in a non Hg-impacted Deer Lake were < 0.4 ng/m$^2$/d (Cox, 2011). However, other non-chamber studies that evaluate MeHg accumulation via seasonal mass balance have shown relatively high net accumulation rates. Watras et al. (1996) reported MeHg production rates in temperate freshwater lakes ranging from 1.4 to 14 ng/m$^2$/d. Specifically in north-central Wisconsin, Watras et al. (1994) examined two non-Hg-contaminated lakes and found MeHg efflux rates (>50 ng/m$^2$/d) in Little Rock Lake and (>54 ng/m$^2$/d) in Mary Lake due to atmospheric deposition of Hg. Both Lakes are dimictic that stratify thermally soon after ice-out in spring and remain stratified until autumn. As noted above, chamber studies from Hg contaminated lakes showed higher MeHg fluxes. This correlated with the growing evidence that sedimentary production of MeHg is influenced strongly by the availability of dissolved inorganic Hg to methylating bacteria. As inorganic Hg(II) is the substrate for the production of MeHg, abundant availability of Hg can promote higher potential for MeHg efflux (Benoit et al., 2003). Several studies have found a positive relation between the methylation rate and the Hg(II) concentration (Hammerschmidt and Fitzgerald 2004, 2006; Fitzgerald et al., 2007; Benoit et al., 2003).

Most of the research quantifying Hg efflux from sediments has focused on coastal marine systems. Release rates measured in this study were lower than those reported for marine systems. Benoit et al (2009) reported MeHg efflux rates range from -0.9 to 41 ng/m$^2$/d for coastal marine sediments. Point et al. (2007) examined the Thau Lagoon in France. This study found MeHg efflux rates from -32 to 68 ng/m$^2$/d. Hammerschmidt and Fitzgerald (2008) examined MeHg
efflux from marine sediment using shipboard benthic flux chambers and reported MeHg efflux from 15 to 19 ng/m$^2$/d. Additional studies from Hg-contaminated marine systems that measured MeHg efflux rates in excess of 100 ng/m$^2$/d (Choe et al., 2004; Covelli et al., 2008). Marine systems have a tendency to have very high levels of biological sulfate reduction that result in elevated rates of Hg methylation and enhanced MeHg efflux rates from sediments (Hammerschmidt et al., 2004). As noted by Lawson and Mason (1998), in marine ecosystems, the chemical speciation of MeHg and its bioavailability are enhanced by an abundance of sulfur. Similar observation were made by a number of studies (Han et al., 2007; Benoit et al., 2003), reinforcing the contention that SRB in anoxic waters and sediments are the major producers of MeHg. These systems are therefore considered to be hot spots for MeHg production. It is important to note that many field studies have found that MeHg efflux varies substantially with different type of systems. Marine systems tend to have higher MeHg fluxes compared to freshwater systems (Table 4-3) (Benoit et al., 2009; Covelli et al., 2008; Lambertsson and Nilsson 2006).

4.4.2. Efflux Rates in Almaden Lake versus Guadalupe Reservoir

Under oxic conditions Almaden showed uptake of both THg and MeHg, as expected (Table 4-2). But an unexpected result of this study was that Guadalupe showed continuous release of these compounds. In Guadalupe chambers, aerobic release of THg was twice the anaerobic release rate, and aerobic release of MeHg did decrease but was still positive. Aerobic release of MeHg was one fourth of anaerobic release (Table 4-2).

Almaden responded conventionally to oxygenation. MeHg efflux was repressed, and this repression corresponded with substantial drops in total Fe, Mn, ammonia, and SRP under oxic versus anoxic conditions. As discussed by Watras (2009), lakes with anoxic hypolimnia tend to
accumulate MeHg at concentrations that are orders of magnitude higher than lakes with oxic bottom waters. Anoxic conditions support the activity of anaerobic microorganisms including sulfate-reducing bacteria that methylate ionic Hg (Fleming et al., 2006; Gilmour et al., 1992). In addition, anoxic conditions can lead to the production of sulfide, which at moderate levels can enhance bioavailability of ionic Hg for methylation (Benoit et al., 2003) and MeHg for uptake into phytoplankton through the formation of neutral lipophilic Hg-S complexes (Masson, 2002). Sulfide may also increase the diffusive efflux of MeHg from anaerobic sediment (Holloweg et al., 2010) and can strip Hg from settling metal oxides before it settles onto the sediments (Morel et al., 1998). Anoxic conditions may also result in reduction and dissolution of Mn and Fe oxides in surficial sediments with corelease of Hg/MeHg-DOC complexes (Feyte et al., 2010; Chadwick et al., 2006; Ullrich et al., 2001).

Mn and Fe are highly sensitive to redox conditions and oxic conditions are typically effective in repressing the release of reduced Mn (II) and Fe (II) from reservoir sediments (Bryant et al., 2011). The observed difference in MeHg efflux rates between oxic and anoxic conditions in Almaden may also be the result of sorption with Fe (III) in surficial sediments during the oxic phase. Under oxic conditions, Fe and Mn oxides have the ability to sorb MeHg, but in contrast to the anoxic phase, the reduction of these oxides can enhance the release of MeHg to overlying water (Regnell et al., 2001). A handful of studies have reported that Fe and Mn oxides play important roles in MeHg accumulation at the sediment water interface through impacts on Hg complexation (Chadwick et al., 2006; Merritt et al., 2008). Sediments can be a source of both Hg (II) and MeHg through the reduction of oxyhydroxides of Fe (III) and Mn (IV) with subsequent diffusion of new dissolved Hg species to the overlying water (Chadwick et al., 2006). Fe (II) and Mn (II) have been detected in anaerobic bottom water as a result of the
reduction of metal oxides in profundal surface sediments and sinking particulates from overlaying surface waters (Davison, 1993; Balistrieri et al., 1992). As observed in our study, Fe and Mn in Almaden indicated a strong redox response to anoxic conditions. Increases in Fe and Mn suggested diffusive flux from sediments into the overlying water. It is equally important to observe that MeHg accumulates only after Fe starts to accumulate, even though Mn accumulation started earlier. Mn increased first in anaerobic chamber water because Mn(IV) is biologically reduced at higher redox potentials than Fe(III). It appears that anaerobic conditions and associated Fe oxide reduction were mainly the source of MeHg in chamber water. Similar responses were observed by a number of researchers (Chadwick et al., 2006; Merritt et al., 2008; Meili, 1997; Davison, 1993; Balistrieri et al., 1992). For example, Chadwick et al. (2006) evaluated Hg cycling in the Experimental Lakes in Ontario, Canada. They found Fe and Mn oxides had an indirect influence on Hg accumulation at the sediment water interface. Under reducing conditions, these metal oxides appeared to release sorbed dissolve organic, which likely contained Hg. As a result, metal oxides can also be a sink for Hg. Meili (1997) also discussed the importance of adsorption of Hg to oxyhydroxides in lakes. His research suggested the enrichment of MeHg in anoxic waters may result from the sedimentation of Hg-laden oxyhydroxides of Fe and Mn from the surface and their dissolution in the anoxic bottom water.

Differences in total ammonia and dissolved orthophosphate between oxic and anoxic conditions in Almaden suggest that oxygen inhibited sediment orthophosphate release, likely by repressing the biological reduction of phosphate-containing iron-oxide complexes in profundal surficial sediments (Golterman, 2001). Oxygen also inhibited sediment release of ammonia, likely by enhancing nitrification of ammonia to nitrate and ammonia assimilation into bacterial biomass (Beutel, 2006).
Guadalupe chambers responded in a surprising way in that THg efflux was high and MeHg efflux was not inhibited under aerobic conditions. For this scenario, we noticed a correlation between the efflux of ionic Hg, Fe and sulfate under oxic conditions. Our results showed that Fe efflux under oxic conditions was still positive; meanwhile the efflux of Mn, which is more resistant to rapid abiotic oxidation, was negative (Table 4-2). This suggests that there was some source of Fe release. Also, in contrast to Almaden where sulfate was negative under both oxic and anoxic conditions, we observed substantial release of sulfate in experimental sediments (Table 4-1) leading to high sulfate efflux rates (Table 4-2) at the sediment-water interface. As discussed by Kusel et al. (2002), lakes near mining areas can be characterized by low pH and nutrient status, and high Hg, Fe and sulfate ions due to the oxidation sulfide minerals in the surrounding mine tailings.

In Guadalupe chambers, these observations were most likely due to the oxidation and dissolution of cinnabar (HgS) and pyrite (FeS$_2$) in profundal sediments. Under oxic conditions, the formation of oxidation products from cinnabar and pyrite can cause liberation of Fe, Hg and sulfur as sulfate ions. This process involves the transfer of several electrons from each sulfur atom in the minerals to an aqueous oxidant. Since cinnabar is unstable in the presence of oxygen, Hg may be released by simple oxidative dissolution (Eq. 1). Likewise, the oxidation of pyrite leads to the oxidation of sulfide to sulfate with the co-release of dissolved ferrous iron. After dissolution the ferrous iron undergoes oxidation to ferric iron, which then hydrolyzes to form insoluble ferric hydroxide (Eq. 2). The overall reactions are commonly written as (Barnett et al., 2001; Rimstidt et al., 2003):
HgS + 2O_2 + 2 H_2O \Rightarrow Hg(OH)_2^0 + SO_4^{2-} + 2H^+ \text{ (cinnabar)} \quad \text{Eq. 1}

FeS_2 + 15/4 O_2 + 7/2H_2O \Rightarrow Fe(OH)_3 + 2SO_4^{2-} + 2H^+ \text{ (pyrite)} \quad \text{Eq. 2}

This phenomenon has been observed in other Hg-contaminated sites (Barnett et al., 2001; Monterroso et al., 1998; Holley et al., 2007). Barnett et al. (2001) investigated cinnabar with respect to oxidative dissolution by dissolved oxygen focusing on Hg cycling in contaminated environments. Results showed cinnabar, owing to the presence of reduced sulfur, is thermodynamically unstable in the presence of oxygen, causing Hg to be released. Therefore, dissolution will have a much larger thermodynamic driving force in the presence of oxygen (Barnett et al., 2001). Monterroso et al. (1998) evaluated the quality of drainage waters from Puentes Lignite Mine Dump in Galicia, Spain, containing sediments with different amounts of carbonaceous material, with pyrite often being present. Results showed the presence of elevated concentrations of Fe, sulfate, and H^+, liberated from the oxidation of Fe-S (Monterroso et al., 1998).

In addition to Hg and Fe release during oxidation of cinnabar and pyrite, there is growing evidence that for many sulfide minerals such as cinnabar and pyrite, the first step in oxidation produces a sulfate-enriched zone in natural waters (Buckley and Woods, 1987; Kartio et al., 1998; Rimstidt et al., 2003). Since sulfide minerals were likely the dominant source of sulfate release in our chamber, it is reasonable to conclude that this evidence confirmed the high levels of sulfate observed in our experiment (Table 4-2).

Holley et al. (2007) discussed Hg mobilization by oxidative dissolution of cinnabar (alpha-HgS) and metacinnabar (beta-HgS) in oxygenated slurry experiments. Results showed
that a great amount of sulfur is oxidized to thiols, which tend to be liberated over time as sulfate; therefore, the initial observation of sulfate production via oxidative dissolution underestimates the actual amount of mineral that has been oxidized.

Much of the Hg that is liberated by oxidative dissolution of cinnabar ends up sorbing to remaining mineral surfaces, and as a result Hg fluxes tend to be 1 to 2 orders of magnitude lower than sulfate fluxes (Holley et al., 2007). Presumably, this scenario was observed on the macro scale in our chambers. Hg fluxes were approximately 1.2 orders of magnitude less than sulfate fluxes. It is reasonable to conclude that source of sulfate and Hg in our chambers was due to oxidative dissolution.

It is important to note that these oxidation reactions, however, are extraordinarily simplistic compared to the realities of chemistry and the actual processes occurring in the environment (Nordstrom, 1997). Because so many electrons must be removed from each sulfur atom to oxidize it to sulfate, this oxidation process is one of the most complex processes in sulfide mineral oxidation. It is unlikely that more than one electron is removed from the sulfur atom at a time, therefore, there must be several steps to this overall process (Rimstidt et al., 2003; Williamson and Rimstidt, 1992; Basolo and Pearson, 1967).

Furthermore, in Guadalupe chambers, MeHg efflux was not reversed even under oxic conditions. The high availability of ionic Hg and sulfate at the sediment-water interface could have enhanced methylation and could account for MeHg release under oxic conditions. As observed in Guadalupe chambers, sulfate, a key substrate for methylation bacteria, was very high in chamber water. Gilmour et al. (1992) reported that high levels of sulfate above intact sediment cores resulted in increased microbial production of MeHg from bioavailability of inorganic Hg. Thus, even though chamber water was oxic, high sulfate levels combined with elevated ionic Hg,
both liberated via oxidative dissolution of Hg-containing minerals, likely stimulated Hg methylation in anoxic sediments. And this MeHg diffused upwards into oxic chamber waters.

4.4.3. Effects of Oxygen and Nitrate in Experimental Chambers

Our experimental efforts confirm that MeHg efflux rates from both oxic and nitrate addition chambers were repressed at the sediment water interface (Fig. 4-6), which correlate well with reported relationship between oxygen and nitrate and sediment MeHg release (Matthews et al., 2013; Austin et al., 2011; Todorova et al., 2009; Effler et al., 2008; Eckley et al., 2006; Benoit et al., 2006; Golterman, 2001). Our study demonstrated oxygen inhibited the release of MeHg from lake sediments in chamber water (Fig. 4-6). Similar dynamics were observed in Benoit et al. (2006). The study reported the penetration of oxygen into sediments with the net result of pushing the site of Hg methylation deeper into sediments and eventually inhibiting MeHg release. In addition, Gilmour et al. (1992) amended intact lake sediment cores with Hg and incubated them under water with various sulfate concentrations. High levels of MeHg in surface sediment correlated with intermediate levels of sulfate but dropped significantly in well-oxygenated sediments. DeLaune et al. (2004) showed that Hg-enriched surface sediments from three Louisiana lakes accumulated less MeHg when water above the sediments was aerated (oxygen). Klein (2006) reported a number of studies in which MeHg production was inhibited from lake sediments by maintaining an oxidized sediment-water interface. Golterman (2001) suggested that oxygen levels and oxidation-reduction potential at the sediment-water interface are particularly critical in regulating the flux of reduced and oxidized substances into and out of profundal sediments.
Our experimental study also demonstrated the presence of nitrate overlaying the sediment-water interface was effective in repressing the release of MeHg in chamber water (Fig. 4-6). Some researchers even argue that nitrate addition may be more effective and ecologically benign than aeration in inhibiting MeHg production in the profundal zone (Effler and Matthews, 2008, Matthews et al. 2013). An interesting potential advantage of nitrate is that at sites with contaminated sediments, nitrate addition can elevate redox without relieving hypoxia, thereby limiting the exposure of aerobic macrobenthos and connected food webs to sediment-based Hg contamination. Similar studies confirmed nitrate’s ability to repress MeHg accumulation in lakes and reservoirs (Mathews et al., 2013; Todorova et al., 2009). Sulfate-reducing bacteria activity in lake sediments and related internal production of MeHg may be inhibited by maintaining adequate concentrations of alternate, energetically favorable electron acceptors, such as oxygen and/or nitrate (Stephan et al., 1988). Due to its effect on redox conditions in the hypolimnion, increased nitrate concentration can reduce the process of methylation. For example, Matthews et al. (2013) conducted a two-year pilot study of nitrate addition to Onondaga Lake, a Hg-contaminated lake in New York. Results showed that maximum concentrations of MeHg and soluble reactive phosphorus in bottom waters decreased by 94% and 95%, respectively. Austin (2011) documented nitrate repression of MeHg accumulation in bottom waters, including one by the consulting firm CH2MHill that added liquid calcium nitrate to a number of small lakes in Minnesota. One potential downside of nitrate addition is the production nitrous oxide. During denitrification, oxidized forms of N such as nitrate and nitrite are converted to dinitrogen and, to a lesser extent, nitrous oxide gas. Nitrous oxide is a major contributor to the atmospheric ozone destruction and a potent greenhouse gas. However, the nitrous oxide yield in fresh water systems
is low, generally ranging between 0.1 and 1.0% of total N gas production (Seitzinger and Kroeze, 1998).

4.4.4. Sediment Ammonia Release

In our research, our first study showed ammonia release under aerobic conditions was lower compared to anaerobic conditions in both Almaden lake (aerobic: $14.9 \pm 13.9$ mg/m$^2$/d; anaerobic: $29.0 \pm 4.1$ mg/m$^2$/d) and Guadalupe reservoir (aerobic: $-1.5 \pm 5.2$ mg/m$^2$/d; anaerobic: $16.6 \pm 1.5$ mg/m$^2$/d) (Table 4-2). In our second study, ammonia release under aerobic treatment was low ($1.7$ mg/m$^2$/d) in comparison to other treatments (Fig. 4-6). Under both nitrate-rich and nitrate-free anoxic (anaerobic) conditions, sediment ammonia release rates were relatively high ($3.2$ mg/m$^2$/d). This indicated that when oxygen is present, biological nitrification of ammonia to nitrate could proceed, but in the absence of oxygen nitrification in sediments was inhibited. Anaerobic microorganisms also have much lower cell yields and growth rates compared to aerobic microorganisms. Thus, anaerobic bacteria take up ammonia liberated from decaying matter at much slower rates than aerobic bacteria (Snoeyink and Jenkins, 1980). This could exacerbate ammonia release under anoxic conditions. Nitrate-rich anoxic conditions can also result in enhanced biological ammonia removal. But this did occur in our chambers. As mentioned by Burgin et al. (2007), nitrate may be reduced either to ammonium, as a form of dissimilatory reduction of nitrate to ammonium (DNRA), or to N$_2$, as a form of denitrification. In this process, the predominant fate of the reduced nitrate may be determined by the ambient concentration of free sulfide, which is known to inhibit the final two reduction steps in the
denitrification sequence. As a result, sulfide inhibition of these terminal steps may drive the reduction to ammonium rather than N₂O and N₂ (Burgin et al., 2007).

Similar dynamics between ammonia, oxic, nitrate-rich and nitrate-free anoxic (anaerobic) conditions were observed in a number of studies (Beutel et al., 2006; Rysgaard et al., 1994; Mengis et al., 1997; Graetz et al., 1973). For example Beutel et al. (2006) evaluated how oxygen and nitrate in overlying water affect nutrient release from profundal sediments in a reservoir (Lake Mathews, CA) and as well as in experimental sediment-water chamber incubations. The study found that under nitrate-free anoxic conditions, ammonia release from sediments in experiment incubations was 2.8 mg/m²/d; this compared to -3.6 mg/m²/d (uptake) under oxic conditions. As observed in our study, only oxic phase and not nitrate-rich and nitrate-free anoxic (anaerobic) phase was successful in repressing sediment ammonia release, perhaps by enhancing biological nitrification and assimilation in surficial sediment under oxic conditions. Cubas (2012) performed extensive experimental and field studies of nitrogen cycling in the Occoquan Reservoir, Virginia. Results showed that ammonia release was higher under anoxic conditions versus oxic conditions. But when nitrate input to the reservoir was increased, sediment ammonium release was decreased. As a result, nitrate was converted to nitrogen gas as it moved through the reservoir. On the other hand, Austin et al. (2015) reported substantial DNRA driven by high sulfide levels in a shallow lake in Minnesota where nitrate was added to repress Hg accumulation. The main nitrate sink was anaerobic oxidation of sulfidic compounds, but some dissimilatory nitrate reduction cannot be ruled out. Sulfate at the time of nitrate addition was 0.99 mg/L, rose to 4.0 mg/L at depletion, and fell to 0.89 mg/L at turnover in early October. This showed sulfide is much more of a sink for nitrate in natural system than organic carbon and plays a critical role in determining the dominant pathway of nitrate reduction.
4.4.5. Effect of Sodium Aluminate in Chamber Water

There is growing interest in the use of sorbents for the in situ treatment of Hg-contaminated sediment. In situ sorbent amendments have attracted recent attention as a low-cost, low-impact approach for remediation of organic-contaminated sediments (Ghosh et al. 2011). Conventional remedial methods for Hg-contaminated aquatic sediment, including dredging, capping and monitoring natural recovery, have shortcomings (Ghosh et al. 2011, Wang et al. 2004).

Dredging and disposal are expensive and inappropriate for large areas of contamination. Capping with clean material can inadvertently enhance MeHg production by promoting conditions that accelerate methylation (Johnson et al. 2010). Recent studies have focused on sorbent amendments such as activated carbon and biochar (Gilmour et al. 2013, Ghosh et al. 2011). These amendments act as a sorption barrier to the efflux of hydrophobic organic compounds (e.g., PCBs, dioxins, pesticides) from surficial sediment, thereby repressing uptake of pollutants into the aquatic food web. Over time the barrier is mixed into sediments via bioturbation and/or covered with clean sediment via deposition. A recent study by Gilmore et al. (2013) showed activated carbon amendments to Hg-contaminated sediments were highly effective in reducing methyl Hg bioaccumulation by benthic invertebrates. In these studies, the effectiveness of activated carbon and other amendments in reducing invertebrate bioaccumulation was well-correlated with their effectiveness in decreasing pore water MeHg concentrations, and increasing sediment: water partition coefficients of MeHg. The mechanism of activated carbon remediation appears to be mainly by reductions in MeHg bioavailability to
worms, rather than by reductions in sediment MeHg production or bulk sediment methyl Hg concentration.

In our experimental chamber study, we evaluated sodium aluminate, a potential sorption sink for MeHg. Aluminum treatment via alum (aluminum sulfate) and/or sodium aluminate is a common lake management strategy to control internal P cycling (Cooke et al., 1993). Aluminum addition results in a floc of aluminum hydroxide overlaying sediment, which is a highly effective technique to reduce internal loading of phosphate in both stratified and unstratified lakes and reservoirs (Welch and Cooke, 1999; Rydin et al. 2000). As noted by a number of authors, native aluminum and manganese oxides have been implicated in the retention of MeHg in surficial sediments (Chadwick et al., 2006; Merritt and Amirbahman, 2008). However, in our study aluminum addition did not impede sediment release of MeHg efflux rates (Fig. 4-6). MeHg efflux rates (> 12 ng/m²/d) appeared to be as high as in anaerobic conditions. The question arises as to why aluminum addition did not repress MeHg release. Presumably, strong ligands such as sulfide and DOC have an effect on the effectiveness of aluminum as a potential sorption to suppress MeHg release. The binding of DOC and sulfide with ionic Hg and MeHg has a major effect on Hg cycling in surface waters (Ullrich et al., 2001). As with inorganic Hg (II), MeHg can form highly stable complexes with sulfide (CH₃HgS⁻) and other ligand (DOC), depending on the concentration of various ligands (More et al., 1998; Ullrich et al., 2001). Sulfide complexation dominated MeHg speciation in anoxic conditions (Han et al., 2007; Zhan et al, 2004). In our laboratory experiment, we observed strong sulfide odor in our chambers during initial and medium points during incubation. Apparently, the availability of sulfide may have controlled metal distribution and solubility. Perhaps in our systems, strong ligand like sulfide may have repressed the effectiveness of aluminum floc. Other studies have reported the binding
of MeHg to other ligand such as DOC may be affected by the presence of other cationic metals. Driscoll et al. (1995) found that MeHg levels in fish were positively correlated with concentration of monomeric aluminum and suggested that this was because aluminum competes with MeHg for binding sites on DOC, thereby increasing the concentration of unbound MeHg.

Our results suggest that aluminum hydroxide, whether it comes from aluminum sulfate (alum) or sodium aluminate, does not impede MeHg efflux. In fact, addition of alum could enhance methylation due to the addition of sulfate. A number of studies have reported addition of sulfate to anoxic sediment above intact sediment cores resulted in increased production of MeHg by SRB activity (Gilmour et al., 1992; Gilmour and Henry, 1991). SRB are major producers of MeHg in anoxic sediments and typically inhabit the anoxic zones of sediment where sulfate is abundant (Gilmour and Henry, 1991). SRB are obligate anaerobes that obtain energy for growth by oxidation of organic substrates. They use sulfate as the terminal electron acceptor and consequently convert sulfate to sulfide (Harmon et al., 2007). Other studies have investigated sulfate limitations in fluvial sediment and wetland systems by testing whether sulfate addition alone can stimulate Hg methylation (Braunfireun et al. 1999; Gilmour et al. 1992; Jeremiason et al. 2006; Harmon, 2004). Since SRB are the primary methylators of Hg in many environmental systems, sulfate addition to an anoxic system starved for sulfate often increases SRB activity, thereby enhancing MeHg production (Benoit et al., 2003). In addition, excess sulfate can also trigger SRB to produce hydrogen sulfide, which can make water undesirable as a raw water source for drinking water (Levine et al., 2004) and can be highly toxic to aquatic biota (Reiffenstein et al., 1992).

In some cases, there could be benefit of using a combination of alum/sodium aluminate instead of alum with buffer, since less sulfate would be available to enhance MeHg production.
This is because in the alum/sodium aluminate application, some of the aluminum is added with sodium rather than sulfate to stimulate methylation. A number of studies have reported a limited availability of sulfate can repress MeHg production at the sediment-water interface because SRB are the dominant methylators of inorganic Hg in freshwater sediments (Benoit et al., 2003; Ullrich et al., 2001; Morel et al., 1998; Gilmour et al., 1992). In this scenario, SRB are unable to use sulfate as the terminal electron acceptor during the oxidation of organic matter to produce hydrogen sulfide, reinforcing the idea that the activity SRB, and consequently the production of MeHg, might be inhibited by adequate limitation of sulfate.

4.4.6. Implications for Lake Management

Best management practices that address MeHg production in Hg-contaminated freshwater and marine sediments need to be implemented toward sustainable remediation alternatives. The input of Hg into the environment and its distribution, behavior and fate in the different environmental compartments need to be further investigated (Ullrich et al., 2001). Furthermore, a detailed understanding of the processes of bioaccumulation and biomagnification of MeHg in the food chain that lead to different levels of MeHg in different fish species and different areas is crucial to gaining a fuller picture of the whole Hg pathway (Benoit et al., 2003). Therefore, it is important to understand Hg chemistry and the corresponding health risks to the entire ecosystem. In this manner, MeHg contamination can be pinpointed and addressed so that a given remediation project can be both effective in reducing health risks and feasible in an economic and technological sense. In Guadalupe Reservoir and Almaden Lake, remediation of MeHg within watersheds is currently being addressed as a major priority in water quality management, but there are several legal and technical obstacles to Hg clean up.
This experimental study can advance lake management practices with respect to the MeHg efflux from sediments. One approach is to increase oxygen in anoxic bottom waters of lakes to inhibit MeHg production. Hypolimnetic oxygenation is a relatively new management strategy that uses pure oxygen gas as oxygen source to release soluble oxygen into the hypolimnion during summer stratification to maintain a well-oxygenated condition in bottom waters while maintaining thermal stratification. Hypolimnetic oxygenation has the potential to decrease sediment release of MeHg, as suggested by Beutel and Horne (1999). In addition, oxygenation systems have successfully oxygenated large multi-purpose reservoirs (Speece, 1994), moderately sized drinking water reservoirs (Jung et al., 2003), and relatively small natural lakes (Moore et al., 1996). Higher oxygen levels at the sediment-water interface could result in lower bioaccumulation in benthic biota by two key mechanisms: (1) deeper oxygen penetration into the sediments could push the site of methylation by SRB downwards and away from the sediment-water interface where benthic biota primarily reside; and (2) deeper oxygen penetration can maintain an well-oxygenated surficial sediment layer rich in metal oxides which may act as a sorption sink for ionic Hg and MeHg, thereby making ionic Hg less bioavailable to get methylated while limiting the diffusion of MeHg out of the sediment and into overlaying water.

Nitrate addition is another attractive management alternative for control of MeHg mobilization as shown in this study. It may represent a viable remediation alternative to dredging, capping, and oxygenation at Hg-contaminated sites (Matthews et al., 2013). For instance, nitrate can inhibit sulfur-reducing bacteria and MeHg efflux from sediment, without providing an oxygen-rich environment for biota. In this experimental study, nitrate addition repressed MeHg efflux rates even lower compared to oxygenated chamber water. Todorova et al. (2009) reported the maintenance of nitrate at concentrations greater than 1 mg-N/L in the water.
column overlying the sediments was sufficient to prevent mobilization of MeHg in Onondaga Lake. Similar dynamics were observed at Round Lake, Minnesota, where the addition of nitrate poised the oxidation-reduction potential and repressed MeHg accumulation in bottom waters (Austin, 2013). Once nitrate was exhausted, MeHg concentrations rapidly returned to pre-treatment levels. A recent study by Mathews et al. (2013) also found in anaerobic bottom waters of Onondaga Lake, nitrate addition inhibited MeHg efflux rates. The reuse of nitrate in wastewater to improve surface water quality is compelling because it lowers treatment costs and complexity while enhancing environmental quality. The case study of Occoquan Reservoir, described in Chapter 2 and 5, was a good example of such an application. Because the added nitrate is mainly converted to harmless dinitrogen gas via biological denitrification, negative impacts related to eutrophication should be minimal if nitrate addition is properly managed. In addition, nitrate addition to freshwater bodies in which P loading is low may not substantially enhance productivity. To ensure minimal impacts, N could be added at stoichiometric amounts relative to environmental levels of P (N:P < 10:1) so that little or no residual N is available to stimulate phytoplankton growth.
4.5. References


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United States Environmental Protection Agency (USEPA). (1993). Method 300.0: Methods for the determination of inorganic anions by ion chromatography. EPA/600/R-93/100.


Wisconsin Department of Natural Resources (WDNR). (1999). Recommended strategy for mercury reductions to the atmosphere in Wisconsin. Madison, WI: Wisconsin Department of Natural Resources. 13 p.


Figure 4-1. Map of the Guadalupe River watershed in San Jose, California. Our study sites include Guadalupe Reservoir in the upper center of the watershed, and Almaden Lake which is downstream of the reservoir. Figure is from the Draft Final Conceptual Model Report, June 2004 by Tetra Tech, Inc.
Figure 4-2. Profiles of temperature, dissolved oxygen (DO), and methylmercury (MeHg). A. Lake Almaden sampled September 13, 2011. B. Guadalupe Reservoir sampled August 16, 2011.
Figure 4-3. 2008 dissolved oxygen (DO), nitrate and methylmercury (MeHg) concentrations in bottom water near the sediment-water interface of Lake Almaden in 2008. Note high levels of MeHg in bottom water are associated with depletion of both DO and nitrate.
Figure 4-4. Total mercury and sulfate efflux from Lake Almaden (left) and Guadalupe Reservoir (right) to aerobic conditions (clear bars) and anaerobic conditions (black bars). Error bars are one standard deviation ($n = 4$).
Figure 4-5. Cumulative mass of methylmercury, manganese and iron in chamber water from anaerobic incubation of Lake Almaden sediment.
Figure 4-6. Efflux rates from Lake Almaden sediment for methylmercury (ng/m²·d), manganese (mg/m²·d), iron (mg/m²·d), and ammonia (mg/m²·d) in anaerobic control and three experimental treatments: aerobic, anoxic with nitrate addition, and anaerobic with sodium aluminate addition. Error bars are one standard error (n = 3-4). Asterisk (*) signifies that efflux rate in experimental treatment is significantly different from control (two-tailed t-test, p < 0.05).
Table 4-1. Sediment Quality in Lake Almaden and Guadalupe Reservoir

<table>
<thead>
<tr>
<th>Site</th>
<th>Loss on ignition, %</th>
<th>Total C, %</th>
<th>Total N, %</th>
<th>Total Hg, µg/g</th>
<th>Total Mn, µg/g</th>
<th>Total Fe, µg/g</th>
<th>Total P, µg/g</th>
<th>Total S, µg/g</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake Almaden</td>
<td></td>
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<td></td>
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<tr>
<td>Station 1</td>
<td>84.2 ± 0.1</td>
<td>2.4</td>
<td>0.27</td>
<td>7.6 ± 0.6</td>
<td>1,100</td>
<td>51,000</td>
<td>840</td>
<td>2,300</td>
<td>7.3</td>
</tr>
<tr>
<td>(12.5 m deep)</td>
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<td></td>
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<tr>
<td>Station 2</td>
<td>85.3 ± 0.1</td>
<td>nm</td>
<td>nm</td>
<td>13.7 ± 0.2</td>
<td>nm</td>
<td>nm</td>
<td>nm</td>
<td>nm</td>
<td>nm</td>
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<tr>
<td>(10.5 m deep)</td>
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<tr>
<td>Station 3</td>
<td>83.1 ± 0.3</td>
<td>nm</td>
<td>nm</td>
<td>7.8 ± 1.7</td>
<td>nm</td>
<td>nm</td>
<td>nm</td>
<td>nm</td>
<td>nm</td>
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<td>(8 m deep)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Station 5</td>
<td>87.8 ± 0.1</td>
<td>nm</td>
<td>nm</td>
<td>34.6 ± 6.2</td>
<td>nm</td>
<td>nm</td>
<td>nm</td>
<td>nm</td>
<td>nm</td>
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<tr>
<td>(8 m deep)</td>
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<tr>
<td>Guadalupe Reservoir</td>
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<td></td>
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</tr>
<tr>
<td>Deep Station</td>
<td>90.8 ± 0.3</td>
<td>5.9</td>
<td>0.70</td>
<td>6.3 ± 0.3</td>
<td>1,100</td>
<td>40,000</td>
<td>1,000</td>
<td>7,000</td>
<td>7.2</td>
</tr>
<tr>
<td>(15 m deep)</td>
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</table>

Values are average plus/minus standard deviation (n = 3-6); concentrations are µg/g dry weight; nm = not measured.
Table 4-2. Fluxes measured in experimental chambers under aerobic and anaerobic conditions in Lake Almaden and Guadalupe Reservoir.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Lake Almaden</th>
<th>Guadalupe Reservoir</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aerobic</td>
<td>Anaerobic</td>
</tr>
<tr>
<td>Total Hg, ng/m²·d</td>
<td>-207 ± 98*</td>
<td>51 ± 35*</td>
</tr>
<tr>
<td>Methyl Hg, ng/m²·d</td>
<td>-2.3 ± 0.8†</td>
<td>11 ± 4.1†</td>
</tr>
<tr>
<td>Total Manganese, mg/m²·d</td>
<td>-1.1 ± 0.9*</td>
<td>17.7 ± 6.7*</td>
</tr>
<tr>
<td>Total Iron, mg/m²·d</td>
<td>-2.2 ± 0.9</td>
<td>6.7 ± 5.6</td>
</tr>
<tr>
<td>Nitrate, mg-N/m²·d</td>
<td>8.3 ± 3.0*</td>
<td>-2.4 ± 1.6*</td>
</tr>
<tr>
<td>Ammonia, mg-N/m²·d</td>
<td>14.9 ± 13.9</td>
<td>29.0 ± 4.1</td>
</tr>
<tr>
<td>Dissolved Orthophosphate, mg-P/m²·d</td>
<td>1.0 ± 0.4*</td>
<td>17.6 ± 5.0*</td>
</tr>
<tr>
<td>Sulfate, mg/m²·d</td>
<td>-90.3 ± 53.1</td>
<td>-167 ± 17.6</td>
</tr>
</tbody>
</table>

Values are mean plus/minus standard deviation (n = 4).
*Aerobic and anaerobic fluxes are significantly different (two-tailed paired t-test, p < 0.05)
*Aerobic and anaerobic fluxes were nearly significantly different (two-tailed paired t-test, p = 0.07)
Table 4-3. Comparison of net flux of methylmercury rates to similar studies in freshwater and marine systems.

<table>
<thead>
<tr>
<th>Site</th>
<th>Net Flux (ng/m²/d)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almaden Lake, CA</td>
<td>-2.3 ~ 11</td>
<td>This research</td>
</tr>
<tr>
<td>Guadalupe Res., CA</td>
<td>5.5 ~ 22.3</td>
<td>This research</td>
</tr>
<tr>
<td>Deer Lake, WA</td>
<td>0.7 ~ 0.4</td>
<td>Beutel et al., 2014</td>
</tr>
<tr>
<td>Camp Far West, CA</td>
<td>1.0 ~ 3.0</td>
<td>Kuwabara et al., 2003a</td>
</tr>
<tr>
<td>Boston Harbor, MA</td>
<td>-0.9 ~ 41</td>
<td>Benoit et al., 2009</td>
</tr>
<tr>
<td>Lahontan Res., CA</td>
<td>-3.9 ~ 27.4</td>
<td>Kuwabara et al., 2002</td>
</tr>
<tr>
<td>Little Rock Lake, WI</td>
<td>~ 50.0</td>
<td>Watras et al., 1994</td>
</tr>
<tr>
<td>Mary Lake, WI</td>
<td>~ 54.0</td>
<td>Watras et al., 1994</td>
</tr>
<tr>
<td>NY/NJ Harbor, NY</td>
<td>15~19</td>
<td>Hammerschmildt et al., 2008</td>
</tr>
<tr>
<td>Thau Lagoon, France</td>
<td>-32 ~ 68</td>
<td>Point et al., 2007</td>
</tr>
<tr>
<td>San Francisco Bay, CA</td>
<td>-20 ~ 183</td>
<td>Choe et al., 2004</td>
</tr>
<tr>
<td>Gulf of Trieste, Italy</td>
<td>-12~ 238</td>
<td>Covelli et al., 2008</td>
</tr>
<tr>
<td>Grado Lagoon, Italy</td>
<td>34 ~ 498</td>
<td>Covelli et al., 2008</td>
</tr>
</tbody>
</table>
CHAPTER 5

Spatial and Temporal Patterns of Nitrate, Oxygen and Methylmercury during Summer Stratification in Occoquan Reservoir, Virginia.

5.1. Introduction

Freshwater quality studies and management have become increasingly important over the past decade (USEPA, 2000). As the global population continues to expand, the demand for freshwater increases yearly by approximately 64 billion m$^3$ (United Nations, 2010). As a result, the global water consumption rate doubles every twenty years, a pace that is twice the rate of population growth (World Water Organizations, 2010). Due to this increased stress on water sources, water quality in lakes and reservoirs has become a chronic problem for some water utilities and has forced many others to embrace best management practices as possible strategies for improving raw water quality and treatability (Wetzel, 2001; USEPA, 1997). Consequently, federal, state and local government agencies have developed plans and laws that protect and preserve the quality of drinking water sources (USGS, 2005). However, USEPA (2004) reported 64% of the 16 million acres of lakes in the nation was found to be impaired and not suitable to support the use for primary contact (aquatic life, fish consumption, and drinking water supply) and secondary contact (swimming and recreation). Nutrients, sediment and metals, including mercury (Hg), were listed as leading pollutants and caused dramatic impact on water quality.

A factor that contributes to poor water quality in productive, moderately deep lakes and reservoirs is stratification in the summertime. During the period of thermal stratification, the hypolimnion is essentially cut off from oxygen exchange with the atmosphere and is often too
dark for plants and algae to grow and produce oxygen by photosynthesis. As a result, bottom water become anaerobic as the summer progresses. This occurs as supply of oxygen is consumed by bacteria and other bottom-dwelling organisms (Beutel et al., 2008). As anaerobic conditions at the sediment-water interface continue, bottom waters accumulate a range of compounds that exacerbate eutrophication, are toxic to aquatic biota, and limit the potential for potable use.

A critical toxic compound that tends to be released from anaerobic sediments is methylmercury (MeHg). MeHg production is primarily mediated by anaerobic bacteria in surface sediments, particularly sulfate reducing bacteria (SRB) in organic-rich sediments. SRB are the key Hg-methylating organisms in nature (King et al., 2001). SRB typically inhabits the anoxic transition zones of sediment where sulfate is present, such as subsurface zones of lakes and anoxic sediments (Gilmour and Henry, 1991). SRB are obligate anaerobes that obtain energy for growth by oxidation of organic substrates. In many cases, they use sulfate as the terminal electron acceptor and consequently convert sulfate to sulfide (Harmon et al., 2007). Methylation of Hg occurs in SRB as an accidental co-process of sulfate reduction (Gilmour et al., 1992). The bioavailability of ionic Hg to get methylated appears to be enhanced at moderate sulfide levels, at which neutral Hg-S complexes are formed, which more easily diffuse across the polar cell walls of SRB and into the cell where methylation occurs (Benoit et al., 2003).

Once released, MeHg can accumulate in the surface sediments since a concentration gradient between sediment pore waters and overlaying waters develops, which drives the diffusive flux of MeHg from the sediment to the overlaying water (Rolfhus et al., 2003). As a result, MeHg, a potent neurotoxin, can quickly bioaccumulates in phytoplankton, which is consumed by the next level in the food chain (Farina et al., 2011). The National Research Council, in its 2010 report on the toxicological effects of MeHg, pointed out that the population
at highest risk is the offspring of women who consume large amounts of fish and seafood (NRC, 2010). MeHg affects the immune system, alters genetic and enzyme systems, and damages the nervous system, including coordination and the senses of touch, taste, and sight (Clifton, 2007). MeHg is particularly damaging to developing embryos, which are five to ten times more sensitive than adults (Clarkson, 1997).

As redox potential continues to decrease, sediments release manganese (Mn (II)) and iron (Fe (II)) via microbiological and chemical reduction of metal oxides (Davison, 1993). Iron and manganese are chemically similar and cause similar problems. Elevated Fe and Mn in raw water reservoirs complicate drinking water treatment and cause build up in pipelines, pressure tanks, water heaters, and water softeners. This reduces the available quantity and pressure of the water supply (Logsdon et al., 1999; USEPA, 2000). In addition, Fe and Mn accumulation become an economic problem when water supply and water softening equipment must be replaced. There also are associated increases in energy costs from pumping water through constricted pipes and heating water with electric heating rods coated with iron and manganese mineral deposits (Gaskill, 1996; O’Connor, 1971). Although Fe and Mn have been considered non-hazardous, excessive concentrations in water supply can cause several health problems. Kawamura et al. (1981) reported health effects resulting from the ingestion of Fe and Mn-contaminated well water for an estimated 2-3 months by 25 individuals. Results showed heath effects included lethargy, increased muscle tonus, tremor and mental disturbances.

The goal of this study was to perform an evaluation of spatial and temporal patterns of nitrate and oxygen on MeHg, as well as the redox sensitive metals Fe and Mn, during the summer stratification period in Occoquan Reservoir, VA. My hypothesis was that MeHg would only be observed in substantial levels in bottom waters if oxygen and nitrate were not present at
the sediment-water interface, that is, when or where water conditions shift from anoxic (no oxygen but some nitrate) to anaerobic (no oxygen or nitrate). The rationale for this hypothesis was that the production of MeHg is usually associated with anoxic conditions and the enhancement of the activity of SRB. These bacteria are important Hg methylators in sediments and are regulated by the flux of organic matter to the sediment and the availability of oxygen or nitrate for oxidation of organic matter. In the absence of oxygen or nitrate, SRB produce hydrogen sulfide, which facilitates the methylation process (Benoit et al., 2003). Our study evaluated both nitrate and oxygen, and more specifically how MeHg accumulation correlates with the accumulation of other redox-sensitive metals such as Fe and Mn. While previous studies have focused on the effect of oxygen addition on hypolimnetic accumulation of nutrients and conventional metals, this study is novel in that it also evaluated the fate of MeHg, a potent and widespread bioaccumulative toxin in aquatic ecosystems.

5.2. Materials and Methods

5.2.1. Study Site

The Occoquan Reservoir is a large, run-of-the-river reservoir (volume = 31.4 x 10^6 m^3; maximum depth = 19.8 m; mean hydraulic retention time = 20 d) located in northern Virginia, southwest of Washington D.C. (Fig.5-1) (Randall and Grizzard, 1995). The reservoir ultimately discharges into the Potomac River, a major tributary to the Chesapeake Bay. The reservoir, which serves as a water source for 2 million people, has a history of excessive nutrient loading from wastewater discharges and storm runoff. In the late 1960s, massive cyanobacteria blooms led to frequent taste and odor problems, depletion of oxygen in bottom waters, and complications
and increased costs to treat reservoir water for potable use. To address water quality concerns, regional authorities evaluated a range of wastewater management options including a moratorium on new sewage connections and the export of wastewater from the basin. Seeing the value in utilizing wastewater as a resource, managers ultimately developed the "Occoquan Policy" – a farsighted program of indirect potable reuse via advanced wastewater reclamation practices. The Upper Occoquan Service Authority (UOSA) discharges $360 \times 10^6 \text{ m}^3/\text{yr}$ of highly treated, low nutrient, reclaimed water to one of the two main tributaries of the reservoir. During the summer, treatment is modified so that the discharge is rich in nitrate. Much of the added nitrate (approximately 80%) flows with the colder stream water to the profundal zone of the reservoir due to density gradients that form at the reservoir inlet. Typical nitrate levels are $> 10 \text{ mg-N/L}$ in the discharge and $< 2 \text{ mg/L}$ in bottom waters near the reservoir outlet 26 km downstream. Fairfax Water, the drinking water authority, withdraws water near the outlet of the reservoir and treats it for potable use.

The Occoquan Reservoir water reuse program, combined with UOSA seasonal nitrate-rich product water, has the multiple benefits (Cubas 2012, Cubas et al. 2014). Treatment costs are reduced by avoiding a denitrification treatment step during the late spring and summer. The water supply yield of the reservoir has also increased. From a water quality perspective, nitrate addition has reduced internal loading of P and other reduced compounds from sediments. In the reservoir, nitrate concentrations greater than $1 \text{ mg-N/L}$ above the sediment-water interface prevented the release of phosphate from sediments during periods of hypolimnetic anoxia (Fig.5-2). When nitrate concentration in water overlaying sediment was higher than $1 \text{ mg-N/L}$, phosphate seldom exceeded $0.03 \text{ mg-P/L}$. At the same nitrate levels, sediment release of total organic carbon was also reduced. Organic carbon is a precursor to the formation of cancer-
causing disinfection byproducts during drinking water treatment. Both field and experimental sediment-water interface chamber work showed that nitrate in the bottom layers of the reservoir also prevented sediment release of Fe and Mn and delayed ammonia release. As detailed in Cubas et al. (2014), decreasing the nitrate load from UOSA to comply with new stringent regional N regulations could inadvertently result in enhanced internal release of undesirable reduced compounds into reservoir waters, thereby impacting water quality in the reservoir and in downstream waters.

5.2.2. Collection of Field Sampling

This study included two summers of sampling events. During the first summer (2012), water samples were collected once a month from July through mid-October at 2-m intervals from stations RE02 (near the dam), RE05, RE10, RE15, RE20 (confluence of Bull Run and Occoquan Creek), RE30 (upstream shallow arm, nitrate-rich effluent), and RE 35 (downstream shallow arm, nitrate-poor effluent) (Fig.5-1). During the second summer (2013), water samples were collected every ten days on average from June through September at 1-m intervals from stations RE20, RE30, and RE 35. For both summer sampling events, vertical profiles of dissolved oxygen and temperature were measured by taking reading in the water column at 1-m intervals using a YSI 600 XL Sonde probe. Nitrate profiles were measured at 1-m vertical intervals with a Satlantic SUNA optical nitrate probe. Surface and bottom samples were collected and analyzed for nitrate in accordance with Standard Methods (APHA, 2005). For summer 2012, MeHg, Fe and Mn samples were collected at 2-m intervals. However, summer 2013, these samples were collected at 1-m intervals. A Teflon Kemmerer sampler was used for sample collection.
5.2.3. Water Quality Analysis

MeHg samples were stored in acid washed 250 ml amber glass bottles with Teflon lined caps. These samples were preserved with trace metal grade hydrochloric acid then were stored 4°C until analysis. MeHg were analyzed using a MERX automated modular Hg system (Brooks Rand Labs, Seattle, WA, USA) using cold vapor atomic fluorescence spectroscopy (CVAFS) based on USEPA method 1630 for MeHg (USEPA, 2001). The method detection limit was 0.02 ng/L for MeHg. Standard quality control procedure were followed including calibration blanks, matrix spikes samples (acceptable range of 70-130 %), and ongoing precision recovery samples (acceptable range of 75-125%).

Fe and Mn samples were stored in pre-washed 60 ml wide neck bottles from Thermo Scientific Nalgene. These samples were preserved with nitric acid and analyzed using inductively coupled plasma mass spectrometry (ICP-MS) (American Public Health Association, 2005). Detection limits were 0.001 mg/L for Mn and 0.05 mg/l for Fe.

5.3. Results

5.3.1. Water Quality

Occoquan Reservoir exhibited spatial and temporal patterns of water quality indicative of summer thermal stratification and oxygen consumption in the hypolimnion (Fig.5-2). In 2012 and 2013, DO was < 1 mg/l near the sediment-water interface during much of the summer. However, nitrate varied according to the outlet of the reservoir and ranged from (0-8 mg/l). DO levels tended to drop as the stratified period progressed. While anaerobic, the hypolimnnetic water column of the reservoir accumulated MeHg, Mn and Fe during the stratified period.
In 2012, a peak of MeHg was observed near the sediment-water interface at station RE-35, the upstream tributary that does not receive nitrate rich discharge (Fig.5-1), early in the summer. The mean hypolimnmonic MeHg concentration was > 1 ng/L where oxygen was depleted (DO < 0.3 mg/L), but MeHg levels were 0.04-0.12 ng/L in the latter part of the stratified period in conjunction with increased oxygen levels (DO < 5 mg/L) (Fig.5-3). Mn and Fe tended to be fairly well correlated with MeHg accumulation throughout the hypolimnion, though mean peak levels were observed near the sediment-water interface. Hypolimnmonic concentrations ranged from 0.15-1.2 mg/L for Mn and 0.5 -2.2 mg/L for Fe (Fig.5-3). Obviously, nitrate concentration was low throughout the hypolimnmonic water column of the reservoir and ranged from 0 – 0.6 mg-N/L) (Fig.5-3).

At Station RE-30, the upstream tributary that does receive nitrate-rich discharge (Fig.5-1), mean hypolimnmonic MeHg concentration ranged from 0.02-0.1 ng/L, and was lower compared to R35; it also showed little variation. The hypolimnion accumulated little Mn and Fe and showed the same trend with MeHg. Values peaked near the sediment-water interface at 0.05-1.2 mg/L for Mn and 0.2-0.4 mg/L for Fe (Fig.5-3). DO and nitrate tended to be higher, and MeHg tended to be lower, at RE-30 relative to RE-35. Mean hypolimnmonic DO ranged from 0.4-9 mg/L and 4-6 mg/L for nitrate (Fig.5-3). Regarding mean hypolimnmonic concentrations of Mn and Fe, the only difference was that Mn levels were statistically significant compared to Fe. Mean hypolimnmonic Mn was, on average, three times higher than Fe.

At Station RE-20, downstream of the confluence of the two tributaries in the upper portion of the reservoir (Fig.5-1), MeHg was consistently lower and showed little to no variation at the sediment-water interface. Mean hypolimnmonic MeHg ranged from non-detect to 0.09 ng/L with peak value early during the stratified period (Fig.5-3).
were near zero, the hypolimnion accumulated Mn and Fe. Hypolimnetic concentrations ranged from 0.07-3.5 mg/L for Mn and 0.2-1.3 mg/L for Fe (Fig.5-3). Peak concentrations of Mn and Fe displayed similar patterns as the mean hypolimnetic concentrations. DO and nitrate started lower but increased dramatically early August. Mean hypolimnetic concentrations ranged from 0.2-8 mg/L for DO and 0.1-4 mg/L for nitrate (Fig.5-3). Similar trends were observed for DO and nitrate.

At Station RE-15, downstream of the reservoir near segment 3 (Fig.5-1), mean hypolimnetic MeHg concentrations ranged from non-detect to 0.3 ng/L, but levels consistently dropped in the latter part of the stratified period coincidental with a substantial increase of DO (0.2-6 mg/L) and slight increases of nitrate (0.06-2 mg/L) (Fig.5-4). Mn and Fe showed similar trends with MeHg accumulation throughout the hypolimnion, though peak levels were observed near the sediment-water interface. Hypolimnetic concentrations ranged from 0.06-5 mg/L for Mn and 0.1-3 mg/L for Fe (Fig.5-4). Peak hypolimnetic concentration of Mn for RE-15 was, on average, the highest compared to other stations throughout the reservoir.

At Station RE-10, downstream of the reservoir near segment 2 (Fig.5-1), mean hypolimnetic concentrations of MeHg ranged from 0.02-0.2 ng/L with a peak above the sediment of 0.3 ng/L (Fig.5-4). Mn and Fe, which correlated to MeHg, showed a similar seasonal trend. Values peaked near the sediment-water interface at 0.08-1.5 mg/L for Mn and 0.05-1.6 mg/L for Fe (Fig.5-4). DO and nitrate exhibited similar seasonal patterns and showed little variation at the end of the stratified period. Mean values ranged from 0.1-5 mg/L for DO and 0-1.2 mg/L for nitrate (Fig.5-4).

At Station RE-05, downstream of the reservoir near the Dam (Fig.5-1), accumulation of MeHg showed no variation for the month of June and July, but levels decreased in the
hypolimnion as thermal stratification progressed. Mean hypolimnetic MeHg ranged from non-detect to 0.16 ng/L (Fig.5-4). Regarding mean hypolimnetic concentration of Mn and Fe, the only difference was that Mn levels were statistically significant relative to Fe at the sediment water interface. Mean values ranged from 0.4-2.3 mg/L for Mn and 0.2-1.4 mg/L for Fe. DO and nitrate displayed similar trends and mean hypolimnetic concentrations ranged from 0.1-5 mg/L for DO and 0-1.4 mg/L for nitrate (Fig.5-4).

At Station RE-02, downstream of the reservoir near the Dam (Fig.5-1), mean hypolimnetic concentrations of MeHg ranged from non-detect to 0.2 ng/L with peak values near the sediment-water interface (Fig.5-4). Mn and Fe, which correlated with MeHg, showed similar patterns. Fe peak concentration was lower compared to Mn. Values peaked near the sediment-water interface at 0.3-1.2 mg/L for Mn and 0.2-0.8 mg/L for Fe (Fig.5-4). DO and nitrate exhibited similar seasonal patterns and showed little variation at the beginning and end of the stratified period. Mean values ranged from 0.1-4 mg/L for DO and 0-1.5 mg/L for nitrate (Fig.5-4).

In 2013, Station RE-35, the upstream tributary that does not receive nitrate rich discharge (Fig.5-1), peak hypolimnetic MeHg was observed between July and September near the sediment-water interface. Mean hypolimnetic MeHg was > 0.5 ng/L where oxygen was depleted (DO < 0.6 mg/L), but levels were 0.03-0.1 ng/L in the latter part of the stratified period when DO increased (DO < 6 mg/L) (Fig.5-5). Clearly, nitrate accumulation was near zero throughout the hypolimnetic water column of the reservoir. Mn and Fe tended to be fairly well correlated with MeHg accumulation throughout the hypolimnion, though mean peak levels were observed near the sediment-water interface. Hypolimnetic concentrations ranged from 0.2-2.7 mg/L for Mn and 0.5-2.3 mg/L for Fe (Fig.5-5).
At Station RE-30, the upstream tributary that does receive nitrate rich discharge (Fig.5-1), mean hypolimnetic for MeHg was ranged from (0.02 – 0.08 ng/L) much lower compared to R-35 and showed little variation. The hypolimnion accumulated little Mn and Fe and showed the same trend with MeHg. Values peaked near the sediment-water interface at 0.04 – 0.6 mg/L for Mn and 0.1 - 0.7 mg/L for Fe (Fig.5-5). DO and nitrate tended to be higher and MeHg tended to be lower in RE-30 relative to RE-35. Mean hypolimnetic DO was ranged from 2 to 8 mg/L and 2 to 9 mg/L for nitrate (Fig.5-5). Regarding peak mean hypolimnetic concentrations of MeHg between RE-30 and RE-35, the only difference was that MeHg levels in RE-35 were statistically significant compared to RE-30. Peak mean hypolimnetic MeHg was, on average, six times higher in RE-35 relative to RE-30.

At Station RE-20, downstream of the confluence of the two tributaries in the upper portion of the reservoir (Fig.5-1), MeHg was consistently lower near the sediment-water interface compared to RE-35 and showed little variation. Mean hypolimnetic concentrations ranged from 0.02-0.2 ng/L with peak value around mid-August (Fig.5-5). In mid-June, while DO and nitrate were near zero, the hypolimnion accumulated Mn and Fe. Hypolimnetic concentrations ranged from 0.2-2 mg/L for Mn and 0.2-3 mg/L for Fe (Fig.5-5). Peak concentrations of Mn and Fe displayed similar patterns. Similar trends were also observed for DO and nitrate throughout the hypolimnion. Mean hypolimnetic concentrations ranged from 0.2-12 mg/L for DO and 0.07-7 mg/L for nitrate (Fig.5-5).

5.3.2 Correlated Variation between Methylmercury, Oxygen, Nitrate, Manganese and Iron

Correlations were observed between DO, nitrate and MeHg in 2013 from stations RE-35, RE-30 and RE-20 (Fig.5-6). At station RE-35, where nitrate and DO were consistently near zero
throughout the hypolimnetic water column of the reservoir, MeHg accumulated up to 0.75 ng/L. However, at the end of the stratified period, DO increased rapidly to 6 mg/L and MeHg decreased to the low levels (0.03 ng/L), even though nitrate was not present. In contrast to RE-30, where nitrate was moderately present near the sediment-water interface and DO was low, MeHg showed little to no accumulation with a peak concentration of 0.07 ng/L. Interestingly, no MeHg release was observed in the presence of nitrate or DO (Fig.5-6), suggesting that DO and nitrate are interrelated. Similar observation continued in RE-20. Nitrate and DO levels above the sediment were higher relative to RE-35 but lower than RE-30. MeHg peak accumulation was between RE-30 and RE-35 [(RE-30) 0.07 ng/L < (RE-20) 0.23 ng/L < (RE-35) 0.75 ng/L)] (Fig.5-6). MeHg were observed in bottom waters in mid-July, and these levels increased to around 0.23 ng/L by August while DO and nitrate was very low.

The distribution of MeHg was correlated closely with the distribution Mn and Fe in all three stations (Fig.5-7). In RE-35, concurrent peaks of mean hypolimnetic concentrations of Mn (3 mg/L) and Fe (2.2 mg/L) were observed at the same time of MeHg (0.75 ng/L). Clearly, MeHg tended to display comparable spatial patterns with Mn and Fe. Similar observation continued in RE-30 and RE-20 (Fig.5-7).

5.4. Discussion

5.4.1 Comparison between Stations RE-30 and RE-35, 2012 and 2013

MeHg production in bottom waters is primarily mediated by anaerobic bacteria. The efficiency of this microbial process is dependent on the activity of the methylating bacteria and the bioavailability of Hg(II) associated with sulfides and dissolved organic matter (Benoit et al.,
Microbial respiration in bottom waters combined with thermal stratification can lead to elevated levels of hydrogen sulfide (Benoit et al., 1999). The sulfide is produced by sulfate reducing bacteria, which have also been shown to be the principal methylators of Hg to MeHg (Gilmour et al., 1992). Surface water concentration of MeHg is generally much lower than in anoxic hypolimnetic water, and since water discharged from most lakes is surface water, it is also generally low in MeHg. However, water discharged from reservoirs frequently comes from lower in the water column. In some cases, this includes water coming directly off the bottom where there is a greater potential for MeHg to exist. The MeHg enriched discharge water can then be transported downstream where it would be bioavailable to accumulate in the aquatic food web below the reservoir (Canavan et al., 2000).

In the case of Occoquan Reservoir, discharges of nitrate rich water resulted in decreased buildup of MeHg in bottom waters in station RE-30 (the upstream tributary that does receive nitrate rich discharge) relative to RE-35 (the upstream tributary that does not receive nitrate rich discharge). This study provided a rare opportunity to further our understanding of Hg cycling in a reservoir and advance management and remediation to improve water quality. Based on the two-year data set, mean hypolimnetic MeHg was lower in RE-30. There was also a modest negative correlation between mean hypolimnetic MeHg and DO/nitrate (2013) in RE-30 (Fig.5-6), reinforcing the contention that higher DO or the presence of nitrate resulted in lower MeHg buildup. Also supporting this assessment was the observation that years with higher DO or the presence of nitrate in RE-30 (2012 and 2013) had lower MeHg. Mean hypolimnetic nitrate in 2012 and 2013 on average remained above 5 mg/L; mean hypolimnetic MeHg remained below 0.1 ng/L in 2012 and 0.08 ng/L in 2013. In 2012, mean hypolimnetic DO stayed below 3 mg/L until the end of the stratified period when it increased to 8 mg/L compared to 2013, when it
remained on average above 5 mg/L. Mean hypolimnetic MeHg did not accumulate even though DO was low at the beginning at the stratification period due to the presence of nitrate. In contrast to RE-35, mean hypolimnetic nitrate (2012 and 2013) remained below 0.6 mg/L and mean hypolimnetic MeHg showed a steady buildup to 1.10 ng/L in 2012 and 0.75 ng/L in 2013. Hypolimnetic buildup of MeHg was clearly associated with anoxia and the absence of nitrate. In both years, mean hypolimnetic MeHg began to increase once hypolimnetic mean DO dropped near zero, and MeHg tended to increase through the stratified period (Fig.5-3; Fig.5-5). A highly significant negative correlation was observed between mean hypolimnetic MeHg and nitrate plus DO for 2013 data set (Fig.5-6). In RE-35, elevated levels of MeHg corresponded with nitrate and DO below 1 mg/L on a mean hypolimnetic basis. In addition, mean hypolimnetic MeHg concentrations were typically higher in RE-35 compared to RE-30.

This is one the few studies to examine the effects of nitrate and DO on the hypolimnion Hg concentrations in a reservoir. Our observation confirmed that the presence of nitrate or DO repressed MeHg accumulation during extended period of hypoxia. This observation correlated well with reported relationships between nitrate/DO and MeHg release at the sediment-water interface (Matthews et al., 2013; Austin et al. 2015; Todorova et al., 2009; Effler et al., 2008; Eckley et al., 2006; Benoit et al., 2006; Golterman, 2001). Similar dynamics were observed by Austin et al. (2015). The study reported the effectiveness of nitrate addition to urban lakes in Saint Paul/Minneapolis, Minnesota, USA in an effort to lower Hg bioaccumulation. A whole-lake pilot study consisting of a slug dose of liquid calcium nitrate in Round Lake, Mn (area= 13 ha; maximum depth = 11 m) was conducted in 2010. Nitrate in bottom water peaked at 4 mg-N/L then dropped to zero over a 2-month period. Bottom water MeHg remained steady at 0.3 ng/L.
when nitrate was present, then rapidly increased to 1 ng/L once nitrate disappeared (Austin et al., 2015).

Matthews et al. (2013) reported results from a whole-lake pilot test to assess the effect of nitrate addition on mobilization of MeHg from the sediments of Onondaga Lake (area = 1 km²; maximum depth = 20 m), a Hg-contaminated urban lake in New York, USA. Eighty-four thousand kilograms of nitrate-nitrogen was added as liquid calcium nitrate to bottom waters from a barge during thermal stratification. Bottom waters were maintained at a nitrate concentration of 2-4 mg-N/L and MeHg, which typically ranged from 2 to 6 ng/L in prior years, was negligible. The probable regulating mechanism was enhanced sorption of MeHg to Mn and Fe in surficial sediment. Recent incubations with sediment from Almaden lake in San Jose, California (maximum depth of 12.5 m; surface area of 3.2 ha), a highly Hg polluted lake from gold mining, showed that while both nitrate and oxygen addition repressed MeHg efflux, nitrate was even more effective than oxygen. Results showed that in the nitrate added chamber, MeHg efflux decreased to 0.2 ng/m²/d compared to 0.5 ng/m²/d in the oxic chamber (Duvil et al., 2013). A potential benefit of nitrate addition compared to oxygenation is exclusion of aerobic macroinvertebrates from polluted profundal sediment (Beutel et al., 2014). Effler and Matthews (2008) suggested that macroinvertebrates could enhance MeHg efflux from Hg-contaminated sediment as a result of bioirrigation or could act as a vector for Hg uptake into the aquatic food web. This potential benefit is likely less of an issue in oxygenation of non-Hg contaminated lakes and reservoirs like Occoquan Reservoir. On the contrary, oxygen addition is more effective than nitrate in repressing Mn buildup in bottom waters, a compound that can greatly complicate potable water treatment (Bryant et al., 2011; Betancourt et al., 2010) while repressing MeHg release at the sediment-water interface.
The magnitude of MeHg release also correlated closely with the distribution Mn and Fe in both stations near the sediment-water interface (2012 and 2013) (Fig.5-4; Fig.5-7). Mean hypolimnetic Mn and Fe was lower in RE-30 compared to RE-35 in 2012 and 2013. In both 2012 and 2013, peak mean hypolimnetic concentrations of Mn and Fe were observed at the same time as MeHg peaks, suggesting that the reduction Mn and Fe oxides were associated with MeHg accumulation and reductive mobilization occurred at the sediment-water interface. Observations of simultaneous increase in hypolimnetic concentrations of MeHg, Fe, and Mn under anoxic conditions suggest that mobilization of MeHg is linked to the dissolution of Fe and Mn oxyhydroxides in surficial sediments (Jacobs et al., 1995; Regnell et al., 2001; Chadwick et al., 2006). As observed in RE-35, there was a rapid and synchronous increase in Fe and MeHg following DO depletion and the absence of nitrate. On the contrary, in RE-30, Fe and MeHg remained low and showed little to no variation, suggesting the presence of nitrate inhibited the reduction of Fe and Mn at the sediment-water interface, hence repressing MeHg release. Todorova et al. (2009) also reported nitrate control MeHg accumulation in Onondaga Lake may result from direct inhibition of dissolution of hydrous ferric and manganese oxides from anoxic sediments.

5.4.2 Variation of Methylmercury, Manganese and Iron in Downstream Region of Reservoir

Mean hypolimnetic MeHg, Mn and Fe concentrations downstream of the reservoir from RE-20 to RE-02 were lower compared to upstream stations (RE-30 and RE-35), except for the month of July in RE-15 where Mn concentration was around 5 mg/L and Fe concentration was around 3 mg/l, the highest throughout the whole reservoir (Fig.5-4). During that month, MeHg
increased to around 0.3 ng/L in RE-15, the second highest MeHg accumulation observed behind RE-35. This also suggested that Mn and Fe oxides were present within the sediments prior to the onset of anoxia. Most of the other stations showed little to no variation at the sediment-water interface and minimal MeHg accumulation. Mean hypolimnetic MeHg in RE-20 was approximately the same in 2012 and 2013, even though mean hypolimnetic Mn and Fe was higher in 2012 relative to 2013 (Figs.5-4; Fig.5-5). RE-02 and RE-05 displayed similar patterns and showed no hotspot of MeHg release compared to RE-10 and RE-15. Mean hypolimnetic MeHg was two times higher in RE-10 and RE-15 relative to RE-02 and RE-05.

The relationships observed between the downstream stations in regard to low levels of MeHg release were surprising. Since most of the nitrate did not reach the downstream of the reservoir, it was expected that some MeHg accumulation would occur in the water column, but the transition zone showed little to no buildup of MeHg release. As discussed by Cubas et al. (2014), one of the unique features of the reservoir is its capability to consume most of the nitrate that entered to the system. The mean nitrate concentration measured in downstream stations of the reservoir never exceeded 2.5 mg-N/L, even though the nitrate concentration in the input exceeded 10 mg-N/L. The nitrate depletion along the length of the reservoir is attributed to high denitrification rates favored by high concentrations of organic matter and low DO concentration at the sediment-water interface (Randall and Grizzard, 1995). At station RE-15, RE-10, RE-05 and RE-02, DO was depleted by the end of June and remained low until early September (Fig.5-4). As a result, most of the nitrate was consumed in the upper reaches of the reservoir during the stratified period. Clearly, it can be concluded downstream stations were not hotspot for MeHg accumulation since methylation process showed little activity above the sediment in the absence of nitrate and DO.
Due to the differences in standard potential, rates of diffusion, and kinetics of oxidation, differences in the water column distribution of Mn and Fe were expected downstream of the reservoir. Because of their differing responses to redox conditions (Davison, 1993), our results showed strong variation of Mn and Fe between stations throughout the stratified period, indicating that Mn and Fe are good indicators of the redox status of surficial sediments. The highest peak of Mn and Fe was observed at RE-15 in 2012, suggesting that both were very persistent in the anoxic portion of the hypolimnion at this station and possibly Mn and Fe oxides were present within the sediment prior to the onset of anoxia. Mean hypolimnetic of Mn was higher than Fe throughout the reservoir, except for the month of July in some stations. Apparently, the rate of reduction of Fe is faster than the rate of reduction of Mn, suggesting that Mn is more resistant to rapid abiotic oxidation (Chadwick et al., 2006). Since Mn does not readily oxidize as quickly as Fe, Mn oxidation and subsequent settling cannot account for lower Mn in the reservoir. The probable sink for Mn in the reservoir was coprecipitation with Fe oxides, a phenomenon that has been observed in other lakes and reservoirs (Hongve, 1997; Davison, 1993). Our results were consistent with this observation in RE-15, RE-20 in 2012 (July-August) and RE-35 in 2013 (September-October)(Fig.5-4; Fig.5-5). In this case, Fe(II) and Mn(II) were released from anoxic surficial sediment and mixed into the upper hypolimnion where Fe(III) coprecipitated with Mn(II). Fe(III) oxides enriched with Mn(II) then settled out of the water column with some of the Fe(III) oxides redissolving at the anoxic sediment-water interface, thereby recycling Fe(II) and Mn(II) back into the water column (Beutel et al., 2014). Dent et al. (2014), in his study at Twin Lakes in Washington, USA, also discussed how Mn(II) coprecipitation with Fe(III) was also the mechanism proposed for a rapid and massive drop in Fe(II) and Mn(II) from the hypolimnetic water during an oxygenation system test. In contrast to
coprecipitation mechanisms observed in RE-15, RE-20 and RE-35, RE-05 in 2012 and RE-20 in 2013 exhibited different patterns. Mn oxidized slower than Fe, reinforcing the contention that Mn is biologically reduced at higher redox potentials than Fe. An example of this phenomenon is the temporal disparity between the accumulation of Mn and Fe in bottom waters of some lakes. Mn accumulation in eutrophic Lake Sammamish, Washington, started around 10 weeks before Fe accumulation (Balistrieri et al., 1992). Redox potential in seasonally anoxic Rostherne Mere, UK never dropped low enough to induce Fe accumulation; only Mn was observed at the sediment-water interface (Davison and Woof, 1984).

5.5. Conclusion

Reservoir water quality is a complex problem and needs advance management and sustainable remediation alternatives (Holland, 1993). Rational planning and operation of water supply systems requires recognition of the cause-effect relationships that influence water quality and, therefore, influence the feasibility and costs of supplying water that meets state and federal standards and criteria. The trend toward increasingly restrictive drinking water standards can be attributed to increased scientific understanding of the relationships between drinking water quality and health and to vast improvements in analytical capabilities, particularly with respect to nutrient and metals (USEPA, 2004). Given this trend and the current and projected waters supply shortages in many areas of the country, the protection or enhancement of source water quality has become increasingly critical.

In the case of Occoquan Reservoir, depletion of oxygen at the sediment-water interface caused the release of Fe, Mn, P and ammonia into the water column, and likely MeHg. However,
the nitrified effluent from the Upper Occoquan Service Authority (UOSA) has had clear beneficial effects on water quality in the Occoquan Reservoir by repressing the release of these redox-sensitive compounds (Cubas, 2012). Regarding MeHg, nitrate addition affected the upper portion of the reservoir, and still kept downstream portion from going severely anaerobic. As a result, MeHg release downstream was lower than expected. This suggested that perhaps if nitrate was not added, downstream would exhibit higher MeHg release at the sediment-water interface. Because this distinctive approach to nutrient and metal cycling treatment, several concerns have been raised about the effects of implementing stringent nitrogen removal regulations in wastewater treatment plant effluents in order to minimize excessive nitrogen to estuarine tributaries of the Chesapeake Bay (Cubas et al., 2014). But data collected by the Occoquan Water Quality Monitoring Laboratory over the last 40 years have demonstrated that there is a well-understood water quality benefit from the maintenance of oxidized nitrogen in the hypolimnion of the reservoir (Randall and Grizzard, 1995; Banchuen, 2003; Cubas et al., 2014). In fact, an important observation is the reservoir’s capacity to consume most the nitrate that enters in the system from the UOSA. As a result, effluents from the UOSA served as a significant supplement to the safe drinking water supply yield of the Occoquan Reservoir, and nitrate was an attractive management alternative for lakes and reservoirs that are subject to extended period of hypoxia.
5.6. References


Figure 5-1. Map of Occoquan Reservoir complements of Francisco Cubas and Tom Grizzard of Virginia Tech. Stations monitored in 2012 include Stations 1 (RE30), 2 (RE35), 3 (RE20), 4 (RE15), 5 (RE10), 6 (RE5) and 7 (RE02). Stations monitored in 2013 include 1 (RE30), 2 (RE35), 3 (RE20). The nitrate-rich effluent from the WWTP is input upstream of Station 1.
Figure 5-2. 2009 dissolved oxygen (DO), nitrate and phosphate in the bottom waters of the Occoquan Reservoir, Virginia. Depletion of DO and nitrate in June and July corresponded with elevated phosphate. Elevated nitrate in August and September, a result of inflow of nitrate-rich tertiary treated wastewater, corresponded with lower levels of phosphate. Data from Cubas (2012).
Figure 5-3. Bottom water quality in Occoquan Reservoir in 2012. RE 35 is the upstream tributary that does not receive nitrate rich discharge. RE 30 is the upstream tributary that does receive nitrate rich discharge. RE 20 is downstream of the confluence of the two tributaries in the upper portion of the reservoir. Stations continuing downstream are shown in the following figure. Values are average of bottom water samples collected below the thermocline (n = 1-5).
Figure 5-4. Bottom water quality in Occoquan Reservoir in 2012. Stations are moving downstream through the reservoir from RE 20 to RE 02 near the dam. This is a continuation of the previous figure. Values are average of bottom water samples collected below the thermocline (n = 1-5).
Figure 5-5. Bottom water quality in Occoquan Reservoir in 2013. RE 35 is the upstream tributary that does not receive nitrate rich discharge. RE 30 is the upstream tributary that does receive nitrate rich discharge. RE 20 is downstream of the confluence of the two tributaries in the upper portion of the reservoir. Values are average of bottom water samples collected below the thermocline (n = 1-5).
Figure 5-6. Correlations between DO/nitrate and methylmercury (MeHg) in bottom waters of station RE-35, RE-30 and RE-20 for 2013. Values are average of bottom water samples collected below the thermocline (n = 1-5).
Figure 5-7. Correlations between iron/manganese and methylmercury (MeHg) in bottom waters of station RE-35, RE-30 and RE-20 for 2013. Values are average of bottom water samples collected below the thermocline (n = 1-5).