Manganese Cycling in the Marine Environment

by

Shannon Marie Owings

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Bachelor of Sciences in Chemistry with Distinction

Spring 2013

© 2013 Shannon M. Owings All Rights Reserved

Manganese Cycling in the Marine Environment

by

Shannon Marie Owings

Approved: George Luther, III, Ph.D. Professor in charge of thesis on behalf of the Advisory Committee

Approved:

Karl Booksh, Ph.D. Committee member from the Department of Chemistry and Biochemistry

Approved:

David Legates, Ph.D Committee member from the Board of Senior Thesis Readers

Approved:

Michelle Provost-Craig, Ph.D. Chair of the University Committee on Student and Faculty Honors

ACKNOWLEDGMENTS

I would like to express great appreciation to Dr. George Luther for being an outstanding mentor and teacher. I would like to thank him for the valuable laboratory experience and the opportunities to learn a variety of field work techniques including trips on small boats, cruises aboard the R/V Sharp and the unique learning experience on the R/V Knorr.

I would also like to thank the graduate students I have worked with for the past four years for being patient and always willing to help and answer questions: Amy Gartman, Alyssa Findlay, Dan MacDonald, Trish Hredzak- Showalter, Andrew Madison and Dave Chu. You all have been great role models for how a graduate student can succeed. I am grateful for all the lessons I have learned as an undergraduate and how I have been able to gain practical knowledge in the field of oceanography to prepare me for my future as a scientist. The guidance and training I received over the years has been exceptional and I could not have imagined working with a better lab group.

The Summer Scholars Program funded by Delaware NSF EPSCoR and the University of Delaware Department of Undergraduate Research has been an important program that has allowed me to dedicate my summers to full time research. Meg Meiman has been a great help and resource. She has answered many logistical questions I have had along the way regarding Summer Scholars and the Senior Thesis process.

iii

The continued support and enthusiasm for my successes as a scientist from my family has made the successes as well as the difficult times of the research process more enjoyable because of my family's love and support. Special thanks to Aunt Cheryl and Uncle Al, Nana and Grandpa, G-dad and Nan, and of course my incredible Mom and Dad and siblings Katie, Patrick and Shane.

TABLE OF CONTENTS

LIST	OF TABLES	vi
LIST	OF FIGURES	vii
ABST	TRACT	ix
1	INTRODUCTION	1
2	METHODS	
3	LAKESIDE DRIVE, MAINE	7
	3.1 Introduction	7
	3.2 Results and Conclusions	
4	MID-ATLANTIC RIDGE, ATLANTIC OCEAN	
	4.1 Introduction	
	4.2 Results and Conclusions	
5	CHESAPEAKE BAY, AUGUST 2012	
	5.1 Introduction and Background	
	5.2 Sample site, Chesapeake Bay 38°58.62 N; 76°21.98 V	<i>W</i> 24
	5.3 Initial Results analyzed on board the <i>R/V Sharp</i>	
	5.4 In lab experiments and results after the research cruis	e29
	5.5 Profiles of original Chesapeake Bay CTD and Mn dat	ia 32
	5.6 Future Directions	
6	SUMMARY OF ACCOMPLISHMENTS	
	REFERENCES	

LIST OF TABLES

Table 4.1 MAR vent sites information	12
Table 4.2.1 Data for Rainbow vent site	17
Table 4.2.2 Data for TAG vent site	18
Table 4.2.3 Data for Snakepit vent site	19
Table 4.2.4 R ² values for plots of [Mn(II)] vs. pH at different vent sites	21
Table 5.5.1 Percentage of Mn(III) of total Mn pool for CTD11	34
Table 5.5.2 Percentage of Mn(III) of total Mn pool from CTD 13	36

LIST OF FIGURES

	rine tapse of porphyrin method
Figure 2.2: 5	5μM Mn(II) standard solution added to different salinities solutions and measured using the Madison et. al porphyrin method
Figure 3.1 P	icture of stream water at Lakeside Drive, ME. The manipulator for the microelectrode is shown on the left
Figure 3.2.1	[Mn(II)] over time at the Lakeside Drive stream9
Figure 3.2.2	: [Fe(II)] and [Fe(III)] over the three day period at Lakeside Drive, ME (figure credit to Sarah Bennett)
Figure 4.1.1	A Hydrothermal vent, also known as a "black smoker"11
Figure 4.1.2	Google Earth image of the three hydrothermal vent sites samples were collected
Figure 4.1.3	<i>R/V Knorr</i>
Figure 4.1.4	ROV Jason II (photo credit David Chu)14
Figure 4.2.1	· Pasaling increase caused by iron interference at 169nm (arrow) where
0	the Mn(III)-T4CPP product from the metal substitution complex is absorbed. The baseline (black) is recorded at 0 seconds when the concentration of Fe=0
Figure 4.2.2	the Mn(III)-T4CPP product from the metal substitution complex is absorbed. The baseline (black) is recorded at 0 seconds when the concentration of Fe=0
Figure 4.2.2 Figure 4.2.3	the Mn(III)-T4CPP product from the metal substitution complex is absorbed. The baseline (black) is recorded at 0 seconds when the concentration of Fe=0
Figure 4.2.2 Figure 4.2.3 Figure 4.2.4	 baseline increase caused by non interference at 400 min (arrow) where the Mn(III)-T4CPP product from the metal substitution complex is absorbed. The baseline (black) is recorded at 0 seconds when the concentration of Fe=0. Increase in the absorbance value of Mn(III)-T(4-CP)P caused by increased Fe concentrations. 16 A linear trend exists between the [Mn(III)-T4(CP)P] versus the Mn:Fe ratio. 17 Purple Major 11 taken from dive J2-669 from the plume at TAG. The purple major was held in right manipulator of <i>ROV Jason II</i>, and the temperature probe was held in the left manipulator.

Figure 5.1.1 Profile of Chesapeake Bay Station in July 2012 and August 2012. (Used with permission of Dr. George Luther)
Figure 3.1.2 Map of Chesapeake Bay with sampling site labeled with a black X 25
Figure 3.1.3 Google image of Chesapeake Bay sampling site near Chesapeake Bay Bridge. (image used with permission from Andrew Madison)25
Figure 5.3.1 These are the samples analyzed at different depths during CTD cast 22, the depths are labeled in the legend. A steady increase in the [Mn(III)-T4CPP] occurs after an initial rise to a maximum
Figure 5.3.2 Standard curves of porphyrin method using Mn(II) standard solution27
Figure 5.3.3 Overlay of 5µM standard Mn(II) in DI, 5µM standard Mn(II) with 17‰ solution, and the Chesapeake Bay samples
Figure 5.3.4 Overlay of 6µM Mn(II) in same salinity solution as Chesapeake Bay sample from CTD13B1 collected from 21.52 m
Figure 5.4.1 Reduction of Mn(III)-DFOB using hydroxylamine (NH ₂ OH)
Figure 5.4.2 Unsuccessful recovery of total Mn species from original sample after treated with HNO ₃ , NaOH and NH ₂ OH32
Figure 5.5.1 CTD11 plot of [Mn(II)], [Mn(III)], [O ₂], temperature and salinity vs. depth
Figure 5.5.1 CTD 13 plot of [Mn(II)], [Mn(III)], [O ₂], temperature and salinity vs. depth
Figure 5.5.3 CTD22 profile of [Mn(II)], [O ₂], temperature and salinity vs. depth 37

ABSTRACT

In this work the newly developed spectrophotometric method for the simultaneous determination of soluble Mn(II), Mn(III) and total Mn species via a metal substitution reaction (Madison et. al. 2011) was applied in different marine and freshwater environments. At an iron rich freshwater stream in East Boothbay Harbor, ME where an iron oxidizing bacteria, Leptrothrix ochrea, thrives Mn(II) was present (~60µM). Mn(III) was not detected in the freshwater stream indicating the bacteria were not oxidizing Mn(II) for an alternative source of energy.

At hydrothermal vents 2,500-3,500 m below the ocean surface along the Mid-Atlantic Ridge high concentrations (~300µM-1.10mM) of Mn(II) were detected, and no Mn(III) was present in the vent fluid samples that were collected. No evidence of microbial or abiotic oxidation of Mn(II) was found due to the absence of Mn(III) and a linear relationship observed from plots of [Mn(II)] vs. temperature, and [Mn(II)] vs. pH plotted for each of the vent sites.. The linear trends indicate a conservative relationship, which explains Mn(II) is diluted with bottom water as it travels away from the vent. Mn(II) does not readily form a MnS solid, and the linear trends are consistent with that fact.

Evidence of Mn(III) species was found in the Chesapeake Bay. The water column profiles show Mn(III) in the suboxic zone and Mn(III) makes up about 45-90% of the total Mn pool. The Mn(III) profiles show Mn(III) peaks consistent with the oxidation of Mn(II) to Mn(III) below the oxic zone, and a reduction peak of Mn(IV) to Mn(III) and leaching from the sediments, or possibly produced by microbial reduction. In the Chesapeake Bay there are high amounts of organic matter and biological activity. These factors lead to the production of Mn(III) via biological activity and stabilization of Mn(III) by organic ligands.

Chapter 1

INTRODUCTION

Manganese (Mn) is an essential component for many chemical and biological processes. Mn(II/III/IV) are found in photosystem II of photosynthetic organisms and Mn(III) is important in bacterially mediated decomposition of organic matter (Trouwborst et. al. 2006). Mn(III) is an important one electron transfer redox species which can act as either a reductant or an oxidant (Trouwborst et. al. 2006). Environmental chemists have assumed that Mn(III) is negligible in water because as a free ion it disproportionates to Mn(II) and MnO₂. But chemical analysis of samples collected from suboxic zones of the Black Sea and the Chesapeake Bay detected low levels of Mn(III) in suboxic zones of the Black Sea and the Chesapeake Bay (Trouwborst et. al. 2006). Using a new spectrophotometric method involving a metal substitution reaction Mn(III) was quantified in suboxic zones in the St. Lawrence Estuary and constituted up to 85% of the total soluble Mn present (Madison et. al. 2011). Luther (2005) describes the thermodynamically favorable reduction of Mn(IV) to Mn(II) and oxidation of Mn(II) to Mn(IV) involving two one-electron transfer steps which involve the formation of Mn(III) as an intermediate, instead of a one-step twoelectron transfer reaction. The production of Mn(III) as an intermediate via bacterial oxidation of Mn(II) to Mn(IV) was supported by Webb et. al. (2005), and the bioreduction of Mn(IV) to Mn(II) involved the formation of Mn(III) as an intermediate determined by Lin et. al. (2012).

In this work, the presence of Mn(III) in oxic waters is investigated using the methods developed by Madison et. al. (2011). Mn(II) is thermodynamically stable at a pH below 9 (Luther et. al. 2010). In oxic waters Mn(II) can be microbially oxidized to Mn(III) and stabilized by organic or inorganic ligands.

This undergraduate senior thesis includes a summary of the Mn chemistry investigated at a freshwater stream in East Boothbay Harbor, ME, hydrothermal vents along the Mid-Atlantic Ridge, and the oxic and anoxic water column of the Chesapeake Bay.

Chapter 2

METHODS

The spectrophotometric method developed by Madison et. al. (2011) was used to determine concentrations of soluble manganese oxidation state species. The water samples collected were filtered through 0.2 micron filters. The filtered samples were added to a cuvette along with reagents and deionized water to reach a total volume of 3mL. The reagents and their final concentrations in a total of 3mL were CdCl₂ (24 μ M), 120 μ L of imidazole/borate buffer, and a meso-substituted porphyrin [α , β , γ , δ -tetrakis(4-carboxyphenyl)porphine (T(4-CP)P)] (24 μ M). The 3mL mixture was monitored over time on a diode ray UV-Vis spectrophotometer and the spectrum from 200-800 nm was collected every five seconds for 15-20 minutes. The metal substitution reaction that occurs in the cuvette if Mn(II) (Reaction 1) and/or Mn(III) (Reaction 2) are present are shown below.

+ Cd

1

+ Cd 2

Reaction 1 occurs when Mn(II) is the only Mn species present in the sample. Reaction 2 occurs when Mn(III) is present in the sample, this is a metal substitution reaction which takes longer to occur due to ligand exchange. The growth of the Mn(III)-T(4-CP)P peak at 468nm is monitored over time as well as the decrease in Cd(II)-T(4-CP)P peak at 433nm (Figure 2.1).



Figure 2.1: Time lapse of porphyrin method.

The Mn(III)-T(4-CP)P peak absorbance values are converted to concentration using Beer's Law and then plotted versus time. Samples with higher salinities were run for 20 minutes because of slower kinetics caused by formation of Cd-chloro complexes which removes Cd from the porphyrin and cause a slower metal substitution reaction between Cd and Mn (Madison et. al. 2011). The samples of different salinities still reach a maximum value of the same absorbance value (Figure 2.2).



Figure 2.2: 5µM Mn(II) standard solution added to different salinities solutions and measured using the Madison et. al porphyrin method.

The plot of concentration vs. time is fit to an exponential rise to a maximum using SigmaPlot 11. With a two parameter fit (reaction 3)

3

the rate constant, k_1 , can be determined for a sample which forms the Mn(III)-T(4-CP)P from Mn(II) (refer to Reaction 1). Using a four parameter fit of an exponential rise to a maximum the rate constants for Mn(II) and Mn(III), k_1 and k_2 , can be determined (Reaction 4) from the formation of Mn(III)-T(4-CP)P from Mn(II) and Mn(III) present in the solution (refer to Reaction 2).

The concentrations of Mn(II) and Mn(III) can also be determined from the model fits of the experimental data points. The porphyrin method and model fit to the experimental data correctly determines the concentration of Mn(II) and Mn(III) within 0.4-2% of the values for known solutions (Madison et. al. 2011).

Chapter 3

LAKESIDE DRIVE, MAINE

3.1 Introduction

The manganese concentrations of a freshwater stream at Lakeside Drive, East Boothbay Harbor, Maine (Figure 3.1) were monitored from July 17-19, 2012. The purpose of this field study was to determine if the autotrophic iron oxidizing bacteria, *Leptothrix ochracea*, was oxidizing manganese as a second source of energy production. The stream had high concentrations of iron and iron oxides, low oxygen concentrations (\leq 80 µM) and a pH of 6.19 ± 0.22. The source of water for the stream was groundwater and the stream displayed variable conditions which included low to no flow, and could dry up quickly (within 24 hours or less). Water samples were collected at two different sites at the stream: upstream and downstream. The upstream site was closer to the ground water source feeding the stream and the downstream site was farther from the groundwater source.



Figure 3.1 Picture of stream water at Lakeside Drive, ME. The manipulator for the microelectrode is shown on the left.

3.2 Results and Conclusions

During the three day period at the upstream site, the Mn(II) concentrations stayed relatively constant (Figure 3.2.1), while the Fe(II) concentrations decreased (Figure 3.2.2). These trends indicate that the bacteria, *Leptothrix*, is only oxidizing Fe(II) and not Mn(II). The decrease in Fe(II) concentrations is caused by the oxidation of Fe(II) by *Leptothrix* to form solid Fe(III).



Figure 3.2.1 [Mn(II)] over time at the Lakeside Drive stream.



Figure 3.2.2: [Fe(II)] and [Fe(III)] over the three day period at Lakeside Drive, ME (figure credit to Sarah Bennett)

The Mn(II) concentrations are lower at the downstream site compared to the upstream site because the upstream site is closer to the groundwater feeding the stream. The downstream portion of the stream dried up on the third day, therefore there is no data point for July 19th for the Mn(II) concentrations at the downstream site. The kinetic curves created from the analysis of the filter water samples using the Madison et. al (2011) porphyrin method show there was no Mn(III) species present in the freshwater stream. This is further evidence that the bacteria are not oxidizing the Mn(II) via two one-electron transfer steps that form Mn(III) as an intermediate described by Luther (2010) and observed by Webb et. al. (2005).

Chapter 4

MID-ATLANTIC RIDGE, ATLANTIC OCEAN

4.1 Introduction

The Mid-Atlantic Ridge is an open-ocean spreading zone where hydrothermal vent fields are present. The hydrothermal vents (Figure 4.1.1) are important sinks for Mg and also sources of Mn, Fe and many other metals to the deep ocean (Tivey 2007).



Figure 4.1.1 A Hydrothermal vent, also known as a "black smoker"

For the purposes of studying the Manganese chemistry at hydrothermal vents, fluid samples were collected at the vent sites TAG, Snake Pit, and Rainbow (Table 4.1.1 and Figure 4.1.2) during a research expedition aboard the *R/V Knorr* (figure 4.1.3) from October 16, 2012- November 9, 2012. The Remotely Operated Vehicle

Jason II (Figure 4.1.4) was controlled by the Jason crew including a pilot, navigator, and engineer and *Jason II* was used to collect the fluid samples using titanium syringes (also known as major samplers) from the vents located 2,400-3,600m below the ocean surface (Figure 4.1.5). The porphyrin method developed by Madison et.al. (2011) described above was used to analyze the samples collected at the MAR vent sites. The fluid samples were filtered through 0.2 micron filters into falcon tubes. Then further diluted 1:10 in another falcon tube and finally diluted to a final volume of 3mL in the cuvette and monitored over 15 minutes on the diode-ray spectrophotometer.

Site	LAT(N)	LON(W)	Depth (m)
Rainbow	36° 13.8'	33° 54.14'	2260-2350
TAG	26° 08'	44° 49.6'	3635-3670
Snake Pit	23°22.18'	44°57.28	3486



Figure 4.1.2 Google Earth image of the three hydrothermal vent sites samples were collected.



Figure 4.1.3 R/V Knorr



Figure 4.1.4 ROV Jason II (photo credit David Chu)

4.2 **Results and Conclusions**

The first vent fluid samples analyzed from the Rainbow vent site showed indications of the presence of Mn(III) in the samples because the experimental data fit the 4 parameter rise to a maximum model with high R^2 values. Further investigation of the possibility of Mn(III) present revealed that there was an iron interference because concentrations of Fe ranged from 23.3mM at Rainbow, 5.3mM at TAG, and 4.3mM at Snakepit (Gartman and Findlay, cruise report). The method was not originally tested to be used in environments with such high concentrations of Fe; the method was developed for samples collected from groundwater, lakes, rivers, Bays, and freshwater marshes and modified for marine porewaters by Madison et al (2011). During the research cruise on the *R/V Knorr*, the porphyrin method was tested to investigate the

interference of iron from the hydrothermal vents. An experiment performed using the same concentration of reagents, no Mn(II), and increasing concentrations of Fe(II) showed an increase in the baseline absorbance occurred as the concentration of Fe increased. (Figure 4.2.1)



Figure 4.2.1: Baseline increase caused by iron interference at 468nm (arrow) where the Mn(III)-T4CPP product from the metal substitution complex is absorbed. The baseline (black) is recorded at 0 seconds when the concentration of Fe=0.

Another experiment was performed where the Mn(II) concentration was held constant (3μ M) and the concentration of Fe(II) was increased from a 1:1 to 1:20 Mn/Fe molar ratio (Figure 4.2.2). The final absorbance of the Mn(III)-T4CPP peak was converted to concentration and plotted versus the ratio of Mn to Fe (figure 4.2.3). A linear relationship existed between the molar ratio of Mn:Fe therefore the final concentrations of the vent fluid samples can be corrected for the iron interference with the equation in Figure 4.2.3. The iron values measured for each major by Gartman and Findlay listed in Tables 4.2.1-4.2.3 were used to correct the Mn values for each major.



Figure 4.2.2 Increase in the absorbance value of Mn(III)-T(4-CP)P caused by increased Fe concentrations.



Figure 4.2.3: A linear trend exists between the [Mn(III)-T4(CP)P] versus the Mn:Fe ratio.

The corrected values for the total Mn concentrations, pH, temp and location of the sample collected are listed in Tables 4.2.1-4.2.3.

	Major	shipboard			[Fe]	
Dive No.	ID	рН	T (°C)	Location	(mM)	$[Mn_{Total}](\mu M)$
J2-664	Black	3.28	366.8	Guillarme	13.60	1222.88
J2-664	Purple	5.34	353	Regner	3.62	89.82
J2-664	Blue	2.87	366.8	Ennyer	15.81	315.25
J2-664	White	4.65	368	Trident	7.57	626.45
J2-664	Red4	3.16	370.7	Jean Luc	18.42	293.73
J2-664	L. Blue	3.37	229.6	Jean Luc plume	14.83	321.65
J2-664	Red8	7.71	31.8	Jean Luc plume	1.30	127.05
J2-664	Yellow	5.43	80	Jean Luc plume	3.22	299.41

Table 4.2.1 Data for Rainbow vent site

	Major	shipboard			[Fe]	
Dive No.	ID	pН	Τ (° C)	Location	(mM)	$[Mn_{Total}] (\mu M)$
				Black Diffuse		
J2-665	Blue	4.36	269.9	Flow	1.64	248.78
J2-665	White	3.03	313.4	Top of Mound	4.34	384.30
J2-665	Purple	3.2	301.5	Top of Mound	4.39	381.05
J2-665	Red8	4.8	354.7	Top of Mound	1.24	227.96
J2-665	Black	4.56	303.4	Top of Mound	1.48	257.29
J2-665	Yellow	2.98	310	Top of Mound	4.38	451.20
J2-665	Green	3.14	365.6	Top of Mound	5.03	363.24
J2-668	Black	3.01	365.4	Top of Mound	5.01	485.51
J2-668	Purple	4.92	359.7	Top of Mound	1.19	215.48
J2-668	Blue	5.11	347	Top of Mound	1.02	188.73
J2-668	Green	4.15	189	Top of Mound	2.22	272.79
				Plume top of		
J2-669	Purple	5.53	247.2	mound	0.52	114.85
				Plume top of		
J2-669	Orange	3.38	346.8	mound	2.70	293.47
				Plume top of		
J2-669	Red4	5.07	93.2	mound	1.15	216.70
				Plume top of		
J2-669	Red8	6.77	37.6	mound	0.18	14.49

Table 4.2.2 Data for TAG vent site

	Major	shipboard			[Fe]	
Dive No.	ID	pН	T (°C)	Location	(mM)	$[Mn_{Total}] (\mu M)$
J2-666	Red4	5.17	340	Beehive top	0.39	143.44
J2-667	Black	4.42	358.4	Beehive 2	0.58	180.98
J2-667	Blue	3.29	341.5	Beehive 2	2.18	360.04
J2-667	Red8	3.45	351.3	Beehive 2	2.46	389.64
J2-667	Orange	3.22	346.6	Beehive 2	1.72	361.29
J2-667	White	3.19	336.4	Moose	2.29	171.66
				Plume above		
J2-667	Purple10	5.64	170	Moose	0.13	54.19
				Plume above		
J2-667	Purple11	6.30	35	Moose	0.13	16.15

Table 4.2.3 Data for Snakepit vent site

The Mn(II) concentrations versus pH and temperature for each of the specific vents were plotted to determine the reactions that Mn may undergo as it travels away from the hydrothermal vent. The ambient water temperature at the bottom of the ocean is 1.8° C. The fluid expelled from the vents was recorded to be around $170-365^{\circ}$ C depending on the vent sampled. Water collected from the plumes about 30 cm above the orifice of the hydrothermal vent was around 35° C and fluid collected from 6 meters above a plume at TAG was 7.6° C. There is a dramatic decrease in temperature a short distance (0.5 meters) away from the orifice of the hydrothermal vents and collected at the orifice had low pH values The chemical explanation of the low pH is the reaction with Fe(II) and H₂S forming FeS and 2H⁺. As the vent fluid mixes with the ambient bottom water the pH increases. Plotting [Mn(II)] vs. temp and pH are proof Mn(II) oxidation to Mn(III) was not occurring as the Mn expelled from the vents travelled away from the vents. When [Mn(II)] was plotted versus temperature for different vent sites, the plots do not exhibit a linear trend with high R² values. This unreliability is caused from the

unsteady measurement of temperature. The temperature probe was held in the left manipulator of the ROV *Jason II* while the sample was collected in the major sampler held in the right manipulator (Figure 4.2.4). Because of the difficult control needed to complete this task, at some points the temperature probe was not held exactly where the vent fluid sample was collected.



Figure 4.2.4 Purple Major 11 taken from dive J2-669 from the plume at TAG. The purple major was held in right manipulator of *ROV Jason II*, and the temperature probe was held in the left manipulator.

The [Mn(II)] vs. pH plots of the vent fluid samples followed a linear relationship, showing the Mn(II) decreases in a linear trend as pH increases (Figure 4.2.4, Table 4.2.5). The linear trend indicates a conservative trend existed between [Mn(II)] and pH, which explains Mn(II) is diluted with bottom water as it travels away from the vent. The R^2 values of the linear relationship range from 0.78 and greater.



Figure 4.2.5 [Mn(II)] vs. pH for "Top of the Mound" site at TAG collected on Dive J2-669.

Table 4.2.4 R² values for plots of [Mn(II)] vs. pH at different vent sites

Dive No.	Vent site	Vent Location	\mathbf{R}^2
J2-664	Rainbow	Jean Luc Vent	0.7814
J2-667	Snakepit	Moose	0.9988
J2-667	Snakepit	Beehive 2	0.9057
J2-669	TAG	Plume top of mound	0.9332

If oxidation were to occur as Mn(II) traveled away from the vent there would be an exponential decrease in the concentration of Mn(II) vs. pH and temperature. An exponential decrease was the trend observed in the concentration of Fe(II) vs. pH and temperature plots, because FeS and FeS₂ precipitation occurred with some oxidation of Fe(II) to Fe(III) as Fe traveled away from the hydrothermal vents. No MnS precipitation or Mn(II) oxidation occurred as Mn(II) was expelled from the hydrothermal vents and diluted on contact with the oxic water at the bottom of the ocean. This is supported by the fact no Mn(III) was detected using the Madison et. al porphyrin method, and the fact a conservative trend is observed when [Mn(II)] versus pH was plotted.

Chapter 5

CHESAPEAKE BAY, AUGUST 2012

5.1 Introduction and Background

In the Chesapeake Bay the difference in salinity, temperature and density between cold, salt water from the ocean and warmer freshwater set-up an interface where there is a decrease in temperature and oxygen concentrations and an increase in salinity that occur around the same depth. This interface can be less defined due to greater mixing and cause less prominent suboxic zone. The comparison of a strong interface versus no distinct interface can be seen by comparing the profile of late July 2011 to August 2012 in Figure 5.1.1.



Figure 5.1.1 Profile of Chesapeake Bay Station in July 2012 and August 2012. (Used with permission of Dr. George Luther)

The heavy rain and snow fall during the spring and winter of 2011 caused runoff of nutrients into the Bay creating a eutrophied system in the summer of 2011. These conditions caused a stratified system in the summer of 2012 with a distinct oxic/anoxic barrier and H₂S present at detectable levels in the anoxic zone. There was 30% more rainfall in the spring of 2011 compared to the spring of 2012 (Madison et. al PowerPoint presentation). Because of the lower nutrient input during the spring of 2012, the system was less eutrophied therefore oxygen was detectable throughout the entire water column, with low concentrations (~1 μ M) at the furthest depth. During the summer of 2012 there was no detectable H₂S. The role of H₂S in the manganese cycle is the reduction of Mn oxides to Mn(III) and/or Mn(II) or the reduction of Mn(III) to Mn(II).

Trouwborst et. al (2006) detected Mn(III) complexes in micromolar concentrations when a strong suboxic zone was set-up in the Chesapeake Bay water column during August 2003 at a location below the Bay Bridge (8°58.10'N; 76°21.43'W). No Mn(III) complexes were detected by Trouwborst et. al. (2006) in July 2002 when the water column was well mixed after a storm.

5.2 Sample site, Chesapeake Bay 38°58.62 N; 76°21.98 W

For the purposes of this study the site sampled on the Chesapeake Bay was a 30 meter deep hole at the east end of the Chesapeake Bay Bridge, $38^{\circ}58.62$ N; $76^{\circ}21.98$ W. (Figure 5.1.2 and 5.1.3). CTD (conductivity, temperature and depth) casts were performed from the *R/V Sharp* to collect the water samples in Niskin bottles. The samples were filtered through 0.2 micron filters and analyzed using the Madison et al. (2011) porphyrin method.



Figure 3.1.2 Map of Chesapeake Bay with sampling site labeled with a black X.



Figure 3.1.3 Google image of Chesapeake Bay sampling site near Chesapeake Bay Bridge. (image used with permission from Andrew Madison)

5.3 Initial Results analyzed on board the *R/V Sharp*

The majority of the analysis of the water samples showed an initial increase to a maximum [Mn(III)-T4CPP] followed by a steady increase that did not level off over time (Figure 5.3.1). Standard Mn(II) solutions show a leveling off after 15 minutes (Figure 5.3.2) because the porphyrin reacts with all the Mn(II) after 15 minutes (Madison et. al. 2011). After the samples were analyzed on the *R/V Sharp* they were frozen and stored to be re-analyzed at a later date.



Figure 5.3.1 These are the samples analyzed at different depths during CTD cast 22, the depths are labeled in the legend. A steady increase in the [Mn(III)-T4CPP] occurs after an initial rise to a maximum.



Figure 5.3.2 Standard curves of porphyrin method using Mn(II) standard solution.

From the comparison of the Chesapeake Bay samples (Figure 5.3.1) and the standard curve in DI water (Figure 5.3.2), the steady increase of the Mn(III)-T4CPP concentration is apparent. Standard curves were also run in a final dilution in the cuvette which matched the salinity of the samples collected from the Chesapeake Bay. The average salinity of the collected samples was 17 ‰. In the final dilution in the cuvette 250μ L of 17% water was added along with standard because 250μ L of Chesapeake Bay water sample was added to cuvette for analysis. The rate constant for Mn(II), k₁, is slower for the standard Mn(II) in saline solutions but the final concentration matches the final concentrations reached in DI water for the same amount of standard Mn(II) solution added (Figure 5.3.3). The metal substitution

reaction between Cd and Mn in the porphyrin complex takes longer because of the chloro-Cd complex formation, but the reaction does occur to completion as seen in Figure 5.3.3.



Figure 5.3.3 Overlay of 5µM standard Mn(II) in DI, 5µM standard Mn(II) with 17‰ solution, and the Chesapeake Bay samples

Comparing a standard curve in 17‰ salinity seawater solution with a Chesapeake Bay water sample analyzed supports the presence of Mn(III) in the sample. The initial part of the curve for the water sample where it begins to level off before the continual incline overlays with the curve of Mn(II) standard solution with the same salinity as the water sample analyzed. Figure 5.2.4 shows the initial curve for CTD13B1 matches the curve of the standard in 17‰ seawater suggesting Mn(II) is present and another stronger Mn species which is strongly bound to a ligand causes the continual incline.



Figure 5.3.4 Overlay of 6µM Mn(II) in same salinity solution as Chesapeake Bay sample from CTD13B1 collected from 21.52 m.

5.4 In lab experiments and results after the research cruise

The first explanation to the continual increase of [Mn(III)-T4CPP] was there was Mn(III) present strongly bound to a ligand that did not release the Mn(III) within the allotted 15 minutes for the reaction to occur. Therefore it was hypothesized, hydroxylamine (NH₂OH) could be added to the samples to reduce the Mn(III) and dissociate the Mn(III) from the strong ligand into solution. In the lab after the research cruise, this method was tested on Mn(III)-desferrioxamine B (Mn(III)-DFOB) to determine if hydroxylamine would reduce the Mn(III) from the strong ligand, DFOB. The hydroxylamine was added in excess (1.5mM) to a solution containing 5 μ M Mn(III)-DFOB, with a recovery of 99.75 \pm 0.18%. To make sure there was no interference with the method, hydroxylamine (1.5mM) was added to 5 μ M Mn(II) standard solution and no interference was observed. Figure 5.4.1 compares only Mn(III)-DFOB (5 μ M) analyzed with the porphyrin method, Mn(III)-DFOB reduced with hydroxylamine, and Mn(II) with hydroxylamine. Without hydroxylamine, Mn(III)-DFOB (green line), does not dissociate readily and a slow linear increase in absorbance occurs. An equivalent amount of 5 μ M of Mn(III)-DFOB was added with reagents but as Figure 5.4.1 shows, the same concentration is not recovered as the Mn(III)-T4CPP during the allotted 15 minutes for the reaction to take place (green line in Figure 5.4.1).



Figure 5.4.1 Reduction of Mn(III)-DFOB using hydroxylamine (NH₂OH).

Before the addition of hydroxylamine to the Chesapeake Bay water samples the frozen samples were thawed and completely melted. To avoid the possibility of Mn bound to the walls of the plastic falcon tubes, nitric acid was added to the thawed sample to bring the sample to a pH around 1.7.

A concern arose as Mn(III) could be bound in humics that precipitated on acidification. Humic material is organic material that can contain many different function groups and bind to metals. Humic acids are insoluble at pH values less than 2 (vanLoon and Duffy 2011). To dissolve the humics in the water sample, the pH was raised to 5-7 using 0.1 M trace metal clean NaOH.

In summary, after the frozen Chesapeake Bay water samples were thawed, nitric acid was added and the samples were untouched for two months. To analyze a sample to reduce any Mn(III) bound to strong ligands hydroxylamine was added for a final concentration of 1.5mM in the sample. After the addition of hydroxylamine, 0.1M NaOH was added to raise the pH to dissolve possible humic material. After following this procedure with the Chesapeake Bay water samples, there was not a successful recovery of Mn species that were larger than the original concentrations determined aboard the *R/V Sharp* and analyzed shortly after collection. (Figure 5.4.2)



Figure 5.4.2 Unsuccessful recovery of total Mn species from original sample after treated with HNO₃, NaOH and NH₂OH.

5.5 Profiles of original Chesapeake Bay CTD and Mn data

The original scans of the Chesapeake water samples that were run aboard the R/V Sharp were analyzed to determine if the experimental data fit a 2 or 4 parameter

model fit to an exponential rise to a maximum. The experimental data were analyzed in the first 100-300 seconds where the concentration appears to remain constant over a period of time and does not begin to increase steadily. If the concentration of Mn(III)-T4CPP did not level off over time, the model fit to the data could not be used because the experiment data did not reach a maximum. From the analysis of these data if a 2 parameter fit to the experimental data was more accurate then only Mn(II) was present in the water sample. If a 4 parameter model fit to the experimental data was more accurate this was evidence of Mn(III) present in the sample. The following depth profiles compare the salinity, temperature, oxygen concentrations, Mn(II) concentration and Mn(III) concentrations.

CTD 11 (Figure 5.5.1) was cast on August 16, 2012 at local time 08:21, GMT 12:21, at the Chesapeake Bay Station during high tide. The Mn concentrations are listed in Table 5.5.1 along with the percentage of Mn(III) of the total Mn pool.



Figure 5.5.1 CTD11 plot of [Mn(II)], [Mn(III)], [O₂], temperature and salinity vs. depth.

Depth (m)	[Mn(II)] (µM)	[Mn(III)] (µM)	Σ [Mn(II)] & [Mn(III)] (μM)	% Mn(III)
18.44	1.431		1.431	
18.98	1.410	1.078	2.488	43.33
20.88	0.219		0.219	
21.83	0.392	3.529	3.921	90.01

Table 5.5.1 Percentage of Mn(III) of total Mn pool for CTD11

The Mn profile of CTD11 follows trends of Mn(III) profiles from the Black Sea collected by Trouwborst et. al. (2006). Two Mn(III) maxima exist, one at the beginning of the suboxic zone and another at the bottom of the suboxic zone. The Mn(III) present where oxygen begins to disappear can be explained by the oneelectron oxidation of Mn(II) to Mn(III) which is then trapped by chelating agents and ligands into Mn(III) complexes. Mn(III) could also be present due to the decomposition of photosynthetic organisms and organic matter causing the release of Mn(III) by the lysis of photosystem center II (Trouwborst et. al. 2006). Mn(III) at the bottom of the suboxic zone could be caused by Mn(III) in the sediment leaching into the water. The one-electron reduction of MnO_2 by Fe(II) or H₂S form Mn(III) in the sediments which diffuses from the sediments. Madison et. al. (2011) found the highest concentrations of Mn(III) in the top 1-5 cm of sediment porewaters of the Lower St. Lawrence Estuary. The sediments at the Chesapeake Bay station were not analyzed but if the same system is set-up in the sediments of the Chesapeake Bay, the Mn(III) in the sediments could be leaching into the lower suboxic zone of the water column attributing to the Mn(III) maximum in the lower suboxic zone.

CTD13 (Figure 5.5.2) also collected at high tide on August 16, 2012 at a local time of 20:40 and GMT 00:40 at the same Chesapeake Bay station shows the same [Mn(III)] trends as CTD11. Two maxima are observed, one at the top of the suboxic layer that appears when oxygen begins to deplete and another at the bottom of the suboxic layer. The percentage of Mn(III) of the total pool of the Mn species is 65-75% (Table 5.5.2).



Figure 5.5.1 CTD 13 plot of [Mn(II)], [Mn(III)], [O₂], temperature and salinity vs. depth.

Depth (m)	[Mn(II)] (uM)	[Mn(III)] (µM)	Σ [Mn(II)] & [Mn(III)] (µM)	% Mn(III)
2.07	0.256		0.256	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,
8.96	0.538		0.538	
14.96	0.608	1.176	1.784	65.93
16.54	0.549		0.549	
19.41	1.484	2.886	4.370	66.04
21.52	1.275	4.644	5.919	78.46

Table 5.5.2 Percentage of Mn(III) of total Mn pool from CTD 13

CTD22 (Figure 5.5.3) was collected during mid-tide (low tide transition to high tide) on August 18, 2012 at local time 1838 and GMT 2238. Because of the transition from low to high tide there was greater mixing of the water column less of an interface but still low levels of oxygen. A 2 parameter fit was the best fit for the experimental data rise to a maximum for the CTD22 samples collected indicating only Mn(II) was present in the water samples.



Figure 5.5.3 CTD22 profile of [Mn(II)], [O₂], temperature and salinity vs. depth.

5.6 Future Directions

In the future water samples collected on a Chesapeake Bay cruise will further conclude the presence of Mn(III) in the water column. This will be done by the addition of hydroxylamine after the sample is analyzed aboard the ship. Further dilution of the samples before analysis will lower the formation of Cd-chloro complexes and speed up the kinetics of Mn(II) ion binding in the porphyrin complex causing the experimental data fit the model of the exponential rise to a maximum better.

Chapter 6

SUMMARY OF ACCOMPLISHMENTS

This report adds to the limited data available regarding Mn cycling in the environment considering Mn(III) as an important species. Tradionally, Mn(III) has been ignored as an important species, but Mn(III) can be stabilized by inorganic and organic ligands and be an important species present in an environment. Madison et. al.(2011) proves Mn(III) is present in up to 85% of the Mn pool in sediment porewaters in the St. Lawrence Estuary. The investigation of the presence of Mn(III) in a freshwater stream in Maine, hydrothermal vents along the Mid-Atlantic Ridge, and the Chesapeake Bay performed by this study explain the importance of the presence or absence of Mn(III) in these varying environments.

At the freshwater stream in Maine no Mn(III) was found. This proved that the iron oxidizing bacteria that thrive in the stream do not oxidize Mn(II) to Mn(III) to produce energy which is a mechanism observed in other bacteria. The pH of the stream was around 6.19 where Mn(II) is a stable species. Another factor not conducive to Mn(II) oxidation at this stream was the relatively low concentrations of oxygen ($\leq 80\mu$ M).

At the hydrothermal vents visited along the MAR, no oxidation of Mn(II) to Mn(III) was expected to occur as Mn was expelled from the vents because the pH is below 9 and no bacterial oxidation should occur. After analysis of the vent fluid samples from multiple sites and multiple plumes along the Mid-Atlantic Ridge, no

39

soluble Mn(III) was present in the water samples, only soluble Mn(II) was detected which was the outcome that was expected.

The Chesapeake Bay water samples collected and analyzed from the water column indicated a presence of Mn(III) in the suboxic zone. Mn(III) consisted of 45-90% of the total Mn pool in cases where Mn(III) was present. There are large amounts of organic matter present in the Chesapeake Bay from run-off. These organic compounds can bind and stabilize Mn(III) in the water column. The profiles of Mn(III) match trends of Mn(II) and Mn(III) trends seen in the Black Sea by Trouwborst et. al. (2006). The presence of Mn(III) in the Chesapeake Bay was reported by Trouwborst et. al. (2006) and the findings of this report support the presence of Mn(III) found using the spectrophotometric method developed by Madison et. al. (2011).

REFERENCES

Gartman, A. and Findlay, A. R/V Knorr Cruise Kn 209-02 SNAPMORE 2012 October 16 – November 10, 2012 Searching for Nanoparticulate Pyrite at the Mid Ocean Ridge, unpublished, Section 4, pg. 11.

Hocking, Rosalie, Brimblecombe, R., Chang, L., Singh, A., Cheah, M., Glover, C., Casey, W., Spiccia, L. (2011) Water-oxidation catalysis by manganese in a geochemical-like cycle. *Nature Chemistry*, **3**, 461-466.

Lin, H., Szeinbaum, N. H., DiChristina, T. J., & Taillefert, M. (2012). Microbial Mn(IV) reduction requires an initial one-electron reductive solubilization step. *Geochimica et Cosmochimica Acta*, **99**, 179–192.

Luther, G. W. (2005). Manganese(II) Oxidation and Mn(IV) Reduction in the Environment—Two One-Electron Transfer Steps Versus a Single Two-Electron Step. *Geomicrobiology Journal*, **22**, 195–203.

Luther, G. W. (2010). The Role of One- and Two-Electron Transfer Reactions in Forming Thermodynamically Unstable Intermediates as Barriers in Multi-Electron Redox Reactions. *Aquatic Geochemistry*, **16**, 395–420.

Madison, A.S., Tebo, B.M., Luther, G.W. (2011) Simultaneous determination of soluble manganese(III), manganese(II) and total manganese in natural (pore)waters. *Talanta*, **84**, 374-381.

PowerPoint Presentation, Coupling in situ CTD, FIRe fluorometry and voltammetry to determine biogeochemical processes in Chesapeake Bay, Andrew Madison, Daniel Macdonald, Michael McGinley, Erick Geiger, Matthew Oliver and George W. Luther III

Tivey, Margaret K. (2007) Generation of Seafloor Hydrothermal Vent Fluids and Associated Mineral Deposits. *Oceanography*, **20**(1), 50-65.

Trouwborst, R.E., Clement, B.G., Tebo, B.M., Glazer, B.T. & Luther, G.W. (2006) Soluble Mn(III) in suboxic zones. *Science (New York, N.Y.)*, **313**, 1955-7

vanLoon, G. W.; Duffy, S. J. Organic Matter in Water, *Environmental Chemistry: A Global Perspective*. 3rd ed; Oxford University Press. New York, 2011, pp 275-283.

Webb, S. M., Dick, G. J., Bargar, J. R., & Tebo, B. M. (2005). Evidence for the presence of Mn(III) intermediates in the bacterial oxidation of Mn(II). *Proceedings of the National Academy of Sciences of the United States of America*, *102*(15), 5558–5563.