Characterizing Manganese Cycling During Estuarine Mixing

Sophie E. Jenness

University of Washington

School of Oceanography, Box 357940

Seattle, WA 98195

Email: sophij3@uw.edu

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Abstract

Manganese (Mn) is a trace element essential for many biological processes and influences the cycling of many other elements. In the past two decades, the previous paradigm for how Mn exists in the environment has evolved to now recognize that soluble Mn(III) exists when stabilized by organic ligands (Mn(III)-L). A product-approach mixing experiment was used to better understand how Mn cycles between dissolved and particulate phases, as well as its three environmentally relevant oxidation states, along the salinity gradient of the of the Mississippi River delta. Trace metal concentrations were analyzed using inductively coupled plasma-mass spectrometry. Dissolved Mn speciation, characteristics of organic matter, and particulate Mn oxides (MnOx) were analyzed with UV-Vis spectrophotometry. Total dissolved Mn was comprised of a majority of Mn(III)-L at nearly all points along the salinity gradient, and included two peaks in Mn(III)-L concentration, one at low salinity, and one at high salinity. MnOx in incubated samples showed significant oxidation of dissolved Mn during this experiment, however, it still conservatively diluted supporting that the salinity gradient had no influence on ligand-promoted reduction. These suggest that the salinity gradient could cause a shift in the Mn-binding ligand pool, with little effect on ligand-promoted reduction of Mn. As nutrient loading to the Northern Gulf of Mexico continues to regularly deplete oxygen, continuing to understand the dynamics of Mn, particularly its solubility that influences transport, is imperative because of the role it plays in redox process of the region.

Plain Language Summary

Manganese (Mn) is a trace element essential for life. In the environment, it can exist in three states (Mn(II, III, IV)), with Mn(II) being dissolved, Mn(IV) being particulate, and Mn(III) being particulate unless stabilized by organic molecules known as ligands. This project aimed to

understand how Mn cycles between its three states during mixing of the Mississippi River and the Gulf of Mexico through a mixing experiment designed to mimic the salinity gradient. The proportion of dissolved Mn(III) may have been influenced by the salinity gradient, although rates of ligand promoted reduction of MnOx did not vary along it. These have broader implications for how the transition from freshwater to seawater impacts the transport of Mn, and establish a better understanding of how the salinity gradient influences the phase of Mn. Having better insight into how Mn cycles in the Mississippi River delta is important because of the broader implications it has for the chemistry of the Northern Gulf of Mexico

Introduction

Manganese (Mn) is a redox active trace element that helps to sustain life. Notably, Mn is a catalyst in photosynthesis, where it assists in the oxidation of H_2O to O_2 (Twining & Baines, 2013). It is estimated that roughly half of the molecular oxygen on Earth is produced in the ocean, specifically from photosynthesis (NOAA, 2023). Because Mn catalyzes the production of oxygen in the ocean and influences the cycling of many other bioactive elements (e.g., carbon, nitrogen, phosphorus, and iron), it is important to understand how Mn cycles in both the coastal and open oceans.

Over the past two decades, knowledge of the redox chemistry of Mn in the environment has evolved. Originally, it was thought that dissolved Mn existed only in its +II oxidation state, and particulate Mn in its +III and +IV oxidation states (Tebo & Luther III, 2019), with dissolved being operationally defined by its ability to pass through a 0.2 μm filter. Soluble Mn(III) was presumed to not be present, as it would immediately oxidize to particulate Mn oxides (MnOx) or reduce to Mn(II) (Stumm & Morgan, 1995). However, soluble Mn(III) had been observed in laboratory studies when complexed by organic ligands (e.g. Klewicki & Morgan, 1998), which

are organic molecules that bind and stabilize metals and help to keep them in the dissolved phase. In the environment, Mn(III) is sourced from the oxidation of Mn(II) because it is most likely to occur in two one-electron transfer steps, as opposed to a single two-electron transfer, creating Mn(III) as an intermediate (Luther III, 2005). The two one-electron transfer steps are more likely because electrons are accepted or donated by orbitals with different spatial characteristics (Luther III, 2005). When organic ligands are present, the intermediate Mn(III) can be stabilized and remain in the dissolved pool of Mn. Mn(III)-L complexes were first measured in the environment in suboxic waters by Trouwborst et al. (2006). Oldham et al. (2017a) first measured Mn(III)-L complexes in oxygenated waters, where they also found that a significant portion of the soluble Mn(III) pool was complexed by humic substances. While the identities of non-humic substance ligands that bind Mn(III) are not well understood, laboratory studies have shown Mn(III) to bind with ligands similar to ligands bound by dissolved iron (Fe) (Duckworth & Sposito, 2005), the vast majority of which is ligand bound in the environment (Gledhill & Buck, 2012). The stabilization of Mn(III) by organic ligands keeps it in the dissolved pool, having broader impacts on the residence time of Mn.

Recent findings about the ubiquitous presence of Mn(III)-L complexes in the water column make it necessary to reevaluate how Mn cycles, especially as Mn(III) can behave as both an oxidant and a reductant. In coastal estuaries, previous studies have shown the removal of 25-45% of dissolved Mn during the mixing of freshwater and seawater (Sholkovitz et al., 1978). The mechanism through which this removal occurs is aggregation of Mn(III,IV) (hydr)oxides, allowing them to sink out of solution. Oldham et al. (2017b) studied Mn cycling in Broadkill River system in Delaware, and found complexation of Mn(III) by humic substances is important in keeping Mn in solution. They further proposed a complex system of cycling mechanisms (Fig.

1) that may be occurring in this river system. The cycling mechanisms proposed consider recent findings about the dissolved pool of Mn, however, the salinity gradient created during estuarine mixing may also influence processes connected with the cycling of Mn, such as the availability of organic ligands.

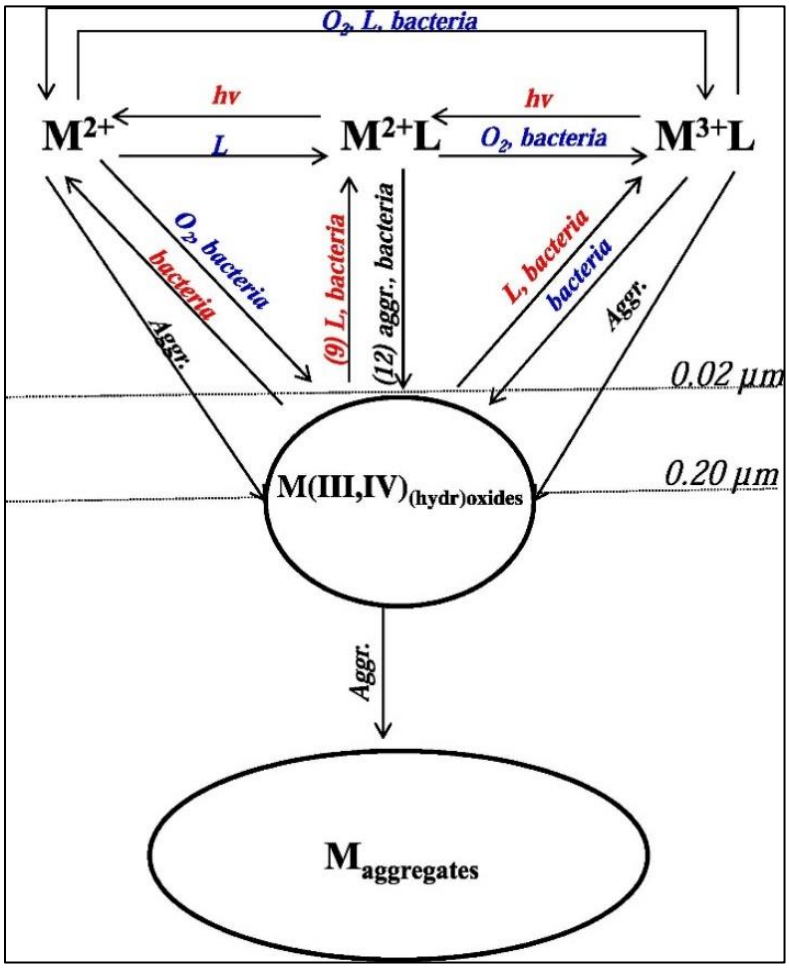

Figure 1: Proposed Mn cycling mechanisms between oxidation states and dissolved and particulate phases in the Broadkill River system. Blue pathways indicate oxidative processes, red pathways indicate reductive processes (Fig. 7, Oldham et al., 2017b)

Rivers are rich in humic substances, which can complex up to 100% of the dissolved Mn(III) pool (Oldham et al., 2017a). Yet along a salinity gradient, humic substances can flocculate (Sholkovitz, 1978), allowing them to be removed from solution and removing ligands

that can stabilize Mn(III). Conversely, some organic matter in river water can be sorbed to suspended minerals, shielding it from degradation (Keil et al., 1997). As these suspended minerals flocculate in response to the increasing ionic strength of seawater, organic matter can potentially be mobilized (Hedges & Keil, 1999), introducing organic ligands that bind dissolved Mn(III).

The Mississippi River delivers large concentrations of inorganic nutrients to the Northern Gulf of Mexico, creating eutrophic conditions in the summer months when the water column is tightly stratified (Rabalais et al., 2009). This results in large algal blooms, the degradation of which depletes oxygen in the water column faster than it is re-aerated (Rabalais et al., 2010). In the absence of oxygen, Mn is one of the most thermodynamically favorable oxidants (Emerson & Hedges, 2008), so as hypoxic events persist, Mn will continue to be an important element in the cycling of many other bioactive elements. Understanding how the Mississippi River acts as a source of Mn to this region, and how the Mn that it introduces cycles along the salinity gradient, is important for understanding the broader redox chemistry of this region.

In this study, I investigated the cycling of Mn between phases and oxidation states via a product approach mixing experiment. I hypothesized that the availability of organic ligands will be influenced by the salinity gradient of the Mississippi River delta, which will promote the oxidation of Mn(II) and the reduction of particulate Mn(IV), making Mn cycling during estuarine mixing more complex than previously established by keeping Mn in solution in the form of Mn(III)-L along the salinity gradient.

Methods

Sample Collection and Experimental Setup

Aboard the *R/V Pelican* in October 2023, I collected 8 L of river water from the Mississippi River that had a salinity as near 0 PSU as I could obtain within the constraints of the research cruise. I also collected and filtered with a 0.2 μm PES filter 8 L of seawater near a salinity of 35 PSU in the Gulf of Mexico. The purpose of collecting water at these salinities was to reflect in situ endmember properties as accurately as possible. Prior to mixing waters, I calculated the volumes of each endmember to mix to represent a range of salinities, capture salinity values of interest based on findings by Sholkovitz (1978), and to have controls for both the river water and seawater (Table 1). During sample collection, seawater was filtered to help preserve its properties prior to the mixing experiment. The river water and seawater endmember samples were mixed in different volumes, in triplicate, shipboard (Table 1) and allowed to equilibrate in a flow-through incubator with surface seawater for 36 hours before having the salinity verified with a handheld refractometer, and being aliquoted for different laboratory analyses.

Table 1: Volumes of river water (3.1 PSU) and seawater (33.7 PSU) mixed to model the salinity gradient of the Mississippi River delta.

Volume River Water (mL)	Volume Seawater (mL)	Calculated Salinity (PSU)
250	0	3.1
215	35	7.4
180	70	11.7
145	105	16
95	155	22.1
60	190	26.4
45	205	28.2
20	230	31.3
0	250	33.7

Laboratory Analyses

To analyze dissolvable and dissolved trace metals, a total of 120 mL of incubated waters was aliquoted, with 60 mL being acidified using Optima™ grade hydrochloric acid prior to filtration through a 0.2 μm PES filter (dissolvable) and the other 60 mL being acidified after filtration (dissolved). I analyzed Mn concentrations with an Element2 High-Resolution Magnetic Sector Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) in an eluent of Trace Metal Grade™ nitric acid with indium (In) and rhodium (Rh) internal standards to correct for drift in intensity counts during data processing. This method is similar to that utilized by Bhatia et al. (2021). Samples were grouped into three salinity categories: low salinity, mid salinity, and high salinity. Concentrations of Mn were calculated using a mixed metal standard, and separate standards were made with different salinities for each category in order to minimize interference from the matrix.

I collected 2 mL of filtered incubated water to analyze the absorbance at a range of wavelengths that indicate properties of organic matter. This was analyzed using a 1 cm quartz cuvette and a UV-Vis spectrophotometer immediately following aliquoting of incubated water samples. These can act as proxies for humic substances, as developed by Purmalis & Kļaviņš (2013). Oldham et al. (2017b) utilized the absorbance measured at 280 nm specifically for this proxy.

Filtered incubated water was also used to analyze dissolved Mn concentrations and speciation in a method originally developed by Ishii et al. (1982), significantly modified by Madison et al. (2013) to detect Mn(III), although in micromolar concentrations. Thibault de Chanvalon & Luther III (2019) further developed the method to detect nanomolar concentrations. I utilized the nanomolar version of this method, specifically using the reduced

and heated treatments described by Thibault de Chanvalon & Luther (2019), only using a 100 cm wave capillary cell instead. Samples were frozen to preserve them until analysis.

Following filtering of water for other analyses, the 0.2 μm syringe filter was saved. I used the same set up and method as Oldham et al. (2017b) for measuring MnOx. The use of Leucoberbelin Blue (LBB) dye to measure MnOx was originally developed by Altmann (1972). In addition to measuring MnOx of incubated waters, I also utilized water collected from the Mississippi River to measure the in situ MnOx, without any incubation, for comparison to incubated samples.

Results

Along the salinity gradient, the concentration of total dissolved Mn more than doubled (Figure 2). While total dissolved Mn increased, Mn(III)-L complexes were the dominant species in nearly all incubated water samples, ranging from 9 to 24 nM. Despite the increase in total dissolved Mn, the percent of dissolved Mn comprised of Mn(III)-L was not consistent, and displayed two peaks in concentration, each around 24 nM, at salinities of 12 and 34 PSU (Figure 2). The only incubated sample where Mn(III)-L was not the dominant species had a salinity of 26 PSU.

Using the absorbance at 280 nm as a proxy for humic substances present, similar to Oldham et al. (2017b), humic substances decreased linearly along the salinity gradient (Figure 2). A linear regression of the absorbance at 280 nm versus salinity returned an R^2 value of 0.997. Concentrations of dissolved Mn(III)-L complexes did not strongly correlate with humic substances, a linear regression of the two returning an R^2 value of 0.169 (Figure 3).

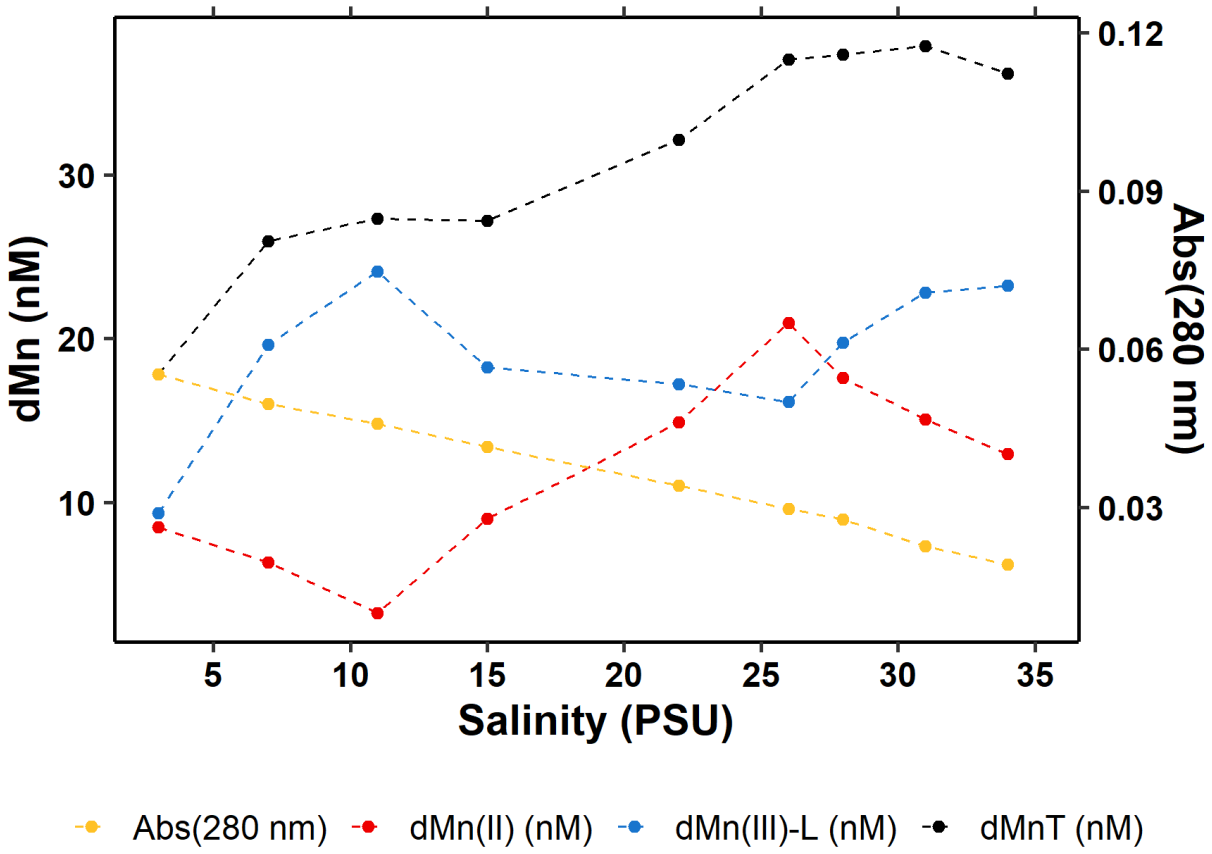

Figure 2. Concentrations of total dissolved Mn (black), dissolved Mn(III)-L complexes (blue), dissolved Mn(II) (red, primary y-axis) and absorbance measured at 280 nm (yellow, secondary y-axis) versus salinity.

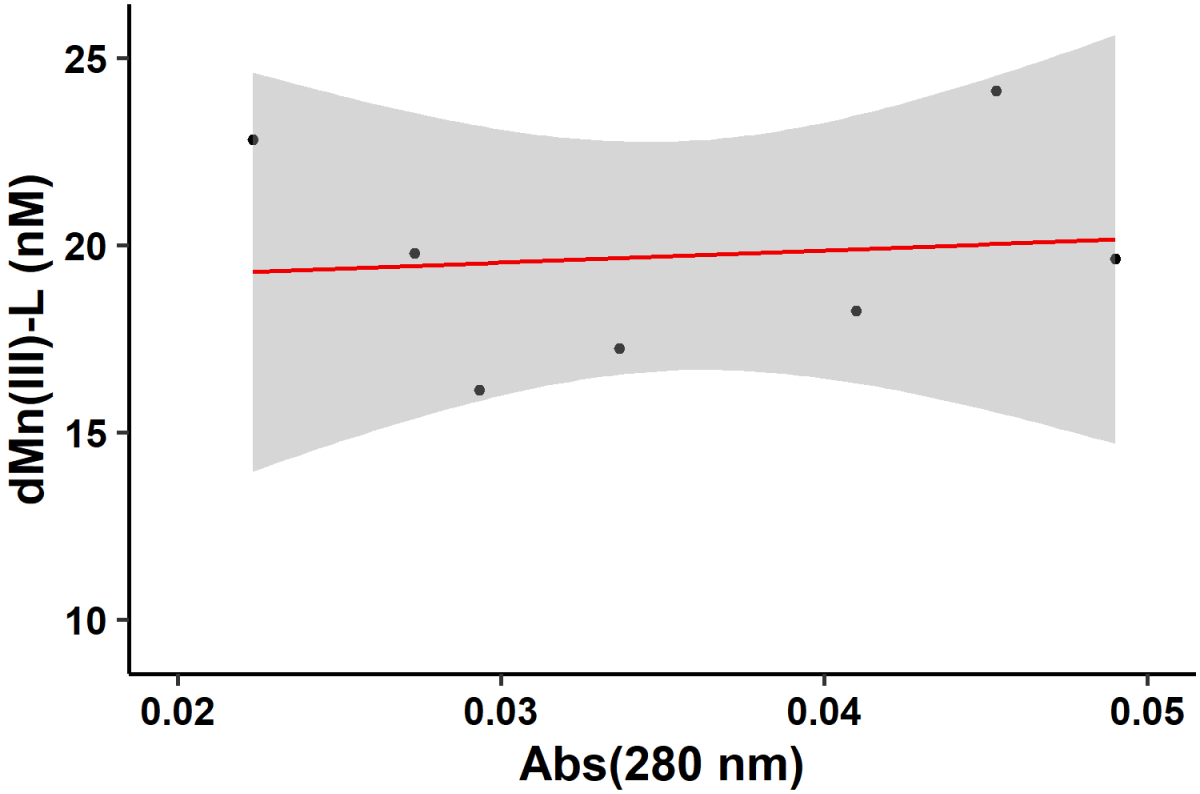

Figure 3. Concentrations of dissolved Mn(III)-L complexes versus the absorbance at 280 nm with a linear regression (red line) and 95% confidence interval (gray shadow) (R^2 = 0.169).

Concentrations of MnOx along the modeled salinity gradient also decreased linearly, with an R^2 value of 0.997 in a regression versus salinity (Figure 4). The concentration of MnOx in incubated samples ranged from 585 nM to less than 1 nM. In comparison, the in situ concentrations of MnOx in the Mississippi River was 91 nM. Particulate Mn, derived from analyses of total dissolvable Mn and total dissolved Mn, was the dominant phase of Mn in samples, before dropping at 26 PSU, leaving dissolved Mn as the dominant phase (Figure 5).

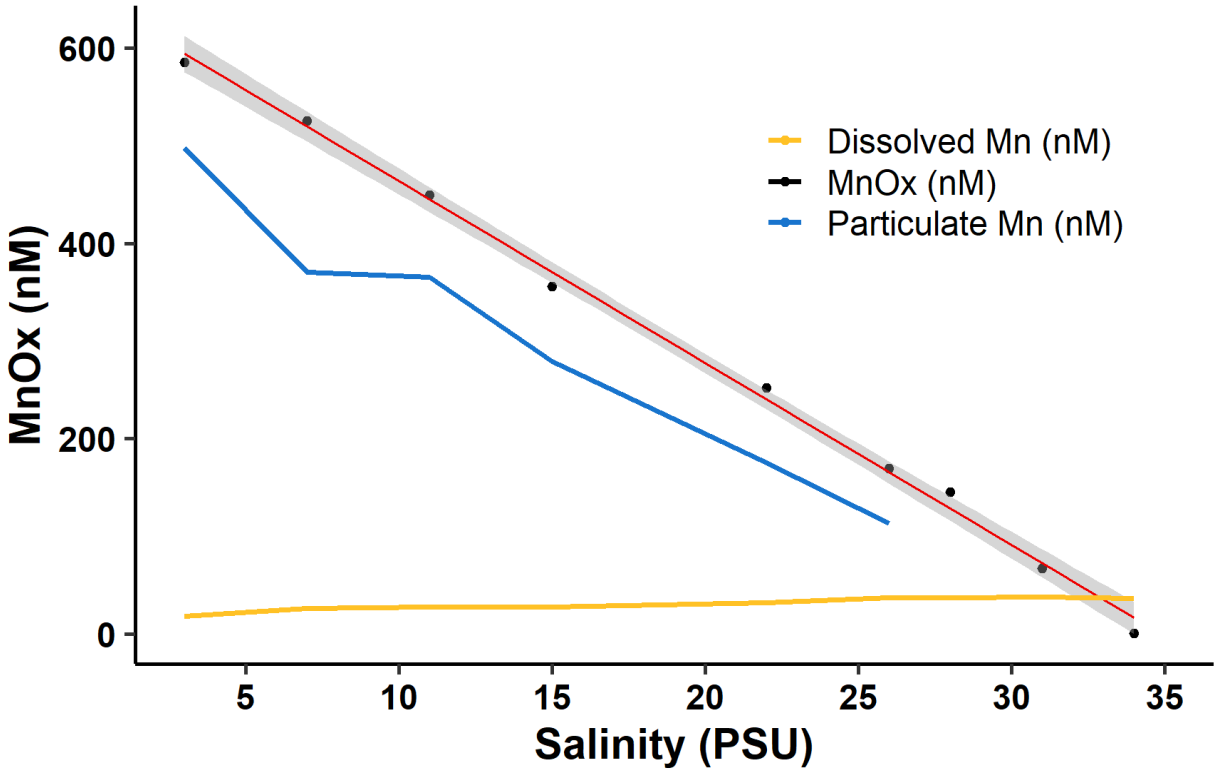

Figure 4. Total dissolved Mn (yellow), total particulate Mn (blue), and MnOx concentrations (black points) versus salinity. Red line is a linear regression of MnOx concentrations (R^2 = 0.997) with a 95% confidence interval (gray shadow).

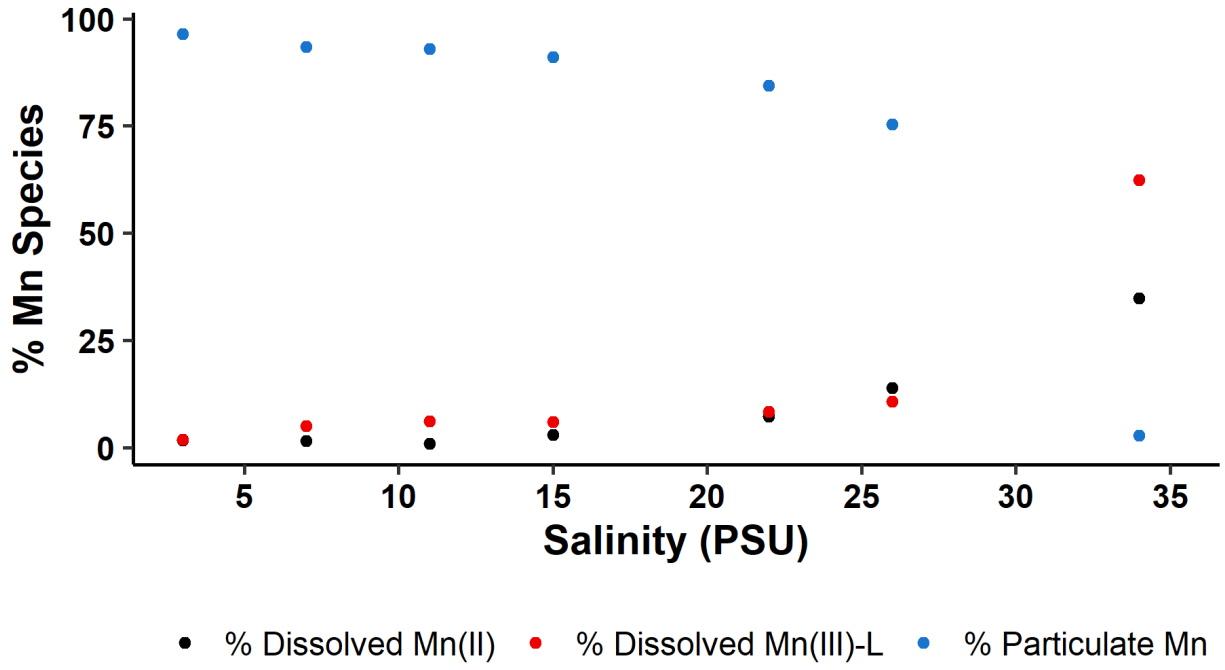

Figure 5. Percent composition of each species in the total Mn pool: particulate (blue), dissolved Mn(III)-L (red), and dissolved Mn(II) (black) versus salinity.

Discussion

Changes in dissolved Mn speciation along salinity gradient

In modeling the salinity gradient, I attempted to observe how the speciation of dissolved Mn changed. Despite a linear decrease in humic absorbance, concentrations of Mn(III)-L did not follow that trend (Figures 2, 3). This is opposite of what was expected based on findings by Oldham et al. (2017b), where seasonal changes in humic absorbances correlated with changes in Mn(III)-L. Rather, even when humic absorbance was low, Mn(III)-L almost always comprised the majority of the dissolved Mn pool. Concentrations of Mn(III)-L peaked twice along the salinity gradient, once at 12 PSU where it was 88% of the total dissolved Mn pool and where humic absorbance is still high, and again at 34 PSU where it was 64% of the pool and there was low humic absorbance. The mid-salinity range where Mn(III)-L concentrations drop is in a

salinity range (12-15 PSU) that Sholkovitz (1978) suggested was the range where flocculation of humic substances plateaus, however, the strong linear decrease in humic absorbance (Figure 2) suggests they only diluted in this experiment and did not flocculate. Nevertheless, the decrease in humic substances, along with a decrease in Mn(III)-L suggests less humic ligands binding Mn(III) due to their decreased availability (Figure 2). However, the presence of a second peak of Mn(III)-L of approximately equal concentration at higher salinities points to additional ligands available in order to bind Mn(III) (Figure 2). Since humic absorbance only decreases (Figure 2), these could be ligands not proxy measured by absorbance at 280 nm and rather are non-humic ligands introduced by seawater that are available to bind dissolved Mn(III), suggesting that the ligand pool that interacts with Mn is variable in the types of ligands along the salinity gradient, and potentially their binding strength as well. While up to 100% of the Mn(III)-L pool can be complexed by humic ligands (Oldham et al., 2017a), this may not be the case in the Northern Gulf of Mexico. A variable ligand pool could explain why Mn(III)-L was not related to humic substances as found in Oldham et al. (2017b). Variance in the ligand pool has broader implications for the transport and availability of Mn because if it is bound by a stronger ligand, it can be transported further and is less bioavailable.

The changes in Mn(III)-L observed support different potential mechanisms through which the salinity gradient may influence the ligand pool. The increase in Mn(III)-L at high salinities could point to the release of ligands, specifically by mobilizing organic matter, as suggested by Hedges and Keil (1999). However, the salinity gradient could also have little impact on ligand availability at higher salinity brackish waters, and simply seawater from the Northern Gulf of Mexico that mixes with river water could be transporting ligands that bind

Mn(III). In either mechanism, the dissolved Mn in the Mississippi River is influenced during mixing with the Northern Gulf of Mexico.

Oxidation of Mn during experimental setup and conservative dilution

MnO_X in my incubated samples, specifically the river water control, existed in a far higher concentration of 585 nM (Figure 4) compared to the in situ concentration of 91 nM. These high concentrations indicate that during the set up of this experiment, a large pool of dissolved Mn was oxidized to particulate MnOx. Because MnOx conservatively diluted (Figure 4), a majority of this oxidation likely occurred prior to the mixing of seawater with river water. Microbial oxidation rates of Mn have been found to be higher in freshwater as compared to seawater (Vojak et al., 1985), which under the assumption that further oxidation occurred post-mixing of waters, could explain why a great pool of dissolved Mn in the river water was oxidized as opposed to in the seawater controls, leaving more dissolved Mn in seawater (Figures 2, 5). However, following collection, river water was stored in a fridge, which would decrease oxidation rates. The more likely explanation is that since the river water was unfiltered, these differences indicate that adsorption of Mn(II) occurred, since the presence of MnOx enhances the kinetics of Mn(II) oxidation (Stumm & Morgan, 1995). Aside from this oxidation, MnOx still conservatively diluting did not support my hypothesis of seeing ligand-promoted reduction of MnOx being influenced by the salinity gradient. Ligand-promoted reduction of MnOx has been observed by Oldham et al. (2017b) in an experiment using low salinity water, however, whereas Oldham et al. (2017b) looked at MnOx reduction promoted by ligands over time, I examined it along a salinity gradient with the prediction of observing differences in ligand-promoted reduction between salinities. This supports the idea that if ligand-promoted reduction is occurring, the rate at which it occurs is not influenced by mixing with seawater.

Conclusion

Because of the role that Mn plays in biological processes and elemental cycling, it is important to understand its cycling and the dynamics of different systems that influence Mn. Although my project was limited by its setup that resulted in Mn oxidation occurring prior to incubation, making it difficult to draw conclusions about cycling in the dissolved pool of Mn, there were signals of salinity and intruding seawater having an influence on the cycling and transport of Mn that should be further studied. A peak in dissolved Mn(III)-L complexes at high salinity brackish waters suggested potential sources of ligands related to either the salinity gradient or transport from the Northern Gulf of Mexico. These could be better examined through in situ measurements that utilize all of the treatment methods outlined by Thibault de Chanvalon & Luther (2019) to understand the properties and qualitative strength of the ligands binding Mn(III). Simultaneous measurements of humic substances would als

o provide a better understanding of how they are influenced by the salinity gradient and interact with the cycling of Mn.

The oxidation of Mn that occurred prior to set up of this mixing experiment demonstrates the difficulty of this method for studying the cycling of Mn between phases, making in situ studies likely a better method. It does, however, also demonstrate that microbial oxidation or more likely adsorption of Mn, could have a major role in the loss of Mn, although the residence time of Mn within the river as it is deposited towards the Gulf of Mexico likely impacts this. Further, the conservative dilution of the MnOx formed prior to experimental set up demonstrates that ligand-promoted reduction likely does not contribute greatly to Mn cycling, or that the introduction of saltwater does not influence the rates at which this occurs.

This project identified multiple areas where seawater from the Northern Gulf of Mexico impacts the cycling of Mn as it is introduced from the freshwater Mississippi River. Especially as knowledge of Mn redox cycling in the environment continues to evolve, further investigation through both in situ and ex situ studies is needed to understand the impacts a dynamic system has on Mn, especially because of the implications that changes in the phase of Mn has for the broader biogeochemistry of the Northern Gulf of Mexico and similar regions.

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