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EFFECTS OF SALINIZATION ON MERCURY BIOAVAILABILITY IN COASTAL WETLANDS AT ALBEMARLE-PAMLICO PENINSULA, NORTH CAROLINA

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Effects of salinization on mercury bioavailability in coastal wetlands at Albemarle-Pamlico Peninsula, North Carolina

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1. Acknowledgments

We would like to thank undergraduate students Aslihan Sakar, Nadia Aitmbarek, and Ph.D. student Prashant Waiker (UNC-Greensboro) and Egemen Ulus (my brother) for assisting in the field to collect samples during our study. We are also thankful to Dr. Marcelo Ardon and his group (NCSU) for water quality analysis as well as the UNC-Greensboro Biology department for providing extra funding. Moreover, we are grateful for the funding from NC WRRI & Sea grant for supporting our study.

2. Abstract

The primary purpose of this study was to assess salinization impact (spatial and temporal) on mercury (Hg) cycling in coastal plain wetlands of North Carolina. We hypothesized that salinization of coastal freshwater wetlands increases methylmercury (MeHg) levels, as the sulfate-reducing bacteria (SRB), dominant groups that promote methylation of an inorganic form of mercury Hg (II) is stimulated by elevated levels of sulfate (SO_4^{2-}) derived from saltwater.

To achieve our goal, we collected water, sediment, and biota (note that biota was not included in the proposal) samples every month, between April and September 2019. We measured total Hg, MeHg, sulfate (SO_4^{2-}), and dissolved organic carbon (DOC) levels along with the necessary field measurements (e.g., salinity, temperature, and dissolved oxygen).

Total mercury (THg) levels for surface water samples ranged widely among the sites, and the highest levels were observed in Freshwater Wetland (at Point Peter site), where the DOC levels were the highest as well. Also, we observed increasing aqueous filtered MeHg concentrations (3.13 and 3.72 ng/L) in May and July in Freshwater Wetland, when the sulfate levels were at the peak (i.e., 4.85 and 4.79 mg/L, respectively). So far, our results for water samples suggest that the salinization of freshwater wetlands elevates MeHg levels in surface water. However, our analyses for sediment and biota samples are still ongoing (will be finalized in summer 2020) and will give us a better understanding of salinization impact on mercury cycling in coastal plain wetlands.

Keywords: Bioaccumulation, Coastal wetlands, Mercury (Hg), Methylation, Salinization, Sea level rise, Sulfate (SO_4^{2-}),

3. Introduction:

Mercury (Hg) is a toxic metal with a global circulation due to its inert gaseous phase (as elemental Hg0) that can travel long distances in the atmosphere and be wet or dry deposited onto the landscapes, including remote regions (Fitzgerald et al., 1998). While deposited Hg is in the inorganic form (as HgII), the most problematic is when HgII is transformed by anaerobic microbes such as sulfate-reducing bacteria to highly bioavailable and neurotoxic methylmercury (MeHg) in sediment and its porewater (Whitacre, 2014). Once produced, MeHg can be concentrated by algae and bacteria for 10^4-10^5 times compared to aqueous levels (Pickhardt & Fisher, 2007), and its levels increase by 2-10 folds as it moves up the food chain, leading to extremely high concentrations of MeHg in the top predators such as piscivorous fish, a process called biomagnification (Wiener et al., 2003). In North Carolina, there is a clear spatial pattern of MeHg levels in piscivorous fish such as largemouth bass with very high concentrations (1-4 ppm or µg/g; note: EPA guideline for fish consumption is only 0.3 ppm; USEPA, 2001) often found in water bodies in the eastern and southeastern parts of the state (Sackett et al., 2009), and such pattern coincides with the distribution of extensive wetlands in this part of the state where HgII methylation is highly favorable in the reducing wetland soils.

Besides, we are facing yet another regional and global challenge – salinization of coastal, low-lying wetlands due to sea-level rise (Herbert et al.,2015), **which I hypothesized that this would lead to enhanced microbial production of MeHg mainly because seawater can provide abundant sulfate (SO_4^{2-}) to further stimulate sulfate- reduction and microbial HgII methylation.**

An earlier model developed by Gilmour & Henry (1991) (Fig.1) predicted that low SO_4^{2-} level ($<10^1$ µM) would limit Hg II methylation (i.e., sulfate-limited; Jeremiason et al., 2006) while high SO_4^{2-}-level ($>10^4$ µM) as found in estuarine waters and seawater may limit HgII methylation due to the buildup of reduced sulfide (S^{2-}), as S^{2-} can extensively sequester HgII and inhibit further bacterial HgII methylation (Benoit et al., 1999). Interestingly, the peak of HgII methylation is predicted to be at intermediate SO_4^{2-}-level (10^2 - 10^3 µM) as this would provide sufficient SO_4^{2-}-for extensive HgII methylation while not leading to an excessive buildup of S^{2-}. In a laboratory microcosm study, we found that freshwater wetland soils from coastal South Carolina incubated with 1 ppt water (contains $10^{2.7}$ µM of SO_4^{2-}) produced almost 10 times more MeHg than with 0 ppt water (or freshwater, with ~10^1 µM of SO_4^{2-}) (Ku et al., 2018), suggesting that salinization of coastal wetland to 1 ppt of salinity is “ideal” in stimulating extensive HgII methylation in organic matter-rich wetland soils.

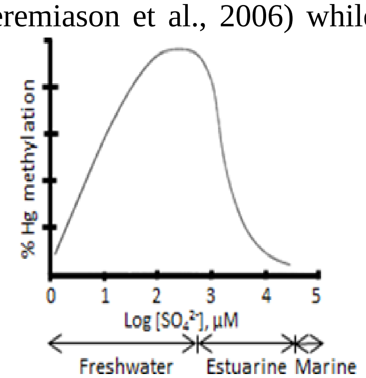

Figure 1: Theoretical relationship between water column sulfate levels and mercury methylation rate in sediments (modified from Gilmour & Henry (1991).

Specific aims and hypotheses

Aim 1: Determine the effects of salinity gradient on the spatial and temporal variation of MeHg in coastal wetlands (Point Peter Site).

Hypothesis 1: Increasing salinity level elevates MeHg concentrations in the water column and sediment.

Aim 2: Determine the impact of episodic seawater intrusion/incursion on spatial variations of MeHg in restored forested wetlands (TOWeR site).

Hypothesis 2: Episodic seawater intrusion/incursion in summer drought provides sea salt for enhancing MeHg levels in restored freshwater wetlands.

4. Methods

Study sites: Sampling sites are located at (Aim 1) Point Peter Site (35°46'08.4"N, 75°45'03.6"W) in Dare County, and (Aim 2) Timberlake Observatory for Wetland Restoration (TOWeR) (35°53'52.8"N, 76°10'01.2"W) in Tyrell County. Point Peter Site consists of a forest-marsh transect, including freshwater wetland (FW), partially degraded wetland (PDW), and salt marsh (SM) (Fig. 2). TOWeR site, restored freshwater wetlands, T-North, T-Middle (note that biota was not included in the proposal) and T-South, consists of 420 ha mature forested wetland, 787 ha of forested wetland, 57.2 ha of drained shrub as well as 440 ha former agricultural fields that underwent stream and wetland restoration (Ardón et al., 2013) These wetlands have waters with deficient dissolved oxygen (DO) and elevated dissolved organic carbon (DOC), have been exposed to extensive saltwater intrusion permanently or episodically, which may lead to degradation of freshwater wetland or dynamic changes in ecosystem biogeochemical cycling (Ardón et al., 2013).

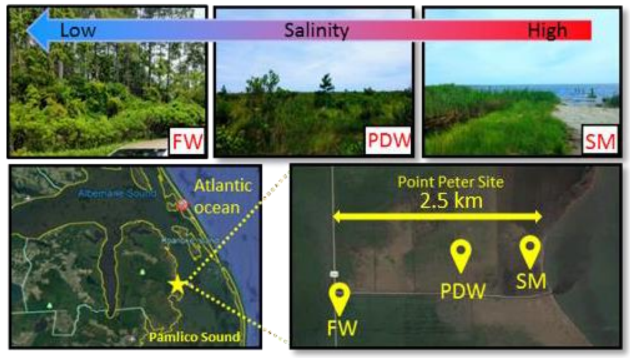

Figure 2: *(Aim 1) Sampling locations in Dare County, North Carolina: Fresh water (FW), partially degraded wetland (PDW), and salt marsh (SM) at Point Peter site.*

Sampling and analysis: Surface sediment and water samples were collected in replicates every month (starting in April 2019 and ending in September 2019) from locations at FW, PDW, and SM sites at Point Peter Site and T-south, T-middle (between T-North and T-South) and T-north sites at TOWeR site. During each field trip, water quality parameters (DO, conductivity, and temperature) were measured in-situ with a handheld YSI probe. Samples were transported to the analytical laboratory at UNCG on ice. Water samples were filtered with a 0.7 µm filter paper (Whatman GF/F) for THg, MeHg, DOC, and SO_4^{2-} analyses. Before the water THg analysis, we digested samples with the mixture of 1 ml acid (HNO_3 & H_2SO_4; trace metal grade, Fisher Scientific) and 1 ml of Potassium Permanganate ($KMnO_4$) and Persulfate ($K_2S_2O_8$) (analytical grade; Fisher Scientific) and kept in 60 °C overnight(Woerndle et al., 2018). For water MeHg analysis, we added 0.4 % HCl (for freshwater) or 0.2 % H_2SO_4 (for high salinity water samples) and kept at 4 °C before further processing. Both THg and MeHg analyzed with Brooks Rand cold-vapor atomic fluorescence spectrometry purge-and-trap manual system at UNCG. SO_4^{2-} and DOC analysis were performed on a Metrohm 930 Flex Ion Brain Chromatograph using chemical suppression and conductivity detection (EPA 300.1) and on a Teledyne Tekmar Torch TOC combustion analyzer with a total nitrogen module in Dr. Marcelo Ardón's lab at North Carolina State University, respectively.

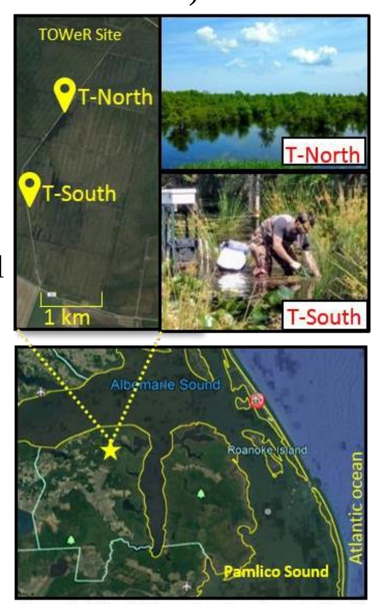

Figure3: (Aim 2) Sampling locations in Tyrell County, North Carolina: TN and TS restored fresh water wetlands

5.Results

During sampling in Point Peter site (SM, PDW, and FW), the sound-side wetland, salinity levels were very dynamic and ranged from 0.09 to 7.83 ppt. In freshwater wetland salinity levels usually were less than 0.3 ppt, however, during the late summer (July and August 2019), as well as after hurricane Dorian (September 13th), salinity increased to 1.83 ppt, 1.63 ppt, and 0.68 ppt, respectively (Table 1). Salinity levels generally were less than 0.4 ppt in the TOWeR site (T-North, T-Middle, and T-South). However, salinity levels increased right after hurricane Dorian hit the coast of North Carolina (e.g., T-North 1.06 ppt) due to storm surges (Table 1).

Table 1: Salinity (ppt) levels for all sites.

Date	SM	PDW	FW	T-North	T-Middle	T-South
22 Apr.	5.11	1.97	0.09	0.02	0.03	0.02
12 May	7.83	2.59	0.17	0.04	0.05	0.03
27 Jun	2.09	1.2	0.11	0.03	0.03	0.02
22 Jul.	2.73	1.62	1.83	0.15	0.1	0.04
23 Aug.	1.24	1.31	1.63	0.34	0.16	0.03
4 Sept.	2.23	1.85	0.21	0.32	0.25	0.03
13 Sept.	1.13	3.53	0.68	1.06	0.52	0.29
Average	3.1±2.25	2.01±0.75	0.67±0.69	0.28±0.34	0.16±0.16	0.06±0.09

Levels of THg and MeHg in filtered water samples: All filtered water samples exceeded the method detection limit (i.e., MDL, 0.2 ng/L) of THg levels. The concentration of THg varied from 0.83 ng/L (T-South) to 14.9 ng/L (Freshwater Wetland) across all sites. While Point Peter site showed mainly higher THg levels (e.g., 2.13 - 14.9 ng/L), TOWeR sites showed relatively lower THg concentrations (e.g., 0.83 - 3.4 ng/L) (Figure 4). Surface water MeHg levels showed similar trends, with higher MeHg concentrations in Point Peter Site than TOWeR sites (Fig.5).

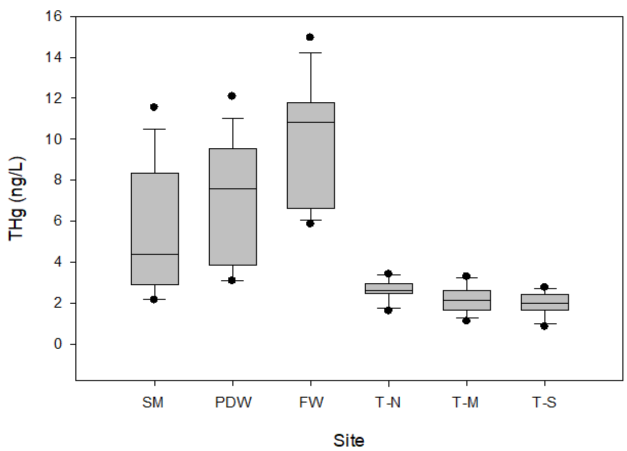

Figure 4: Surface water total mercury (THg) levels in Point Peter site, saltmarsh (SM), partially degraded wetland (PDW), freshwater wetland(FW), and TOWeR site, T-North (T-N), T-Middle(T-M), and T-South(T-S). n = 14 for each site except T-S (n = 12).

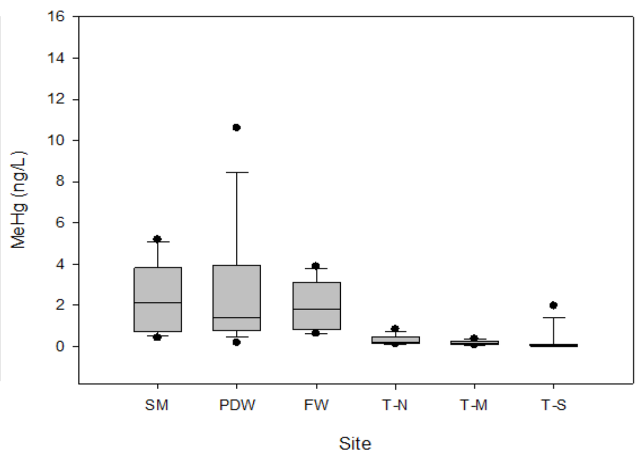

Figure 5: Surface water methylmercury (MeHg) levels in Point Peter site, saltmarsh (SM), partially degraded wetland (PDW), freshwater wetland(FW), and TOWeR site, T-North (T-N), T-Middle(T-M), and T-South(T-S). n = 14 for each site except T-S (n =

With this study, our primary goal was to understand the effects of salinity on Hg methylation in coastal freshwater wetlands. In Figure 6, we show a positive correlation, increasing saltwater derived sulfate concentrations on May 12th, and July 22nd led to an increasing MeHg levels in freshwater wetland. Interestingly, total Hg levels increased until July, but after that, total Hg levels decrease toward the early September and eventually sharply increased in mid-September after hurricane Dorian (Fig. 6).

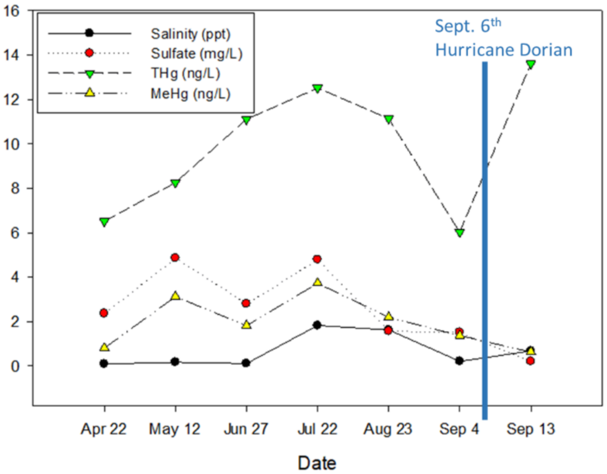

Figure 6: Temporal variations of salinity, sulfate, THg and MeHg levels in Freshwater wetland.

<u>Relationship between THg and DOC:</u> DOC levels varied between the sites with the highest in the freshwater wetland (e.g., 99.99 mg/L). Aqueous total Hg concentrations exhibited a positive relationship with the DOC levels, with a higher concentration of DOC, resulting in higher levels of THg in water (Fig. 7).

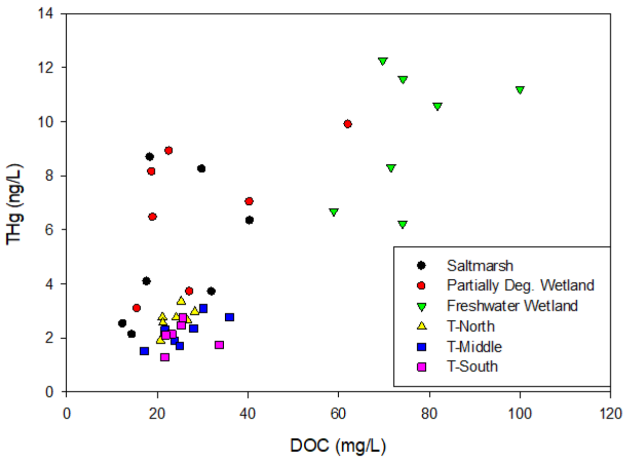

Figure 7: Relationship between total Hg and DOC samples across all sites (r^2 = 0.467).

6. SUMMARY, CONCLUSION, AND RECOMMENDATIONS

To the best of our knowledge, our study is the first to systematically investigate salinity impact on Hg cycling in coastal plain wetlands in North Carolina. Aqueous THg and MeHg concentrations were high (especially sound side wetlands) compared to coastal wetlands in Georgetown South Carolina (Ulus et al., unpublished data). We also observed an increasing trend on MeHg levels with the increasing levels of sulfate in freshwater wetland, which supported our hypothesis (Fig. 8). These results suggest that the salinization of freshwater wetlands may increase the MeHg levels in North Carolina's coastal plain wetlands. Higher MeHg levels, in sediment and water, due to salinity gradient, can promote higher entry of MeHg into the base of food webs that can lead to higher bioaccumulation and biomagnification of MeHg into the aquatic food webs, leading to higher levels of MeHg in fish, and wildlife and human who consume fish (Mergler et al., 2007). This would ultimately affect the health of the coastal ecosystem, including wildlife and human residing, and can have an impact on the sustainability of fisheries and aquaculture. Our findings are still preliminary (additional analyses including sediment/biota are underway and will be finalized by summer), and results from WRRI/Sea Grant will be combined with the previous data collection of 2018 in order to have a long-term data.

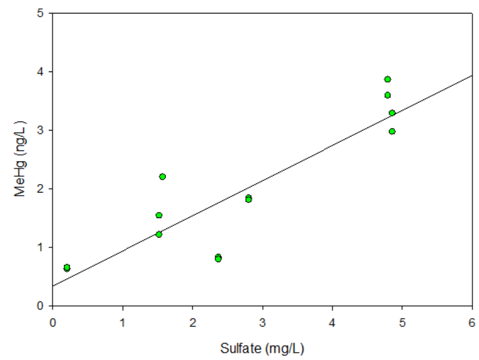

Figure 8: Relationship of sulfate and MeHg in freshwater wetland (r^2 = 0.78)

7. Outreach/Information Dissemination

The town manager of Columbia, Mr. Rhett White, was contacted to obtain a general understanding of people of the town about how salinization could induce the change in Hg levels and thus negatively impact their community. The information he shared with me was that the general residents were not aware of high Hg levels in fish, i.e., they even do not know about fish consumption advisories against Hg in the region. I have arranged a meeting with the mayor, aldermen, press, and other members of the town of Columbia to present the work I have conducted in their town. We have talked about mercury pollution, salinization of freshwater wetlands and Hg contaminated fish consumption. In this project, I have collaborated with several undergraduate students (including minority and international students) at UNC-Greensboro.

I have included an undergraduate research assistant (i.e., underrepresented group in STEM education) who is majoring in environmental biology as well as some other undergraduate students who voluntarily assist during field sampling, which showed students that science is not only something you study but also something you do.

In my institution, I participated in the Science Everywhere Festival and introduced mercury cycling to K-12 students by playing games. Finally, I have shared some of the outcomes from this study through presenting at professional conferences, including the WRRI 2020 Annual Conference (abstract accepted).

In my outreach and dissemination plan, I have reached into the K-12 students (through Science Everywhere Festival), undergraduate students (UNCG), and the community at the local levels (Town of Columbia, NC).

8. References

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Appendix 1:

DOC = Dissolved organic carbon

FW = Freshwater wetland

Hg (II) = inorganic mercury

MeHg = methylmercury

PDW = partially degraded wetland

PPT = part per thousand

SM = salt marsh

SO_4^{2-} = sulfate

SRB = sulfate-reducing bacteria

THg = total mercury

T-Middle = between T- North and T- South

T-North = north of Timberlake Observatory for Wetland Restoration

TOWeR = Timberlake Observatory for Wetland Restoration

T-South = south of Timberlake Observatory for Wetland Restoration

Appendix 2:

Presentations

1. Ulus Y., Tsui M., Ardon M., Sakar A., & Cohen T. (2019, September) Changes of aqueous mercury concentrations in a coastal freshwater wetland at North Carolina (USA) as impacted by storm surge due to Hurricane Florence in 2018. 14th International Conference on Mercury as a Global Pollutant. Krakow, Poland (Sep 8 - 13, 2019)

2. Ulus Y., Tsui M., Sakar A., Nyarko P., & Ardon M. (2020, March) Effects of seawater intrusion on mercury cycling in coastal wetlands at Albemarle-Pamlico Peninsula, North Carolina. WRRI Annual Conference. Raleigh, North Carolina (March 18 - 19, 2020)

Dissertations

Yener Ulus (Ph.D. dissertation, ongoing) Mercury cycling in coastal wetlands affected by saltwater. Advised by Dr. Martin Tsui.