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LINKAGES OF MERCURY AND METHANE CYCLES IN PIEDMONT STREAMS AND
RIVERS IN NORTH CAROLINA, AND IMPLICATIONS FOR MERCURY
BIOACCUMULATION IN FOOD WEBS

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ABSTRACT

Project title: Linkages of mercury and methane cycles in Piedmont streams and rivers in North Carolina, and implications for mercury bioaccumulation in food webs

Mercury (Hg) is a global contaminant, and its inorganic form has been thought to be methylated by sulfate-reducing bacteria to become highly toxic methylmercury (MeHg). However, recent evidence showed that methanogens, which produces methane (CH₄), can also methylate inorganic Hg. In twelve Piedmont streams, we examined the levels of total Hg, MeHg and CH₄ in different compartments (water, sediment, and/or biota). In general, we found very low levels of total Hg and MeHg, but high and variable levels of CH₄ in sediment porewater among sites. The direct relationship of Hg methylation and CH₄ production was not observed due to the fact that many stream samples had very low or undetectable levels of MeHg. In a control experiment using specific microbial inhibitors, our results demonstrated that Hg methylation in Piedmont stream sediment is mainly mediated by sulfate-reducing bacteria but there is a small but consistent role of methanogens in methylating Hg. Overall, this project is the first to conduct comprehensive sampling and analysis of Piedmont streams (within or near Greensboro, NC) for Hg and CH₄ cycling.

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Peter Blum (M.S. student) and Josh Brigham (Ph.D. student), both at UNC-Greensboro, completed the majority of the work outlined in this project, as part of their thesis/dissertation. We thank Kimber Corson and Matthew Monteverde (UNC-Greensboro) for assisting in the field/lab. We appreciate the assistance of Katherine Broadwater (UNC-CH) for methane analysis in Whalen lab. We also acknowledge the help from Dr. Chad Hammerschmidt and his group for performing mercury isotope analysis for our microcosm experiments. UNC-Greensboro Biology provided funding for P. Blum and J. Brigham to present parts of this project in regional and national conferences. Lastly, the funding from NC WRI for supporting this work is gratefully acknowledged.

1. INTRODUCTION

Atmospheric transport and deposition of mercury (Hg) contaminates the majority of natural ecosystems while a small fraction of systems are impaired by local point sources such as mining and industrial discharges (Morel et al., 1998). More importantly, the deposited Hg is mostly inorganic form [abbreviated as Hg(II)] and is not very bioavailable to organismal uptake (Tsui and Wang, 2004). However, Hg(II) can be efficiently methylated to become highly toxic methylmercury (MeHg), especially under anoxic conditions via anaerobic microbial communities (Gilmour et al., 1992), such as sulfate-reducing bacteria. MeHg can extensively bioaccumulate and biomagnify in aquatic food webs, leading to widespread fish consumption advisories (e.g., statewide fish consumption advisory in North Carolina) across the nation due to high Hg found in fish tissues (Mergler et al., 2007).

Methane (CH₄), a potent greenhouse gas, is produced by methanogens through methanogenesis, which is an important pathway for organic matter decomposition that occurs universally in anoxic aquatic sediments (Whalen and Reeburgh, 2000). Streams typically are supersaturated in methane (CH₄) and can result from allochthonous inputs as well as autochthonous methanogenesis. Studies have shown significant methanogenic potential and high abundance of methanogens on the streambed in Piedmont streams (Smith 2013), suggesting that zones of methanogenesis are widespread in stream ecosystems. Furthermore, methane concentrations may change radically from high to low gradient reaches, and with season (Jones and Mulholland 1998), reflecting in-stream conditions as well as lateral and upslope processes. Methanogen populations in streams are not well studied, but current studies in Piedmont streams indicate strong seasonal patterns and seasonal differences between streams in forested compared to urban landscapes (Smith 2013).

Recent experimental studies have shown the capability of certain groups of methanogens to methylate in both lab and field settings (Hamelin et al., 2011; Yu et al., 2013), suggesting that it is possible for the CH₄ and Hg cycles to be coupled in natural settings. In this work, we test the idea that the extensive methanogenesis in Piedmont streams may be coupled to Hg methylation. We hypothesized that: (1) streams and rivers with higher dissolved CH₄ will have higher levels of MeHg in water and sediment (porewater and solid phase), and high MeHg levels in consumers; (2) Hg methylation and methanogenesis are interrelated processes that affect bioavailability and bioaccumulation of MeHg. Our objectives are to: (i) examine spatial and temporal patterns of MeHg and CH₄ concentration in water, sediment and food webs; (ii) use experimental approaches to probe whether methanogens are important Hg methylators in controlled assays. We propose to use field surveys and experimental manipulation to assess the relationships between CH₄ and Hg methylation and demethylation in Piedmont streams.

2. METHODS

2.1. Field survey

A total of twelve Piedmont streams/sites were examined for mercury and methane distribution within or near the city of Greensboro, NC (**Fig. 1**). Study sites were chosen based on the variability in channel sizes and land use covers in the watershed. Intensive sampling was performed during low flow period in the summer of 2014.

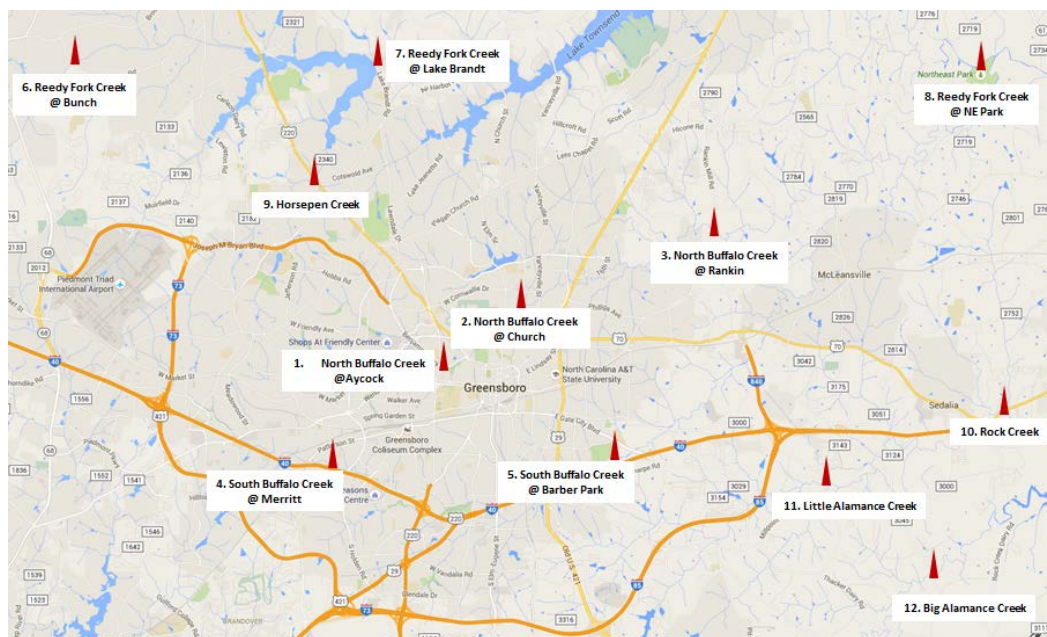


Fig. 1 Study sites for the field survey in the summer 2014.

At each site, a suite of abiotic and biotic samples were collected, including surface water, bulk sediment (< 1-mm sieved), porewater, seston, and dominant macroinvertebrates (including Asian clams (*Corbicula fluminea*) and hydropsychid caddisflies). **Table 1** summarized the specific sample types and analyses performed, in three different analytical laboratories.

Table 1 Summary of sample type collected and parameters analyzed in this study.

Sample type/analytical methods	THg and MeHg (UNCG Tsui lab)	CH ₄ (UNC-CH Whalen lab)	δ ¹³ C and δ ¹⁵ N (CPSIL) ^{a,b}
Surface water	UF and FIL fractions	Dissolved gas	Seston only
Bulk sediment	< 1-mm sieved	-	< 1-mm sieved
Porewater	FIL fraction	Dissolved gas	-
Macroinvertebrates	Tissues	-	Tissues
<i>Analytical methods</i>	<i>Cold vapor atomic fluorescence spectroscopy</i>	<i>Gas chromatography flame ionization detector</i>	<i>Isotope ratio mass spectrometry</i>

^a Colorado Plateau Stable Isotope Laboratory, Northern Arizona University (Flagstaff, AZ)

^b Data for stable C/N isotopes are not shown in this report, as further interpretation will be required.

2.2. Specific microbial inhibitor experiments

In late January 2015, we set up control experiments (a total of 80 individual microcosms) with the addition of different microbial inhibitors for stream sediments from four different sites with contrasting land uses and sediment properties within or around Greensboro, NC (i.e., #1 North Buffalo Creek, #4 South Buffalo Creek, #8 Reedy Fork Creek, and #11 Little Alamance Creek). For each microcosm bottle, we use 200 ml glass serum bottle with a gas impermeable stopper, in which we added 100 g of wet and unsieved stream sediment with 100 ml of reconstituted soft freshwater (USEPA, 2002) (**Fig. 2**).



Fig. 2 Picture of microcosm set up in this study (by P. Blum).

Specific microbial inhibitors were added according to **Table 2**, each treatment had a total of four replicates. The selection of microbial inhibitors and their final concentrations were based on the previously published studies on Hg(II) methylation (e.g., Fleming et al., 2006; Hamelin et al., 2011).

Table 2 Summary of treatments of microbial inhibitor experiment.

Treatment	Inhibited Microbes	Unaffected Microbes
Control	None	All types
BESA ^a	Methanogens	Sulfate-reducing bacteria, other types ^b
Na ₂ MoO ₄	Sulfate-reducing bacteria	Methanogens, others*
Na ₂ MoO ₄ + BESA	Sulfate-reducing bacteria, and methanogens	Other types
Chloramphenicol	All types	None

^a (2-Bromoethanesulfonic acid)

^b Other types especially refer to iron-reducing bacteria that can also methylate Hg(II) (Fleming et al., 2006)

All microcosms were pre-incubated for 10 days to allow the development of the natural microbial communities, and on day 10, we spiked isotopically enriched ²⁰⁰Hg(II) (96.4% purity; Oak Ridge National Laboratory) into each microcosm so as to double the total Hg in each microcosm, and microcosms were left to run for 8 more days before we sampled the sediment to analyze the fraction of ²⁰⁰Hg(II) being methylated to become Me²⁰⁰Hg by CV-GC-ICP-MS at Chad Hammerschmidt's Lab at Wright State University (Dayton, OH). The Hg methylation potential would be calculated as %Me²⁰⁰Hg (i.e., percent of ²⁰⁰Hg as Me²⁰⁰Hg). Meanwhile, we collected gas in the headspace from each microcosm over time (0, 0.5, 1, 1.5, 2, 3, 5, 7, 10, 13, 18 days) to measure CH₄ produced by the microbes.

3. RESULTS AND DISCUSSION

3.1. Dissolved methane data in field survey

All surface water and porewater samples contain measurable concentrations of dissolved CH₄. However, while the surface water represents a well-mixed pool of dissolved CH₄, the porewater pool of CH₄ is very heterogeneous and the sample CH₄ concentration varied widely, over 2-3 orders of magnitude. **Fig. 3** shows CH₄ data from two urban streams at different locations within the city of Greensboro. As shown, porewater CH₄ can be highly variable, and can be higher or lower than that in surface (overlying) water.

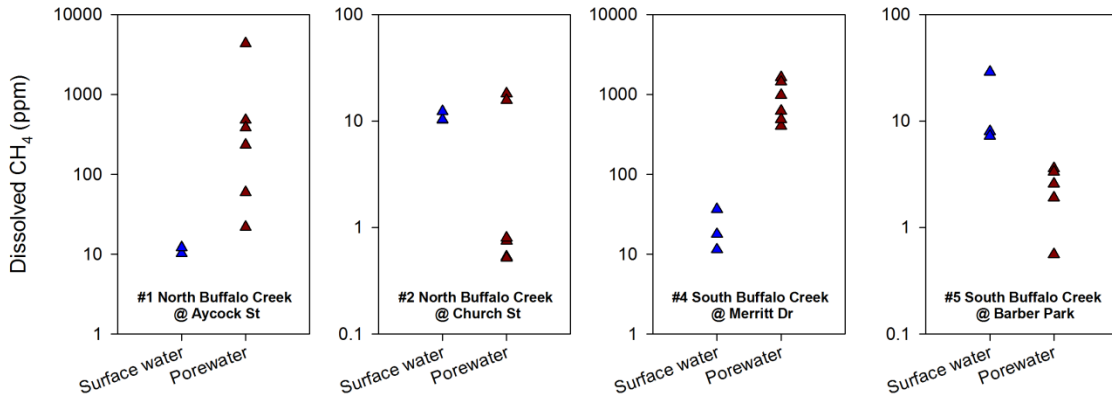


Fig. 3 Concentrations of dissolved CH₄ in surface water (collected in a fast-flowing portion within the channel) and porewater (5-10 cm in sediment) in two major urban streams in Greensboro.

3.2. Aqueous mercury data in field survey

All surface water (both UF and FIL fractions) and porewater samples have relatively low, but consistently above method detection limit (MDL, 0.2 ng/L), of THg concentrations (**Fig. 4**). FIL samples had about 50% of THg of UF samples. Porewater samples in most cases had lower THg than FIL samples. However, many sites had FIL surface water and FIL porewater with MeHg below our MDL of 0.04 ng/L (**Fig. 4**).

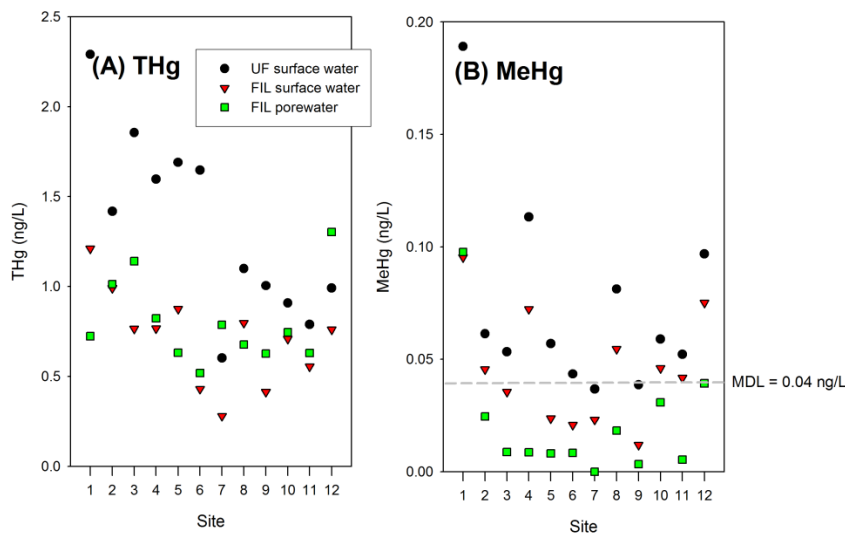


Fig. 4 Aqueous THg and MeHg data.

Interestingly, there was some relationship between THg and MeHg in UF surface water samples across sites (**Fig. 5**), being highest for both at an urban stream (#1 North Buffalo Creek @ Aycocock). The slope represents the mean fraction of THg as MeHg (or %MeHg) in these water samples, being 5.1%, and is at the low end of stream water %MeHg among other stream ecosystems (Brigham et al., 2009; Tsui et al., 2010).

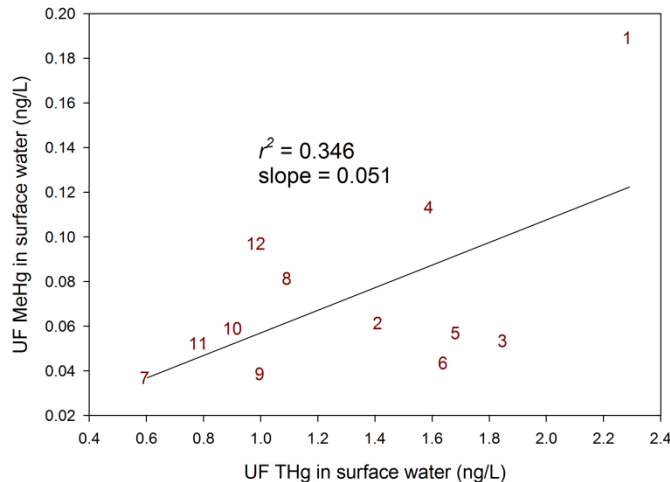


Fig. 5 Relationship between UF THg and UF MeHg across study streams.

3.3. Sediment mercury data in field survey

In stream sediments (<1-mm sieved), THg concentrations are very low but variable among streams, however, the majority of samples had MeHg levels (*data not shown*) below our MDL (~0.1 ng/g dw). It should be noted that the stream sediments in our study sites are in general very sandy and coarse, and low in organic content, but Hg is often positively correlated with organic matter content in surface sediment (Chakraborty et al., 2015), thus it is not surprising to observe low Hg contents in sediment across sites.

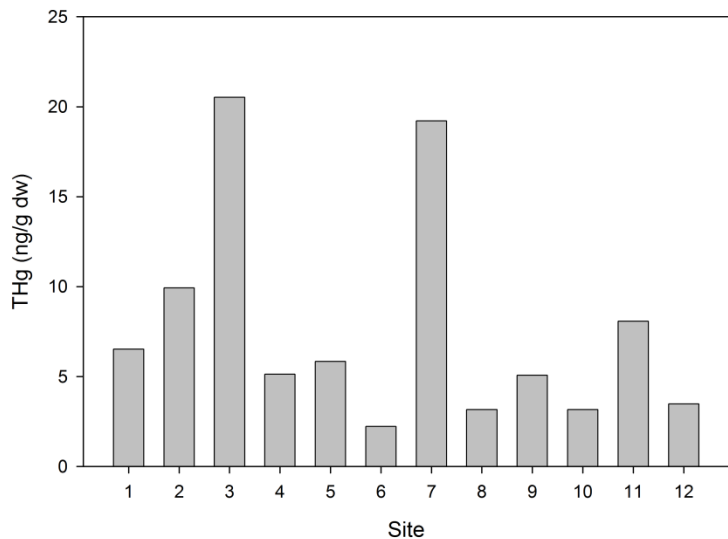


Fig. 5 Sediment (<1-mm) THg data.

In each site, we collected two major macroinvertebrates, Asian clams and hydropsychid caddisflies, when available (except two sites where we did not collect any clam samples). In general, these macroinvertebrates had %MeHg from 31-100%, and clams had variable but generally higher MeHg tissue concentrations than hydropsychids in the same site (**Fig. 6**).

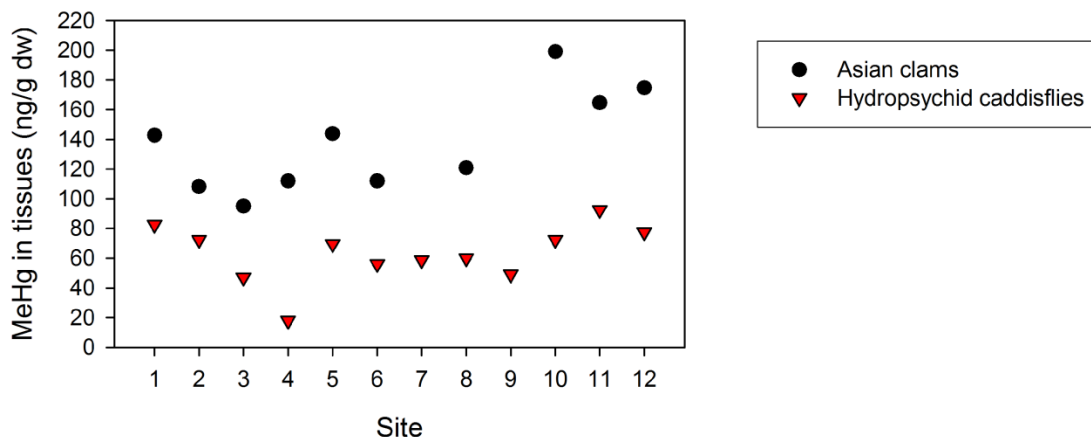


Fig. 6 MeHg in two macroinvertebrate tissues among study sites

3.4. Relationship between methylmercury and methane in field survey

Since a lot of FIL surface water, FIL porewater and bulk sediment had MeHg below our MDL, and thus we rely on the data of UF MeHg in surface water and MeHg in tissues of two macroinvertebrates to represent MeHg levels among study sites. For CH₄ data, we use the mean values of replicated analyses of surface water data but we only select the “highest” CH₄ data from porewater since they were very variable. As shown in **Fig 7A**, we did not observe significant relationship between CH₄ and MeHg in surface water, but we found that increasing CH₄ in surface water would have decreasing MeHg in tissue of biota (**Fig 7B**). As largely driven by a single data point, we found positive relationship between porewater CH₄ and UF MeHg in surface water among sites (**Fig 7C**). The relationship may be non-existing. However, increasing porewater CH₄ we found weak increases of MeHg in clams but not hydropsychids (**Fig 7D**).

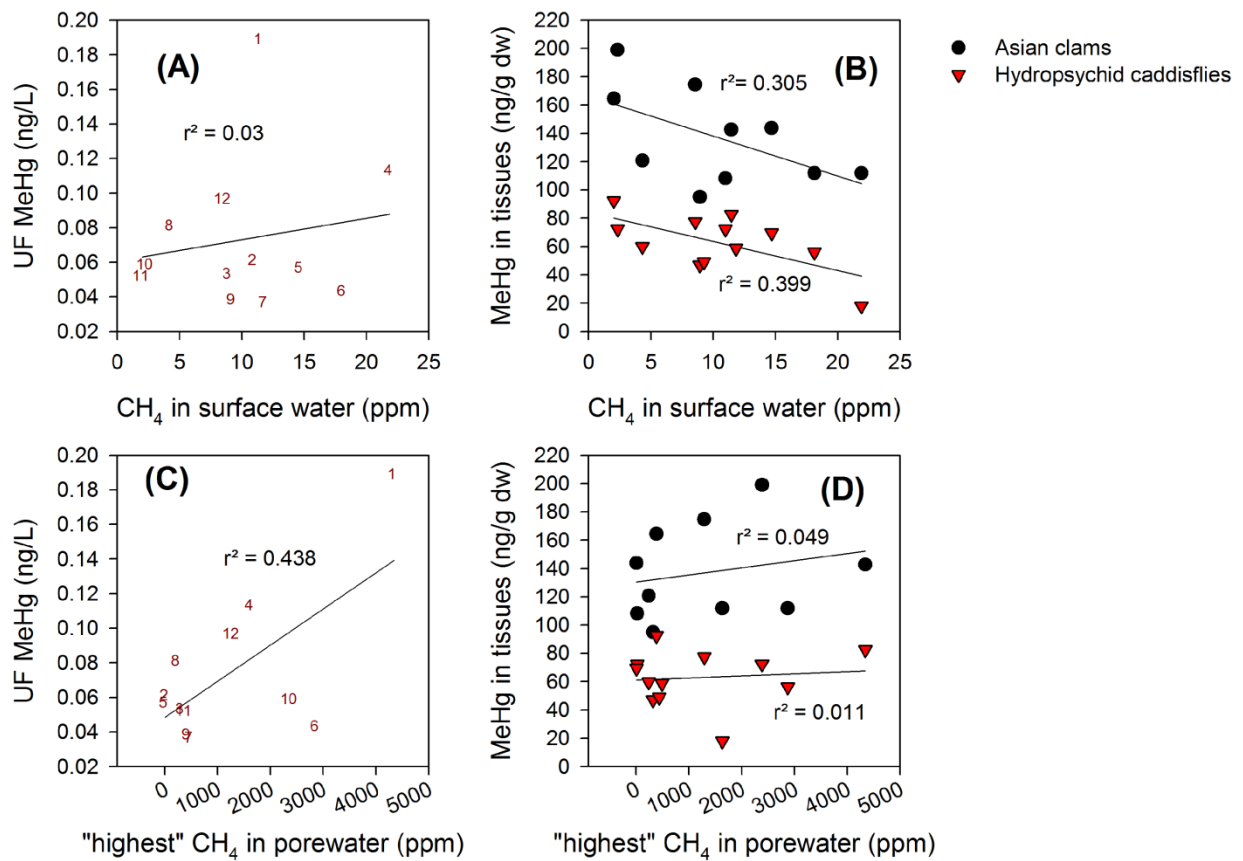


Fig. 7 Multiple relationships explored between CH₄ and MeHg among sites.

3.5. Microbial inhibition experiments

Our microcosm experiments have been analyzed for CH₄ samples over time and Me²⁰⁰Hg on the last day of incubation (i.e., day 18), and the data on %Me²⁰⁰Hg at the end of the incubation for the four stream sediments were shown in in **Fig. 8**. As shown, except site 11 sediments all sites had the highest mean %Me²⁰⁰Hg in the control without any microbial inhibitor, in most cases, by adding BESA (an inhibitor for methanogens) may decrease slightly %MeHg compared to the control. However, by adding Na₂Mo₄ with or without BESA, or just chloramphenicol (general bacterial inhibitor) we observed significantly lower %MeHg ($p < 0.05$). All these data strongly suggest that methanogens (inhibited by BESA) are not principal microbial groups in methylating Hg in these piedmont stream sediments, while we believe that sulfate-reducing bacteria (inhibited by Na₂Mo₄ and/or chloramphenicol) are the main methylators of Hg, similar to the many other studies on freshwater wetland and lakes.

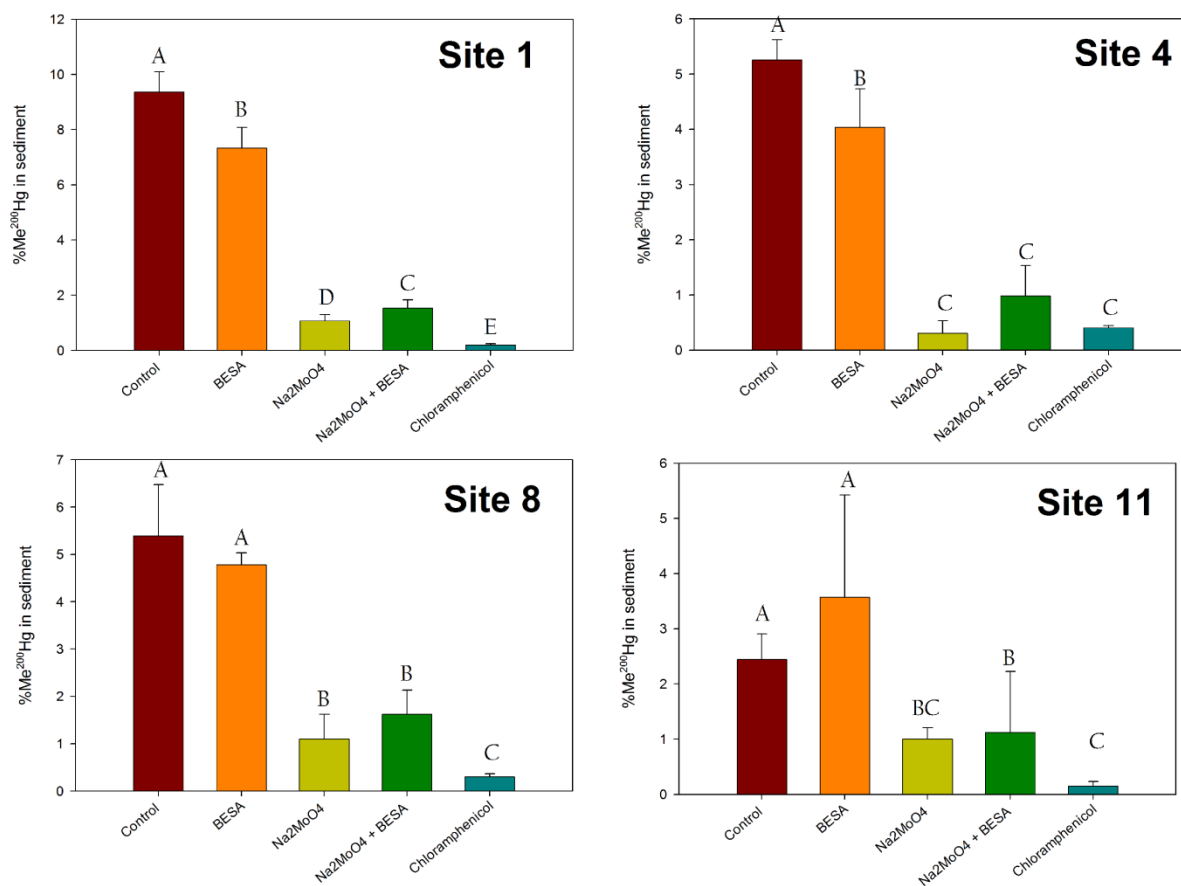


Fig. 8 %Me²⁰⁰Hg in sediment microcosms of different stream sediments on day 18, without any microbial inhibitors (control) or with different types of microbial inhibitors .

Interestingly, we found that for all uninhibited microcosms (i.e., control) there are a positive and strong relationship between methane produced and %Me²⁰⁰Hg in sediment (**Fig. 9**), such results may contradict with our above findings that methanogens are not important in methylating Hg but instead should suggest that the higher microbial activities (i.e., higher methane production) would be associated also with higher Hg methylation activities (potentially mediated by sulfate-reducing bacteria, methanogens and other microbes).

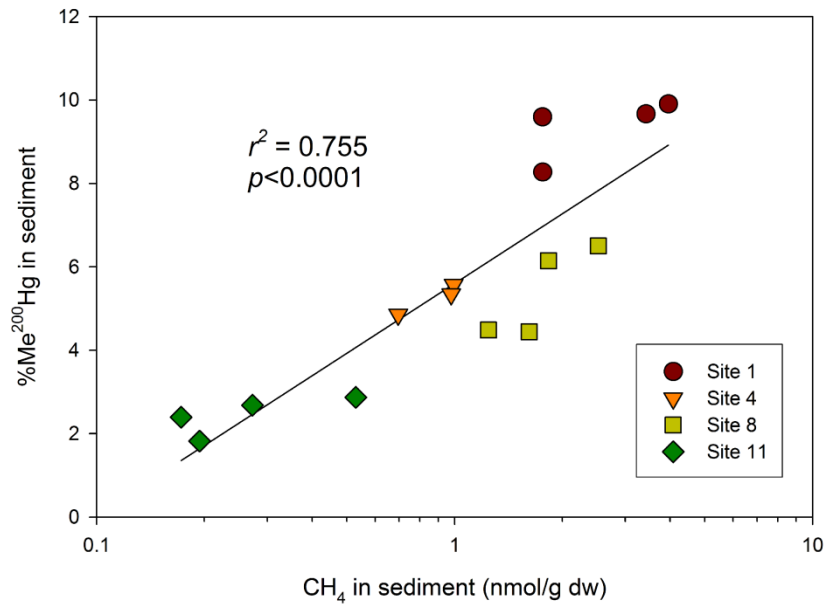


Fig. 9 Relationship between %Me²⁰⁰Hg and methane produced in sediment on day 18 of incubation without any microbial inhibitors (i.e., controls) among the four study sediments.

4. SUMMARY, CONCLUSION AND RECOMMENDATIONS

To our knowledge, it is the first comprehensive study examining Hg distribution in a variety of streams in Piedmont of North Carolina, especially within or near the city of Greensboro. THg and MeHg concentrations are in general at the low end of stream ecosystems across the United States receiving mainly atmospheric deposition (Brigham et al., 2009; Chasar et al., 2009; Tsui et al., 2009). Due to the fact that MeHg levels are very low, it reduces our capability to explore the relationship between MeHg with CH₄ cycling. However, we did observe very variable levels of CH₄ concentrations in porewater samples, suggesting active methanogenesis within the stream sediment, result not previously reported in streams in the region.

Based on our control experiments, the research results suggest that methanogenesis plays a minor role in methylating Hg(II) while sulfate-reducing bacteria are the dominant groups in methylating Hg(II), consistent with reports on other lake and wetland ecosystems (Gilmour et al., 1992). Finally, we conclude that Hg contamination is not very serious in Piedmont streams but more future sampling would be needed to examine the temporal variability.

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APPENDIX 1 (ABBREVIATIONS AND SYMBOLS)

THg = total mercury

MeHg = monomethylmercury

UF = unfiltered (water samples)

FIL = filtered (water samples)

CH₄ = methane

APPENDIX 2 (OUTPUTS FROM THE PROJECT)

Presentations

Blum PW, Hershey AE, Tsui MTK (2015) Methylmercury production from microbes in North Carolina piedmont streams. Society of Environmental Toxicology and Chemistry - Carolinas Chapter Annual Meeting. Raleigh, North Carolina.

Blum PW, Hershey AE, Tsui MTK (2015) Methylmercury production from microbes in North Carolina piedmont streams. Society of Freshwater Science Annual Meeting. Milwaukee, Wisconsin.

Brigham JS, Hershey AE, Tsui MTK (2015) Examining methane processes and methane derived carbon in food webs in North Carolina piedmont streams. Society of Freshwater Science Annual Meeting. Milwaukee, Wisconsin.

Theses/dissertations

Peter Blum (M.S. thesis, in progress) Methylmercury production from microbes in North Carolina piedmont streams. Co-advised by MTK Tsui and AE Hershey

Josh Brigham (Ph.D. dissertation, in progress) Examining methane processes and methane derived carbon in food webs in North Carolina piedmont streams. Advised by AE Hershey