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Title: Interactive Roles of Microbial and *Spartina* Populations in Mercury Methylation Processes in Bioremediation of Contaminated Sediments in Salt-Marsh Systems

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Objectives/Hypothesis

The goal of the project is remediation of mercury-contaminated sediments is being examined to establish the coupled roles and effects of *Spartina*, microbial activity and sulfate reducing bacteria (SRB) on mercury methylation in saltmarsh sediments. The fundamental conceptual model (see Figure 1) is that the incidence of mercury methylation is biochemically driven by sulfate-reducing bacteria (SRB) in the top layers of sediments. Furthermore in *Spartina* systems the production of methyl mercury is potentially lowered in the root zones or altered by biologically mediated demethylation of methylmercury in marsh sediments by the microbial community. Mercury toxicity and availability in the biological food chain is driven by methylmercury and its production is controlled by the net rate of microbial formation and removal within sediments.

The growth of *Spartina* and the associated injection of root-zone organic exudates and molecular oxygen into microbially active sediments influence sulfate reduction, mercury methylation and demethylation processes. Hence the bioavailability and ultimately the risk associated with mercury contamination in saltmarsh sediments is related to presence and growth of *Spartina*. Thus, understanding the relationships among sulfate reducing bacteria (SRB), demethylating processes, *Spartina* and sediment-based mercury will be useful for assessing sediment quality and developing and assessing in-situ remediation strategies and methods for risk assessment.

Approach

The research plan for the current growth season (March '00 _ February '01) include an enhanced focus on methylation and demethylation processes in sediments and pursuit of the effects of *Spartina* on the stabilization of mercury-contaminated sediments. The earlier research (year 01) focused on assessment of the biogeochemical properties of sediments has been reduced to focus on critical parameters directly linked to mercury availability and sulfate reducing populations. Earlier work in this area over the initial 18 months of the project clearly indicated the full equilibration of the sediments and the relevance of the current research to sediments systems in natural saltmarshes.

As described in our initial work plan, mercury demethylation rates will be determined in sediments by incubation with methylmercury using μg level injections of methylmercury in 1 cm, sediment core lifts and in homogenized slurries from sediment cores using an incubation time of 12 hr in both systems. Demethylation rates will be determined by the loss of methylmercury after incubation. Methylmercury will be determined by distillation, derivatization, chromatographic separation and thermal decomposition using a Tekron 2500 CVAFS (Smith, 1993), as with all current measurements. Recent improvements in the isolation of methylmercury from sediment and water involve distillation into a Teflon collection vessel with detection limits for methylmercury using this method of 2 ng/L for porewaters and 0.01 $\mu\text{g}/\text{kg}$ in sediments in SkIO laboratories.

These methods will provide potential demethylation rates because of the artificially high methylmercury concentrations involved in this experimental procedure. Therefore, in addition to these measurements, we are coordinating a multi_lab approach to make mercury demethylation measurements using radiotracer approaches using radioactive mercury and C_{14} labeled methylmercury. These methods are projected to provide the most sensitive and accurate means to estimate demethylation rates. Technical and scientific details and radiotracer expense associated with conducting such tracer studies call for collaborative efforts with others who are conducting these measurements and are interested in their application to controlled mesocosm systems. We are currently organizing a joint exercise to make mercury demethylation rate measurements in a variety of mercury contaminated sediment types using both multiple radiotracer and conventional methods. This exercise will provide for calibration of radiotracer methods with other methods and will provide a context for interpreting data generated in this study. In addition, these studies will provide a set of high quality demethylation rate measurements that would otherwise not be available. Currently we are organizing these studies with Drs. Eric Roden (University of Alabama), Ron Oremland (USGS), and Mark Hines (University of Alaska) and anticipate this to be an integral component of the year 03 plan.

An additional objective for this period will be to investigate the influence of *Spartina* on sulfate reducing bacteria (SRB) populations in contaminated marsh sediments. The influence of *Spartina* on methylation (see Figure 2) indicates that the resident SRB population could be influenced such that lower levels of methylmercury are produced in these sediments. Our previous investigations have demonstrated that the primary source of bioavailable methylmercury in mercury contaminated marine sediments is derived from the activity of SRB. Furthermore, our recent HSRC funded studies have shown that genetically distinct sulfate reducing bacteria (SRB) methylate mercury with various incidence rates, relative to sulfate reduction activity (King et al., 2000). In particular, the *Desulfobacterium* group of the SRB appears to be the mercury methylators with the highest incidence rate for methylation in marsh sediments. *Desulfobacterium* methylated mercury at an incidence rate that was 92 fold higher than the lowest mercury methylating group *Desulfobulbus* that was studied (Table 1). Based on these studies, it was concluded that the composition of SRB populations in mercury contaminated sediments is a primary

parameter responsible for controlling the biological availability of mercury in contaminated marine sediments.

Table 1. Incidence of Mercury Methylation in Various Sulfate Reducing Bacteria

SRB Taxon	MMR/SRR (pmole [Hg]/mole[S ₀])	Relative rate (normal to <i>Desulfobulbus</i>)
<i>Desulfobulbus</i>	1.40 ± 0.4	1
<i>Desulfovibrio</i>	6.83 ± 1.0	4.9
<i>Desulfobacter</i>	20.44 ± 4.7	14.6
<i>Desulfococcus</i>	22.93 ± 0.65	16.4
<i>Desulfobacterium</i>	128.62 ± 15.0	91.9

Through our HSRC sponsored activities we have utilized these data to develop a kinetic model that can be used to predict the formation rate of methyl mercury in contaminated sediments. This model incorporates measurements of sulfate reduction activity, mercury concentration, and the structure of SRB populations. Initial studies suggest that this model significantly improve our ability to predict mercury methylation activity in contaminated marine sediments.

As a component of our studies during this phase of the project, we will pursue and utilize these findings (and methods developed during the course of these studies) to explore the influence of *Spartina* on SRB populations and activity. In addition, we will determine the species composition of *Desulfobacterium* species present in the BERM mesocosm systems. This information will be used to refine existing models and to extend our understanding of how complex biogeochemical and plant processes interact to influence mercury cycling in contaminated salt marsh systems. Specifically, our objectives are twofold. First, we will determine the structure of SRB communities in sediment cores from pristine and contaminated mesocosm systems. These studies will be conducted in the BERM mesocosms over a full annual cycle to capture the influence of plant growth on SRB populations. These studies will utilize SRB group-specific 16S rRNA targeted oligonucleotide probes and methods utilized in our previous studies (Frischer et al., 2000). This information will be used as model input so that methylation rate can be estimated in the contaminated marsh sediments. Second, we will determine the species composition (richness) of *Desulfobacterium* species in each of the three mesocosms. In these studies 16S rDNA targeted oligonucleotide PCR primers specific for the *Desulfobacterium* group of SRB will be utilized to amplify, clone, and sequence representative *Desulfobacterium* species from LCP marsh sediments in the presence or absence of *Spartina*.

In summary, the results of these studies will add to our continued understanding of the interactions between plant processes and mercury cycling, particularly with respect to methylmercury formation and cycling. These insights will then form the basis for

improved risk assessment and the development of innovative in situ bioremediation strategies for mercury contaminated marine sediments. Understanding controlling processes for methylmercury production in sediments is the critical question being addressed to understand the processes, which control mercury endpoints in evaluating risk and designing and evaluating in_situ bioremediation technologies. Our research is directed at a fundamental assessment of methylation/demethylation processes and their application to sediments contaminated with hazardous wastes.

Expected Results: The project is being conducted at the Bioremediation Environmental Research Mesocosm facility (BERM) at the Skidaway Institute of Oceanography (SkIO). Three mesocosms are in their second season of operation with sediment placement and equilibration having been completed. Sediments in the three mesocosms were from the contaminated tidal saltmarsh at the LCP site (EPA IV Superfund site in Brunswick GA) and an uncontaminated Skidaway saltmarsh. One contaminated saltmarsh mesocosm and an uncontaminated saltmarsh mesocosm contain *Spartina*, while the remaining contaminated saltmarsh mesocosm contains LCP sediment without *Spartina*.

Based on our year_to_date quarterly measurements in 10_cm, sediment cores, the mercury levels of the sediments are as indicated in Table 2. The LCP sediments contain 13.3 mg/kg of total mercury and are generally representative of the extensive saltmarsh area associated with the LCP site, while the uncontaminated Skidaway (Priests Landing site) sediments contain 51 :mg/kg of total mercury. Whole_sediment methylmercury levels are 3.62 and 0.27 :g/kg, respectively for the LCP and Skidaway sediments.

Table 2. Mercury content of LCP and Skidaway (Priests Landing) saltmarsh sediments for period of June '99 to March '00.

	Sediment		Porewater
	Total Hg	Methyl Hg	Total Hg
Priests Landing	0.051 mg/kg	0.27 :g/kg	227 ng/L
LCP Site	13.3 mg/kg	3.62 :g/kg	220 ng/L

Of critical relevance are the porewater levels of total mercury species in the sediments. The total mercury in porewater samples, inclusive of data for 10_cm cores and parallel in_situ sipper samples, in the mesocosm systems is 227 ng/L and 220 ng/L for the Skidaway and LCP sediments, respectively. These results are indicative of the potential similar bioavailability of mercury for methylation in the two sediments. In that porewater concentrations of mercury are virtually equal in both contaminated and uncontaminated sediments, containing total mercury at levels that are different by a factor of over 200 (i.e., 260:1), indicates the controlling link for methylmercury production may be related to factors controlling and influencing biological activity.

The current data for the systems are those through March '00 of the current growth season. The results to date for porewater methylmercury concentrations are presented in Figure 2 and indicate the correlation of porewater methylmercury levels with sulfate reduction rates (SRR). The indicated SRR values are 10 cm_{integrated} averages for intact core analyses for the sediments and porewater methylmercury levels are averaged values for 10_{cm} cores and parallel in situ sipper analyses. The SRRs indicate activities ranging from 100 to 500 nmole/cm³_{day} over the season and reflect in part, the temperature variations for the period.

Sediments with *Spartina* contain background levels of methylmercury in the range of 1₁ ng/L and indicate no significant correlation with SRR. This is the outcome for both of the uncontaminated and contaminated sediments with *Spartina*. For the contaminated LCP sediment without *Spartina*, there is a positive correlation indicated for porewater methylmercury levels and SRRs. In *Spartina*_{free} systems, sulfate reduction is therefore paralleled by the elevation of porewater methylmercury in the sediment. This correlation is as expected through the stimulation of sulfate reducing bacteria found previously in related HSRC/S&SW research. The concentration values for porewater methylmercury are well above the background levels for vegetated *Spartina* mesocosms.

The lack of stimulation of methylmercury formation in the contaminated mesocosm with *Spartina* and its close correlation with the pristine sediment are projected to be related to (i) a low level of methylation in both *Spartina* sediments due to the phylogenetic composition of the microbial communities and (ii) the enhancement of demethylation of in situ methylmercury in parallel with production. The equal levels of total available mercury in the sediment porewaters indicate a similar potential for methylation in the three sediment systems, independent of "contamination" status. The assessment of the positive impact of *Spartina* growth on remediation of mercury_{contaminated} sediments is being pursued in the current growing season. Furthermore, an enhanced understanding of the microbial role in the methylation and associated demethylation of mercury is planned.

Supplemental Keywords

Methylmercury, mercury toxicity, and demethylation

Publications and Presentations

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For Further Information

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