Report as of FY2007 for 2006WA149B: "Effects of Sediment Oxygenation on Methylmercury Bioaccumulation in Benthic Biota"

Publications

- Water Resources Research Institute Reports:
  - Dent, Stephen and Marc Beutel. 2007, Effects of Sediment Oxygenation on Methylmercury Bioaccumulation in Benthic Biota, State of Washington Water Research Center, Washington State University, Pullman, Washington. State of Washington Water Research Center Report WRR-28, 11 pages.
- Other Publications:
  - Dent, Stephen and Marc Beutel, 2006, Effects of Hypolimnetic Oxygenation on the Bioaccumulation of Hg in Benthic Organisms, Platform Presentation at the Eighth International Conference on Hg as a Global Pollutant, August 2006, Madison, Wisconsin.

Report Follows

PROBLEM AND RESEARCH OBJECTIVES

Mercury (Hg) contamination of fish in lakes and reservoirs is a serious, widespread threat to wildlife and humans that eat lake fish. Hg is a widespread contaminant due in part to its long residence time in the atmosphere. Hg discharged to the atmosphere - power plants are a common source - travels long distances before entering aquatic systems via atmospheric deposition, thereby contaminating relatively pristine ecosystems far from the discharge source. Another key to Hg's potency as a contaminant is its potential to bioaccumulate in biota. Inorganic Hg from atmospheric deposition can be microbiologically transformed to methylmercury (MeHg) by sulfate reducing bacteria (SRB). MeHg accumulates in organisms at the base of the food chain (eg. worms, algae) at concentrations many times higher than in the water. MeHg biomagnifies in tissue of biota at higher trophic levels (eg. insects, fish, water fowl) as they consume contaminated prey.

Anaerobic bottom sediments occur in many productive, moderately deep lakes during the summer and fall, and provide an ideal environment for SRB activity and the concurrent formation of MeHg. MeHg accumulates in sediment pore water and then diffuses into overlaying lake water. Once mixed into the lake, the MeHg bioaccumulates in plankton, then biomagnifies through upper trophic level biota that consume contaminated prey. This phenomenon has been observed in a number of lake ecosystems, where studies have correlated anaerobic conditions with MeHg accumulation in bottom water, and lake mixing with bioaccumulation in lower trophic level biota. Thus, bottom water anoxia coupled with lake mixing is a key entry mechanism for getting Hg into aquatic food webs.

The preliminary goal for this research effort is to evaluate a key concern regarding lake oxygenation; essentially, will the recolonization of previously "dead" anoxic sediments exacerbate the accumulation of Hg up the food web by exposing a lower trophic organism to contaminated sediments. To fulfill this goal several objectives need to be met. First, the selected test organism, the benthic oligochaete *Tubifex tubifex*, needs to be successfully grown in large enough colonies to obtain a broad sample population. Second, an analytical method capable of discerning between total Hg and MeHg is required considering only the MeHg fraction will be transferred up the food web. Third, undisturbed sediment water interfaces from the designated study site must be obtained. Ideally, the oligochaetes would be placed in undisturbed sediment/water interface incubations with known sediment Hg concentrations at varying degrees of oxygenation. The degree of MeHg accumulation in organism would then either validate or disqualify the concern described above.

Early on in our efforts it was realized that colonization of our selected benthic oligochaetes to significant numbers would not be possible within the time frame of this project. This was due to three factors: 1) the unexpected die off of our original starter colony; 2) the slow turnaround from stock *Tubifex tubifex* provider; and 3) the time needed to grow a new starter colony to ample numbers. In order to proceed, our focus shifted to evaluating a synthetic biomimetic device to imitate the oligochaetes interaction with Hg. The methodology section below describes in detail our process for developing a biomimetic device utilizing solid-phase micro extraction (SPME) fibers. Although SPME fibers have been used successfully as biomimetic devices for compounds such as TNT and PCBs, this is the first case of using these devices as a biomimetic device for Hg.

METHODOLOGY

Benthic Oligochaetes: Benthic oligochaetes can be harvested by sieving sediment and isolating the organisms. Once isolated outside their protective sediment the worms come together and form a protective ball. These balls are typically used for starter colonies. Our lab received a ball of *Tubifex tubifex* from Dr. Billie Kerans of Montana State University's Department of Ecology in August of 2006. The organism strain had been cultured in MSU's lab since 1997, originating from a hatchery in California. It was important to get a strain that was acclimated to laboratory conditions and far removed from background Hg contamination present in most aquatic systems. Worms were cultured using the "Efficient method for culturing sludgeworms (*Tubifex tubifex*) for use in sediment toxicity tests" (Browen, Conder and La Point; 2002). Worms were placed in an aquarium with quartz sand and DI water. The prescribed food for the organisms was a combination of distilled water, cleaned iceberg lettuce, and flake fish food blended into a paste. Food was dispensed out in 50 mL aliquots every two weeks.

Biomimetic Devices: Solid-phase microextration fibers were selected as potential surrogates for the interaction of *Tubifex tubifex* with Hg in contaminated sediments. Our approach was based on a modified method from Dr. Jason Conder of the University of North Texas who successfully used SPME fibers as a biomimetic device for benthic oligochaete interactions with TNT. There are many different types of SPME fibers, each one of them very specific to the constituents that are being sampled. We chose a polydimethylsiloxane (PDMS) coating for its affinity for organic Hg species and its use in some Hg analytical techniques. Fibers were obtained through Supelco in 50 cm raw fiber lengths and then cut into a more manageable 2 cm pieces. PDMS is a hydrophobic material which requires a holder that could physically hold the fibers in the solution. Fiber holders were constructed out of pure Teflon disks. The disks were folded in half and slits were cut along the seam. Fibers were successfully held within the slits in the disk by friction and the density of the Teflon disk kept the fibers submerged. All fibers were pretreated by soaking in reagent grade methanol, rinsed in DI water, and let dry in a clean hood for 12 hours.

Chemical Extraction Method: In order to begin calibrating the fibers for a new in situ monitoring technique, a non-solvent based liquid extraction method was developed. This new method was compatible with our current Hg analytical device, a Tekran 2600. The methods included: (1) a "hard" complete digestion in aqua reaga followed by the addition of Bromine Monochloride (BrCl); and (2) a "soft" digestion using BrCl alone, which is typically used for water sample digestion. Fibers were placed three to a holder and soaked in DI, 0.5 ug MeHg/L, and 1.0 ug MeHg/L spiked water for 24 hours to ensure equilibrium was reached. The MeHg stock used was prepared by Frontier Geoscience at a concentration of 1 ug/L unpreserved. Literature values for Hg equilibrium with fibers are on the range of 1 to 2 hours; equilibrium time was not further explored in this experiment. Using a technique we coined as "clean tweezers/dirty tweezers", the individual assigned the dirty tweezers removed the soaking holder with fibers from the incubation vessel and dipped it in a DI bath. The individual assigned the clean tweezers then removed the fiber from the holder and placed them in a digestion vessel containing one of the two digestion solutions. Fibers were digested over night for a period of 12 hours each. Digestion was ceased by removing the fibers from solution with acid washed tweezers and adding BrCl to the aqua reaga vials.

Species Extraction: Using the same procedure listed in the Chemical Extraction Method above with only the soft digestion, the effect of Hg species sorption to the fiber was evaluated. Fibers in their holders were soaked in DI, 0.5 ug/L, and 1.0 ug/L inorganic Hg (Hg^{2+}) and MeHg solutions. The

Hg^{2+} solution was made from a 1,000 mg/L preserved stock used for calibrating the Tekran 2600. Fibers were extracted three to a digestion vial to increase detection sensitivity.

Extraction Time Series: Hg extraction from the fibers over time was evaluated using the same methods described above with varying times of fiber exposure to the digesting solution. Fibers were all soaked in a 1.0 ug/L unpreserved MeHg solution and digested in increments of 12, 24, 36, 48 hours. This procedure was then repeated with the addition of a 4 and 8 hour extraction and a parallel run of fibers soaked in an unpreserved 1 ug/L Hg^{2+} solution. The unpreserved Hg^{2+} solution was made by dissolving 1 g of Mercuric Chloride in 1 L of DI and then brought to the desired concentrations by further dilution with DI.

Natural Water Extractions: Incubations were again repeated using the above procedure, this time substituting natural filtered water from a local wetland for DI water. Natural water was spiked to 0.5 ug/L, half with preserved Hg^{2+} and half with unpreserved MeHg. Extraction time was adjusted to 10 hours based on results from the Extraction Time Series (See Principal Findings and Significance).

Mercury Analysis: Hg analysis was performed following EPA's method 1631 for total Hg using our in house Tekran 2600 Cold Vapor Atomic Fluorescence Spectroscopy (CVAFS) Autoanalyzer. All glassware and devices used were cleaned and soaked in a 50% HNO_3 acid bath for two days. Clean hands/dirty hands techniques were used when ever handling fibers, samples, and standards.

PRINCIPAL FINDINGS AND SIGNIFICANCE

Culturing benthic oligochaetes takes time and experience. After the initial die off of our original culture it became apparent that growing a large enough population in the time window of this project would not be possible. Instead, the process of developing an in situ biomimetic sampling device to take the place of the benthic oligochaetes is presented here.

Chemical Extraction Method: Although PDMS fibers have been used in Hg analytical techniques, they are typically not used as in situ sampling devices and based on our analytical capabilities it was not possible to use the standard thermal or solvent extraction methods that the fibers were designed for. As described above, a hard and soft digestion method were developed and evaluated. The assumption was made that the hard, soil type digestion would release all of the Hg from the PDMS matrix. Both the hard and the soft digests performed identically and in a linear fashion between the DI, 0.5 ug/L, and 1.0 ug/L MeHg solution soak. Based on the performance of this extraction series the soft digest was selected for all subsequent extraction series due to its compatibility with Method 1631.

Species Extraction: SPME fibers are hydrophobic and designed to sorb organic compounds. Historically SPME has been used for MeHg analysis. To verify the selectivity of the PDMS fiber, parallel incubations of both MeHg and Hg^{2+} were conducted using the soft digestion method in soaks of 0, 0.5, and 1.0 ug/L of each species. The fibers per digestion vial were increased from 1 to 3 in order to increase detection sensitivity. Both MeHg and Hg^{2+} had linear extraction slopes; however, the Hg^{2+} had nearly double the sorption capacity. This demonstrated that the fibers were not specifically selective for MeHg and that a speciation analytical technique would be required when using the fibers in heterogeneous environmental Hg solutions.

Extraction Time Series: A time series extraction test was used to determine the efficiency of the extraction. Ideally, a mass balance would have been preferred but as the fibers extract such a minute amount out of solution, our analytical techniques are not sensitive enough to "close the loop". Fibers soaked in 1 ug/L MeHg solution were extracted in 12, 24, 36, and 48 hour time intervals. The 12 hour digestion resulted in the most Hg extracted; the amount extracted dropped by half at 36 hours. This may be due to the creation of sorption sites in the siloxane matrix as it is oxidized by BrCl. The subsequent increase in the Hg release at hour 48 may indicate that the oxidizing power of BrCl was spent. The time series was repeated again with the added time points 4 and 8 hours, and with a parallel series with unpreserved Hg^{2+}. Unpreserved Hg^{2+} was used this time to see if the acidic preservative had any type of influence on the sorption. MeHg extractions at 4 hours had higher yield in one vial but less in another; however, the 8 hour extraction was fairly consistent with the 12 hour extraction from the previous run. The rest of the time series was on par with the previous run. The extraction of Hg^{2+} however, was consistently lower than the MeHg extractions, indicating that the preservative may have enhanced the sorption of the inorganic species. The trend over time with the Hg^{2+} was similar to that of MeHg, however Hg^{2+} sorption reached its lowest point one time step earlier at hour 24.

Natural Water Extractions: To determine the effect of competing ions and complexes on the sorption of Hg to the fibers, filtered wetland water was used in place of DI water. Fibers were soaked in 0.5 ug/L Hg^{2+} and MeHg spiked natural water followed by extraction. Results showed a greater than four fold decrease in the fibers ability to sorb both forms of Hg. This implies that either other cations are out competing Hg for negative sites on the fiber or anions are complexing with the Hg species and creating neutral or negative complexes. Either way it would appear that the sensitivity of the fiber decreases with the presence of other ions and complexes in solution.

Concluding Thoughts: It was unexpected to have as much inorganic Hg sorb to the fiber as observed, even considering the unpreserved Hg^{2+} extraction. It is apparent that for this technology to be a successful Hg biomimetic device, analytical speciation capabilities must be utilized. Our research group has solicited the help of Dr. Gary Gill and the Battelle Marine Sciences Laboratory in Sequim, Washington. The analytical laboratory has full Hg analysis capabilities. The natural water portion of this project will be carried out in the summer of 2007 at the Battelle facility. Another issue that will be addressed, based on these preliminary results, is that SPME fibers have a very small volume of coating, which is proportional to how much Hg will be sorbed. In order to be applicable in a natural setting our research group will use PDMS coated disks, with an order of magnitude greater volume, that can be inserted directly in the sediment. By collaborating with Battelle's Hg laboratory and increasing the volume of PDMS per sampling unit, we intend to produce a fully functional Hg biomimetic device within the near future.