Assay of Sediment Sorbent Amendment Efficacy and Effect
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Overview

One potential remediation option for the South River involves sediment sorbent amendment to deduce sediment mercury bioavailability without unintentionally diminishing important benthos processes. Candidate sorbents range from expensive nanoparticles such as thiol SAMM®, to Exponent's moderately priced Sedimite®, to various lower-cost biochars. Given this diversity of sorbents, it is sensible to develop a tool to quantify their relative sequestration efficacies and unintended impact on the benthos. To that end, laboratory bioassays are being done to measure the influence of two top candidate remediation materials on bioaccumulation (reflecting sequestration efficacy) and an important biological effect, detrital processing. Sediments from above and below the historic mercury source are being tested without sorbent amendment, with Sedimite® amendment, and with Cowboy® biochar amendment. Treatment adverse effect on benthic detrital processing is any decrease in amphipod feeding rate in the assays. Detrital feeding/processing is both a measure of effect on individual organisms and an effect on a crucial process in lotic systems such as the South River study reach.

The freshwater amphipod, *Hyalella azteca*, was selected as a convenient representative of the collector/shredder guild. It is a common North American collector/shredder, is used widely for ecotoxicological studies, and was used previously by VIMS to study South River sediment remediation with Sedimite®. It represents the epibenthic invertebrate guild that dominates the base consumer level of the South River study reach food web.

Differences among treatments on both bioaccumulation and detrital feeding/processing are being quantified 0, 1.5, 3, and 6 months after sorbent blending with sediment. Preliminary results from the first three time periods are discussed below.

Study Treatments

Sediments and sediment/amendment mixtures to be used in the bioassays were held in separate 25 mL loosely-covered cups of water. In amended sediment treatments, 0.11 g dw of sorbent was blended in 1.0 g dw of sediment. Using the biochar treatment for the Dooms Crossing sediments as an example, biochar was blended with dry Dooms Crossing sediment and then 1.11 gm portions added to each of 120+ cups of aged tap water. A loose cap was then snapped onto each cup and enough cups for all four assays were stored together at room temperature until used. For the first assay, 30 cups of each blend were used to fill 30 separate wells into which one

amphipod and one leaf disc were later added. Before the leaf disc and amphipod were added, sediments were allowed a day and a half to settle. After settling and before the addition of leaf and amphipod, the sediment in each well was gently stirred to uniformly mix the material that had size fractionated during settling. The 30 wells were the feeding rate replicates for each treatment, e.g., the "Dooms Crossing Biochar" treatment.

Ten-day bioassays were conducted in 6-well tissue culture plates (Figures 1 and 2) incubated at 23.5 C in the dark. The following treatments were included in the design with 30 observations (amphipods) per treatment for feeding rate. In practice, some observations in each treatment were lost because a few amphipods died and others burrowed so effectively into sediments that they could not be found at the end of the assay. The realized number of observations was generally in the range of 25-30. These treatments were established with sediments from either North Oak Lane and Dooms Crossing. Each amphipod was placed into a well of a 6-well plate and provided a leaf disc (circa 1 mg dry weight, dw) to consume during the 10 day assay. Thirty wells containing only leaf disc and amphipod were used in each assay as a positive reference. Thirty wells with only water and leaf discs were used to mathematically correct for leaf disc weight loss due to leaching during the 10 day assays. The following additional treatments were created for each of the two sediments

- Leaf disc plus 1.1 gm (dw) of unamended sediment
- Leaf disc plus 1 gm (dw) sediment into which 0.11 g Sedimite® had been mixed
- Leaf disc plus 1 gm (dw) sediment into which 0.11 biochar had been mixed

At the end of each assay, amphipods were transferred to separate cups containing only aged tap water so that they could void any materials from their gut prior to mercury analyses. Because single amphipods could not be reliably analyzed for mercury concentration, 4-6 amphipods were pooled for analysis, resulting in 4-6 pooled samples for mercury concentration determination per treatment.

Some sediment or sediment/amendment aliquots were used immediately after blending (0 to 10 days post-blending, designated "5 days" in figures below). Other cups of material were used 40 and 95 days after being blended and hydrated. A final assay will be done with such materials 6 months (180 days) after blending and hydration. The table below summarizes the qualities of the materials used in these assays.

GENERAL CHARACTERISTICS OF MATERIALS				
Mercury Concentration (ug/kg dry wgt)				
Material	Mean	Std Dev	95% CI	n
Leaf Disc	0.0044	0.0013	0.0030-0.0060	5
Biochar	0.4693	0.0978	0.348-0.591	5
Sedimite	3.875	0.0562	3.805-3.945	5
North Oak Sediment	37.8	1.9	36.4-39.1	10
Dooms Sediment	8121	286	6991.0-9251.0	14
Sediment Ash Wgt (%)				
North Oak Sediment	9.02	0.86	7.95-10.09	5
Dooms Sediment	8.79	2.61	5.55-12.03	5

Results to Date and Preliminary Speculation

The mercury concentrations in amphipods exposed to the North Oak-related sediment treatments (top panel of Figure 3) had no apparent trends from the first assay (5 days) through the second assay (40 days) to the third assay (95 days). Those for amphipods consuming only leaf (top panel, black symbol/line) were generally not different from those of the three North Oak sediment treatments (top panel, red, blue, green symbols/lines). The amphipod mercury concentrations remained lower for the Sedimite® treatment than those of the unamended Dooms sediment treatment. The mean amphipod mercury concentration for the biochar Dooms sediment treatment was lower than the untreated sediment only for the first assay. It seems that the unamended and biochar-amended Dooms sediment treatments had similar amphipod mercury concentrations in the two latest assays. This was disappointing but a final assay remains to be done before any definitive conclusions can be made.

In the 30 wells in which only leaf material was available for the amphipod to consume (black symbol/line in top panel of Figure 4.), the amphipod feeding rate was highest of all treatments, being approximately 0.2 mg of leaf/mg of amphipod each day. The lowest feeding rates were consistently those for amphipods in the Sedimite®-amended treatments (red symbol/line), being 0.1 mg/mg-day or lower. When Sedimite® was absent and amphipods could feed on both leaf and sediment, the feeding rates of the biochar treatment were consistently similar to those of the unamended sediment treatment. The biochar did not seem to influence feeding rate. Visual observations (e.g., Figure 5) revealed that the Sedimite® is so fine that the amphipods stir it up from the sediments during their normal activities. The Sedimite® then settles back partially, or sometimes completely, covering the leaf disc. This creates a physical barrier that inhibits feeding on the leaf.

Summary of Results to Date

Results gathered to date suggest that Sedimite® might reduce mercury accumulation in the amphipods longer than does biochar. Speculating from the VIMS trophic biomagnification models, it seems that the reduction in mercury bioaccumulation would not enough to bring concentrations in bass or other game fish below consumption advisory concentrations.

In field conditions in which resettling of Sedimite® might occur, there could be an impact on detrital processing/feeding of the shredder guild. It remains unclear whether the Sedimite® impact would be substantially different from that occurring for natural fines in this South River reach. An important determinant would be the amount of Sedimite® fines expected to enter the river during normal and peak flow events.

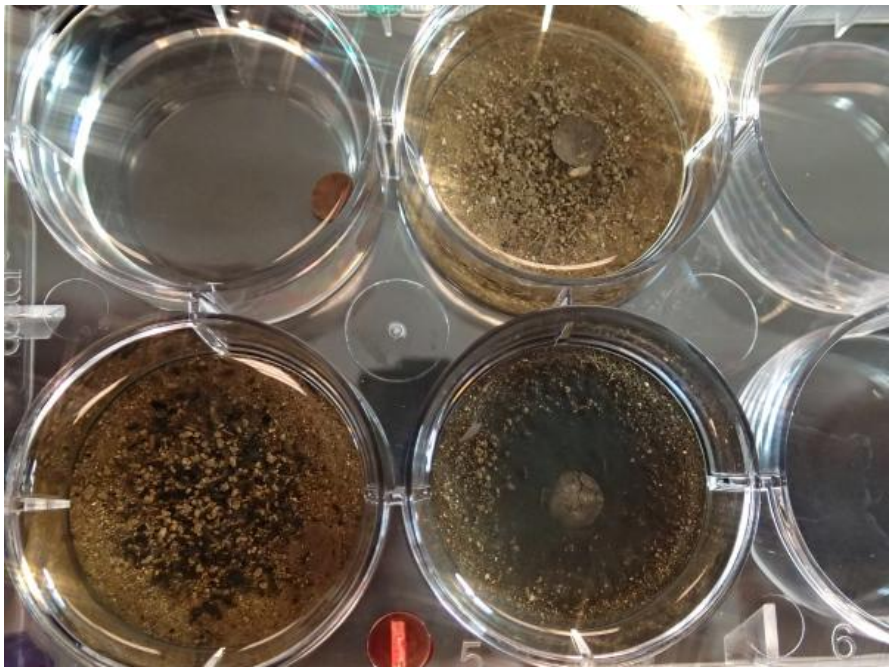

Figure 1. Photograph of one 6-well tissue culture plate with wells containing only one amphipod and leaf disc (top left); one amphipod, a leaf disc and unamended North Oak Lane sediments (top right); one amphipod, a leaf disc and biochar-amended sediment (bottom left); and one amphipod, a leaf disc and Sedimite®-amended sediment (bottom right).

Figure 2. Photograph of leaf disc and amphipod in well after 10 days of feeding. Most of the leaf material has been eaten away between the leaf veins and rendered to small fecal pellets.

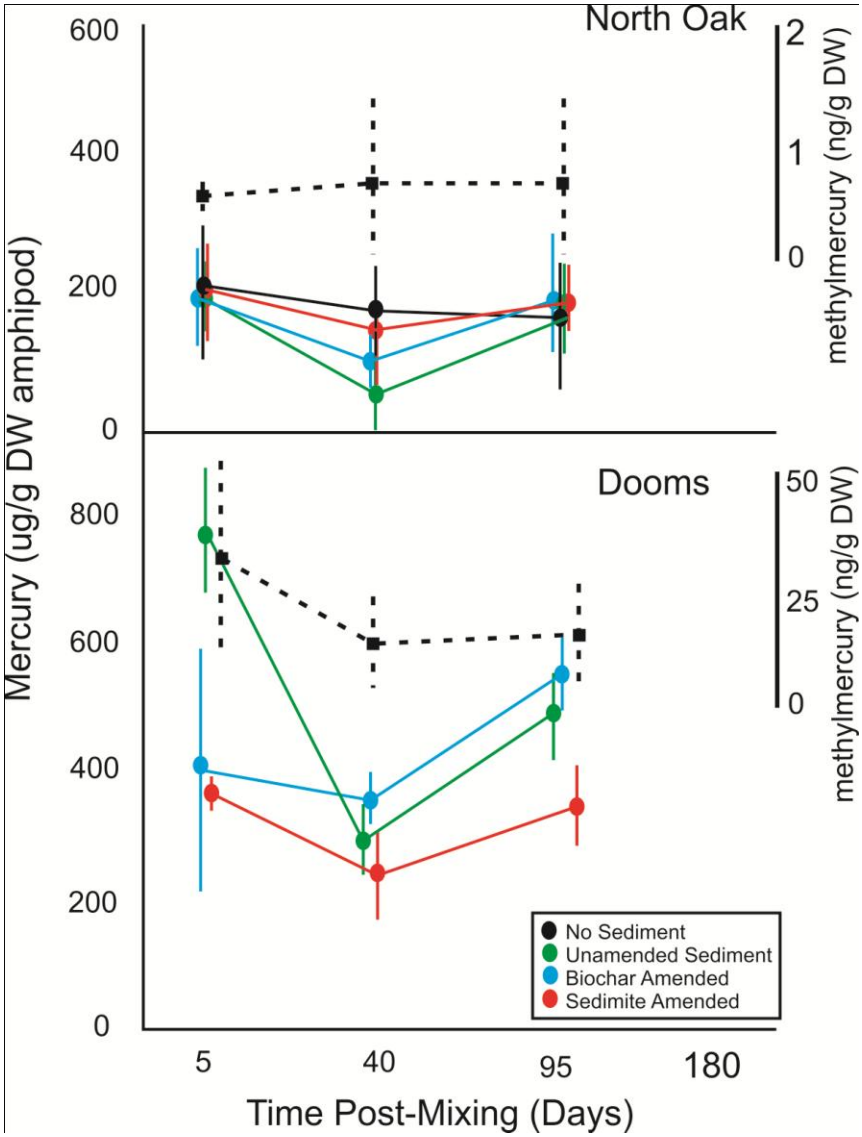

Figure 3. Mercury concentrations in amphipods from each treatment. Top panel = North Oak sediment assays; Bottom panel = Dooms Crossing sediment assays. Error bars are 95% confidence intervals of means. Generally, if the error bars for two treatment means just touch, then the two means are significantly different with a p-value of approximately 0.01. If the error bars for two means overlap by 50% or less, the means are significantly different with a p-value of 0.05 or less. In all other cases, the only logical inference that can be made is that no evidence was produced that contradicts the null hypothesis of no difference. Methylmercury concentrations in the assay sediments are also shown (black square with dashed line). The methylmercury concentrations did decrease in the Dooms sediment/sediment mixtures after the first assay but not in the North Oak materials. The amount of mercury accumulating in the amphipods reflect this decrease.

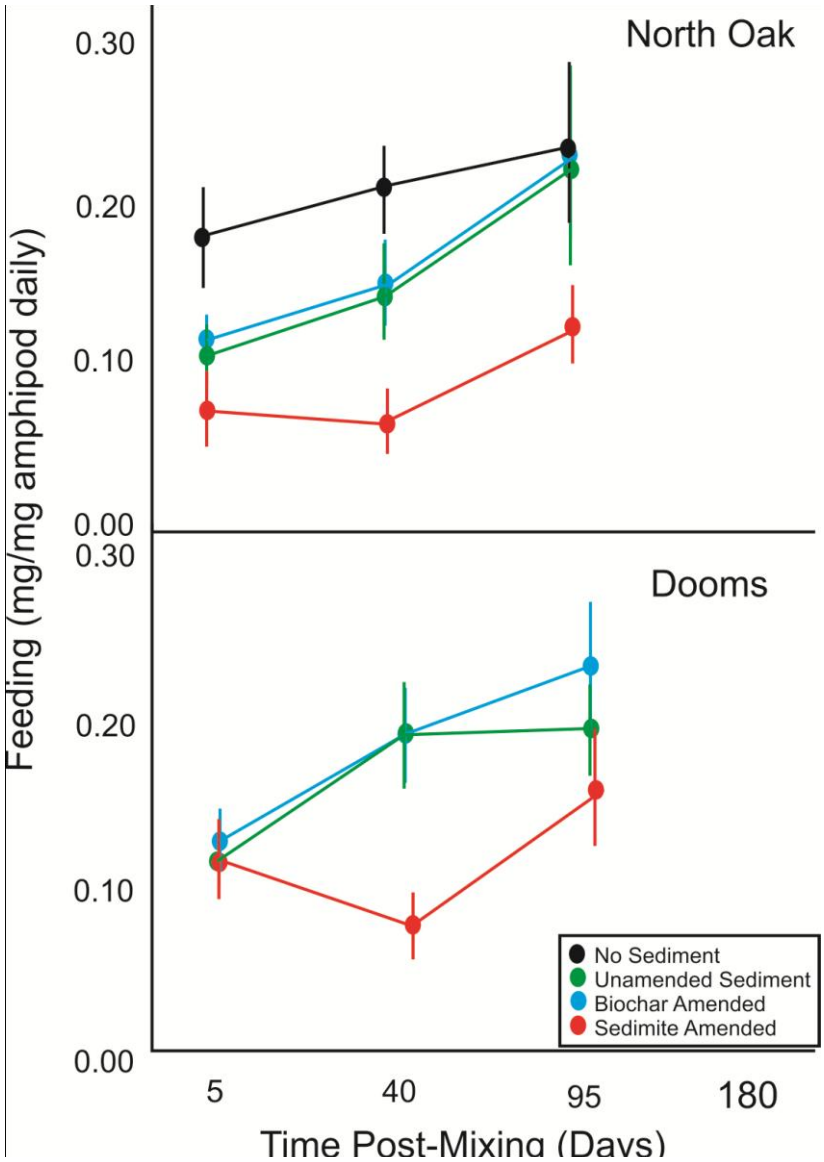

Figure 4. Amphipod feeding rates from each treatment (mg leaf/mg of amphipod daily). Error bars are 95% confidence intervals of means. Generally, if the error bars for two treatment means just touch, then the two means are significantly different with a p-value of 0.01. If the error bars for two means overlap by 50% or less, the means are significantly different with a p-value of 0.05 or less. In all other cases, , the only logical inference that can be made is that no evidence was produced that contradicts the null hypothesis of no difference.

Figure 5. Close-up photograph of a leaf disc partially covered with Sedimite® at the end of a 10-day assay. Normal amphipod bioturbation of sediments caused Sedimite® to cover the sediment surfaces and often the leaf disc.