Lowering Net Methyl Mercury Production in Lower East Fork Poplar Creek, Oak Ridge Reservation, Tennessee - 9155

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ABSTRACT

The U.S. Department of Energy's (DOE) Western Environmental Technology Office is supporting remediation efforts on the DOE Oak Ridge Reservation located in Oak Ridge, Tennessee through this study. MSE Technology Applications, Inc. (MSE) completed a series of literature reviews and bench-scale testing to further evaluate the mercury (Hg) problem in the Lower East Fork Poplar Creek (LEFPC) at Oak Ridge. The primary issue was the decrease in acid extractable mercury (HgT) levels in LEFPC water, while HgT levels in sunfish muscle tissue increased with distance away from the National Security Complex (NSC), despite extensive source control efforts and within downstream riparian zones. Furthermore, dissolved monomethyl mercury (d-MeHg) levels increase downstream from the NSC, especially during warm weather and/or high flow events.

MSE performed a series of mesocosm-scale tests that focused on lowering net MeHg production by bacterial, algal and fungal biofilm on or within sediments from LEFPC. A plexiglass and plastic flume-pump system was designed and constructed to simulate the water/streambed interface. Shakedown Test F1 involved spiking clean sediment and water from Hinds Creek with mercuric ion (Hg^{2+}) concentration of 20-nanograms per liter (ng/L) and periodically measuring d-MeHg levels relative to those in the Flume 2 (control). Treatability testing of the LEFPC sediments proceeded from the results of the shakedown testing. Small quantities of 10-milligrams per liter (mg/L) of L-cysteine or EHC-M, from Adventus Group, were added periodically to treatment Flumes 1 and 3. The EHC-M results from Test F3 were further evaluated in Test F4.

Neither 0.45-micrometer (µm) filterable mercury (d-Hg) nor d-MeHg results for treated flumes in Test F1 differed statistically at probability (p) = 0.05. However, both parameters were slightly higher in the treated flumes, that is 4.4 versus 3.2 ng/L and 0.21 versus 0.06 ng/L, respectively. Such differences indicate a subtle stimulation of net MeHg production. The d-Hg concentration was lower in the Test F2 Flume 2, even though all three flumes received equal additions of Hg^{+2} 20 ng/L. L-cysteine addition of approximately 10 mg/L had no effect on lowering d-MeHg levels. Application of two-tailed Student's t-test to pooled treatment Flumes 1 and 3 for the F3 test showed no differences at p = 0.05 for either d-Hg or d-MeHg. However, the d-MeHg trend indicated that Flume 2 was greater than Flume 1 greater than Flume 3. Thus, the hypothesis evaluated in the F4 test was that daily as compared to one-time addition of EHC-M could lower net MeHg production.

Levels of d-MeHg associated with the daily addition of 10-mg/L EHC-M remained low in Flume 3 of the F4 test, despite higher concentrations of d-Hg, relative to those in the F3 test. The d-MeHg levels in warm season samples at water quality monitoring station EFK6.3 typically range between 0.15 and 0.25 ng/L. However, apparent matrix interferences diminished the quantity, if not quality, of the EPA Method 1630 results for EHC-M treatment. Furthermore, d-MeHg concentrations associated with the daily addition of 20-mg/L EHC-M in Flume 1 imply that such dosing increases net MeHg production by an unknown mechanism. Therefore, further assessment of the 10-mg/L EHC-M treatment would be

necessary before proceeding with a field-scale evaluation of this material for Hg-related remediation of the LEFPC watershed.

A complete account of the mesocosm-scale treatability study is summarized in *Technical Support Document Number 11- Investigation of Increased Mercury Levels in the Fisheries of Lower East Fork Poplar Creek* [1].

INTRODUCTION

Over the past 12 years, Hg source control actions within the East Fork Poplar Creek (EFPC) watershed have resulted in decreased concentrations of aqueous phase inorganic Hg with increasing distance from the Y-12 NSC. During this time, Hg levels in muscle tissue of adult redbreast sunfish (*Lepomis auritus*) sampled from within the NSC had declined and stabilized at concentrations slightly above 0.7 microgram per gram (μg/g) wet weight. Furthermore, Hg levels in sunfish residing in the lower reaches of EFPC are similar to those observed within the NSC, which implies that the bioavailable species of aqueous Hg are not decreasing downstream from the NSC [2].

Over the past 2½ years, MSE has evaluated nutrient-microbial-Hg methylation relationships by conducting a series of static, small volume, 250 milliliter (mL) bench tests. The results indicated that less than or equal to 20 ng/L concentrations of d-Hg can produce d-MeHg levels of environmental concern at greater than or equal to 0.3 ng/L. Despite the artificial nature of the experimental design, this observation correlates with observations made at monitoring station EFK6.3 within the LEFPC watershed.

Attempts at lowering net MeHg production by the addition of less than or equal to 4-μg/g selenite (Se^{4+}) ion or less than or equal to 20-mg/L thiosulfate ($S_2O_3^{2-}$) ion were inconclusive. Such results were most likely confounded by the environmental metastability of these agents; both of these ions were probably converted to less effective species (i.e., selenate (Se^{6+}) and sulfate (SO_4^{2-}) for complexing with aqueous Hg^{2+} [3]. Thus, maintenance of effective dosing conditions with these ions may be problematic in actual conditions.

TEST OBJECTIVES

The major objectives for this project were to:

- Design and implement a recirculating flow, flume-type mesocosm that approximates the sediment-water interface, but not necessarily a stream bottom food web, existing at EFK6.3.
- Select and evaluate acceptably stable and benign treatment agents that may lower d-MeHg levels present in the water column.
- Perform literature review regarding theory and practice of constructing and operating the previously mentioned test apparatus.
- Provide literature-based rationale for selecting the types and respective concentrations of candidate treatment agents.
- Construct and evaluate performance of the prototype apparatus, to assure it meets volumetric discharge in cubic meters per meter (m^3/m) and flow meters per minute (m/min) specifications.
- Following a 7-day equilibration period, perform 30-day evaluations for each of the DOE-approved treatment agents.
- Evaluate, as quantitatively as possible, the test-specific bench and analytical laboratory data to assess the magnitude of lowering d-MeHg levels in solution.

EXPERIMENTAL DESIGN

MSE personnel obtained water, near surface sediment, and periphyton from the vicinities of the Hinds Creek and EFK6.3 monitoring stations as shown in Figure 1. These activities occurred one day before the start of each test series. Each test included one control and two treatment channels. Given the present test apparatus as shown in Figure 2, approximately 57 liters (L) of water, 57 kilograms (kg) of sediment, and 50g of wet periphyton were added to each flume at the beginning of each test; additional makeup water shipments occurred at 10-15 day intervals after the beginning of each test. These materials were shipped by overnight express to the MSE Mike Mansfield Technology Testing Facility located in Butte, Montana. Residual quantities of evaporation makeup water were kept sealed at room temperature and aerated prior to use. Samples of the as-received sediment and biofilm materials were tested for baseline Hg characterizations.

Fig. 1. Sampling site at EFK6.3.

Flume Design

The major components of the test apparatus as shown in Figure 2 included:

- a plexiglass channel with a 15.2-centimeter (cm) diameter head tank and a 100-cm test section;
- a 76-L capacity reservoir that received flow from the channel, where the water was aerated and then returned to the head tank by way of a 1/6 horsepower (hp) sump pump; and
- a 1000-watt metal halide lamp that delivered a quasi-solar spectrum at intensities approaching photosynthetic light saturation.

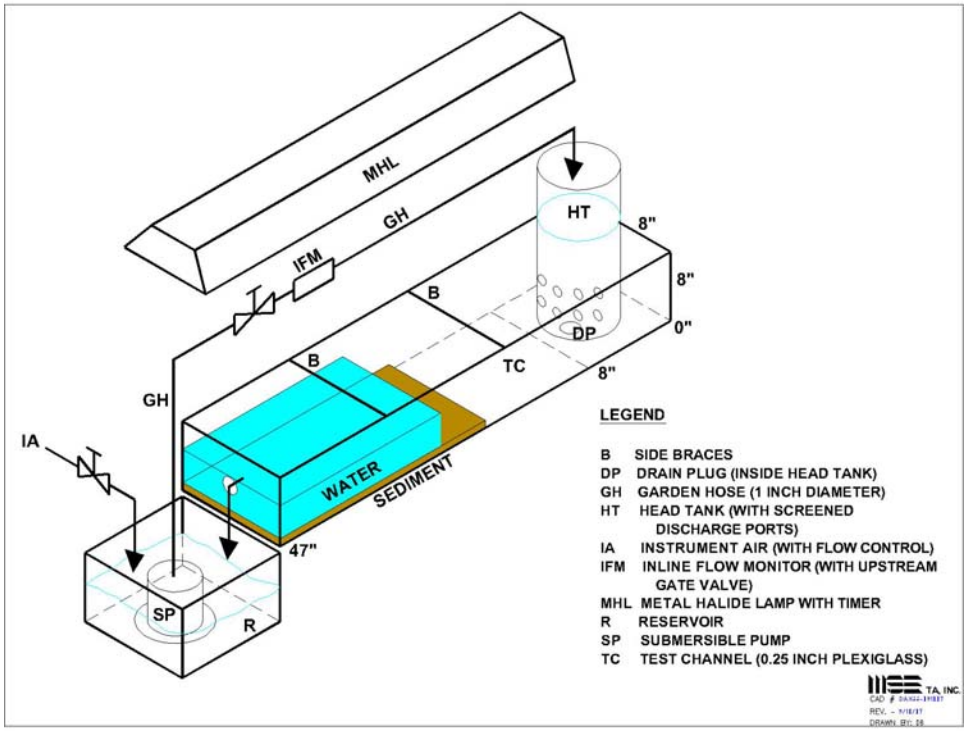


Fig. 2. Test apparatus.

The prepared channels were allowed to equilibrate for 7 days prior to addition of the appropriate treatment agent. Flume operations as set up for laboratory testing are shown in Figure 3. Creek water was circulated at about 42 liters per minute (L/min); the channels were subject to a 17 dark: 7 light photoperiod, with a lighting intensity of about 150-micromole (µM) photons per square meter per second. Given the variation in room temperature, plus heat input from the pumps and MHL, circulating water temperatures ranged from 21 to 30 degrees Celsius (°C) throughout each day. MSE recognized these values could exceed those for a typical summer day at EFK6.3, and may not be compatible with the watershed's warm water fisheries/biotic community. An immersion chiller was used when required to maintain water temperatures in the flumes below 30 °C.

During the first two tests, an effort was made to keep d-Hg levels in the range typical for LEFPC of 15-25 ng/L throughout the entire 37-day test interval. This was accomplished by as-needed additions of Hg^{2+} to the circulating water volume to stimulate contaminant release from upstream sources, and provide a continuous supply of bioavailable Hg for potential methylation by sulfate-reducing bacteria (SRB).

Fig. 3. Flume operations.

WORK DESCRIPTION

MSE performed a series of mesocosm-scale tests that focused on lowering net MeHg production by bacterial, algal and fungal biofilms present on or within sediments from LEFPC. A plexiglass and plastic flume-pump system was designed and constructed to simulate the water/streambed interface. Shakedown Test F1 involved spiking clean sediment and water from Hinds Creek with Hg^{2+} of 20-ng/L and periodically measuring d-MeHg levels relative to those in control Flume 2. Because of the positive test results, the work proceeded to treatability testing of LEFPC materials. Small quantities of 10 mg/L target concentration of L-cysteine or EHC-M were added periodically to treatment Flumes 1 and 3, while Flume 2 was used as the control. The EHC-M results from Test F3 were further evaluated in Test F4.

Insight regarding microbial community structure and function in sediments was obtained from functional gene microarray (GeoChip 2.0) results provided by the University of Oklahoma's Institute for Environmental Genomics [4, 5].

RESULTS

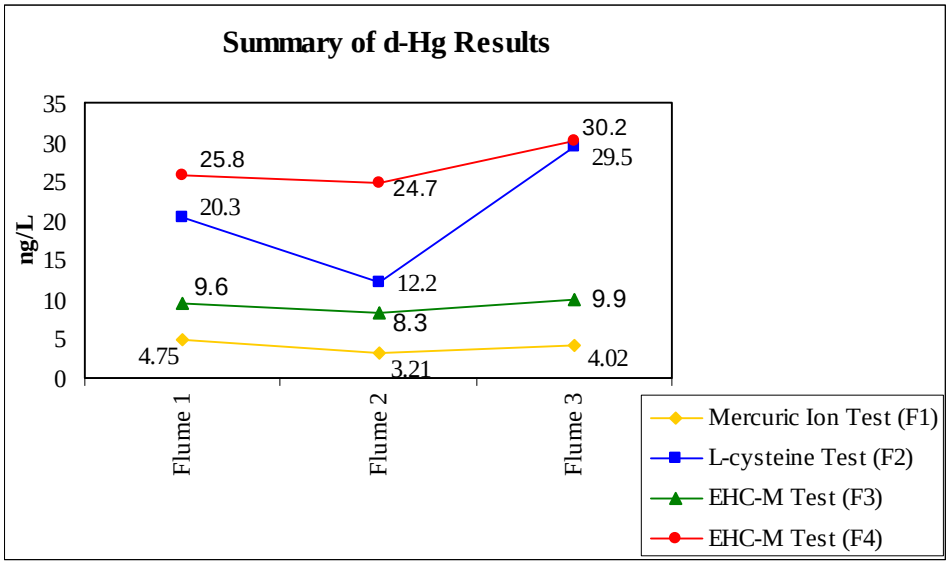
The results associated with each of the four test series are summarized below. Emphasis was placed on assessing changes in net MeHg levels and in microbial community-level gene expression over the course of each 30-day treatment period.

Mercury Treatability Assessments

Results for d-Hg and d-MeHg generated by this testing are presented in Table I and graphically represented in Figures 4 and 5. This data provided the basis for the following test-specific discussions.

Table I. Summary of d-Hg and d-MeHg Results

	Flume Number					
	1		2 (Control)		3	
Treatment Agent (Test)	d-Hg (ng/L)	d-MeHg ng/L	d-Hg ng/L	d-MeHg ng/L	d-Hg ng/L	d-MeHg ng/L
Hg^{2+} (F1)	4.75 ± 0.22	0.208 ± 0.084	3.21 ± 0.97	0.059 ± 0.048	4.02 ± 0.15	0.212 ± 0.266
L-Cysteine (F2)	20.3 ± 13.3	0.331 ± 0.099	12.2 ± 2.8	0.355 ± 0.121	29.5 ± 27.6	0.232 ± 0.155



EHC-M (F3)	9.6 ± 1.6	0.141 ± 0.101	8.3 ± 0.7	0.205 ± 0.087	9.9 ± 0.4	0.070 ± 0.103
EHC-M (F4)	25.8 ± 2.5	0.572 ± 0.767	24.7 ± 1.5	0.457 ± 0.069	30.2 ± 2.9	0.060 ± 0.004

Fig. 4. Summary of d-Hg Results

Fig. 5. Summary of d-MeHg Results

Hinds Creek Shakedown Testing (F1)

The objective of the F1 test was to raise "warm season" d-Hg levels commonly observed in Hinds Creek (5 ± 2 ng/L) to those more typical at LEFPC/EFK6.3 (20 ± 2 ng/L). This increase was to be achieved by the addition of mercuric chloride ion ($HgCl_2$) to Flumes 1 and 3 on days 0, 10, and 20 of the 30-day treatment period. The results indicated that:

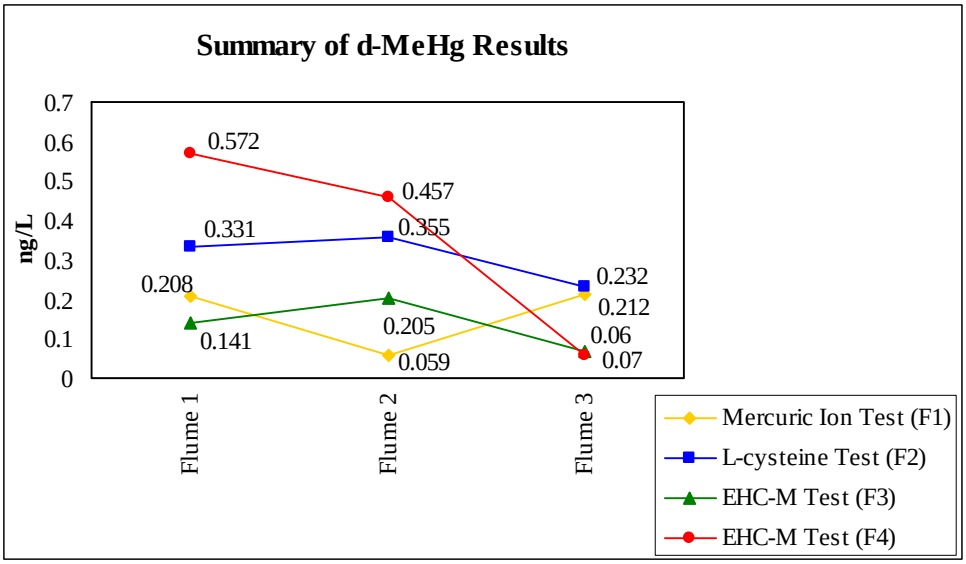
- the dosing regimen (i.e., Hg^{2+} concentration and/or interval) used was not sufficient for simulation of d-Hg conditions at monitoring station EFK6.3; while
- the spike(s) may have resulted in transient increases in net Hg^{2+} methylation activity in the treated flumes; although
- Student's t-test results did not indicate statistically significant (p less than or equal to 0.05) differences in either d-Hg or d-MeHg concentrations between the Hg^{2+} control and treated flumes over the duration of testing.

Such differences could be due to rapid reduction in the bioavailability of the added Hg^{2+}. Potential mechanisms include ad(b)sorption to inorganic and bio-organic particulate matter, and possibly photoreduction-evasion of aqueous phase elemental Hg to the atmosphere.

Although the test objective was not strictly met, the overall results indicated that flume design and operations more closely simulated the water column/sediment interface of lotic aquatic environments than did the previous flask-scale studies [3].

L-Cysteine Treatment of LEFPC Water and Sediment (F2)

The objective of the F2 test was to assess L-cysteine's ability to lower d-MeHg levels under warm weather conditions in LEFPC. All three flumes received Hg^{2+} spikes every five days during the 30-day treatment period, at the same concentration as for F1 testing. Reagent grade amino acid powder was



added every five days to Flumes 1 and 3 to achieve a 10-mg/L target concentration.

The results indicated that:

- decreasing the Hg^{2+} dosing interval from every ten days to five days did not raise d-Hg levels over the testing period. This was probably due to rapid immobilization and/or loss of Hg^{2+}; while
- two-tailed Student's t-test analysis of pooled treated versus control data sets showed no difference (at $p = 0.05$) for either d-Hg or d-MeHg levels.

Thus, intermittent dosing with L-cysteine at the 10-mg/L target concentration did not lower d-MeHg levels over the 30-day test period. It was difficult to understand the variability in d-Hg concentrations because acid extractable and leachable Hg levels in sediment were similar between treated and control flumes.

First Stage EHC-M Treatment of LEFPC Water and Sediment (F3)

The object of the F3 test was to assess whether the addition of EHC-M, added either daily or all-at-once during the treatment period, would lower d-MeHg levels under warm weather conditions at LEFPC. EHC-M treatment levels were as follows:

- daily addition of about 0.58 g powder (equivalent to 10 mg/L target concentration in solution) to Flume 3; versus
- spreading 17.4 g (i.e., $0.58 * 30$) powder throughout the sediment surface in Flume 1 on Day 0.

As application of Hg^{2+} appeared to have little effect on d-Hg levels, as discussed for tests F1 and F2, none of the flumes received Hg^{2+} spikes.

Application of two-tailed Student's t-test to pooled treatment versus control data sets showed no differences, at $p = 0.05$, for either d-Hg or d-MeHg levels. The difference in d-Hg levels between tests F3 and F4 represents natural variability over time, rather than indicating a spike effect in the F3 data. Despite lack of statistical differences in d-MeHg levels between treatments, the following concentration trend appeared: Flume 2 greater than Flume 1 (bulk addition) greater than Flume 3 (daily addition). The working hypothesis was that incremental addition of EHC-M produced a slow increase in SO_4^{2-} level(s) to the degree of lowering net MeHg production (i.e., MeHg demethylation rate greater than Hg^{2+} methylation rate). This concept served as the basis for follow-on evaluation of EHC-M treatment effectiveness.

Second Stage EHC-M Treatment of LEFPC Water and Sediment (F4)

Results of the F3 test indicated qualitatively that daily addition of 0.58 g EHC-M, to achieve a target concentration of 10 mg/L in solution, lowered d-MeHg concentrations to below those observed in the control flume. Therefore, the principal objective of the F4 test was to duplicate the F3 test. The other objective was to evaluate the effect of doubling treatment concentration to 20 mg/L on d-MeHg levels.

Application of two-tailed Student's t-test to the data indicated significant (p less than 0.05) differences in both d-Hg and d-MeHg levels between Flumes 2 (0 mg/L EHC-M) and 3 (0.58 mg/L EHC-M). Although d-Hg levels were elevated relative to those in F3, the F4 concentrations were of similar magnitude to those observed during the F2 L-cysteine testing. This observation supports the hypothesis that d-Hg levels at EFK6.3 can vary ± 100 percent of the average warm weather value of 20 ng/L (i.e., from 10-40 ng/L over this season).

More importantly, d-MeHg levels in EFK6.3 water treated with 10 mg/L EHC-M were generally less than 0.10 ng/L. Such concentrations were relatively low for warm weather samples collected from this particular water quality monitoring station, which typically range between 0.15 and 0.25 ng/L.

Importantly, matrix interferences have diminished at least the quantity, if not also the quality, of the EPA Method 1630 results for EHC-M treatment. Furthermore, the d-MeHg results for the 20-mg/L treatment imply that this addition may actually stimulate net MeHg production by an unknown mechanism.

Therefore, further assessment of the 10-mg/L EHC-M treatment is necessary, under better controlled flume operating and analytical conditions, before proceeding with field-scale evaluation of this material for Hg-related remediation of the LEFPC watershed.

GeoChip Technology Assessments

As net MeHg production is largely driven by microbiological activity, assessment of remediation progress would benefit from monitoring comprehensive changes in microbial community structure and function over the course of site-specific cleanup. This concept was evaluated during the present study by use of the GeoChip 2.0 technology, a form of FGMA developed jointly by Oak Ridge National Laboratory and the University of Oklahoma's Institute for Environmental Genomics (IEG).

Composite sediment samples from treated and control flumes were collected on days 1 and 30 of each of the first three tests. The sediments were prepared and analyzed by Drs. He, Zhou et al. at the IEG using standard biotechnology methods [5]. The test-specific results are summarized below.

Hinds Creek Shakedown Testing (F1)

Findings most relevant to bioavailable Hg treatment included:

- Hg^{2+} addition, at the 20-ng/L d-Hg target concentration, did not appear to stimulate either Hg resistance (merA) or dissimilatory sulfite reduction (dsrAB) activity.
- dissimilatory sulfate reducing (DSR) community function appeared to change with Hg treatment over time; and
- principal component analysis indicated that SO_4^{2-} ion exerted significant effect on overall community composition and function, as well as a relatively close association of dissolved organic carbon and SO_4^{2-} levels with d-MeHg concentrations.

The hierarchy analysis results also show a general trend wherein DSR and metal reduction and resistance (MET) gene expressions differ between the Hinds Creek and LEFPC/EFK6.3 sites.

LEFPC/L-Cysteine Testing (F2)

Findings most relevant to bioavailable Hg treatment included:

- L-cysteine addition, at the 10-mg/L target concentration, stimulated and maintained relatively high expression of carbon degradation, DSR, nitrogen reduction/denitrification, and organic contamination degradation (ORG) genes throughout the 30-day test period;
- the organisms associated with, and respective magnitudes of, merA gene expression did not appear to be affected by L-cysteine addition; and
- the persistently strong presence of tellurium resistant (Te^R) gene expression by *Rhodobacter sphaeroides* may affect the bioavailable Te-Hg interactions, and subsequently net Hg methylation activity.

LEFPC/EHC-M Testing (F3)

Findings most relevant to bioavailable Hg treatment included that:

- a bulk one-time addition of EHC-M produced a large, but short-lived, stimulation of overall microbiological activity that did not appear to affect net Hg methylation activity; and
- daily addition of EHC-M, at the 10-mg/L target concentration, resulted in modest increases in DSR, MET and ORG gene expressions that possibly lowered net Hg methylation activity.

FLUME OPERATING CONDITIONS

Extensive monitoring of in-flume environmental conditions throughout each of the four test series indicated that:

- heat additions from the submerged 0.17-hp pumps and 1000-watt metal halide lamp raised circulating water temperatures into the 27 to 29 °C range, with excursions up to 32 °C;
- the evaporative concentration of dissolved solutes over each 30-day test period (e.g., total dissolved solids increased to 140% of day-1 levels); while
- flume water height was affected by clogging of pump intakes and valves by fine sediment and algae.

Labor-intensive monitoring and application of a non-thermostat controlled immersion chiller and/or use of the ventilation fan, kept water temperatures generally below the 30 °C action limit. This limit is the assumed upper bound for temperature preference for aquatic biota residing in the Oak Ridge vicinity [6, 7]. Acclimatized organisms can tolerate a few degrees greater than 30 °C without significant physiological stress [8, 9]. Realistic bulk water chemistry was maintained by addition of site-specific makeup water to each flume's reservoir at least once per day throughout each test series, as documented in project data sheets and logbook. The pumps were shut down for less than or equal to 10 minutes at a time, when water height dropped by greater than or equal to 3.8 cm to clean the plastic mesh intake screens. The ball valves were adjusted for flow control to prevent sediment buildup in the valve seats. However, this did not prevent plugging of the associated flowmeters associated with Flumes 1 and 2.

Flume operations and temperature monitoring continued for 3.5 weeks after completing the F4 test on June 6, 2008. The raw data sheets are contained in MSE project files.

Evaluation of these results indicated that:

- room air temperature gradually increased along with flume water temperatures; and
- flume water temperatures were 5 to 6 °C higher than room temperature, despite efforts at water-cooling.

Water temperatures exceeding 34 °C for greater than or equal to 12 hours appeared to be stressful to the green algae (i.e., pigment losses), and adversely affected the native clams and snails in Flume 1.

Therefore, greater control of water temperature in the flumes would be necessary during the summer months to maintain water temperatures at approximately 5 °C below air temperature. This differential would also reduce evaporative losses of water from the reservoirs, and achieve uniform, and realistic, water chemistry throughout each test period.

CONCLUSIONS

The results document the achievement of the following project objectives:

- the flume-type microcosm simulates the sediment-water column interface present at Hinds Creek and EFPC;
- the effect of Hg^{2+} addition on bioavailable Hg methylation in an otherwise clean environment (i.e., Hinds Creek microcosm) ;
- the effectiveness of L-cysteine and EHC-M additions on net d-MeHg production in the LEFPC/EFK6.3 microcosm; and
- the use of GeoChip for monitoring gross changes in microbial community structure and function, in response to dosing with the above treatment agents.

Flume design was acceptable for performing short-term, screening-level Hg treatability studies. The Hg^{2+} concentration used and/or dosing interval applied did not elevate d-MeHg levels in the treated flumes, relative to d-MeHg concentrations observed in the control flume. However, these results indicated that low level Hg treatability studies can be performed at MSE's facility, without concern regarding background Hg contamination levels.

L-cysteine powder was added to the treatment flumes in sufficient quantity to initially achieve a 10-mg/L target concentration in solution; this level was maintained by repeated dosing every five days within the 30-day test period. Results to date indicate that this approach did not lower d-MeHg levels, relative to those seen in the control flume. However, L-cysteine's metastability in aqueous environments [10] can result in levels of degradation products that are insufficient to react with the various species of bioavailable Hg. Thus, the potential for L-cysteine's lowering of d-MeHg levels remains, albeit at higher and steady concentrations over time.

Daily addition of 0.58 g EHC-M powder, to create, and ideally maintain, a target concentration of 10 mg/L in solution, appeared to lower d-MeHg levels, relative to those concentrations seen in both the control flume and as commonly observed at LEFPC/EFK6.3. Treatment effectiveness was credible, given the presence of zero valent iron, SO_4^{2-}, and carbon of various solubilities; potential treatment mechanisms are presented in the project work plan [10]. However, the EHC-M treatment regimen tested appeared to exert severe matrix interferences on the conventional analyzed method (i.e., EPA Method 1630). Thus, future replication of this or similar work should consider "parallel" d-MeHg analyses using analytical methods based on different physicochemical principles or procedures [11].

Translation of the GeoChip data to biological results required an intensive, extensive review of the microbiological and microarray literatures. Once accomplished, interpretations of this data provided important insights into community-level biochemical functions in all sediment samples, despite lack of bioreplications of the analyses.

RECOMMENDATIONS

Certain upgrades to flume design and operations should be completed before further treatability studies are performed at MSE. These changes include:

- installation of a thermostat controlled, recirculating chillers in each reservoir;
- continuous delivery of treatment agent stock solution, via a peristaltic pump, to the appropriate flume(s), at a rate that assures maintenance of the target concentration throughout the 30-day treatment period; and
- continuous addition of Hg^{2+} and/or aqueous elemental Hg stock solution, via a peristaltic pump, to all three flumes, to increase the likelihood of a continuous presence of bioavailable Hg species for subsequent methylation.

All of these modifications would enhance the flume's capability of simulating the creek(s). Shakedown testing would then document time course concentrations of HgT and d-Hg plus total and dissolved-MeHg. This data should be supplemented by bioavailable Hg^{2+} estimates in sediment extracts, using whole cell-based [12] or isolated enzyme-based [13] methods. Once relatively stable and representative season-specific concentrations are achieved for the watershed under simulation, treatability studies would commence.

Initial efforts should revisit the L-cysteine and/or EHC-M treatment concentrations used in the present study. Given the strong bonding between Hg and selenium (Se), the concept of organo-Se addition (e.g., as Se-rich yeast biomass) should also be considered [14, 15]. This work would progress to documenting treatment agent(s) and concentration(s) that indeed lower d-MeHg levels in LEFPC, at mesocosm scale. While then proceeding to field-scale studies, the flumes could be used to assess treatment mechanisms and kinetics. Such efforts would be accomplished with frequent analysis of Hg species (d-Hg, d-MeHg) and key treatment agent degradation products (i.e., aqueous sulfur, iron, nitrogen, and carbon species).

Given the difference in d-MeHg analytical results and lack of bioreplication for the GeoChip analysis, no rigorous assessment can be made regarding treatment agent-microbial community interactions and subsequent effect(s) on net Hg methylation. However, the GeoChip technology provides useful insight into microbial community function, structure, and response(s) to environmental change, due to treatment agent addition over time. Replication of analysis under more controlled experimental conditions would greatly improve the use of GeoChip in monitoring bioremediation progress and success. Quantitative polymerase chain reaction based identification of metals-tolerant species, especially those with the merA and merB gene, would increase the understanding of net MeHg production within the LEFPC watershed. Such information would allow assessment of dissolved elemental Hg production by merA species and MeHg production by merA plus B species [16], plus SRB-related taxonomic effects on Hg methylation rates [17]. A model approach for integrating such GeoChip and quantitative polymerase chain reaction data sets is discussed by Lee et al. [18]. Evaluation of net methylation by use of radiolabeled Hg tracers ($^{199}Hg^{2+}$ and $H_3C^{202}Hg^{+}$) [19] would support these treatability studies.

ACKNOWLEDGEMENTS

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